The Expression of TLR2 and TLR4 in the Kidneys and Heart of Mice Infected with Acanthamoeba Spp.

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Research

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Abstract

Background: *Acanthamoeba* spp. are cosmopolitan protozoa that cause infections in the brain, as well as extracerebral infections in the cornea, lungs and skin. Little is known about the mechanisms of the immunological response to these parasites in organs which are not their main biotope. Therefore, the purpose of this study was to determine the expression of TLR2 and TLR4 in the kidneys and heart of *Acanthamoeba* spp. infected mice, with respect to the host's immunological status.

Results: In the kidneys, we observed a higher expression of TLR2 in immunosuppressed mice at 24 days post *Acanthamoeba* spp. infection (dpi) compared to the uninfected mice. There were no statistically significant differences in TLR4 expression in the kidneys between the immunocompetent and immunosuppressed mice, both of infected and uninfected mice. In the heart, we observed a difference in TLR2 expression in immunocompetent mice at 24 dpi compared to immunocompetent mice at 8 dpi. The immunocompetent *Acanthamoeba* spp. infected mice had higher TLR4 expression at 8 dpi compared to the immunocompetent uninfected mice.

Conclusions: Our results indicate that TLR2 is involved in response to *Acanthamoeba* spp. infection in the kidneys, whereas in the heart, both studied TLRs are involved.

Background

Acanthamoebiasis caused by protozoa of the *Acanthamoeba* spp. is an infection that is more frequently found in patients with low immune response. Development of this opportunistic infection is enhanced by chronic stress, coexisting diseases, and immunosuppressive drugs which are used to inhibit the rejection of transplanted organs [1]. It has been shown that corticosteroid therapy increases the number of parasites in the host [2]. Due to immunosuppression of the host organism, some parasitosis from asymptomatic or scarcely symptomatic leads to disseminated parasitic infections. These sudden multi-organ and multi-symptomatic changes may lead to the patient's death [3].

The *Acanthamoeba* spp. trophozoites enter into the tissue or organs and induce granulomatous amoebic encephalitis (GAE), *Acanthamoeba* keratitis (AK) or disseminated acanthamoebiasis [3, 4]. Disseminated acanthamoebiasis is predominantly confirmed *post-mortem* by histopathological examination of the organs and/or re-isolation of amoebae from tissue fragments [5] because biochemical and hematological tests are difficult to interpret; blood parameters may be elevated, lower or not deviating from the norm [6]. In our earlier study concerning hematological and biochemical profiles in the blood of experimentally *Acanthamoeba* spp. infected mice, we found only higher level of lymphocytes, monocytes, thrombocytes and aspartate aminotransferase [7, 8], despite the re-isolation of these amoebae from the brain, eyeball, lungs, kidneys, spleen and heart of the mice [7–11, unpublished data]. In another study concerning histopathological changes in the kidneys and heart of mice infected with *Acanthamoeba* spp., we observed some histopathological changes, including relaxation of muscle fibres, elevated proliferation of cellular nuclei in the proximal/distal tubule epithelium and areas with less acidic cytoplasm

[unpublished data]. Mechanisms of renal and heart invasion in acanthamoebiasis are still unknown. The immune response in renal and cardiac muscle cells to circulating antigens can be augmented by Toll-like receptors (TLRs), which play a key role in the nonspecific immune response, recognizing pathogen-associated molecular patterns (PAMPs) common to most pathogenic microorganisms [12]. The PAMPs on the membrane of *Acanthamoeba* spp. comprise proteins (33%), phospholipids (25%), sterols (13%) and lipophosphoglycan (29%) [13]. Moreover, TLR-signaling pathways may also be activated by components released by tissue damage or inflammation, or so-called damage-associated molecular patterns (DAMPs), and heat shock proteins HSP60 and HSP70, which are revealed in *Acanthamoeba* spp. [14]. The recognition of these patterns by TLRs initiates the migration and aggregation of immunes cells at the site of infection, which in turn leads to the development of inflammation [15]. TLRs activate signaling pathways that lead to activation of the transcription factors NF-κB and IRFs, which control the expression of genes encoding proinflammatory cytokines, such as TNF- α, IL-8 and IFN-γ [16, 17]. A lack of TLR2 and TLR4 on the immune cells leads to a delayed phagocytosis of pathogens, including bacteria such as *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* [18].

TLRs are involved in the response to several parasites, including *Acanthamoeba* spp. Alizadeh et al. [19] reported that *Acanthamoeba* spp. is recognized by TLR4, whereas other authors observed changes in the expression of TLR2 and TLR4 in the brain, lungs and eyes of *Acanthamoeba* spp. infected mice [11, 20, 21]. However, no data exist on TLR activation in the organs and tissues which are not the main biotope of these protozoans. Therefore, the purpose of this study was to determine the expression of TLR2 and TLR4 in the kidneys and heart of *Acanthamoeba* spp. infected mice, with respect to the host's immunological status.

Materials And Methods

Ethics statement

The study comprised organs from immunocompetent and immunosuppressed uninfected and *Acanthamoeba* spp. infected mice. Consents from the Local Ethics Committee for Scientific Experiments on Animals in Szczecin (No. 29/2015 of 22 June 2015) and Poznań (No. 64/2016 of 9 September 2016) were obtained to conduct the experiment on laboratory animals. All animal experiments were performed in strict agreement with good animal practice with the recommendations in the Guide for Care and Use of Laboratory Animals.

Animal model

Adult male Balb/c mice (6–10 months, ~ 23 g, Center of Experimental Medicine, Medical University in Białystok, Poland) were housed on a 12 h:12 h light/dark cycle under controlled temperature with free access to food and water.

The mice were divided into 4 groups:

- immunocompetent uninfected mice - immunocompetent control group (C, *n* = 18);
- immunocompetent *Acanthamoeba* spp. infected mice (A, *n* = 30);
- immunosuppressed by methylprednisolone sodium succinate uninfected mice - immunosuppressed control group (CS, *n* = 18),
- immunosuppressed by methylprednisolone sodium succinate *Acanthamoeba* spp. infected mice (AS, *n* = 30).

