

## Research Article

# Dynamics of Cytokine Regulation of Immunopathology in Mice Infected with Type I RH and Type II ME49 *Toxoplasma gondii* Strains

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- Tissue pathology
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## Abstract

A variety of host and parasite factors determine the outcome of *Toxoplasma gondii* infection and mice are generally used as an animal model to study these outcomes. In the present study, we studied the immunopathogenic characteristics of type I RH and type II ME49 *T. gondii* parasites in MF1 mice by correlating the dynamics of the pro-inflammatory cytokines IFN- $\gamma$ , TNF- $\alpha$ , IL-18 and anti-inflammatory cytokines IL-4 and IL-10 in the blood and spleen tissue with the tissue pathology. All the mice died within 7 days post-infection with 100 tachyzoites of RH parasites however, the majority of mice inoculated orally with 10 cysts of ME49 developed a chronic infection and survived >84 days. Mice infected with RH parasites showed a strong Th2 response followed by a Th1 response characterized by extensive tissue damage. No IFN- $\gamma$  was detected in the blood during the early stages of infection by day 3 which then increased sharply on day 4 and remained high till day 7 when the mice died. All pro-inflammatory cytokines measured in the spleen tissue on day 3 post-RH infection showed an increase. In mice infected with type II ME49 *T. gondii* parasites, there was a significant increase in the pro-inflammatory cytokines in the spleen tissue during the early stages (day3-7) of infection however, in contrast to most earlier studies using BALB/c and C57BL mice, no increase in TNF- $\alpha$  and IFN- $\gamma$  was observed in the blood during the 1<sup>st</sup> week which then showed a marked increase during the 2<sup>nd</sup> week of infection. The cytokine profile switched to an anti-inflammatory Th2 response during the intermediate stages (day 14-28) of the infection characterized by chronicity of infection with minimal tissue damage. During the late stages (day 42-84) of infection, a pro-inflammatory Th1 immune response was observed, associated with marked tissue damage.

## ABBREVIATIONS

i.p: Intraperitoneal; *T. gondii*: *Toxoplasma gondii*

## INTRODUCTION

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii* parasite and has been found worldwide in nearly one third of the human population. The infection is generally acquired by ingestion of *T. gondii* tissue cysts present in the under-cooked meat or by ingestion of infectious oocysts passed into the environment by infected cat feces [1,2]. Serological studies show a considerable variation in the prevalence of Toxoplasma infection from 7.5 to 85% among different countries, different population and ethnic groups within the same country [1-3]. Recent estimates have shown a decrease in the prevalence of infection in the USA and Europe due to comprehensive health education and health care programs however, in sub-Saharan Africa the prevalence of *T. gondii* infection has increased, ranging

from 34% in Sudan to 75.4% in Nigeria, mainly in association with HIV [4,5]. A number of parasite and host factors determine the outcome of infection. The most important factors appear to be the mode of infection, parasite strain and host immunogenic characteristics [6,7]. The majority of infected immunocompetent healthy adults are asymptomatic however; it may cause serious destructive inflammatory consequences in congenitally infected infants [8] and in individuals with a weakened immune system [9].

Though *T. gondii* parasite structure has been shown to have a wide diversity but there is a growing evidence that the three genetically different type-I, type-II and type-III parasite strains may determine the outcome of infection and tissue pathology in the host. Type I strains are shown to be highly virulent and cause rapid death in mice, whereas the outcome of infections with type II and III strains depends on the challenge dose and genotype of the host[10-12].

A complex network of cytokines produced by interacting immune cells significantly contribute to immunity against the parasite, pathologic hypersensitivity reactions leading to tissue pathology and the course of infection in the host. Earlier, the studies have documented that the control of toxoplasma infection in mouse with non-virulent strains is achieved by a vigorous IFN- $\gamma$ -dependent Th1 cytokine response by Toxoplasma-specific T cells [13,14]. These cells either produce protective cytokines inducing toxoplasma activity or cytokines that are involved in T-cell proliferation in the infected organs [6]. In fact, there is growing experimental evidence that these two aspects of T-cell function may be differentially regulated in different organs by different strains of *Toxoplasma gondii*.

It is well documented that the genotype of the infecting parasite shows clear differences in mouse virulence and cerebral tissue pathology [12,15] however, its role is less clear in the tissue alterations of other organs. In the present study, immunopathogenic characteristics of Type I RH and Type II ME49 toxoplasma infection in MF1 mice were studied by correlating sequentially the kinetics of pro- and anti-inflammatory cytokines in the blood and spleen tissue with the tissue pathology during the course of infection.

## MATERIALS AND METHODS

### Animals

Two hundred stable outbred MF1 female mice at 6-8 weeks of age, weighing 25-30 g were provided by the Animal Resource Center at the Faculty of Medicine, Kuwait University, Kuwait. The original strains of mice were supplied by B & K Universal, the United Kingdom. The mice were housed in cages with sterile bedding of wood shavings and fed a standard pelleted diet (Special Diet Services, UK) and filtered tap water. They were kept under specific pathogen-free conditions before and throughout the study.

