Chapter 2

Organ Pathology and Associated IFN-γ and IL-10 Variations in Mice Infected with *Toxoplasma gondii* Isolate from Kenya

John Mokua Mose, David Muchina Kamau, John Maina Kagira, Naomi Maina, Maina Ngotho, Lucy Mutharia and Simon Muturi Karanja

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Abstract

Toxoplasma gondii is an important foodborne opportunistic pathogen that causes a severe disease in immunocompromised patients. The pathology and immune responses associated with the ensuing disease have not been well described in strains from different parts of the world. The aim of the present study is to determine the IFN-γ and IL-10 variations and organ pathology in immunocompetent and immunocompromised mice infected with *T. gondii* isolated from a Kenyan chicken. Two groups of BALB/c mice were infected with *T. gondii* cysts and administered with dexamethasone (DXM) in drinking water. Other two groups: infected untreated and uninfected mice were kept as controls. The mice were euthanized at various time points: blood collected for serum and assayed for IFN-γ and IL-10 variations. After infection, significant ($p<0.05$) elevated levels of IFN-γ and IL-10 were observed. A significant decline in IFN-γ and IL-10 levels ($p<0.05$) was observed after dexamethasone treatment. Histological sections in the liver, heart, and spleen of the mice administered with DXM revealed various degrees of inflammation characterized by infiltration of inflammatory cells. The dexamethasone-treated mice presented with progressively increased ($p<0.001$) inflammatory responses is compared with the infected untreated mice.

Keywords: *Toxoplasma gondii*, dexamethasone, IFN-γ, IL-10, organ pathology

1. Introduction

Toxoplasmasmosis, cause by *Toxoplasma gondii*, is rated the most prevalent parasitic zoonotic disease infecting nearly 2 billion people in the world [1]. The infection may be acquired by oral ingestion of food or water contaminated with oocysts present in the feces of members of the cat family, the definitive hosts for *T. gondii.* Other routes of infections include ingestion of tissue cysts found in undercooked meat and congenitally by transplacental transmission [2].

Cases of toxoplasmosis have been reported in Kenya with the earliest study documented in 1968 [3]. Since then, *T. gondii* has been detected in the general Kenyan population as well as susceptible groups with reduced immunity. A serological survey of 127 children revealed a significant rise of prevalence of the *T. gondii*-specific antibodies from 35% in pre-school to 60% in the early school age group [4]. Screening results for blood donors at Kenyatta National Referral Hospital in Nairobi, Kenya indicated high seroprevalence [5]. Fifty four percent (54%) of HIV positive patients attending Kenyatta National Hospital, Nairobi had *Toxoplasma* specific IgG in contrast to 1% of the HIV negative group [6]. A clinical case report of toxoplasmosis was documented in a patient with HIV infection [7]. About 12.7% of hospitalized HIV positive patients with neurological complications at a private hospital in Nairobi, had *T. gondii* infection [8]. Co-infection of *T. gondii* and other parasites such as *Toxocara canis* has been investigated using samples from Kenyans. *Toxoplasma gondii* was detected in five of seven *T. canis*–positive sera from Maasailand [9]. Chunge and colleagues [10] showed that a moderate number of pregnant women attending a Kenyan referral hospital had *T. gondii* antibodies [10]. Such publications and clinical case reports show that there is widespread distribution toxoplamsois in Kenya.

Natural *T. gondii* infection has been detected in free-living and captive animals [11]. Of these 8 of 8 (100%) captive carnivores, 14 of 19 (74%) captive herbivores, 11 of 14 (79%) free-living carnivores and 97 of 118 (82%) free-living herbivores were found to have *Toxoplasma* antibodies. The detection of *Toxoplasma gondii* in free-range chickens is a good indicator of possible risk to human beings. In a study carried out in Thika region, Kenya, the prevalence of *T. gondii* in the chicken was 79.0% indicating high environmental contamination with *T. gondii* oocysts [12]. In another study carried out by Adele et al. [12] in Thika region, *Toxoplasma gondii* oocysts were detected in 7.8% of the cat samples collected. In the same region of Kenya, up to 39% of the slaughterhouse workers were infected with *T. gondii* as detected using nPCR [13]. Several studies have shown the circulation of various strains of *T. gondii* in Kenya, with the most abundant being type II strain [14, 15].