The experimental animal model has been described in previous research [9, 11]. Briefly, mice from groups A and AS were infected by intranasal inoculation with 3 µl of suspension containing 10–20 thousand strain AM22 *Acanthamoeba* spp., T16 genotype [22]. Mice from groups C and CS were given 3 µl of 0.9% NaCl. To suppress immunity, mice from groups AS and CS were intraperitoneally given (i.p.) 0.22 mg (10 mg/kg body weight) methylprednisolone sodium succinate (MPS, Solu-Medrol, Pfizer, Europe MA EEIG) dissolved in 0.1 ml 0.9% saline daily, for four days before inoculation with *Acanthamoeba* spp. [11, 23]. Euthanasia of the infected mice were at 8, 16, and 24 days post *Acanthamoeba* spp. infection (dpi). The animals were sacrificed with a peritoneal overdose of pentobarbital sodium (Euthasol vet, FATRO, Raamsdonksveer, The Netherlands) (2 ml/kg body weight) and subsequently necropsied. The virulence of the amoebae was determined by the degree of infestation. Fragments (5 mm × 5 mm) of the kidneys and heart were inoculated on NN agar and incubated at 41 °C to assess the infestation intensity [24]. The plates were monitored daily by microscope for 10 days at low magnification.

Expression of TLRs

TLR2 and TLR4 gene expression in the eyes was measured by quantitative real-time polymerase chain reaction (Q-PCR) carried out in a LightCycler real-time PCR detection system (Roche Diagnostic GmbH, Mannheim, Germany) using SYBR Green I as detection dye, and the target cDNA by relative quantification, using a calibrator prepared as a cDNA mix from all samples. The housekeeping gene porphobilinogen deaminase (PBGD) was amplified as the reference gene for mRNA quantification. The quantity of TLR2 and TLR4 transcripts in each sample was standardized by the geometric mean of PBGD transcript level; more analytical procedures are given by Wojtkowiak-Giera et al. [20]. The amounts of TLR2 and TLR4 mRNA are expressed as the multiplicity of these cDNA concentrations in the calibrator.

Immunohistochemical staining

Samples were fixed in 4% buffered formalin solution (Avantor, Poland) and were subsequently embedded in paraffin and cut into 4 µm sections. These sectioned tissues were deparaffinized in a microwave with citrate buffer (pH 6.0) to induce epitope retrieval. After slow cooling to room temperature, the slides were washed in PBS twice for 5 min and then incubated with primary antibodies overnight (4 °C). Immunohistochemistry was performed using specific primary rabbit polyclonal antibodies against TLR2 and TLR4 (Santa Cruz Biotechnology) at a final 1:500 dilution. Sections were stained with an avidin-biotin-peroxidase system with diaminobenzidine as the chromogen (DakoCytomation, Code K0679), according to the staining procedure instructions included. Sections were washed in distilled H_2O and counterstained with hematoxylin. For a negative control, specimens were processed in the absence of

primary antibodies. Positive staining was defined by visual identification of brown pigmentation using a light microscope (Leica, DM5000B, Germany).

Statistical analysis

Statistical analysis was performed using StatSoft Statistica v10.0 and Microsoft Excel 2016. Intergroup comparisons were performed using Mann-Whitney U tests. The significance level was $p < 0.05$.

Results

TLR2 and TLR4 expressions in the kidneys

In the kidneys of the immunocompetent *Acanthamoeba* spp. infected mice at 8, 16 and 24 days post infection (dpi), we found no differences in the TLR2 expression compared to the immunocompetent uninfected mice. The TLR2 mRNA expression level in the immunosuppressed *Acanthamoeba* spp. infected mice was significantly higher at 24 dpi compared to the immunosuppressed uninfected mice (0 dpi; $U = 24$, $p < 0.05$) and the level at 8 dpi ($U = 16$, $p < 0.05$; Fig. 1). Moreover, we found a statistically significant difference in the level of mRNA expression of TLR2 between the immunocompetent and immunosuppressed *Acanthamoeba* spp. infected mice at 24 dpi ($U = 20$, $p < 0.05$; Fig. 1). The TLR4 expression in the immunocompetent uninfected mice and mice at 8 dpi, 16 dpi and 24 dpi were similar. The TLR4 mRNA expression in the immunosuppressed *Acanthamoeba* spp. infected mice was significantly higher at 16 dpi and 24 dpi compared to the immunosuppressed uninfected mice, although the differences were not statistically significant. There were statistically significant differences in the expression of TLR4 between the immunocompetent and immunosuppressed mice at 16 dpi and at 24 dpi ($U = 12$ and $U = 15$, $p < 0.05$, respectively; Fig. 2).

Analysis of the immunohistochemical reaction results showed changes in the intensity of immunoexpression of TLR2 and TLR4 in the kidneys of *Acanthamoeba* spp. infected mice compared to uninfected animals (Fig. 3). It was noted that TLR2 was expressed in the proximal and distal tubules (white and black arrows, respectively; Fig. 3) and collecting ducts (red arrows; Fig. 3), while TLR4 was expressed in the proximal and distal tubules (white and black arrows, respectively; Fig. 3), collecting ducts (red arrows; Fig. 3) and in the renal corpuscles (yellow arrows; Fig. 3). Only the immunosuppressed *Acanthamoeba* spp. infected mice at 16 dpi showed immunoexpression of TLR4 in the nuclei of the distal canal epithelial cells (Fig. 3). In the immunocompetent uninfected mice, TLR2 expression was found in the proximal tubules, while after *Acanthamoeba* spp. infection brown pigmentation was observed in the distal tubules and collecting ducts. TLR2 expression in the kidneys of the immunocompetent *Acanthamoeba* spp. infected mice remained at a similar level during the infection. The highest TLR2 expression level was observed in the immunosuppressed mice at 24 days post *Acanthamoeba* spp. infection. Expression intensity and the number of immunopositive cells were similar in immunosuppressed uninfected mice and immunosuppressed *Acanthamoeba* spp. infected mice at 8 dpi and 16 dpi (Fig. 3). TLR4 expression in the immunocompetent *Acanthamoeba* spp. infected mice was similar to TLR2 expression and it remained at a similar level during the infection. In the

immunosuppressed *Acanthamoeba* spp. infected mice, the number of TLR4 immunopositive cells and intensity of TLR4 immunoreaction increased at 16 dpi and then decreased at 24 dpi (Fig. 3). The highest intensity of TLR4 immunoreaction was observed in the immunosuppressed mice at 16 days post *Acanthamoeba* spp. infection.