### Parasite strain

Both Type I RH and Type II ME49 *T. gondii* strains were maintained in BALB/c mice by serial intraperitoneal (i.p.) passage of *T. gondii* tachyzoites and cysts respectively. These strains originally were received from Dr. David Guy, Singleton Hospital, Swansea, UK in 2003.

### Experimental schedule

The MF1 mice were infected with different parasite concentrations of RH and ME49 *T. gondii* parasites to determine the challenge inoculum i.e. 100 tachyzoites of RH parasites and 10 cysts of ME49 parasites. The mice were divided into three groups: group 1, 75 mice inoculated i.p. with 100 RH tachyzoite; group 2, 75 mice inoculated with 10 *T. gondii* ME49 cysts by oral gavage; group 3-control group, fifty mice inoculated i.p. with PBS alone. All the three groups of mice were kept in separate cages.

### Tissue processing

As RH parasites cause lethal infection in mice so the blood and spleen tissue was harvested from the infected mice on a daily basis until all the mice died however, ME49 parasites cause a chronic infection so the specimen were taken from the

infected mice on day 3, 7, 14, 21, 28, 42, 56, 70, and 84 post-inoculation. At each time point, 3 infected and 3 control mice were sacrificed by cervical dislocation, blood was collected after cardiac puncture and spleen tissue harvested. Serum was stored in small aliquots at -80 °C until analysis. A part of the spleen tissue was immediately fixed in 10% neutral buffered formalin before further tissue processing and rest of the spleen tissue was homogenized and suspended in phosphate buffered solution (PBS) containing 10% Triton X-100 (Sigma chemicals Co., MO, USA). To ensure the spleen tissue cells are stimulated with the corresponding infecting antigen, the tachyzoite lysate antigen (TLA) was prepared from RH and ME49 parasites as described earlier [16]. Briefly, the TLA was prepared from RH and ME49 tachyzoites taken from the peritoneal aspirate of the infected mice. The tachyzoites were washed twice in RPMI 1640 and suspended at a concentration of  $2 \times 10^8$  tachyzoites/ml in distilled water. The suspension was sonicated at 4°C for 6 times. The sonicated antigen (TLA) was passed through a 0.45  $\mu$ m pore size filter and adjusted to a protein concentration of 25  $\mu$ g/ml.

The cells obtained from individual spleens of infected mice in each experimental group were suspended in RPMI 1640 containing antibiotics (Sigma Chemical Co., St. Louis, MO). Cells were placed in flat-bottomed wells of 96 well plates at cell density of  $5 \times 10^5$  cells in a final volume of 150  $\mu$ l and stimulated with the corresponding TLA. The spleen cells from uninfected mice were used as control. After 72 h of incubation, the plates were centrifuged at 1200 rpm for 10 min and supernatants collected and stored at -80°C for cytokine analysis.

### Cytokine analysis in blood and spleen tissue

The IFN- $\gamma$ , TNF- $\alpha$ , IL-4, IL-10 and IL-18 levels were determined in the serum and supernatant from the spleen tissue of the infected and control mice at the selected time points by colorimetric sandwich ELISA using commercial assay kits (eBioscience® Bender MedSystems GmbH, Austria) as described earlier [17]. Briefly, the cytokine assay plate layout consisted of a standard series in duplicate, four blank wells and 20  $\mu$ l duplicates of serum and tissue supernatant samples, and diluted to 50  $\mu$ l with Bioscience mouse serum diluent. The quantification of the assay was done by the use of a standard curve following manufacturer's instructions and presented in pg/ml. The sensitivities of detection in the ELISA were 31.3 pg/ml for IFN- $\gamma$ , IL-4 and 62.5 pg/ml for IL-10, TNF- $\alpha$  and IL-18.

### Histopathological examination of the spleen tissue sections

After chemical fixation, the spleen tissue was dehydrated by a series of graded ethanol (70%-100%) using an automated tissue processor (Shandon Citadel 2000™, Thermo Fisher Scientific Inc., USA) and embedded in paraffin using a histoembedder (Leica Instrument GmbH, Germany). Three-micrometer-thick spleen sections from each mouse were cut using a microtome (Microm HM 325, Thermo Fisher Scientific Inc., USA) and stained with hematoxylin and eosin (H & E). The sections were examined for inflammatory changes and tissue damage using a light microscope (BX61-TRF, Olympus Optical Co., Japan) with camera (DP70, Olympus Optical Co., Japan).

## Ethics statement

The study was reviewed and approved by the Faculty of Medicine, Kuwait University and Kuwait Institute for Medical Specialization (KIMS) joint Research Ethics Committee. The Committee reviewed experimental protocols involving animals as per international standardized ethical guidelines (Federation of European Laboratory Animal Science Association (FALASA).

