Parasitology

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Tissue eosinophilia correlates with mice susceptibility, granuloma formation and damage during *Toxocara canis* infection

Thaís Leal-Silva[1,2], Camila de Almeida Lopes[1], Flaviane Vieira-Santos[1], Fabrício Marcus Silva Oliveira[1], Lucas Kraemer[1], Luiza de Lima Silva Padrão[1,2], Chiara Cássia Oliveira Amorim[1], Jorge Lucas Nascimento Souza[1], Fernando Sérgio Barbosa[1], Milene Alvarenga Rachid[3], Remo Castro Russo[4], Ricardo Toshio Fujiwara[1,2] and Lilian Lacerda Bueno[1,2]

[1]Laboratory of Immunology and Genomics of Parasites, Department of Parasitology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil; [2]Post-graduation Program in Health Sciences: Infectious Diseases and Tropical Medicine, Faculdade de Medicina, Federal University of Minas Gerais, Belo Horizonte, Brazil; [3]Laboratory of Protozooses, Department of General Pathology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil and [4]Laboratory of Pulmonary Immunology and Mechanics, Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil

Research Article

Cite this article: Leal-Silva T *et al* (2022). Tissue eosinophilia correlates with mice susceptibility, granuloma formation and damage during *Toxocara canis* infection. *Parasitology* **149**, 893–904. https://doi.org/10.1017/S0031182022000075

Received: 11 November 2021
Revised: 11 January 2022
Accepted: 13 January 2022
First published online: 10 February 2022

Key words:
Eosinophil; GATA-1; granuloma; immunology; tissue inflammation; toxocariasis

Author for correspondence:
Lilian Lacerda Bueno,
E-mail: llbueno@icb.ufmg.br

Abstract

An increase in peripheral blood eosinophils in helminth infections is expected, and these cells are known to promote immunity against these parasites. However, studies have suggested that in some specific helminths, eosinophils may promote the needs and longevity of these parasites, and their role in these infections remains undefined, including in *Toxocara canis* infection. Thus, this study aimed to investigate the role of eosinophils in the context of larval migration of *T. canis* and the immunopathological aspects of infection. For this, we used wild-type mice and mice genetically deficient for the transcription factor GATA-binding factor 1 (GATA1$^{-/-}$), infected with 1000 eggs of *T. canis*. At 0, 3, 14 and 63 days post-infection, parasite load, tissue cytokine production, leucocyte profile, bronchoalveolar lavage cells and histopathological analyses were carried out. Collectively, our results demonstrate that the presence of eosinophils mediates susceptibility to *T. canis*, inducing leucocytosis and the formation of granulomas, increasing the pulmonary and cerebral parasite load, and reducing the number of neutrophils, which may be necessary to control the infection.

Introduction

Eosinophils are granulocytes present in bone marrow are derived from pluripotent progenitors and represent 5% of the total leucocytes in the peripheral blood circulation. These leucocytes secrete diverse granules composed of cationic eosinophil proteins, eosinophil peroxidases and eosinophil-derived neurotoxins. Eosinophils are activated and recruited into the tissue in response to stimuli such as the cytokine interleukin 5 (IL-5) and chemokines (Rosenberg *et al*., 2013; Obata-Ninomiya *et al*., 2020). In response to allergic insult or some types of infections, eosinophils are recruited to the sites of inflammation secreting their granules, contributing as a source of multiple cytokines, such as IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-16, IL-18, transforming growth factor-β1 and lipid mediators, in addition to being able to induce tissue damage and dysfunction (Rothenberg and Hogan, 2006). In allergic processes, an increase in eosinophils in the peripheral blood is typical as in helminth infections. These cells are known to promote effector immunity against these parasites (Weller and Spencer, 2017). However, studies suggest that in some specific helminths, eosinophils may subsidize the permanence and favour the longevity of parasites in the host, but the role of eosinophils in these infections remains undefined (Swartz *et al*., 2006; Rosenberg *et al*., 2013).

During cell development in the bone marrow, eosinophils have properties shared with basophils, and their differentiation occurs due to the presence of transcription factors PU.1, CCAAT/enhancer-binding protein and GATA-binding factor 1 (GATA-1). Eosinophils have a high affinity palindromic (or double) site of GATA-1 located in the gene regulatory region. In mice, it is possible to perform the deletion of the GATA-1 transcription factor interrupting eosinophilic cell maturation, resulting in complete or selective deficiency for eosinophil differentiation. In this sense, these mice are essential for studying eosinophil-related functions and pathologies (Yu *et al*., 2002; Rothenberg and Hogan, 2006).

In murine models, the protective role of eosinophils has been shown to prevent secondary infection by *Trichinella spiralis*, and in humans with Schistosomiasis, an increase in eosinophils after treatment with praziquantel has been correlated with resistance to reinfection (Hagan *et al*., 1985; Huang *et al*., 2015; Huang and Appleton, 2016). However, in mice infected with *Schistosoma mansoni*, ablation of eosinophil had no significant impact on disease

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development during infection (Swartz *et al.*, 2006). In eosinophil genetically deficient mice, it was shown that *T. spiralis* larvae died in large numbers showing that death of the parasites was correlated with a reduced recruitment of T-helper 2 (Th2) cells and a concomitant increased activity of the inducible nitric oxide synthase and synthesis of nitric oxide by macrophages (Fabre *et al.*, 2009; Gebreselassie *et al.*, 2012).