TLR2 and TLR4 expressions in the heart

A higher TLR4 expression compared to TLR2 was observed in the immunocompetent mice at 24 days post *Acanthamoeba* spp. infection ($U = 0$, $p < 0.01$), and in the immunosuppressed uninfected and in immunosuppressed infected mice at 8, 16 and 24 dpi ($U = 2.5$, $U = 0$, $U = 0$, $p < 0.01$, respectively). In the heart of the immunocompetent *Acanthamoeba* spp. infected mice, the highest TLR2 expression was observed at 8 dpi. In the following days TLR2 expression decreased in the *Acanthamoeba* spp. infected mice. Statistically significant difference was found in the TLR2 expression level between the immunocompetent mice at 8 dpi and 24 dpi ($U = 31.5$, $p < 0.05$; Fig. 4). In immunocompetent and immunosuppressed *Acanthamoeba* spp. infected mice, no statistically significant differences were found in the TLR2 expression level at 8 dpi, 16 dpi and 24 dpi and respective control groups. Comparing the immunoexpression of TLR4 between immunocompetent and immunocompromised mice, we found the differences between uninfected animals and at 16 days post *Acanthamoeba* spp. infection ($U = 23$ and $U = 23$, $p < 0.05$, respectively; Fig.e 5). We observed increased TLR4 expression in immunocompetent mice at 8 dpi compared to uninfected animals and decreased TLR4 expression in immunocompetent mice at 16 dpi compared to mice at 8 dpi ($U = 23.5$ and $U = 22.5$, $p < 0.05$, respectively; Fig. 5). There were no statistically significant differences in the TLR4 expression level between immunosuppressed *Acanthamoeba* spp. infected and uninfected mice.

Changes in the TLR2 and TLR4 immunoexpression in the heart of mice were seen in immunocompetent animals at 8 and 16 days post *Acanthamoeba* spp. infection compared to uninfected hosts (Fig. 6). In the hearts of immunocompetent and immunosuppressed uninfected mice only some cardiomyocytes showed TLR2 expression (Fig. 6A and 6B). TLR2 expression in immunocompetent mice at 8 and 16 dpi was observed in most cardiomyocytes (Fig. 6E and 6I, respectively). The lowest immunohistochemical reaction in the cardiac muscles of immunocompetent *Acanthamoeba* spp. mice was found at 24 dpi (Fig. 6M). In the immunosuppressed *Acanthamoeba* spp. mice, the highest intensity of TLR2 expression was noted at 8 and 16 dpi as brown pigmentation in the most cardiomyocytes (Fig. 6F and 6J, respectively). In the immunosuppressed mice at 24 dpi, TLR4 expression was observed only in some cardiomyocytes (Fig. 6N). We did not find immunopositive cardiomyocyte nuclei in the hearts of immunocompetent and immunosuppressed *Acanthamoeba* spp. infected mice (Fig. 6). The number of immunopositive cells and intensity of immunohistochemical reaction indicate a higher TLR4 expression in immunosuppressed uninfected mice than in immunocompetent uninfected animals (Fig. 6C and 6D). In the immunocompetent *Acanthamoeba* spp. infected mice, we observed increased TLR4 expression at 8 dpi, while at 16 dpi, we observed decreased number of TLR4-positive cells (Fig. 6). In the immunosuppressed *Acanthamoeba* spp. infected mice, the highest TLR4 expression was noted in the hearts of mice at 8 and 16 dpi, while at 24 dpi there was a decrease in TLR4 expression (Fig. 6P).

Discussion

The immune response against parasitic infection is complex, and it involves many effectors and regulators components. *Acanthamoeba* spp. activate the classical TLR signaling pathway inducing NF-kB activation and increased secretion of inflammatory cytokines [25]. Induction of an inflammatory response by amoebas has been proposed as an important factor to determine the course of the parasite infection [8]. Nevertheless, little is known about the innate immune response induced by *Acanthamoeba* spp. in the kidneys and heart. To our knowledge, this is the first report showing TLRs expression in the kidneys and heart of hosts with disseminated acanthamoebiasis. Hence, further knowledge about the molecular and immunologic mechanisms induced by amoebas are important aspects to understand the course of infections and tissue invasion.

The occurrence of *Acanthamoeba* spp. infection has been described in patients following kidney transplantation [26–29], although in just one instance the amoebae were re-isolated from the kidney of patient with probable *Acanthamoeba* meningoencephalitis [30]. Despite the re-isolation of *Acanthamoeba* spp. from the mouse kidneys, the kidney profile performed in mouse serum indicated normal renal function [7]. Therefore, based on previous studies, the affinity of *Acanthamoeba* spp. to the kidneys can neither be confirmed nor excluded.

The factors involved in kidney damage and abnormal kidney function in parasitic diseases are still unknown. Based on studies on the role of Toll-like receptors in nephropathy induced by a *Toxoplasma gondii* infection, it was found that mainly TLR2 plays a role in kidney protection against *T. gondii* infection. Histopathological studies showed larger kidney damage in TLR2 deficient mice compared to those TLR4 deficient [31]. Studies on visceral leishmaniasis associated with renal abnormalities, suggest that *Leishmania* spp. antigens induce kidney inflammation by activating TLR2 and TLR4 receptors. The results indicate that kidney inflammatory processes and apoptosis involving TGF-β have a significant role in the pathomechanism of kidney damage in *Leishmania donovani* infection [32]. In the presented study, statistically significant differences in TLR2 expression occurred only between immunocompromised *Acanthamoeba* spp. infected mice and immunocompromised uninfected mice. We also noted differences in TLR4 expression between immunocompromised infected mice at 16 and 24 dpi and the control group, but the differences were not statistically significant ($p = 0.06$). Mun et al. [33] observed that TLR is not an essential molecule for protective immunity to low-dose of *T. gondii* cysts. In our study, the mice were infected with 10–20 thousands of amoebas, but *Acanthamoeba* spp. were re-isolated from 4 kidneys of immunosuppressed mice at 8 dpi, 6 kidneys at 16 dpi and 7 kidney's mice at 24 dpi. Therefore, the higher expression of TLRs at 16 and 24 dpi may be a result of the low immunity level of the animals and/or the number of parasites that entered the kidneys.