## Statistical analysis

The standard Mann-Whitney U test was used for non-parametric comparison of median cytokine ratios, as the data were not normally distributed. Using SPSS 12.0.1 (SPSS Inc. Chicago, USA), comparisons were drawn between control and test mice groups, and between test groups. Differences were considered significant with  $p < 0.05$ .

## RESULTS

The mice inoculated i.p. with 100 RH tachyzoites became very sick by day 3, developed ascites and all the mice died by day 7-8. The mice infected orally with 10 cysts of ME49 parasites became sick and developed ascites 14 days post-infection however, they recovered a week later but continued to have mild ascites with actively motile tachyzoites till day 84. The spleen tissues harvested at the selected time points showed a significant increase in size and weight of the spleen between day 3-21 post-ME 49 infection, but thereafter the spleen size decreased gradually to about half the normal size. No significant change in spleen size was noted in mice infected with RH parasites.

### Cytokine kinetics in blood and spleen tissue

To study the immunopathogenic characteristics of the infection induced by RH and ME49 parasites, the cytokine kinetics of IFN- $\gamma$ , TNF- $\alpha$ , IL-4, IL-10, and IL-18 were analyzed sequentially in the blood and spleen tissue at each time point during the course of infection.

In mice infected with RH parasites, the cytokine levels in the blood were variable during the course of infection (Figure 1); no IFN- $\gamma$  was detected during the early stages of infection by day 3 but it then increased 100-fold on day 4 and further increased 1000-fold on day 5 and remained at that level till day 7 when the mice died however, no TNF- $\alpha$  was detected in the blood throughout the infection period. IL-18, IL-10 and IL-4 showed only a moderate increase during the early phase of infection (day 3 and 4), but then IL-18 level peaked during the later stages of the infection and remained high till day 7. IL-10 sharply decreased on day 4 and then remained at the same level till day 7 (Figure 1).

All pro-inflammatory cytokines measured in the spleen tissue on day 3 post-RH infection showed an increase; IFN- $\gamma$  showed the highest increase by 40-fold on day 3 followed by IL-18 (10-fold increase) and TNF- $\alpha$  (4-fold). The levels decreased gradually as the infection progressed but remained significantly higher than the base levels till day 7. Both IL-10 and IL-4 increased 2-fold and remained at that level throughout the infection (Figure 1).

As observed in mice infected with RH parasites, the mice infected with ME49 parasites also showed variable levels of the pro-inflammatory cytokines, IFN- $\gamma$ , TNF- $\alpha$ , and IL-18 in the blood

but with a different kinetics (Figure 2); TNF- $\alpha$  was undetectable during the 1st week of infection and then showed a >700-fold increase on day 14-21 and thereafter it was undetectable. The IFN- $\gamma$  level remained almost at the base level till day 28 and then it increased gradually by 20-fold ( $p < 0.01$ ) till day 72 and then dropped to the base level by day 84. IL-18 showed a 20-fold increase during the initial phase of infection (day 3-14) and then dropped gradually thereafter (Figure 2). The anti-inflammatory cytokine (IL-10, IL-4) levels in blood and spleen showed a milder 2-fold increase during the whole course of infection, however IL-4 showed a transient 30-fold increase in the blood on day 14 and 21 (Figure 2).

There was a significant increase in the pro-inflammatory cytokines (IFN- $\gamma$ , TNF- $\alpha$  and IL-18) in the spleen tissue by day 3 and 7 ( $p < 0.02$ ); TNF- $\alpha$  level peaked to almost 500-fold during the first 7-day period of infection. IFN- $\gamma$  and TNF- $\alpha$  showed a 5-fold drop on day 14 and remained almost at that level till day 84. However, IL-18 levels dropped gradually till day 84 (Figure 2).