Toxocariasis is a zoonosis whose primary aetiological agent is *Toxocara canis*. It is a neglected, cosmopolitan disease with a 19% seroprevalence worldwide (Rostami *et al.*, 2019). Humans are infected by accidental ingestion of eggs containing infective *T. canis* larvae, which penetrate the mucosa and migrate to various tissues. The immunological mechanism capable of eliminating *T. canis* larvae remains unclear. However, studies showed that Th2 and Th17 immune responses are triggered during infection, and there is an increase in innate immunity cells, especially eosinophils (Resende *et al.*, 2015; Leal-Silva *et al.*, 2021). We have recently demonstrated that $ST2^{-/-}$ receptor deficiency reduced the parasite load combined with reduced eosinophils during *T. canis* infection in mice (Leal-Silva *et al.*, 2021), suggesting that eosinophils may be relevant in the framework of *T. canis* infection.

Studies regarding immune responses in toxocariasis are scarce, and the role of eosinophils during this infection has not yet been defined. In this context, the present study aimed to determine the role of eosinophils during the immunopathology caused by *T. canis* infection using a murine experimental model. Our results revealed that eosinophils favour *T. canis* larvae tropism during infection, increasing the tissue damage with induction of hepatic and pulmonary granulomas and brain haemorrhage in mice. We concluded that eosinophil cells are deleterious in the context of *T. canis* infection in mice.

Materials and methods

Parasites

Adult *T. canis* worms were collected from the feces of naturally infected puppies and were treated with anthelmintics (Drontal Puppy, Brazil) at a dosage of 1 mL kg^{-1} in the Zoonoses Control Center (Belo Horizonte, Minas Gerais, Brazil). The adult worms were placed in water and taken to the Laboratory of Immunology and Genomics of Parasites at the Federal University of Minas Gerais for processing. The eggs were isolated from the uteri of female adult worms by mechanical maceration, purified by filtration using 100 μm nylon strainers, placed in culture flasks with 50 mL of 0.2 M sulphuric acid and maintained in a Bio-Oxygen Demand incubator at 26 C. The eggs remained in the culture until their embryonic period, which took approximately 6 weeks (Leal-Silva *et al.*, 2021).

Experimental design

Eight-week-old female mice of wild-type (WT) BALB/c and mice genetically deficient for the transcription factor $GATA1^{-/-}$ were maintained in an animal facility of the Department of Parasitology of the Federal University of Minas Gerais under controlled conditions of temperature (24 ± 1 C) and lighting (12-h light–dark cycle), and were provided with filtered water and commercial food (Nuvital Nutrients, Brazil) *ad libitum*.

Each of the two WT and $GATA1^{-/-}$ mice strains was randomly divided into four experimental groups: 0 days post-infection (dpi) (control), 3, 14 and 63 dpi. Each group used a total of 12 mice, divided into two groups containing six mice for immunological and histological analyses and six for parasitological and biochemical analyses (Fig. 1). All mice were euthanized with a lethal injection of xylazine/ketamine (8.5 and 130 mg kg^{-1}).

Toxocara canis *infection*

For *Toxocara* infection, before inoculation, the fully embryonated eggs were incubated with a 5% sodium hypochlorite solution in an incubator [37 C and 5% carbon dioxide (CO_2)] for 1 h 40 min to disrupt the outer layer of the eggs and facilitate *in vivo* larval hatching. After incubation, eggs were resuspended and washed with phosphate-buffered saline (PBS) (0.4 M NaCl and 10 mM $NaPO_4$) three times. All mice, except the 0 dpi group (control), were inoculated by oral gavage with 0.2 mL of the solution containing 1000 embryonated eggs.

Parasitological analysis

Parasite burdens from infected mice were evaluated by counting the total number of larvae recovered from the liver, lungs and brain. Tissues were collected, punctured with surgical scissors and placed in a modified Baermann-Moraes apparatus for 4 h in PBS at 37 C and 5% CO_2. The larvae recovered from the pellet of the device were fixed in 10% formalin and quantified by light microscopy.

Analysis of leucocytes and liver enzymes

For the total and differential leucocyte counts, blood samples of the mice were collected by cardiac puncture, placed in tubes with ethylenediamine tetraacetic acid (EDTA) and were analysed in an automatic haematology counter (Bio-2900 Vet, Bioeasy, USA) for a global leucocyte count. Blood smears stained with Panotic (Laborclin, Brazil) were used for differential cell count under microscopy.

The blood was centrifuged, and the plasma was collected to quantify the liver enzyme activity, aspartate aminotransferase (AST), using a colorimetric enzymatic assay employing a commercial kit (Bioclin Quibasa, Brazil) according to the manufacturer's instructions.

Bronchoalveolar lavage (BAL)

BAL was collected from mice with the assistance of a 1.7 mm catheter inserted into the trachea, and then 2 mL PBS was added through the catheter to collect the BAL fluid. The lavage fluid was centrifuged at 300 ***g*** for 10 min, and the supernatants were collected to estimate protein and haemoglobin levels by using a commercial kit according to the manufacturer's instructions. The pellets were used to quantify total and differential cellularity using light microscopy as previously described (Oliveira *et al.*, 2019).

Cytokine profile

The right lobe of the mice lung tissue was removed to determine tissue cytokine concentration. First, 100 mg of tissue was weighed and then homogenized (TissueLyser LT-Qiagen, Germany) in 1 mL of extraction solution with protease inhibitors (0.4 M NaCl, 0.05% Tween 20, 0.5% bovine serum albumin, 0.1 mM phenylmethylsulphonyl fluoride, 0.1 mM benzethonium chloride, 10 mM EDTA and 20 IU aprotinin A). The homogenate was centrifuged at 800 ***g*** for 10 min at 4 C and the supernatant was used to determine the cytokines IL-1β, tumour necrosis factor-α, IL-6, IL-4, IL-33, IL-13, IL-5, IL-10 and IL-17A by enzyme-linked immunoassay (ELISA) (R&D Systems, USA) according to the manufacturer's protocol.