Infection of immunologically competent persons with *T. gondii* most often results in asymptomatic infection where the parasite forms tissue cysts containing bradyzoites in a variety of organs, particularly the brain, heart, and skeletal muscle. However, in immunosuppressed hosts such as those with AIDS, organ transplantation and radiotherapy, there is a high risk for severe infection [16, 17]. In these individuals, the bradyzoite gets reactivated and gets transformed to tachyzoites which cause severe pathology in the heart, liver and spleen [18]. Cellular immunity plays key role in the host's immune reaction against toxoplasmosis [19]. The macrophages and "natural killer" (NK) cells exert their function via a cytotoxic activity and/or the secretion of cytokines involved in the regulation of immune response [20]. *In vivo* studies have shown that IFN-γ is a major

cytokine, which is produced by CD4 and CD8 T cells, which mediates resistance against *T. gondii* infection [21]. Thus, IFN-γis the main type one cytokine involved in toxoplamosis, although other cytokines such as TNF-α, IL-18, IL-22, and the macrophage migration inhibitory factor (MIF) have also been reported in mediating the observed pathology [22]. As the disease progresses, some studies have reported that IL-10 counters the harmful effect of an exaggerated type-1 inflammatory response [23]. From the foregoing, it is clear that the development of a strong cellular immune response is critical for the control of the *T. gondii* infections in the intermediate hosts.

In a study carried out in Kenya, a neurological murine model of chronic toxoplasmosis in BALB/c was developed in BALB/c mice using *T. gondii* isolated from free range chicken [24]. The brain of toxoplasmosis infected mice showed cellular inflammatory infiltrations, neuronal necrosis, and cuffing. Other studies have showed lymphocytes and plasma cells to be the predominant cells in brains of patients having a coinfection of HIV and toxoplasmosis [25]. The severity of pathology was higher in mice immunosuppressed with dexamethasone compared to the control groups. The findings demonstrated that a dexamethasone-induced reactivation of chronic toxoplasmosis may be useful development of laboratory animal model in outbred mice used for in vivo studies.

Despite the fact that there is a high burden of toxoplasmosis and transmission in Africa [13], there are no studies which have evaluated the immunopathology of Toxoplasma isolates from these countries. Further, there is little information available regarding the immune responses inherent to reactivated toxoplasmosis. Acute and chronic infections in the neurological model described above [24] was associated with increase in both IgM and IgG levels but following dexamethasone treatment, IgM levels declined but IgG levels continued on rising. The current study therefore sought to determine the profile of IFN-γ and IL-10, and organ pathology in immunocompetent and immunosuppressed mice infected with *T. gondii* isolated from a chicken in Kenya.

2. Materials and methods

2.1. Laboratory animals and ethical clearance

Prior to commencement of the study, all protocols and procedures used were reviewed and approved by the Institute of Primate Research, Institutional Animal Care and Use committee (Approval number: IRC/21/11). A total of 84 female BALB/c white mice were obtained from the rodent breeding facility Institute of Primate Research, Nairobi, Kenya. The mice were 6–8 weeks old and weighed 20–30 g. The mice were housed under standard laboratory conditions, in plastic cages (medium size cages; length 16.9 inches, width 10.5 inches, and height 5 inches) with wood shaving bedding and nesting material. Food (Mice Pellets®, Unga Feeds Ltd., Kenya), and drinking water were provided ad libitum.

2.2. *T. gondii* isolate and expansion

The *T. gondii* isolate used in this study was obtained from the brain of a free range chicken from Thika region, Kenya [26]. Briefly, the hen was sacrificed by cervical dislocation and the

brain tissue collected under sterile conditions and processed for experimental infections. The brain was grounded and homogenized using tissue homogenizer. Enumeration of cysts was done as previously described [27], and the suspension was serially diluted with PBS (pH 7.2) to adjust to a desired final concentration of 15 tissue cysts/200 μl [24]. Three BALB/c mice were intraperitoneally injected each with 15 tissue cysts to allow for expansion of *T. gondii* cysts for use in experimental infection described below. The mice were monitored for 6 weeks post infection, euthanized using $CO_{2,}$ and parasites isolated as stated earlier.

Prior to commencement of experimental work, the presence of *T. gondii* in the chicken samples was determined by extracting DNA from the brain sample using a Quick-gDNA™ MiniPrep Kit (Zymo research, USA) and nested PCR undertaken as previously described [13, 26]. Secondary amplification products were electrophoresed on 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet (UV) light.