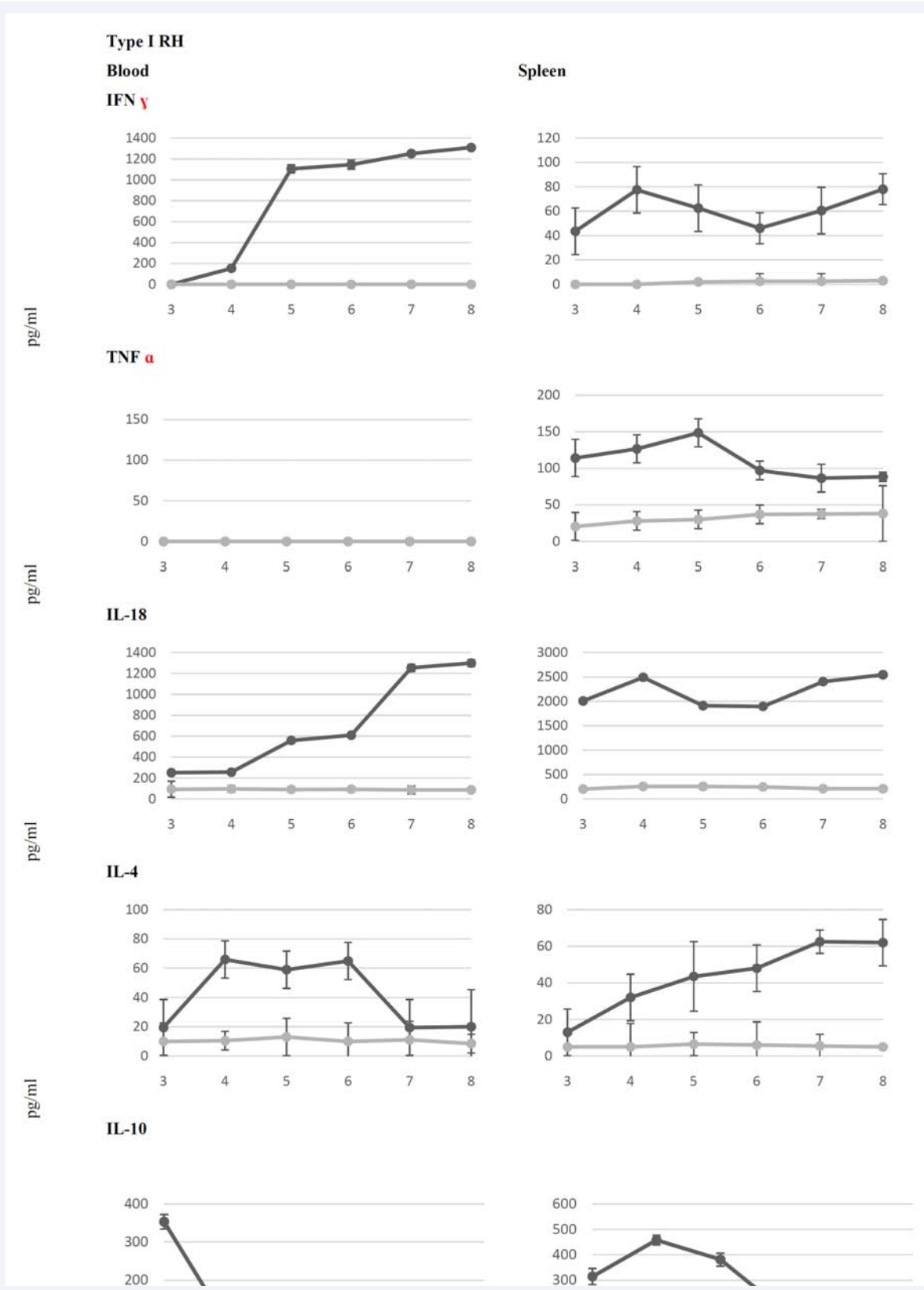
### Pro-and anti-inflammatory cytokine balance during the course of infection

The cytokine balance measured as a ratio of Th2/Th1 provides an indication of an overall cytokine response and thus an indication of Th1 or Th2 cytokine shift at a given time point during the course of infection. The cytokine response in the blood and spleen tissue was measured as a ratio of IL-10 to IFN- $\gamma$ , -TNF- $\alpha$  and -IL-18; a higher value was suggestive of an anti-inflammatory (Th2) cytokine response, and a lower value indicated a pro-inflammatory (Th1) response.