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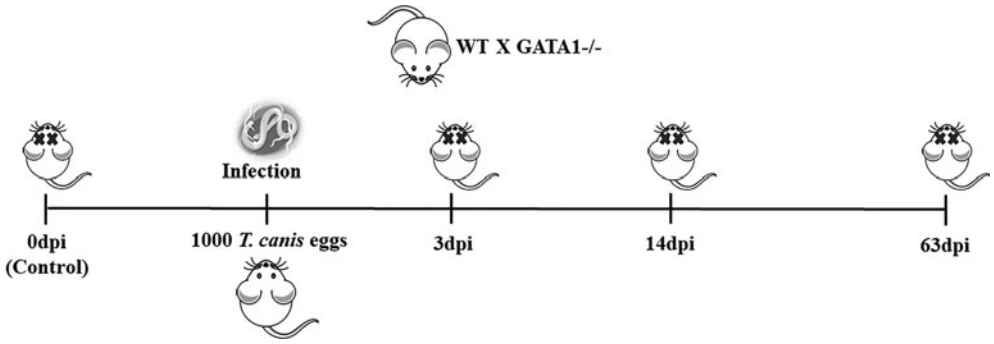

Fig. 1. Characterization of the experimental design of *Toxocara canis* infection. WT and GATA1$^{-/-}$ mice were infected with 1000 eggs of *T. canis* and euthanized with 0, 3, 14 and 63 dpi.

Neutrophil myeloperoxidase (MPO) and macrophage N-acetylglucosaminidase (NAG) assays

The indirect activity of macrophages and neutrophils was assessed by using the concentrations of NAG and MPO, respectively, in the liver, lungs and brain homogenates according to the method previously described (Barcelos *et al*., 2005; Nogueira *et al*., 2016). After the tissues were homogenized (TissueLyser LT-Qiagen, Hilden, Germany), the homogenate was centrifuged at 800 ***g*** for 10 min at 4 C, and the pellet was used to determine NAG and MPO activity. Absorbance was expressed by using a VersaMax ELISA microplate reader (Molecular Devices, USA) according to each assay, and the results were expressed as optical densities.

Histopathological analysis

The left lung, right liver lobe and left half of the brain were collected from mice to analyse the histological changes. The organs were fixed in a 4% formalin solution, gradually dehydrated in ethanol before being cleared in xylene and embedded in paraffin blocks that were cut 4–5 μm thick and fixed on the microscope slide. The tissue slides were stained with haematoxylin and eosin to assess tissue damage under microscopy, and all histopathological analyses were subjected to a blind study..

For the analysis of liver lesions, ten random images per mouse were captured at 20× magnification. The score was based on four grades for liver parenchymal damage: grade 0, no inflammatory cells; grade 1, regions of the liver parenchyma with small inflammatory foci and reduced number of inflammatory cells with small areas of necrosis; grade 2, the liver parenchyma showed inflammatory foci with a moderate number of cells, perivascular inflammatory infiltrate and around the ducts with small areas of necrosis dispersed throughout the parenchyma; grade 3, the liver parenchyma often had larger inflammatory foci, diffuse inflammatory infiltrate, exuberant perivascular inflammation and around the ducts, areas of necrosis dispersed throughout the parenchyma (Leal-Silva *et al*., 2021). Hepatic and pulmonary granuloma counts were also obtained.

For the airway inflammation score, ten random images per mouse were captured at 20× magnification and analysed for perivascular inflammation, peribronchial inflammation, parenchymal damage and haemorrhage (Gazzinelli-Guimarães *et al*., 2018). For the analysis of cellular lesions of the whole brain, the score was based on the intensity of the lesion: 0: none; 1: minimum; 2: light; 3: moderate; 4: intense. All semi-quantitative analyses of the slides were conducted under microscopy coupled with a digital image capture system (Motic 2.0, Xiam, China).

Statistical analysis

For statistical analysis, Prism 8.0 software (GraphPad Inc., USA) was used. To detect possible outliers, the Grubb test was used. To verify data distribution, the Shapiro–Wilk test was used. An analysis of variance (ANOVA) test was used for the comparison between the control groups (0 dpi) and each time of infection, followed by the Tukey multiple comparisons post-test for parametric data or the non-parametric Kruskal–Wallis test followed by the Dunn's post-test. All values were considered significant when the *P* value was $\leq$0.05.

Results

Tissue eosinophilia induces granuloma formation but does not control the T. canis *larval migration in mice liver*

In this study, we demonstrate the role of eosinophils in the immunopathology of *T. canis* infection. After passing through the intestine, *Toxocara* larvae migrates to the liver tissue. No significant differences were observed between the parasite load in the liver and the AST enzyme throughout the infection (Fig. 2A and B). However, the uninfected GATA1$^{-/-}$ mice showed a basal increase in NAG at 0 dpi persisting during the course of infection (Fig. 2C). The neutrophilic activity was reduced in the acute phase of infection, and remains until the chronic phase compared to WT mice (Fig. 2D). In the score analysis, granulomas were observed only in WT mice at 14 and 63 dpi, and no difference was observed between strains in the total inflammation score (Fig. 2E and F).