2.3. Experimental design

The BALB/c mice were intraperitoneally infected with 15 *T. gondii* cysts in a 200 μl inoculum [24, 28]. In the first part of the experiment, 32 infected mice in groups of four were randomly chosen and euthanized by concentrated CO_2 inhalation on 3, 5 and 7 dpi for acute infection and 14, 21, 28, 35, 42 dpi for chronic infection. Sixteen BALB/C mice were controls and not infected with *T. gondii*.. After euthanasia, sampling for blood from the heart was done as previously described [23]. The liver, heart and spleen were also collected and preserved in 10% formalin and used for histology as described below.

At 42 dpi, 48 BALB/c mice previously infected with 15 cysts each, were divided into three groups of 16 mice each. The mice were treated with Dexamethasone (Decadron DexPak PHARMA Links, India) at dosages of 2.66 mg/kg (Group 1) and 5.32 mg/kg (Group 2) daily in drinking water over a period of 6 weeks [24, 29, 30]. Sixteen infected nontreated mice were used as controls (Group 3). Another 16 uninfected control mice were given untreated water (Group 4). The mice were monitored daily over 6 weeks for survival analysis and any clinical signs and mortalities were recorded. After every 2 weeks, four mice from each group were serially euthanized using concentrated carbon dioxide and sampling done as previously described above. Mice that showed any severe clinical signs of toxoplasmosis were anesthetized immediately using concentrated carbon dioxide and sampling of blood, done. The liver, heart and spleen were collected and preserved in 10% formalin.

2.4. IFN-γ and IL-10 levels

Serum for cytokine activities was prepared as previously described by Parasuraman *et al.* [31]. Cytokine production was evaluated using commercial ELISA kits according to the manufacturer's instructions (MABTECH AB, Augustendalsvagen 19, Sweden). Briefly, each well of a 96-well high protein binding microtiter plate was coated with 100 μl/well of the respective monoclonal antibody diluted in PBS, pH 7.4 and incubated overnight at 4–8°C. The plates were washed twice with PBS (200 μl/well) and blocked by adding 200 μl/well of PBS with 0.05% Tween 20 containing 0.1% BSA (incubation buffer) and incubated for 1 hour at room temperature. Serum

samples or recombinant mouse IFN-γ and IL-10 standards were then applied to the plates, and incubated for 2 h at 37°C. After washing, the respective biotinylated monoclonal antibody for IFN-γ and IL-10 was added and the plates incubated for an additional 1 h at 37°C. One hundred microliters of Streptavidin-ALP was then added to each microtiter well and incubated for 1 h at 37°C. After washing, 100 μl of p-nitrophenyl phosphate substrate was added to each well and the optical density measured at 405 nm for pNPP in an ELISA reader after suitable developing time. Cytokine concentrations were determined by reference to standard curves generated with murine recombinant cytokines. The sensitivity limits of the assays were 20 pg/ml for IL-10 and 4 pg/ml for IFN-γ as per the instructions of the manufacturer.

2.5. Histological analysis

Liver, spleen and heart were processed for paraffin embedding and sectioning. To determine the histological changes, tissue sections were stained with hematoxylin and eosin and observed under light microscope. The inflammation was assessed and scored histologically. The severity of the histopathological lesions in the heart was evaluated by grading the lesions using a modified random scale as previously described [32].

In the liver, the inflammatory lesions were quantified based on the degree of lymphocyte infiltration and hepatocyte necrosis as previously described [33]. Segments of spleen were scored for the enlargement of lymphocyte infiltrated areas and for the increased numbers of macrophages and necrotic cells previously described [34].

In these organs, the inflammatory changes were examined in two noncontinuous sections (40 μ distance between them) from each mouse in 25 microscopic fields using a 40× objective. The total inflammation score was determined from the summed scores of each mouse from each group or sampling time point and used for data analysis.

2.6. Data analysis

The results were entered into MS Excel program (Microsoft, USA) before being exported to GraphPad prism version 5.0 (GraphPad Software, USA) for statistical analysis. Statistical differences between the mice groups were determined by ANOVA; groups were considered statistically different if $P \leq 0.05$.