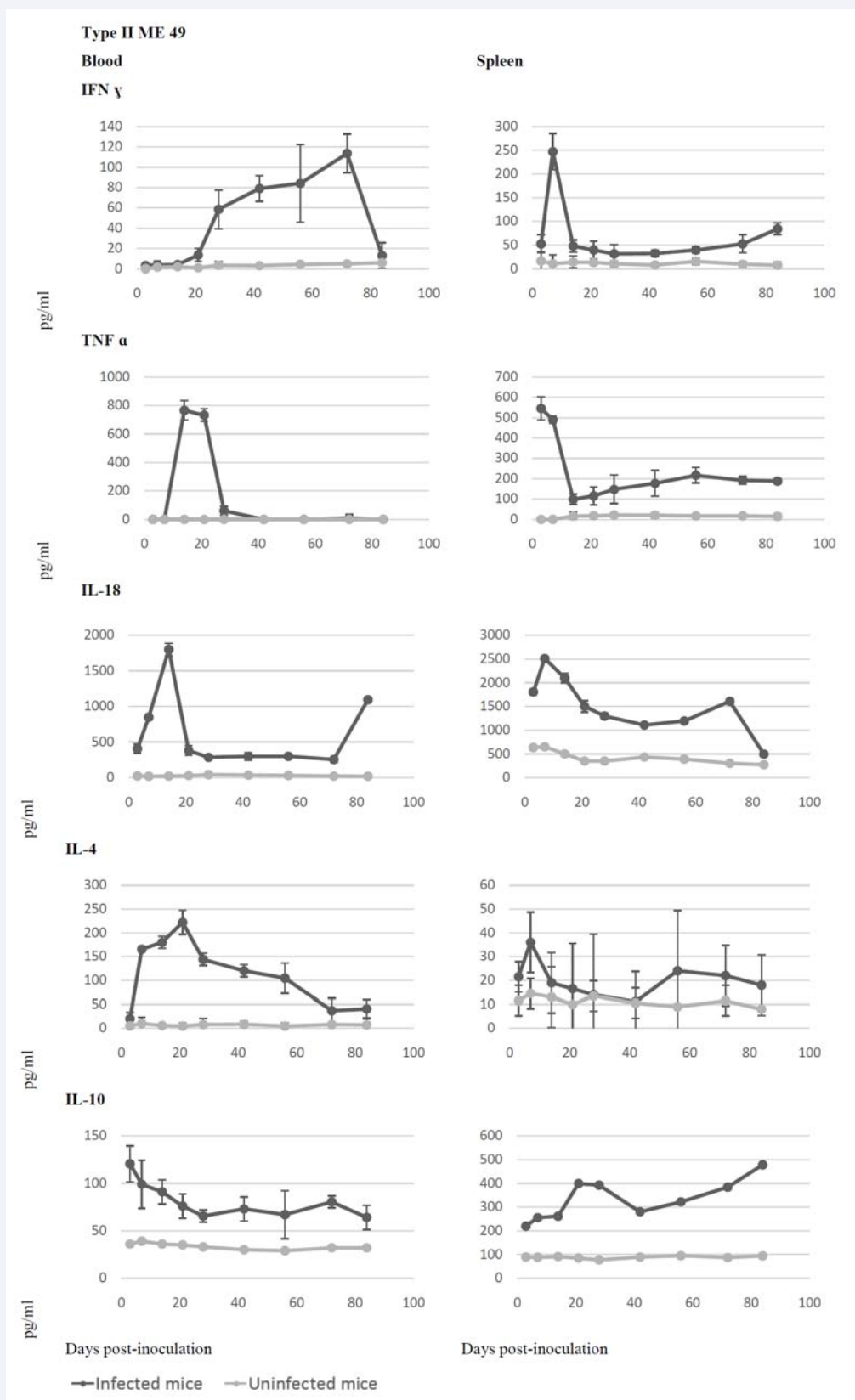
In mice infected with RH parasites, the IL-10/IFN- $\gamma$  ratio showed a strong Th2 response in the blood during the early stages of infection on day 3. The cytokine balance quickly shifted to a very strong Th2 response (>300) on day 3 to Th1 on day 4 which then lasted throughout the infection period till day 7 when the mice died. The IL-10/IFN- $\gamma$  and IL-10/TNF- $\alpha$  ratios in the spleen showed a Th2 response throughout the infection.

In mice infected with ME49 parasites, the IL-10/IFN- $\gamma$  ratio showed a biphasic pattern in the blood, a very strong anti-inflammatory Th2 response (>30.5 + 0.23) till day 21 which then gradually shifted to Th1 till day 72 and then shifted back to Th2 on day 84. IL-10/TNF- $\alpha$  ratios showed a very strong Th2 response (>80 + 0.77) during the early stages of infection (day 3-7) and then shifted to a strong Th1 response during the intermediate stages of the infection (day 14-28) which then shifted back to Th2 during the late stages of the infection (day 42-84). IL-10/IL-18 ratios showed a very strong Th1 response throughout the infection with a ratio of >50 on day 7-14.

However, a different pattern of cytokine response was observed in the spleen; the IL-10/IFN- $\gamma$  ratio showed a strong anti-inflammatory Th2 response throughout the infection, it peaked to >12.3 + 0.45 on day 28-42. However, the IL-10/TNF- $\alpha$  ratio showed a pro-inflammatory Th1 response during the early stages of infection at day 3-7 which then shifted to Th2 response on day 14 and thereafter till day 84. IL-10/IL-18 showed a predominant pro-inflammatory levels throughout the course of infection.



**Figure 1** *In vivo* production of cytokines (IFN-  $\gamma$ , TNF-  $\alpha$ , IL-4, IL-10 and IL-18) in blood and spleen tissue of MF1 mice following intraperitoneal inoculation of 100 tachyzoites of type I RH *T. gondii* parasites. The control mice were inoculated with sterile PBS. Blood and spleen tissues were harvested on a daily basis from mice infected with RH parasites till all infected mice died during the course of infection. The cytokines levels were measured by colorimetric sandwich ELISA. Three mice were sacrificed and samples collected at each time point. Values are the mean  $\pm$  SE of two independent experiments.



**Figure 2** *In vivo* production of cytokines (IFN-  $\gamma$ , TNF-  $\alpha$ , IL-4, IL-10 and IL-18) in blood and spleen tissue of MF1 mice following inoculation of 10 cysts of type II ME49 *T. gondii* parasites by oral gavage. The control mice were inoculated with sterile PBS. Blood and spleen tissues were harvested on day 3, 7, 14, 21, 28, 42, 56, 70 and 84 post-inoculation. The cytokines levels were measured by colorimetric sandwich ELISA. Three mice were sacrificed and samples collected at each time point. Values are the means  $\pm$  SE of two independent experiments.