The histopathological analysis of the liver showed that, in the uninfected mice, both strains retained the hepatocytes with a morphological aspect compatible with normality (Fig. 2G). At 3 dpi, it was observed in some mice in both strains, the larvae dispersed throughout the parenchyma. In WT mice, there were inflammatory foci comprising eosinophils, neutrophils, macrophages and lymphocytes dispersed throughout the parenchyma and around larvae and the blood vessels, in addition to abundant necrosis zones and parenchyma haemorrhage. In GATA1$^{-/-}$ mice, the inflammatory foci comprised of a significant proportion of neutrophils, scarce macrophages and dispersed lymphocytes, and inflammatory infiltrate around larvae, blood vessels, bile ducts and permeating areas of necrosis were also frequently identified. In both strains, the blood vessel congestion induced by infection was observed throughout the hepatic parenchyma. At 14 dpi in WT mice, the presence of granulomas was found, which were in the exudative phase, composed mostly of eosinophils, followed by macrophages, necrotic-exudative and necrotic zones. In both strains of mice, it was possible to identify the presence of inflammatory foci dispersed throughout the hepatic and perivascular parenchyma, consisting of macrophages, low lymphocytes and eosinophils, the latter being observed only in WT mice. In the GATA1$^{-/-}$ group at 14 dpi, the presence of small foci of necrosis near ducts and larger blood vessels, and the

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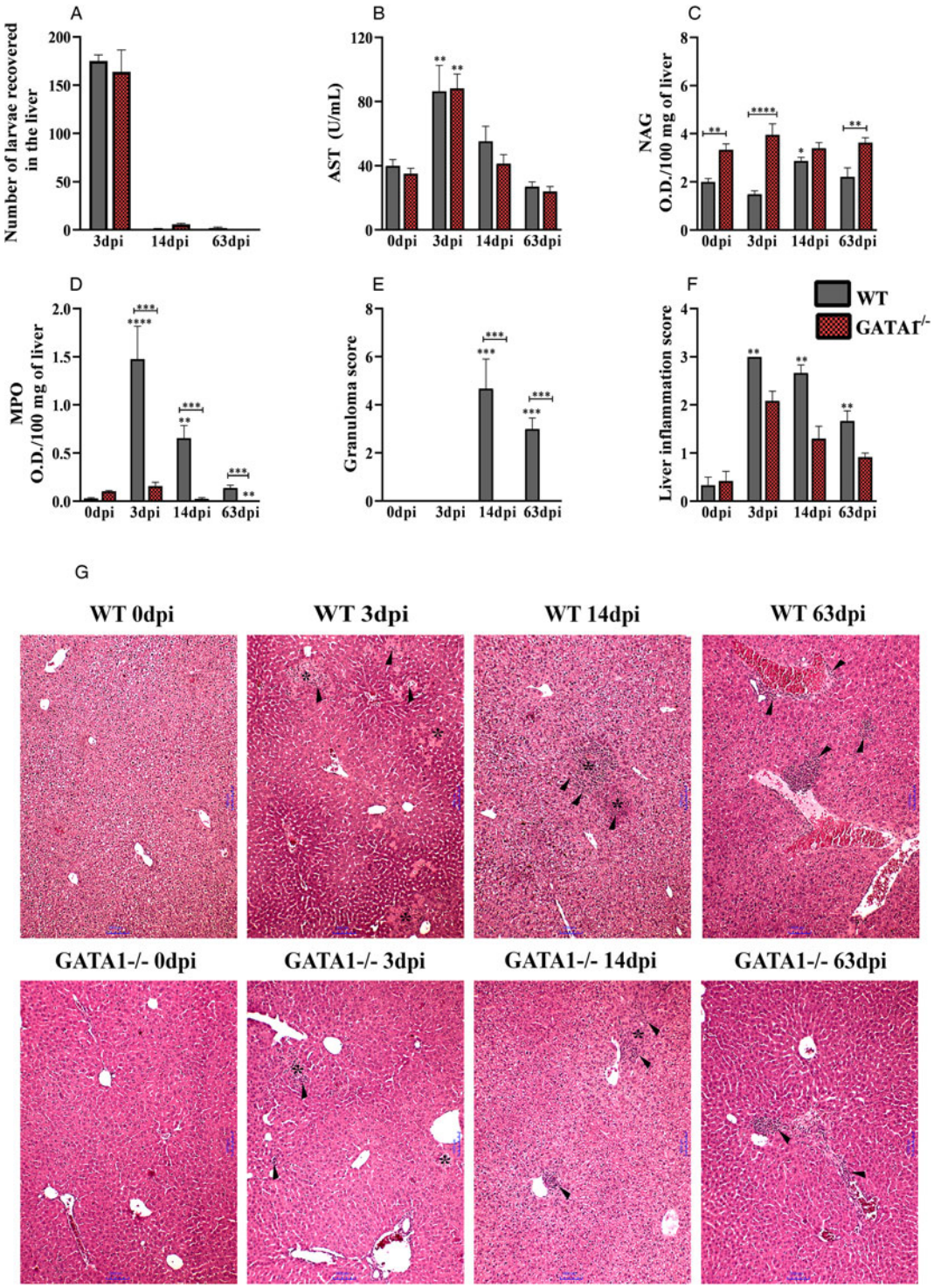

Fig. 2. Liver alterations in WT and GATA1$^{-/-}$ mice infected with *T. canis*. (A) The number of larvae recovered from the liver; (B) analysis of the enzymatic activity of plasma AST; (C) NAG activity in the liver; (D) MPO activity in the liver; (E) number of granulomas; (F) analysis of liver inflammation by score and (G) representative haematoxylin and eosin staining of liver sections, foci of inflammatory infiltration (arrowheads), area of necrosis (*). Bar = 1000 μm. Statistical comparisons were carried out between each strain with its specific uninfected group (0 dpi) represented by the asterisk without the bar and between the strains at the same time of infection represented by the asterisk with the bar. The results represent the mean ± S.E.M., *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. One-way ANOVA test and Kruskal–Wallis test followed by Dunn's test were used.

presence of fibroblasts and collagen fibre deposition were found, characterizing early tissue fibroplasia. In the chronic phase of infection, in WT mice at 63 dpi, the inflammatory foci were scattered throughout the hepatic and perivascular parenchyma. The presence of granulomas was also found, which were in the exudative and productive phases, consisting of macrophages, giant cells, epithelioid cells, fibroblasts and collagen. While in the GATA1$^{-/-}$ mice at 63 dpi, it was possible to identify only the presence of small inflammatory foci dispersed throughout the liver parenchyma, with macrophages and low lymphocyte numbers, and no exudative phenomena or foci of necrosis were observed. Collectively, these data suggest that eosinophils are primarily associated with the presence of hepatic granulomas during *T. canis* infection, in addition to influencing neutrophil activity.