Leemans et al. [34] have shown that TLR2 plays a critical role in the initiation of acute renal inflammation and early tubular injury. In histological preparations of kidneys of immunosuppressed mice, we found no inflammatory foci, but at the same we observed a lighter color of the kidney parenchyma [unpublished data]. In the present study, statistically significant changes in TLR2 expression were observed only at 24

dpi, the last day of the experiment. It is possible that histopathological changes in the form of inflammatory infiltrates could have been visible in the kidneys of mice in a longer-lasting acanthamoebiasis. In future studies, the experiment could be extended to 30–40 days post *Acanthamoeba* spp. infection and include determination of TGF-β expression, a cytokine which may mediate the progression of parasitic diseases in kidneys. Moreover, TGF-β induces transformation of kidney tubule cells to proliferating fibroblasts, causing fibrous changes in kidney parenchyma [35]. Such changes may be visible in histological preparations in the form of a lighter color of the kidney parenchyma.

Literature presents three cases of cardiovascular patients with acanthamoebiasis [6, 36, 37], although no parasite forms have been found in cardiac muscle cells [37]. Also, in this study no trophozoites or cysts were found in myocardial histological preparations from *Acanthamoeba* spp. infected mice, despite the fact that developmental forms of *Acanthamoeba* spp. were isolated from these samples. Therefore, it is not clear whether the amoebae were re-isolated from cardiac fragments or from residual blood in the heart, as the amoebae spread throughout the host organism via the bloodstream.

The Toll-like receptors expression has been reported in epithelium, endothelium and other cardiovascular cells [38, 39]. The results of studies conducted so far suggest that short-term activation of TLR receptors has a protective effect on the cardiovascular system, while long-term or excessive activation of these receptors induces chronic inflammation [38–40]. Cardiomyocytes in response to inflammatory stimuli are capable of secreting pro- and anti-inflammatory cytokines capable of initiating and regulating the inflammatory response, as well as chemokines, which recruit and activate appropriate inflammatory cells [41]. There is little data on TLR expression in the *cardiac* muscle *cells* of parasite-infected hosts. Ponce et al. [42] observed increased *tlr2* gene expression in the heart of BALB/c neonatal mice infected with *Trypanosoma cruzi*. The authors suggested that these parasites may activate the host's innate immune response via different Toll-like receptors to protect cardiomyocytes from parasites. In contrast, Pereira et al. [43] suggest that a high TLR2 expression in patients with chronic Chagas cardiomyopathy may induce an increase in IL-1β, IL-12, and TNF-α, thereby elevating cardiac inflammation and contributing to heart dysfunction. Oliveira et al. [44] observed that a deficiency of TLR4 leads mice to being more susceptible to *T. cruzi* infection, as evidenced by a higher parasitemia and earlier mortality. However, it is important to point out that *T. cruzi* has an affinity for the heart, which is not the main biotope of *Acanthamoeba* spp. In this study, a statistically significant increase in TLR2 expression was seen in the heart of immunocompetent mice at 8 days post *Acanthamoeba* spp. infection compared to 24 dpi. When it comes to TLR4, statistically significant differences were found in the immunocompetent mice between 8 dpi and the uninfected mice, as well as at 8 dpi and 16 dpi. This shows that TLR2 and TLR4 induced an immune response at 8 days post *Acanthamoeba* spp. infection and thus protected cardiomyocytes from parasites, as confirmed by histological studies which did not show morphological changes [unpublished data]. Mogensen et al. [45] concluded that dexamethasone, an immunosuppressive drug, inhibits TLR-receptor signaling in *Neisseria meningitidis* and *Streptococcus pneumoniae* invasion. In our study, increased TLR4 expression in mice treated with the immunosuppressive drug at 16 days post *Acanthamoeba* spp. infection could be associated with delayed activation of this receptor by amoebae. In histological studies,

mice treated with an immunosuppressive drug showed morphological changes in the form of hemorrhages and vacuolized cardiomyocytes with less acidic cytoplasm at 8 dpi and 16 dpi. In immunosuppressed mice at 24 dpi, we did not observe any morphological changes in the cardiac muscle [unpublished data].

Conclusions

The immunological mechanisms preventing renal and cardiomyocyte pathomechanisms in *Acanthamoeba* spp. infection remain unknown. The present study showed upregulation in TLRs in the studied organs of hosts with disseminated acanthamoebiasis. Our results indicate that TLR2 is involved in response to *Acanthamoeba* spp. infection in the kidneys, whereas in the heart, both studied TLRs are involved in response to *Acanthamoeba* spp. infection. In future studies, it will be important to analyze the cytokine profile in the heart and kidneys of hosts with disseminated acanthamoebiasis to better understand the course of infection and tissue invasion.

Abbreviations

A: Immunocompetent *Acanthamoeba* spp.-infected mice; AK: *Acanthamoeba* keratitis; AM 22: Amoebic strain no. 22; AM: Arithmetic mean; AS: Immunosuppressed *Acanthamoeba* spp. infected mice; C: Immunocompetent uninfected control group mice; CS: Immunosuppressed uninfected control group mice; DAMPs: Damage-associated molecular patterns; dpi: Days post-infection; GAE: Granulomatous amebic encephalitis; HSP60: heat shock protein 60; HSP70: heat shock protein 70; IC: immunocompetent mice; IS: immunosuppressed mice; IL-1β: Interleukin 1β; IL-8: Interleukin 8; IL-12: Interleukin 12; IFN–γ: Interferon gamma; IRF: Interferon regulatory factor; MPS: Methylprednisolone sodium succinate; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; NN: Non-nutrient agar; p: Level of significance; PAMPs: Pathogen-associated molecular patterns; PBS: Phosphate-buffered saline; Q-PCR: quantitative real-time polymerase chain reaction; SD: Standard deviation; TLR2: Toll-like receptor 2; TLR4: Toll-like receptor 4; TGF- β: Transforming growth factor β; TNF-α: Tumor necrosis factor alpha.

Declarations

Ethics declaration

Ethics approval and consent to participate

All procedures involving animals were approved by the Local Ethics Committee for Scientific Experiments on Animals in Szczecin (No. 29/2015 of 22 June 2015) and Poznań (No. 64/2016 of 9 September 2016).

Consent of publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Availability of data and materials

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

Authors' contributions

K.K., D.K-B. and N.Ł-A. conceived and designed research; K.K., D.K-B., A.W-G., A.K-W. and N.Ł-A. performed the experiments; K.K., A.W-G. and A.K-W. analyzed the data; K.K. and D.K-B contributed to writing the manuscript. D.K-B. and N.Ł-A provided scientific supervision of the study. All authors have read and agreed to the published version of the manuscript.