## Tissue pathology

*T. gondii* infection led to a marked generalized lymphoid cellular changes and loss of tissue architecture of red and white pulp in the spleen however, no parasites were detected within the red or white pulp region.

In mice infected with RH parasites, the tissue damage was acute and severe with loss of cells in the white pulp regions and loss of tissue architecture. By day 3, areas of acute inflammation with loss of lymphoid cells were seen in the white pulp (Figure 3A), small apoptotic cells were seen to invade and destroy lymphocytes in the white pulp (Figure 3B). The pale area around the white pulp was narrow. By day 6 and 7, the white pulp architecture was completely lost and a large number of damaged lymphocytes were seen. The clear zones in the white pulp were almost equal to the regions containing lymphoid cells and drainage rate of the damaged lymphocytes from the white pulp to the red pulp was very high (Figure 3C & D).

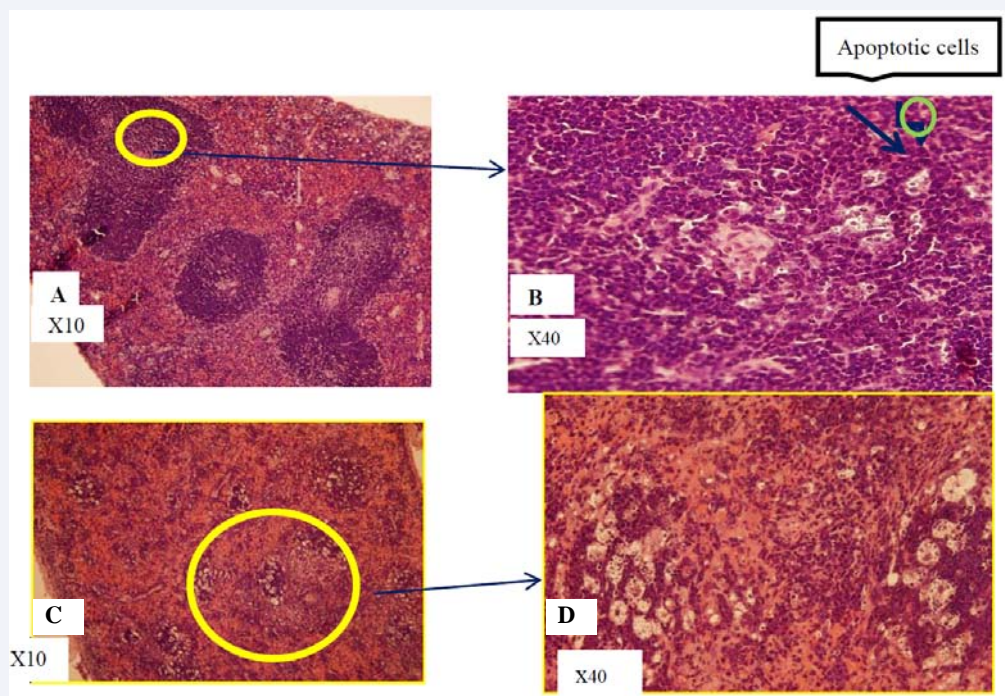
The microscopy of serial spleen tissue sections of mice infected with ME49 parasites showed lymphoid cell damage in the white pulp seen as clear zones appearing within the white pulp during the early stages of infection (day 3-14), the cell damage was considerably less during day 21-42 (Figure 4A & B). During the late stages of infection (day 56-84), the pale area around the white pulp decreased and there was a marked loss of cells in the white pulp, however aggregates of lymphoid cells were seen in the red pulp region and the tissue architecture was completely lost (Figures 4C & D).

## DISCUSSION

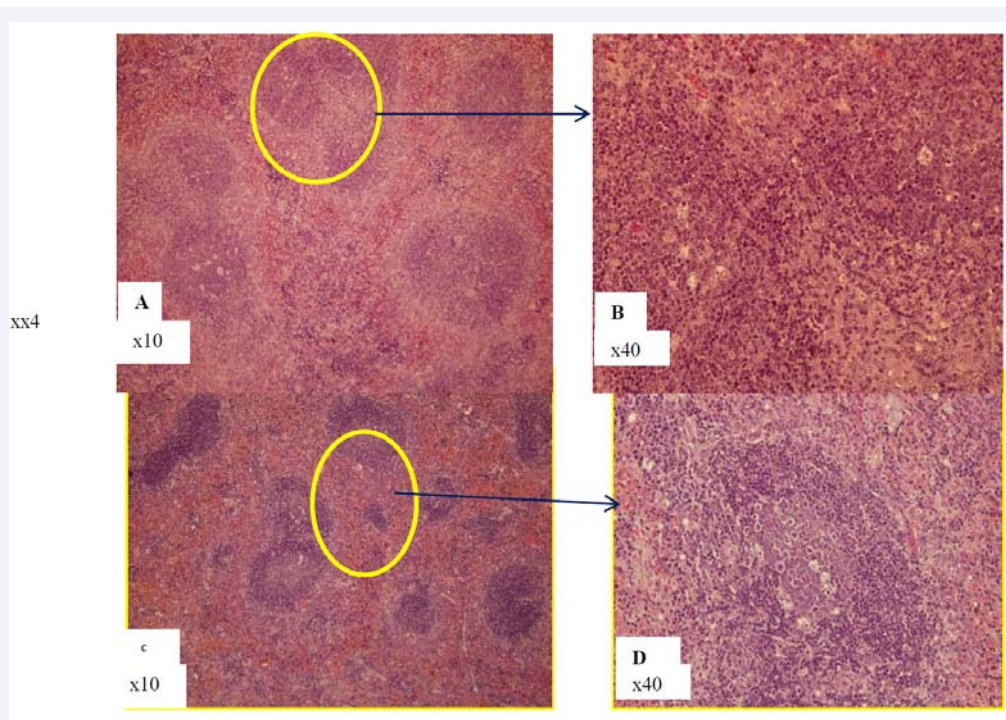
The virulence of different *T. gondii* strains is generally based on the outcome of infection in mice. Studies of cytokine profiles induced by different *T. gondii* strains have shown that while lethal infections were associated with over-induction of inflammatory cytokines, non-lethal infections elicited a modest cytokine production which led to infection control [18,19].