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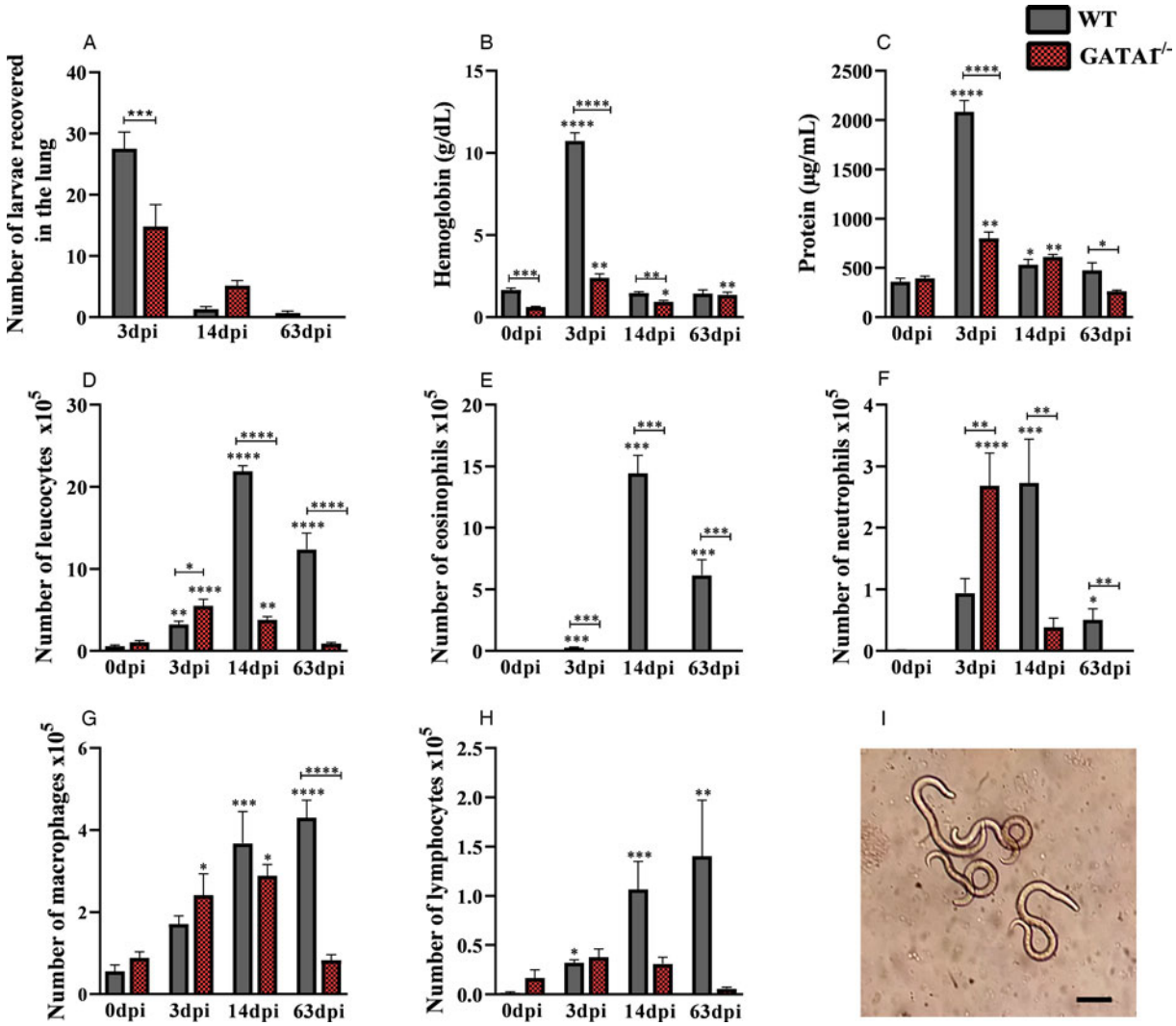

Fig. 3. Number of larvae recovered from lungs and airway inflammation. (A) The number of larvae recovered from the lung; (B) haemoglobin concentration in the BAL; (C) total protein concentration in BAL; (D) number of total leucocytes in BAL; (E) number of eosinophils in BAL; (F) number of neutrophils in BAL; (G) number of macrophages in BAL; (H) number of lymphocytes in BAL and (I) larvae recovered from the lung tissue of WT mice infected with *T. canis*. Bar = 100 μm. Statistical comparisons were carried out between each strain with its specific uninfected group (0 dpi) represented by the asterisk without the bar and between the strains at the same time of infection represented by the asterisk with the bar. The results represent the mean ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. One-way ANOVA test and Kruskal–Wallis test followed by Dunn's test were used.