3. Results

3.1. IFN-γ Levels

The mean of IFN-γ cytokine levels in the infected mice are as shown in **Figure 1**. There was a progressively significant ($p < 0.001$) increase in IFN-γ from 3.5 pg/ml (95%; CI: 2.93–4.07 at day 0 reaching 10.59 pg/ml, (95% CI: 9.03–12.15) at 35 dpi. The noninfected control group did not display any significant increase in IFN-γ cytokine levels and remained decreased at all time points compared to the infected group.

After treatment with dexamethasone, IFN-γ productions levels progressively declined at time points between 42 and 84 dpi. The decline in the 2.66 mg/kg/day of dexamethasone treated mice (Group 1) was from 17.84 pg/ml (95% CI: 1.60–34.08) at 42 dpi to 10.02 pg/ml (95% CI: 2.98–17.07) at 84 dpi (**Figure 2**).

The corresponding decline in the 2.66 mg/kg/day of dexamethasone treated mice (Group 2) was from 15.51 pg/ml (95% CI:−0.64–31.66) at 42 dpi to 7.89 pg/ml (95%; CI: 3.02–12.73.50) at

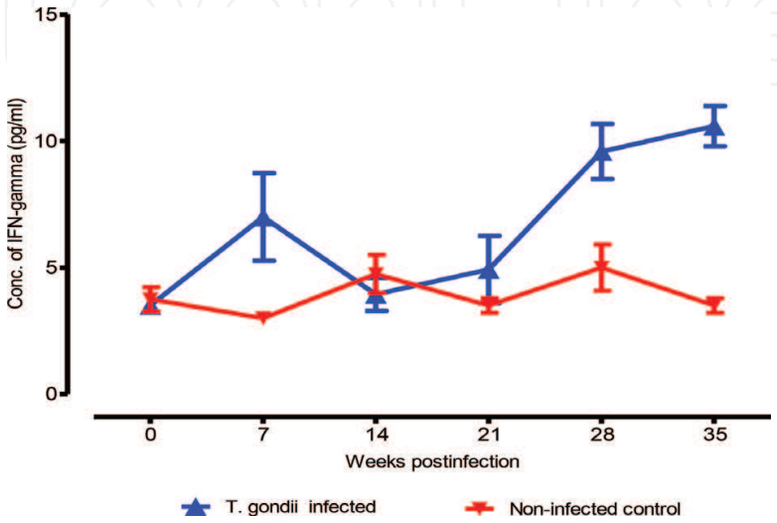

Figure 1. Levels of IFN-γ in serum of BALB/c infected with *T. gondii* during the early (7–14 dpi) and late stages (21–35 dpi) of infection. The data are expressed as the means ± SEM.

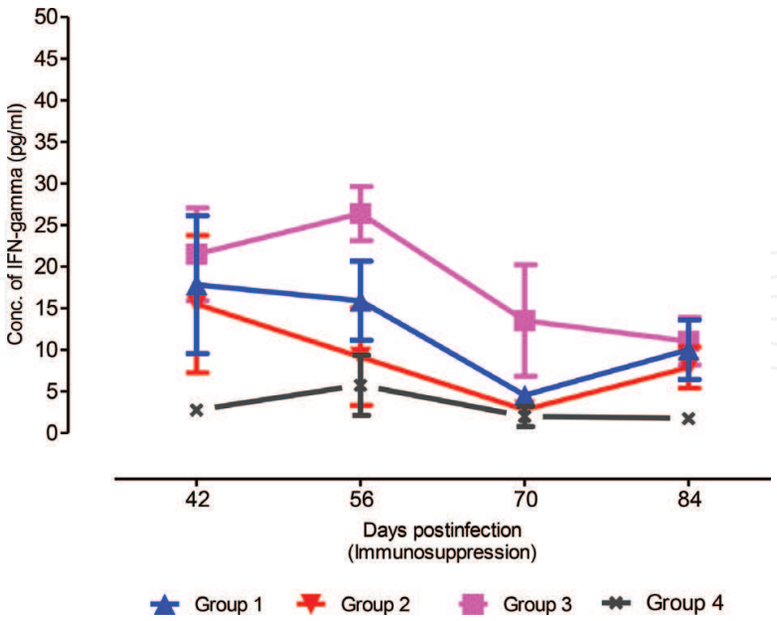

Figure 2. Mean levels of IFN-γ in serum in BALB/c infected with *T. gondii* and after dexamethasone treatment. The results are expressed as the means ± SEM of 4 mice. Group 1 = *T. gondii* infected dexamethasone treated (2.66 mg/kg/day); Group 2 = *T. gondii* infected dexamethasone treated (5.32 mg/kg/day); Group 3 = *T. gondii* infected; Group 4 = Noninfected control.