In this study, immunopathogenic characteristics of type I RH and type II ME49 toxoplasma infection in stable outbred MF1 mice were studied by correlating sequentially the production of pro- (Th1) and anti-inflammatory (Th2) cytokines in the blood and spleen with tissue alterations. Our study demonstrates that MF1 mice were susceptible to infection with RH and ME49 Toxoplasma parasites; i.p inoculation with RH parasites resulted in a rapid death of all mice within 7-8 days whereas oral infection with ME49 parasites led to a chronic infection with majority of the infected mice surviving >84 days .

The lethal infection with RH strain was associated with systemic induction of very high IFN- $\gamma$  levels in blood (1300 pg/ml) and IL-18 levels both in blood (1300 pg/ml) and spleen (2550 pg/ml). The host response to produce such increased levels of IFN- $\gamma$  and IL-18 is probably due to the ability of RH parasites to rapidly reach high tissue burden within 4 days, whereas ME49 parasites reach relatively low levels and takes much longer time. The reason for the dramatic difference in virulence between RH and ME49 strain, although unknown, is probably due to the underlying genetic differences between these strains [10].



**Figure 3** Light micrographs of spleen tissues, stained with hematoxylin and eosin, of MF1 mice infected with type I RH *T. gondii* parasites at day 3 (A & B) and Day 7 (C & D) post-infection. Three mice were sacrificed at the selected time point post-infection, spleen tissue harvested, chemically fixed and processed as described in materials and methods. Figures B & D are the magnified view of the area marked by the circle in A and C respectively. Areas of acute inflammation with loss of lymphoid cells and presence of apoptotic cells in the white pulp (arrow) are visible (B). There is loss of damaged lymphoid cells from the white pulp and complete loss of splenic white pulp architecture (D).



**Figure 4** Light micrographs of spleen tissues, stained with hematoxylin and eosin, from MF1 mice infected with type II ME49 *T. gondii* parasites at day 21 (A & B) and Day 84 (C & D) post-infection. Three mice were sacrificed at the selected time point post-infection, spleen tissue harvested, chemically fixed and processed as described in materials and methods. Figures B & D are the magnified view of the area marked by the circle in A and C respectively. Areas of lymphoid cell damage in the white pulp are seen as clear zones (A) and a marked decrease in pale areas around the white pulp (C) is visible. There is packed lymphoid cells in the red pulp and marked damage of splenic architecture is seen (D).

Interestingly, MF1 mice exhibited significantly higher ( $p < 0.01$ ) amounts of IFN- $\gamma$  and IL-18 cytokines in the blood compared to CD1 and BALB/c mice as reported earlier [10,20] however, both CD1 and MF1 mice showed similar mortality at day 7. Earlier studies and our data demonstrate that host-parasite genes interact in unpredictable ways in mice and that variation in pathogenicity of type I and II parasites depends in part on the strain of mouse.

In RH infection, TNF- $\alpha$  levels increased significantly in the spleen during days 0-5, then the levels decreased gradually till the last day of observation when the mice died. This marked decrease in the levels on day 5 and 6 was unexpectedly low as IL-10 which has been shown to be the dominant immunoregulatory anti-inflammatory cytokine preventing host immunopathology was also low at this time point. It may be due to the delayed production of TNF- $\alpha$  in the spleen tissue. IFN- $\gamma$  stimulates the macrophages to produce TNF- $\alpha$  [21]. Spleen tissue damage led to severe depletion of macrophages and lymphocytes in the spleen. In addition, it has been shown earlier that a significant fraction of IFN- $\gamma$  producing CD4 cells are also IL-10 producers [13]. Thus the low level of IL-10 may have led to slow IFN- $\gamma$  production thus allowing increased parasite multiplication and increased pathology. Mordue et al., 2001 had earlier reported that IL-10 level increased gradually to 450 pg/ml till day 7 in CD1 mice infected with RH strain.