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Figures

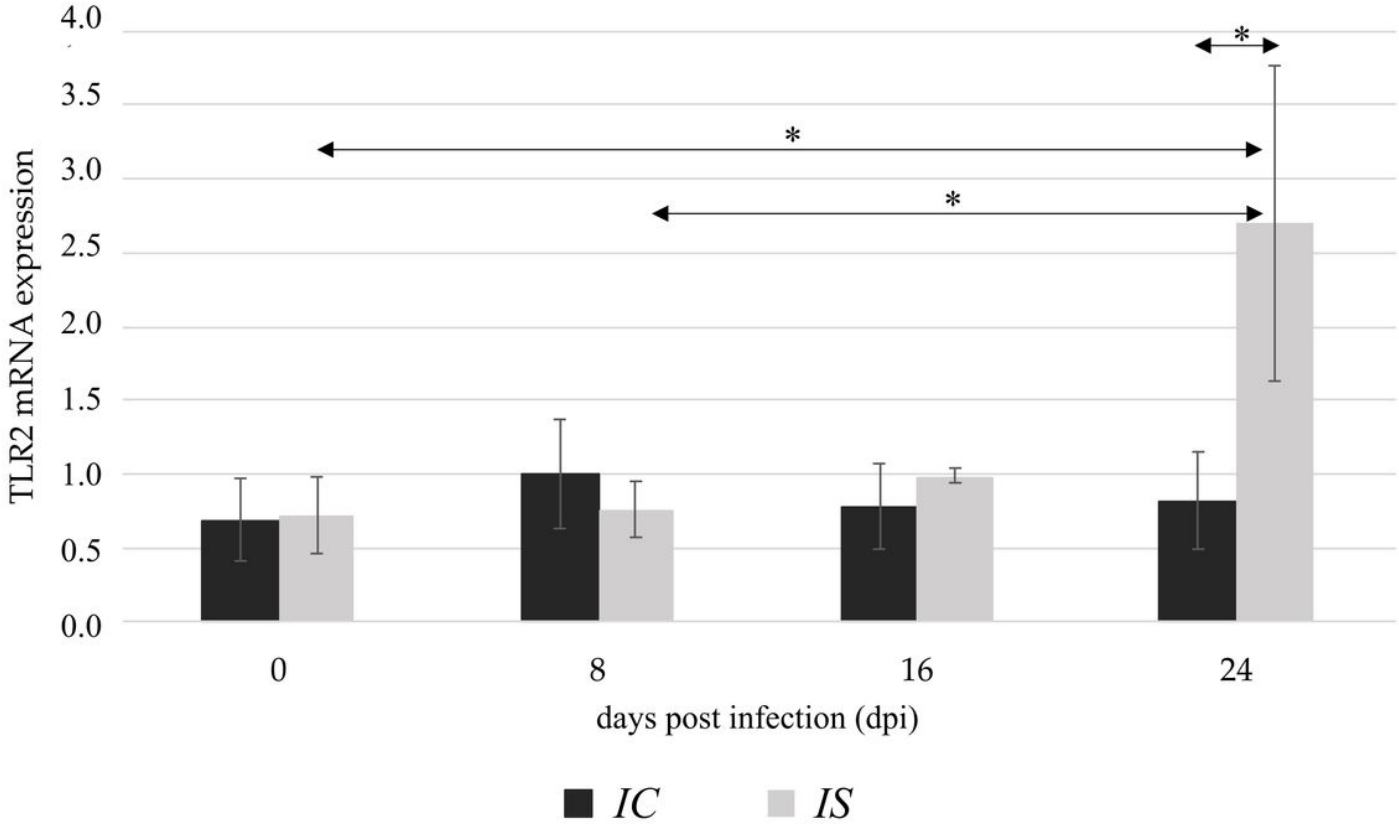

Figure 1

The mRNA expression of the tlr2 gene in the kidneys of uninfected (0 dpi) and infected mice at 8, 16 and 24 days post Acanthamoeba spp. infection (dpi), according to the immunological status of hosts (IC, immunocompetent mice; IS, immunosuppressed mice). The data represent mean±standard deviation (SD) for six independent experiments; *$p<0.05$ using a Mann-Whitney U test.