Eosinophil deficiency is related to increased neutrophil and reduced pulmonary parasite load leading to secondary pulmonary tissue damage during the acute phase of the T. canis *infection in mice*

The larvae migrate from the liver to the lung tissue *via* blood after 3 days of infection (Fig. 3A–I). By analysing the pulmonary parasite load, we observed that GATA1$^{-/-}$ mice showed a reduction in the number of larvae recovered from the lungs at 3 dpi and consequently reduced the concentrations of haemoglobin and protein in BAL when compared to WT mice (Fig. 3A–C). Regarding the leucocytes in BAL (Fig. 3D–H), we observed an increase in the number of total leucocytes in the GATA1$^{-/-}$ mice, due to the increased number of neutrophils. However, at 14 and 63 dpi, there was a reduction in leucocytes in GATA1$^{-/-}$ compared to the WT mice due to the reduced number of neutrophils, macrophages and the absence of eosinophils. In the lung parenchyma, aspects related to tissue inflammation were evaluated (Fig. 4A–H). A reduction in macrophage activity at 3 dpi, haemorrhage score at 63 dpi and granuloma score at 14 and 63 dpi were observed in GATA1$^{-/-}$ mice when compared to WT mice, demonstrating reduced tissue inflammation.

The analysis of lungs of the uninfected mice showed compatibility with normality (Fig. 4I). In the WT mice group at 3 dpi, thickening of the interalveolar septa was observed, with the presence of the mixed inflammatory infiltrate mostly composed of eosinophils and neutrophils, macrophages and to a lesser extent by lymphocytes. In some mice, granuloma was observed in the exudative phase evidenced by perivascular oedema and extensive bleeding areas. Most mice had larvae dispersed in the lung parenchyma and often close to the haemorrhagic zones. In the GATA1$^{-/-}$ mice group at 3 dpi, a significant thickening of the interalveolar septa was observed due to the predominantly mononuclear inflammatory infiltrate characterized by macrophages and lymphocytes, with a lower frequency of neutrophils. Exudative phenomena were also evidenced, such as oedema present in the alveolar and perivascular lumen, large areas of haemorrhage and congested vessels. At 14 dpi, we observed interalveolar septal thickening in both lineages due to the presence of inflammatory infiltrate, and the bronchial and bronchiolar epithelium cells showed intense hypertrophy and hyperplasia, and the formation of a mucus plug obstructing the bronchial lumen was frequently observed. In WT mice at 14 dpi, some macrophages presented

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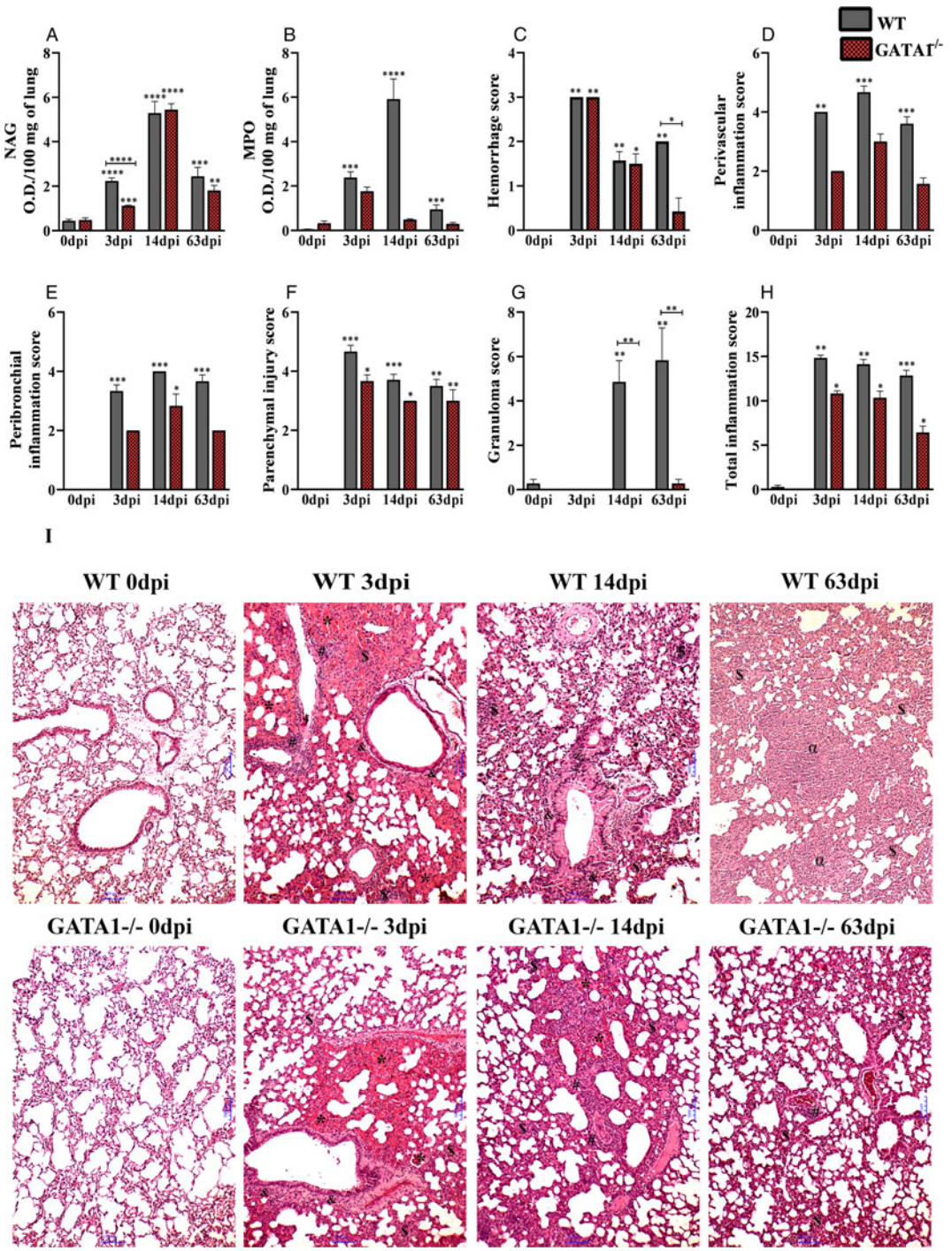