84 dpi. The decrease in IFN-γ levels was associated with increased dose, although the difference between the 2 doses were not significant ($P > 0.05$). The IFN-γ levels in the infected nontreated mice (Group 3) increased from 21.48 pg/ml (95%CI: 10.59–32.38) at 42 dpi to 26.38 pg/ml (95% CI: 20.01–32.75) at 56 dpi and thereafter, a progressive decline in IFN-γ levels reaching 13.53 pg/ml (95% CI: 0.42–26.64) and 11.03 pg/ml (95% CI: 5.43–16.64) at 70 and 84 dpi, respectively. Mice in the infected nontreated group (Group 3) maintained significantly ($P < 0.001$) increased levels of IFN-γ compared to the infected treated mice (**Figure 2**).

3.2. IL-10 levels

The levels of IL10 also increased following *T. gondii* infection. The levels significantly ($P < 0.001$) increased from 3.5 pg/ml (95%; CI: 2.93–4.07) at day 0 post-infection reaching 99.6 pg/ml (95% CI: 83.62–115.58) at 7 dpi and remained elevated up to day 35 dpi (119.6 pg/ml; 95%; CI: 106.27–124.45) (**Figure 3**).

Following dexamethasone treatment, the levels of IL-10 maintained a downward trend (**Figure 4**). In the mice treated with 2.66 mg/kg/day of DXM, the levels ranged between 135.66 pg/ml (95% CI: 82.79–188.54) at 42 dpi and dropped to 71.73 pg/ml (95% CI: 45.67–97.79) at 84 dpi. In the group treated with 5.32 mg/kg/day, the IL-10 level was 116.92 pg/ml (95% CI: 89.69–144.15) at 42 dpi and dropped to 55.59 pg/ml (95% CI: 40.77–70.43) at 84 dpi. The infected group (group 3) recorded a decreased IL-10 concentration ranging between 141.97 pg/ml (95% CI: 134.26–149.68) at 42 dpi and 99.71 pg/ml (95% CI: 77.16–122.27) at 84 dpi. Mice in the infected group recorded significantly ($P < 0.01$) elevated IL-10 levels compared to the treated groups at all time points.

3.3. Histological changes in the peripheral organs of BALB/c mice infected with *T. gondii*

In general, the histopathological changes in the liver, heart and spleen of infected mice consisted of mild-to-moderate congestion and detectable multifocal or focal inflammatory infiltrate. Between 3 and 14 dpi, the liver showed increased pathology characterized by hepatic necrosis, infiltration of lymphocytes and macrophages scattered in portal triad areas (**Figure 5**). The inflammatory scores increased from 1.2 (±0.49) at 3 dpi to 2.0 (±0.316) at 7 dpi. The highest inflammatory score was recorded at 14 dpi (2.8 ± 0.2) and thereafter, a progressive significant decline in inflammatory score ($P < 0.001$) at 42 dpi (1.4 ± 0.4) was observed.

Following dexamethasone treatment, the mice treated with 2.66 mg/kg/day (Group 1) and 5.32 mg/kg/day (Group 2) of dexamethasone showed varied degrees of inflammatory responses. For the mice treated with 2.66 mg/kg/day of dexamethasone, an inflammatory score of 1.4 (±0.245) and 2.0 (±0.00) was observed between 56 and 84 dpi, respectively, while the mice treated with 5.32 mg/kg/day (Group 2) of dexamethasone recorded an inflammatory score of 1.6 (±0.245) and 2.6 (±0.25) at 56 and 84 dpi, respectively. On the other hand, the infected nontreated mice presented an inflammatory score of 0.6 (±0.245) at 42 dpi but did not significantly ($P > 0.05$ change with the progression of the infection maintaining at 0.8 (±0.2) at 56, 70 and 84 dpi. Although the treated mice presented with progressively increased inflammatory scores there was no significant difference ($P > 0.05$) in the liver inflammatory response between the same groups.