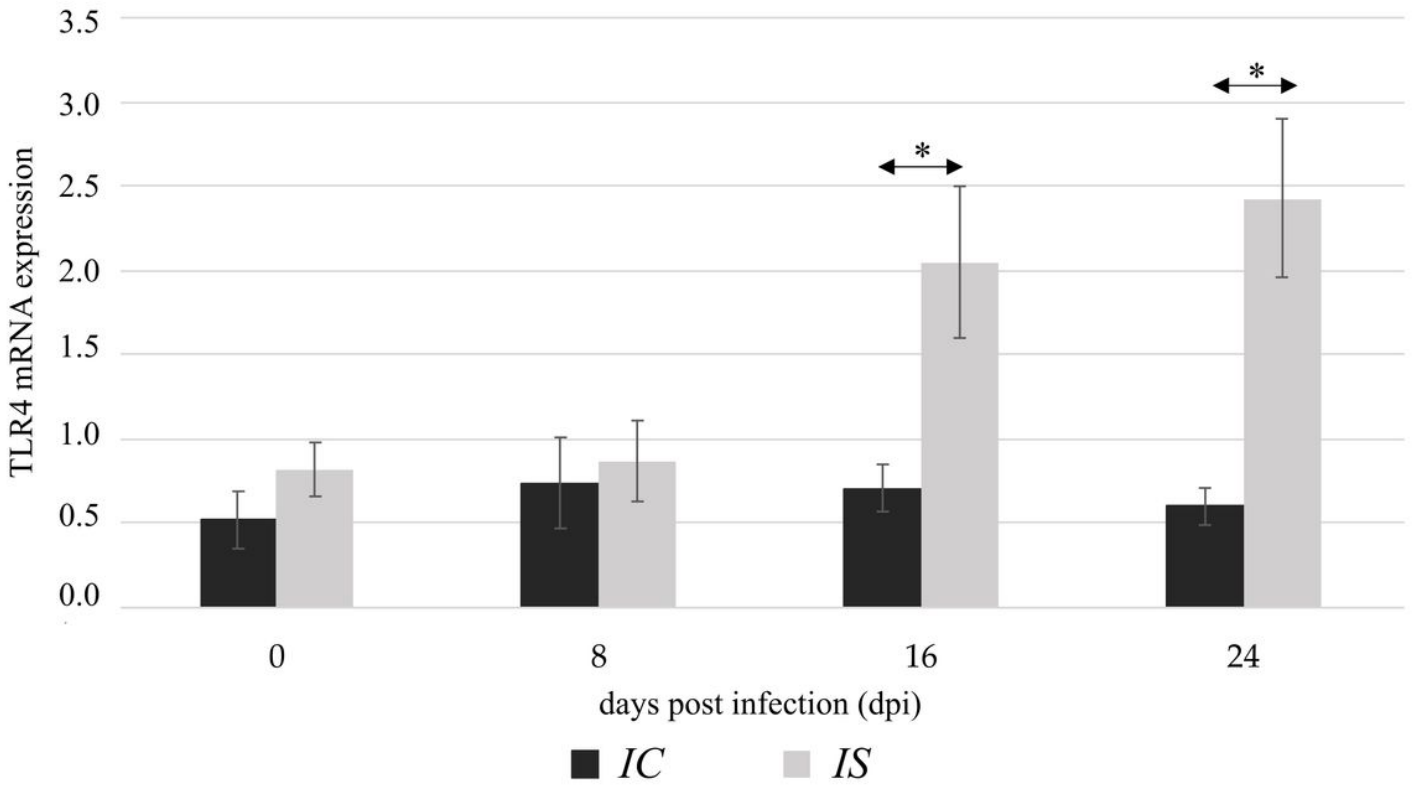

Figure 2

The mRNA expression of the tlr4 gene in the kidneys of uninfected (0 dpi) and infected mice at 8, 16 and 24 days post Acanthamoeba spp. infection (dpi), according to the immunological status of hosts (IC, immunocompetent mice; IS, immunosuppressed mice). The data represent mean±standard deviation (SD) for six independent experiments; *$p<0.05$ using a Mann-Whitney U test.

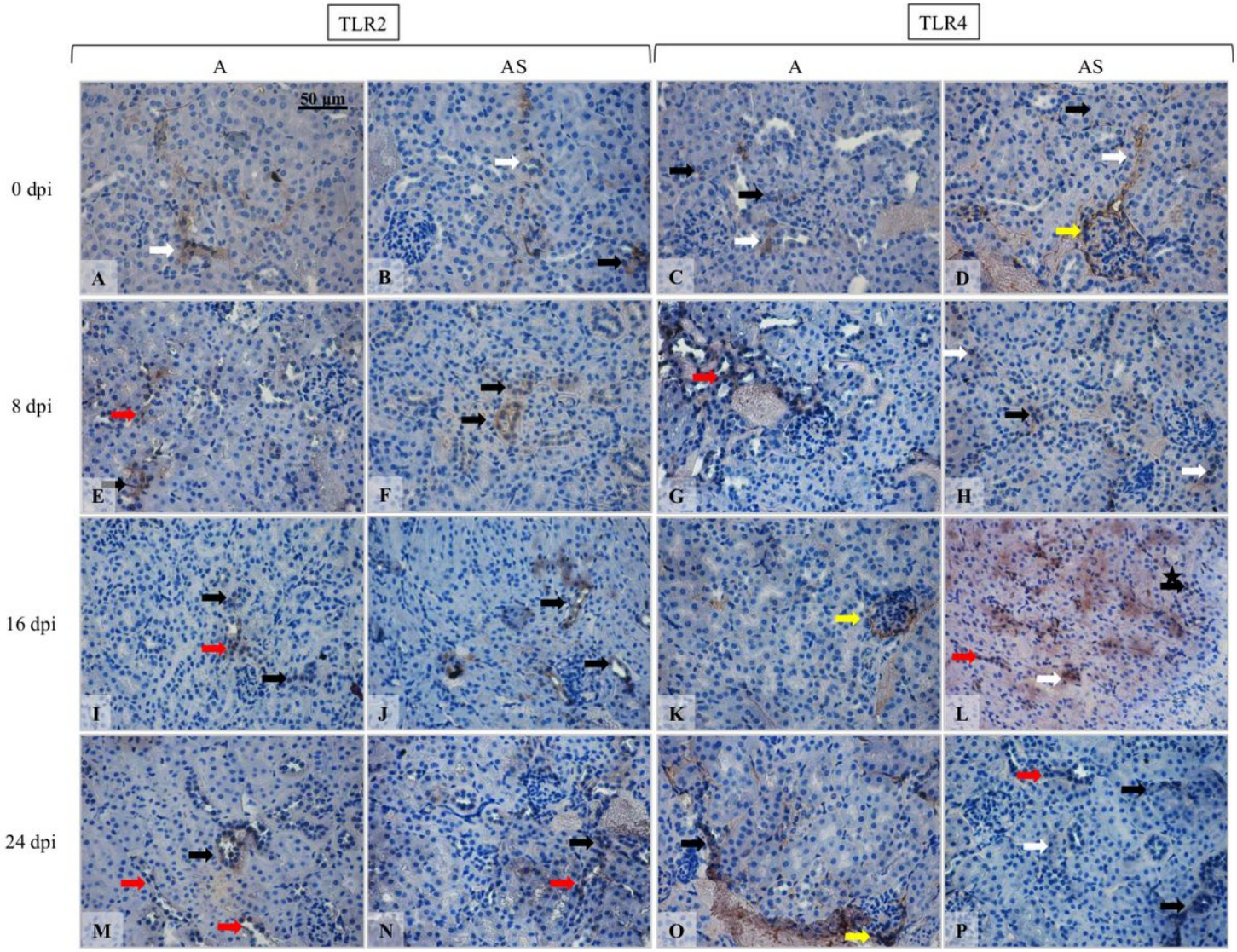

Figure 3

Immunohistochemical staining with primary anti-TLR2 and anti-TLR4 antibodies in the kidneys of immunocompetent and immunosuppressed mice from control groups (0 dpi) and at 8, 16 and 24 days post Acanthamoeba spp. infection (dpi). Magnification x40 (black arrows, brown pigmentation in the distal tubules; white arrows, brown pigmentation in the proximal tubules; red arrows, brown pigmentation in the collecting ducts; yellow arrows, brown pigmentation in the renal corpuscles; black asterisk, immunopositive nuclei in the epithelial cells of the distal tubule).