However in marked contrast to RH infection, our results indicate that there was a minimal increase in IFN- $\gamma$  levels in blood during the first two weeks post-ME49 infection during which

the infected mice became lethargic, developed ascites and had enlarged spleen. However, IFN- $\gamma$  levels peaked 20-fold thereafter and the mice also recovered sufficiently supporting the major role played by IFN- $\gamma$  in the control of parasite multiplication [20,22]. The discrepancy in delayed increase of IFN- $\gamma$  levels in MF1 mice is probably due to the different host genotype and relatively low infecting dose of 10 cysts. In contrast to relatively delayed IFN- $\gamma$  production in the blood, the peak TNF- $\alpha$  levels occurred on day 14 and 21, consistent with most other studies which indicate an important role for these cytokines in resistance to *Toxoplasma* infection by inhibiting or controlling parasite multiplication [20], reducing hemorrhage, and promoting tissue repair [6]. Recently, we have documented that a significantly high TGF- $\beta$ 1 level in the blood as well as in the aqueous humour during the acute intra-ocular infection of MF1 mice with RH parasites may have adversely interfered with an effective immune response leading to an increased mortality and extensive ocular tissue damage [11].

The spleen is considered to be the major reticuloendothelial organ however, only a limited studies have determined cytokine levels in the spleen [23] and correlated it with tissue damage during the course of infection. Though we observed predominance of Th2 cytokines in the spleen tissue during the first three days of the post-RH parasite infection, however, there was hyper-induction of IFN- $\gamma$  and IL-18 causing gradual lymphoid cell death leading to spleen tissue damage. During the later stages of the infection, day 6-8, there was a strong pro-inflammatory Th1 response causing increased cell death

accompanied with extensive cell drainage and severe reduction in the white pulp size. There was a complete breakdown of the splenic tissue architecture leading to the death of all mice. IFN- $\gamma$  and TNF- $\alpha$  are the crucial mediators of *T. gondii*-specific immunity and are thus involved in resistance to *T. gondii* [24,25]. We observed a predominance of pro-inflammatory cytokines in the spleen during the early stage of infection (days 3-7) with ME49 infection which was associated with tissue damage and marked infiltration of lymphocytes causing an increase in the pale areas. The kinetics of IFN- $\gamma$ , TNF- $\alpha$  and IL-18 measured in the spleen were remarkably similar in mice infected with the ME49 strain during the first week with high levels which then dropped significantly during the 2nd week of infection. During the intermediate stage of infection (days 14-56), the Th2 cytokines dominated and the infection became chronic with a gradual increase in the infiltration of inflammatory cells and minimal tissue damage seen in the spleen. High levels (>2500 pg/ml) of IL-18 were detected in blood during the first week of infection. IL-18 is a pleiotropic cytokine secreted by various cells, including macrophages [26] and it has been shown that IL-18 is crucial for IFN- $\gamma$  production [13,27]. Thus the surge in IL-18 level observed during the first week of infection led to an increase in tissue IFN- $\gamma$  levels which is important for the control of infection during the initial stages of infection. IFN- $\gamma$  has been reported to be the major cytokine in resistance to *T. gondii* [2,28]. Our data showed high IFN- $\gamma$  levels in the spleen during the initial stages of infection (week 1-3) however, it is of interest to note the minimal increase in IFN- $\gamma$  levels in blood during the first two weeks post infection but, as the levels decreased in the spleen the IFN- $\gamma$  gradually increased in the serum and peaked during the late stages (days 56-70) of the infection. Mordue et al., 2001 detected the same kinetics in CD1 mice but the levels were relatively low compared to our study while much higher levels were detected in Swiss-Webster mice infected with ME49 [29]. This difference in IFN- $\gamma$  levels is probably due to the different genotype of the mice used in our study. A dominant Th1 response during the late stages of the infection was associated with increased cell death and tissue damage. Central to the host resistance is the generation of IFN- $\gamma$  by CD4 and CD8 lymphocytes and it plays an important role in immune protection however, it may also be pathologic when it is dysregulated [13]. The size of the white pulp was too small to induce significant Th2 response to down-regulate Th1 cytokines associated with complete loss of spleen architecture due to the susceptibility of MF1 mice to ME49 *T. gondii* strain.

## CONCLUSION

Our data shows that MF1 mice infected with type I RH parasites showed a Th2-dominant response followed by a Th1 response characterized by extensive tissue damage leading to a very high mortality of infected mice by day7 post-infection. However, mice infected with type II ME49 parasites showed a strong pro-inflammatory Th1 immune response during the early stages of infection which then switched to an anti-inflammatory Th2 response during the intermediate stages of the infection characterized by chronicity of infection and minimal tissue damage. During the late stages of the infection, a strong pro-inflammatory Th1 immune response was associated with marked tissue damage.

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