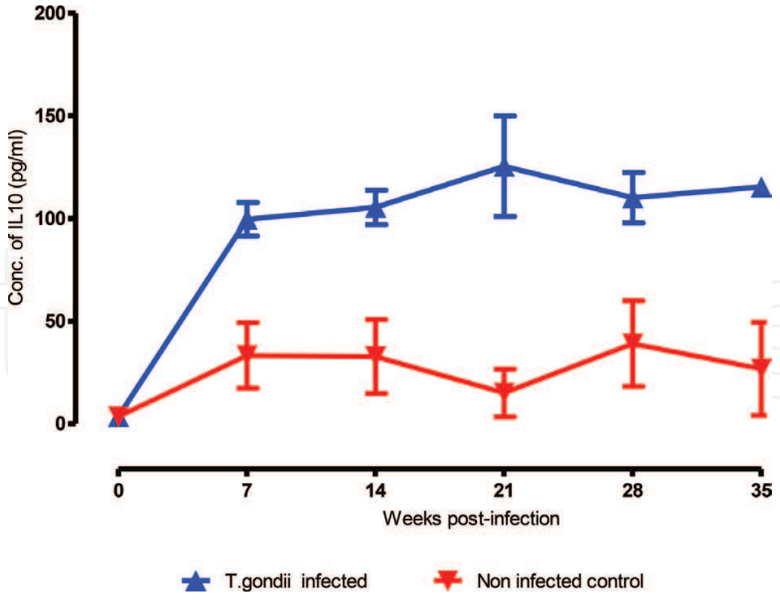

Figure 3. Mean levels of IL-10 in serum of BALB/c infected with *T. gondii* during the early (7–14 dpi) and late stages (21–35 dpi) of infection before treatment. The data are expressed as the means ± SEM.

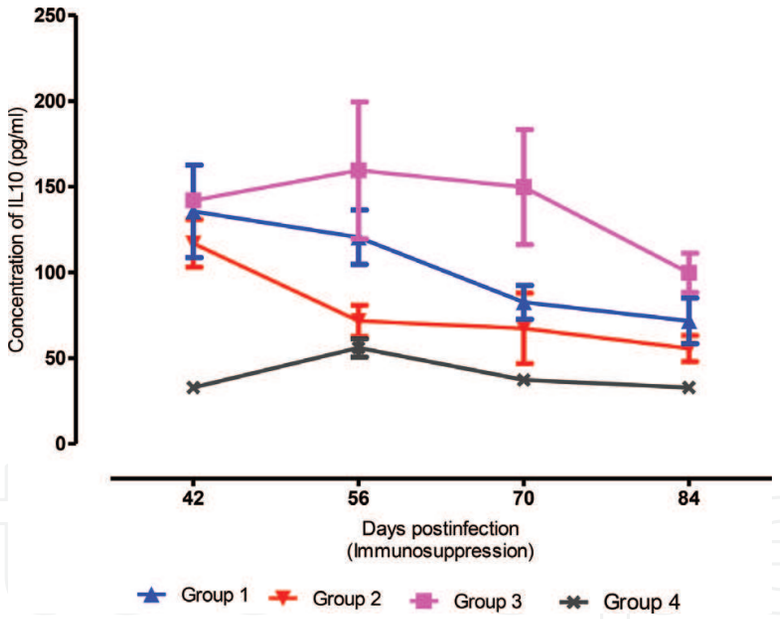

Figure 4. Levels of IL-10 in serum from BALB/c mice infected with *T. gondii* and after dexamethasone treatment. The data are expressed as the means ± SEM of 4 mice. Group 1 = *T. gondii* infected dexamethasone treated (2.66 mg/kg/day); Group 2 = *T. gondii* infected dexamethasone treated (5.32 mg/kg/day); Group 3 = *T. gondii* infected; Group 4 = Noninfected mice.

In the heart of infected mice, the histopathological lesions were relatively fewer compared to those in liver and were characterized by inflammatory infiltrates (**Figure 6**). The inflammatory score at 7 dpi was 1.75 (±0.25) and this was followed by a significant ($P < 0.001$) decrease reaching the lowest inflammatory score of 1.25 (±0.25) at 35 dpi. However, treatment with dexamethasone markedly increased the severity and number of myocardial lesions in these infected animals. The toxoplasma infected group (Group 3) presented with