Fig. 4. Inflammation of the lung parenchyma in WT and GATA1$^{-/-}$ mice infected with *T. canis*. (A) NAG activity in lung tissue; (B) MPO activity in lung tissue; (C) bleeding score; (D) perivascular inflammation score; (E) peribronchial inflammation score; (F) parenchymal injury score; (G) granuloma score; (H) total inflammation score and (I) representation of haematoxylin and eosin staining of lung sections, haemorrhage area (*), parenchymal inflammation ($), airway inflammation (&), vascular inflammation (#), granuloma (α), bar = 1000 μm. Statistical comparisons were carried out between each strain with its specific uninfected group (0 dpi) represented by the asterisk without the bar and between the strains at the same time of infection represented by the asterisk with the bar. The results represent the mean ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. One-way ANOVA test and Kruskal–Wallis test followed by Dunn's test were used.

with a brownish pigment in their cytoplasm suggestive of haemosiderin, and the presence of parenchyma granuloma in the exudative phase was frequently observed. In WT mice at 63 dpi, the presence of diffuse inflammatory infiltrate was observed, and formation of bronchus-associated lymphoid tissue was evidenced in some of them. In all mice, it was possible to identify the presence of granulomas in the productive phase in the lung parenchyma and exudative and vascular phenomena such as small haemorrhagic foci and capillary congestion. While in GATA1$^{-/-}$ mice at 63 dpi, the substantial presence of lymphocytes was observed around the vessels, and in some vessels, the presence of hypertrophy and hyperplasia of bronchial epithelial cells was observed. Sometimes, in some parenchyma regions, the presence of extravasated red blood cells was observed in a small proportion. These results indicate that eosinophils are ineffective at controlling the migration of *T. canis* larvae in lung tissue and contribute to attenuating the lung inflammation reducing the number of neutrophils and secondary tissue damage. However, the neutrophils may be important to control the infection.

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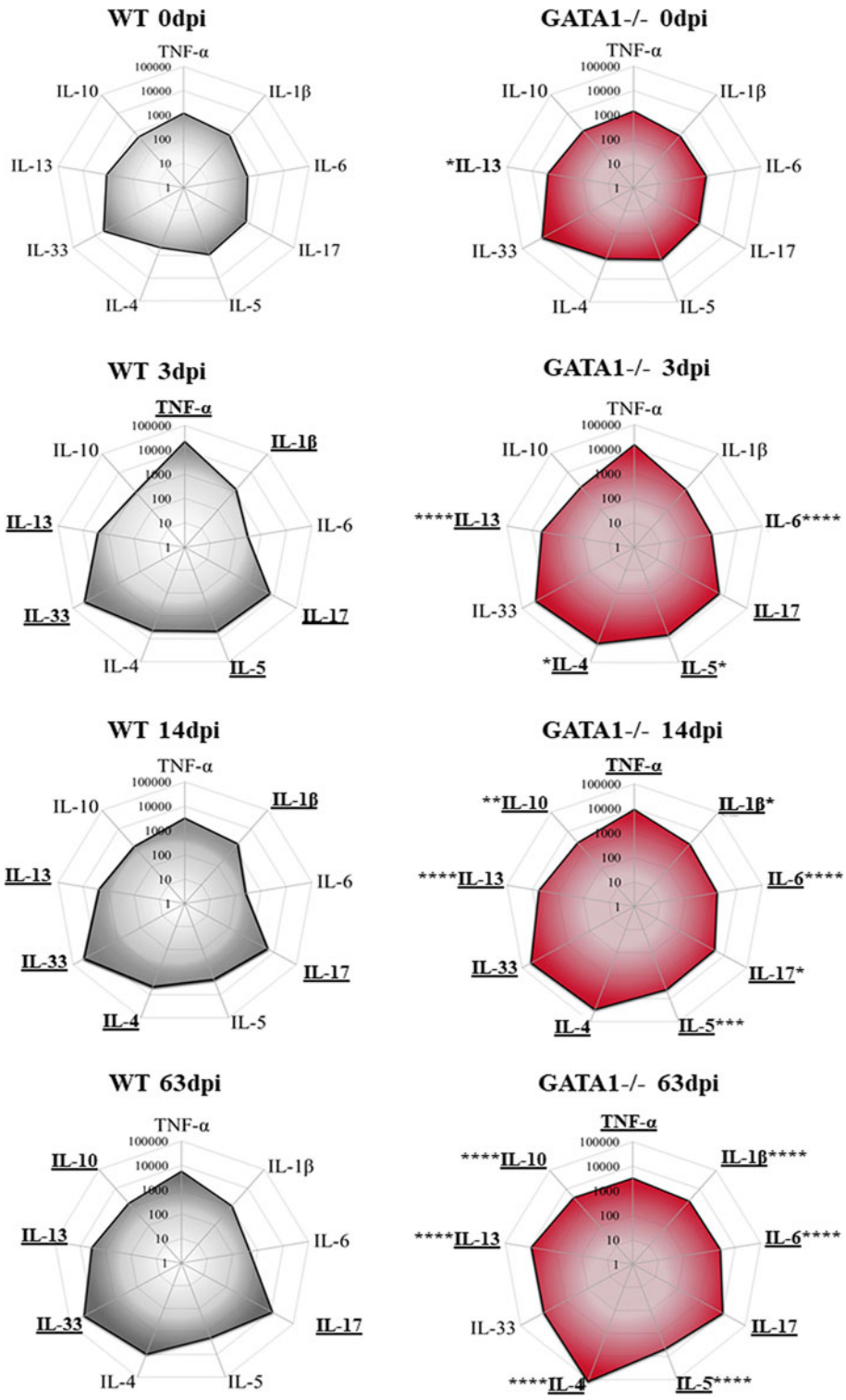

Fig. 5. Concentration of cytokines present in the lung tissue of WT and GATA1$^{-/-}$ mice during *T. canis* infection. Radar graphics express the concentration of pulmonary cytokines throughout the infection. The results represent the mean ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ when compared to the corresponding WT mice groups. Cytokines were underlined when they presented statistical differences with their respective control group (0 dpi). One-way ANOVA test and Kruskal–Wallis test followed by Dunn's test were used.