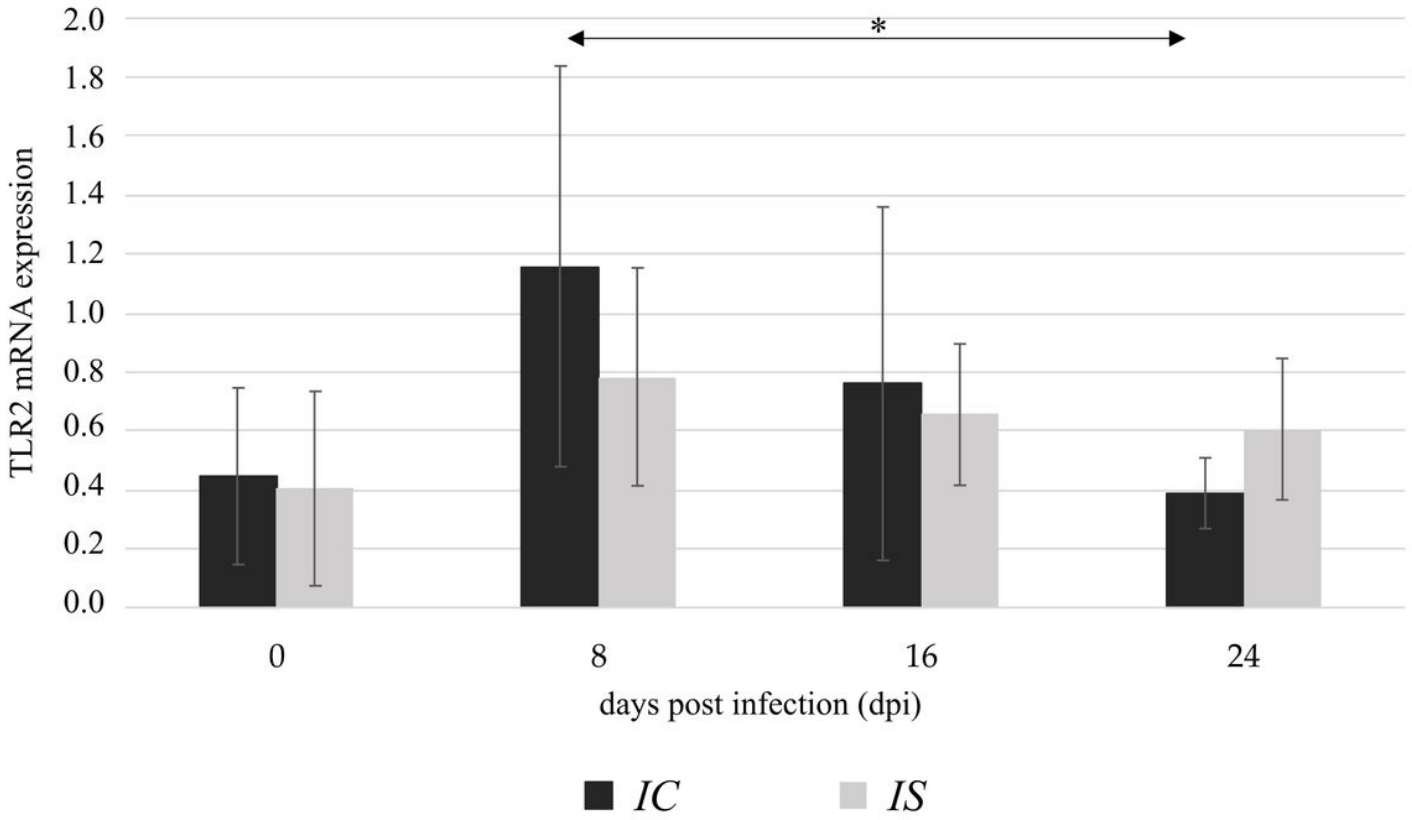

Figure 4

The mRNA expression of the tlr2 gene in the heart of uninfected (0 dpi) and infected mice at 8, 16 and 24 days post Acanthamoeba spp. infection (dpi), according to the immunological status of hosts (IC, immunocompetent mice; IS, immunosuppressed mice). The data represent mean±standard deviation (SD) for six independent experiments; *$p<0.05$ using a Mann-Whitney U test.