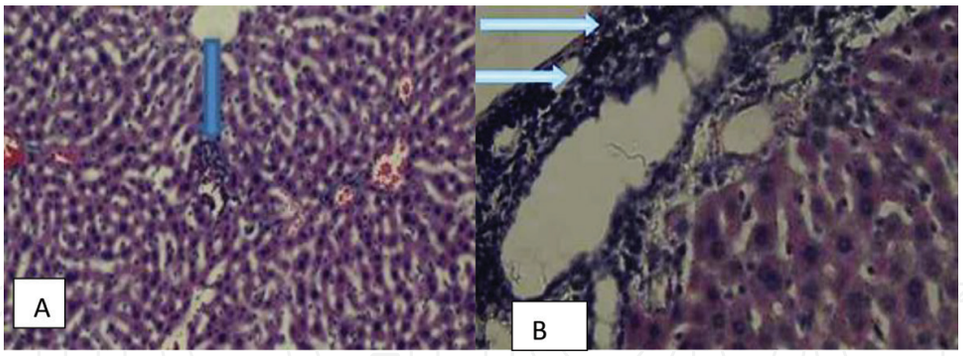

Figure 5. Liver of infected and treated mice showing dense granulomas, irregularly distributed (arrows) (A) and infiltrations of inflammatory cells (arrows) at the portal triad (B).

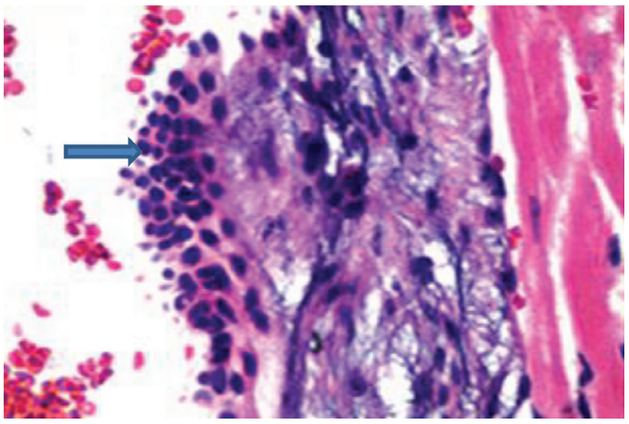

Figure 6. Heart of BALB/c mice showing inflammatory cell infiltrations (arrow).

higher inflammatory lesions at the time of treatment (day 42 dpi; $P < 0.01$). However, at 56, 70 and 84 dpi, an increasing inflammatory score was noted although there was no significant difference ($P > 0.05$). All the heart tissues of mice from group 1 recorded an inflammatory score of 1.25 (±0.25) at 56, 70 and 84 dpi ($P > 0.05$) while group 2 recorded a significant ($p < 0.01$) inflammatory score of 1.25 (±0.25); 1.5 (±0.289) and 2.5 (±0.289) at 56, 70 and 84 dpi, respectively. The uninfected control group (Groups 4) did not show any myocardial lesions at all time points.

The spleen was also affected by *T. gondii* but unlike the liver, the inflammatory response started from 5 dpi. The infected mice spleens from the infected treated mice presented general disorganization of the germinal centers at 70 dpi. The marginal zone disappeared and the limits between the disorganized germinal center and the red pulp were blurred. The noninfected mice spleens exhibited no change in the organizational of the germinal centers.

4. Discussion

In the present study, BALB/c mice infected with *T. gondii* showed that IFN-γ productions were markedly increased after *T. gondii* infection. This observation is consistent with a

previous study in mice by Gazzinelli *et al.* [35], where equally, IFN-γ levels were exceedingly elevated at the disease onset. Once released, IFNγ binds to the IFNγ receptor (IFN-γR), which eventually leads to the activation of IFN-γ signals "signal transducer and activator of transcription 1" (STAT1); [36]. These factors acts on macrophages and monocytes inducing the transcription of various genes involved in anti-parasitic responses including production of toxic reactive-oxygen species [37, 38]. The high levels of IFN-γ production levels are suggestive of its early involvement in parasite clearance [39]. The secretion of IFN-γ increases the phagocyte activity of macrophages and also triggers the conversion of tachyzoites into bradyzoites leading to chronicity [40, 41, 42]. The cytokine also prevents bradyzoite rupture, allowing long specific protection against new parasite infections and is hence responsible for regulation of *T. gondii* load and distribution in the tissues [43]. Although IFN-γ-dependent pro - inflammatory cytokines are essential for resistance to *T. gondii* infection, an over-production of inflammatory cytokine, IFN-γ can result in serious tissue damage [38] . Therefore, the intensity of the immune responses mounted against *T. gondii* just like any other infection must be regulated to avoid exaggerated immune-pathologic effects due to excessive inflammation.

In the current study, the IFN-γ levels were significantly depressed in the dexamethasone-treated *T. gondii* infected mice [44].). Dexamethasone administration have been shown to induce programmed cell death in developing lymphocytes. Harold *et al.* [45] has shown that dexamethasone is a potent suppressor of cytokine production in T cells. This drug, just like other glucocorticoids, act by binding to the glucocorticoid receptor, which blocks the expression of pro-inflammatory cytokines and adhesion molecules. Previous early studies done by Hunter *et al*. [20] showed that mice lacking T cells do not survive latent infection while depletion in T cells during the chronic phase or as a result of immunosuppression re-activates the disease [35].

In the current study, the IL-10 levels were also elevated during the acute and chronic infection and there was also a decline in immunosuppressed mice (42–84 dpi). This anti-inflammatory cytokine, has the ability to antagonize T helper 1 (Th1) responses [46]. IL-10 is considered to be an inhibitor of Th1 and Th2 immune responses [47, 48, 49]. Therefore, the role of IL-10 cytokines secreted by macrophages, monocytes, B cells, and CD4+ and CD8+ T cells during both the acute and the chronic phases of infection in both immunocompetent and immunosuppressed mice is to acts broadly on accessory cells and adaptive cells responses to downregulate or limit the consequences of an exaggerated inflammatory response and major histocompatibility complex and costimulatory molecule expression [20, 47, 50, 51]. This cytokine also prevents tissue immune destruction through immunomodulation [18] and has been identified as a factor induced by *T. gondii* infection [35, 52] that can contribute to the suppression of T cell function [53, 54].

Toxoplasma gondii infection caused different pathological manifestations as shown in this study. In the early infection, BALB/c mice displayed intense inflammatory lesions in the liver, heart as well as disorganization of the germinal centers of the spleen, suggesting a strong immune response in the pathogenesis of the disease. In the spleen, the white pulp appeared enlarged due to cellular proliferation and its limit with the red pulp started to disappear.

The detectable changes in the splenic architecture of the structures in the spleen of dexamethasone treated mice have been associated with a decreased ability to mount an immune response against the toxoplasma parasites [55]. Multiple mechanisms have been implicated in splenic disorganization, including $CD8^+$ T cell-mediated cytolysis of infected stromal cells or follicular dendritic cells and marginal-zone macrophages [56].

The results of the present study showed that chronically infected nontreated mice had an increase in mononuclear cells organ infiltrations upon infection. The recruitment of inflammatory cells as was the case in these organs, is one of the most important immune mechanisms induced by IFN-γ and is geared towards control of parasite multiplication. These cells could also be responsible for the higher levels of cytokines observed in the initial stage of *T. gondii* infection observed in the study. However, although there was a decline in the cytokine levels in the immunosuppressed mice, there was marked infiltration of mononuclear cells in the organs, resulting in myocarditis and hepatitis. This could be a reflection of reactivation and spread of toxoplasma parasites following decline in inflammatory response hindering the control and proliferation of the parasite [57].

5. Conclusions

The results of this study indicates that immunological and pathological features of *T. gondii* in immunosuppressed BALB/c mice mimic toxoplasmosis in immunosuppressed humans as it occurs during advanced HIV infection when CD4+ counts are low. The infection in immunocompetent host was associated with elevated IFN-γ and IL-10 which declined after immunosuppression. However, in both competent and immunocompetent mice, the pathological signs evident in the study were myocarditis, hepatitis characterized by mononuclear cell infiltration. Splenic exhaustion characterized by loss of normal spleen architecture also characterized the infection.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this book chapter.

Author details

John Mokua Mose[1,2], David Muchina Kamau[2], John Maina Kagira[3]*, Naomi Maina[4], Maina Ngotho[5], Lucy Mutharia[6] and Simon Muturi Karanja[2]

*Address all correspondence to: jkagira@gmail.com

1 Department of Medical Laboratory Science, School of Medicine and Health Sciences, Kenya Methodist University, Nairobi, Kenya

2 Department of Public Health, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya

3 Department of Animal Sciences, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya

4 Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya

5 Department of Animal Health and Production, Mount Kenya University, Thika, Kenya

6 Department of Molecular and Cellular Biology, Guelph University, Guelph, ON, Canada

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