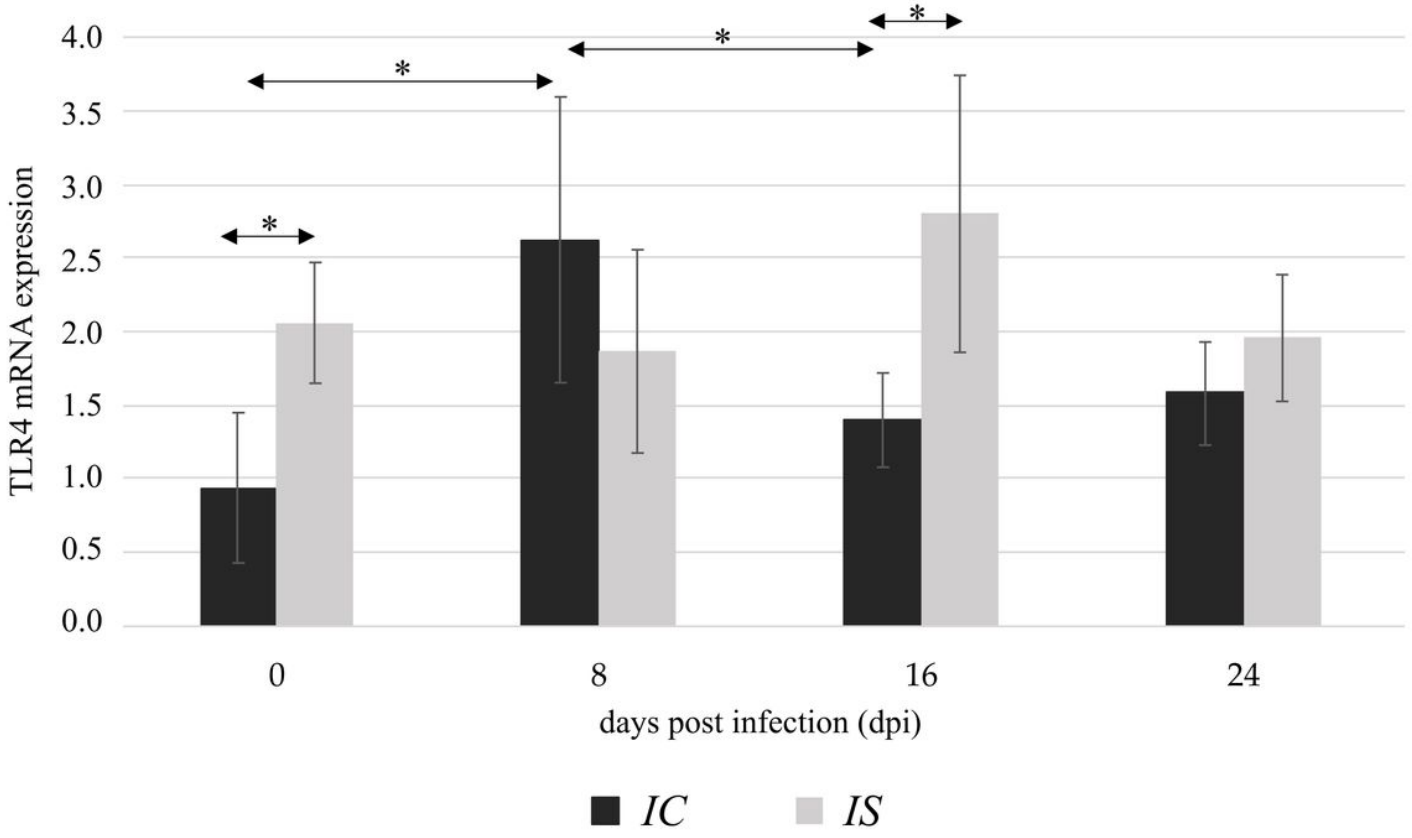

Figure 5

The mRNA expression of the tlr4 gene in the heart of uninfected (0 dpi) and infected mice at 8, 16 and 24 days post Acanthamoeba spp. infection (dpi), according to the immunological status of hosts (IC, immunocompetent mice; IS, immunosuppressed mice). The data represent mean±standard deviation (SD) for six independent experiments; * $p < 0.05$ using a Mann-Whitney U test.

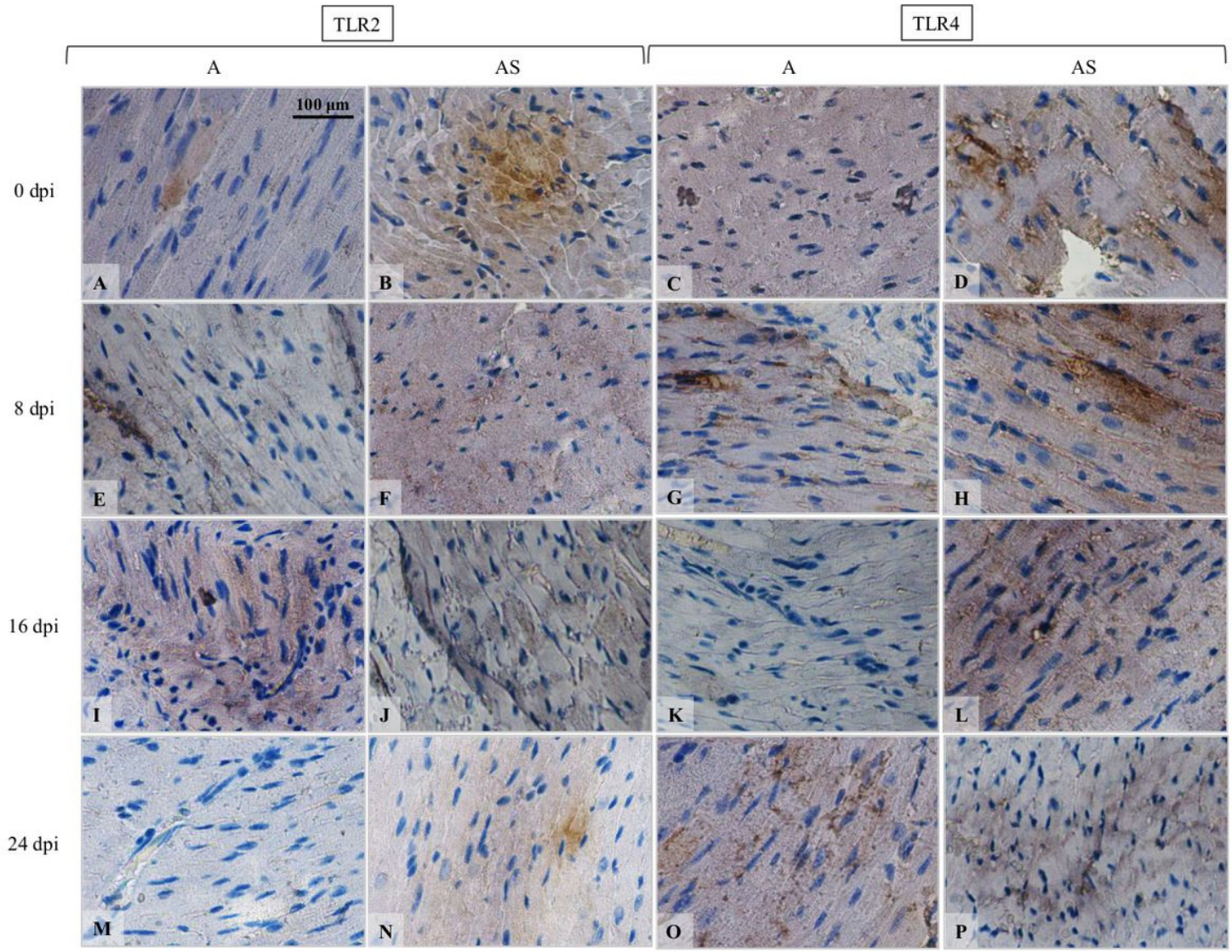

Figure 6

Immunohistochemical staining with primary anti-TLR2 and anti-TLR4 antibodies in the heart of immunocompetent and immunosuppressed mice from control group (0 dpi) and at 8, 16 and 24 days post Acanthamoeba spp. infection (dpi). Magnification x100.

Supplementary Files

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