Eosinophil influences the pulmonary immune profile during T. canis *infection*

We analysed the immune response in the lung parenchyma by measuring cytokines from the Th1, Th2, Th17 and regulatory T (Treg) profiles (Fig. 5). Both mice strains show a mixed type of immune response, with increased Th1, Th2 and Th17 cytokines compared to their respective controls. When comparing the two strains, we observed that the IL-13 cytokine increased in the uninfected GATA1$^{-/-}$ mice (0 dpi) compared to WT mice. At 3 dpi, it was observed that the cytokines IL-6, IL-4, IL-5 and IL-13 increased in the GATA1$^{-/-}$ mice compared to WT mice. In the later stages of infection, GATA1$^{-/-}$ mice showed an increased IL-1*β*, IL-6, IL-4, IL-5, IL-13 and IL-10 cytokines and reduced Th17 response at 14 dpi compared to WT mice. Thus, the results demonstrate that the absence of eosinophils favours secretion of Th2 cytokines in special IL-13 which explains the excessive mucus production. Finally, eosinophil deficiency increases the pro-inflammatory cytokines IL-1*β* and IL-6 during *T. canis* infection and may sustain the neutrophilic inflammation and tissue damage in mice.

Eosinophil deficiency changes the peripheral blood leucocytosis during the T. canis *infection in mice*

To understand the influence of eosinophils on systemic inflammation, the leucocyte profile in peripheral blood throughout the

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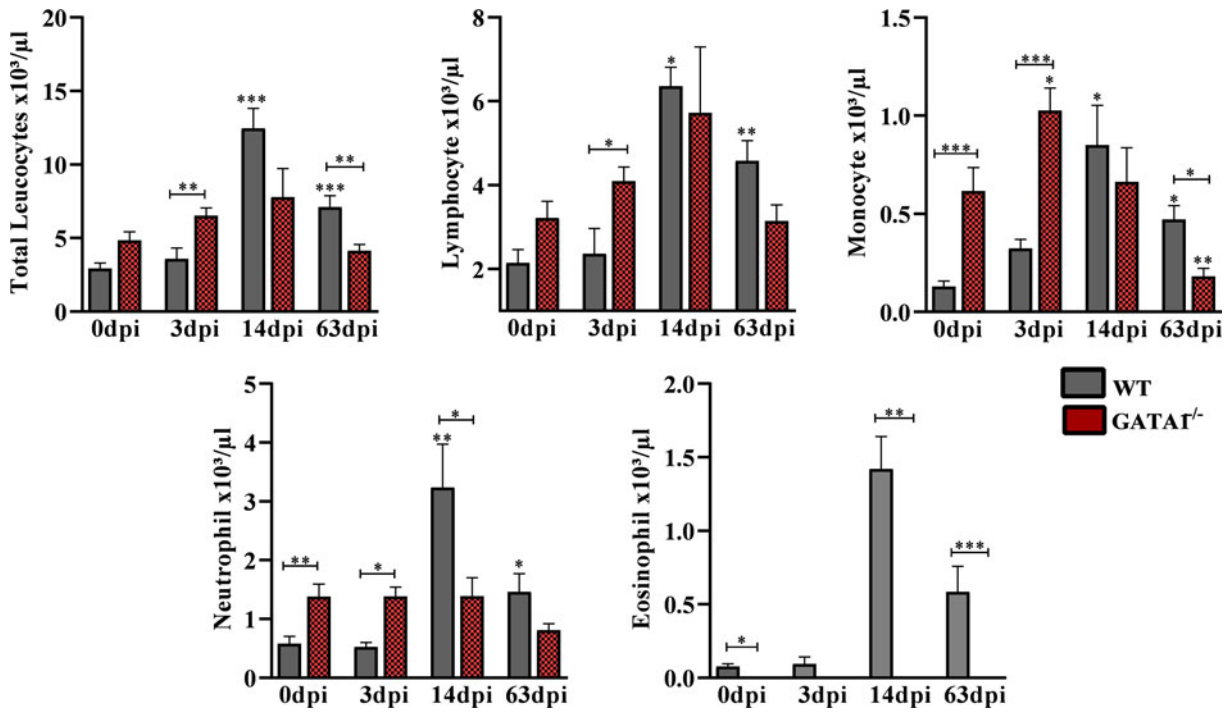

Fig. 6. White blood cells of WT and GATA1$^{-/-}$ mice infected with *T. canis*. Leucogram were shown after 0, 3, 14 and 63 dpi by *T. canis*. Statistical comparisons were carried out between each strain with its specific uninfected group (0 dpi) represented by the asterisk without the bar and between the strains at the same time of infection represented by the asterisk with the bar. The results represent the mean ± S.E.M., *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. One-way ANOVA test and Kruskal–Wallis test followed by Dunn's test were used.

infection was evaluated (Fig. 6). GATA1$^{-/-}$ mice demonstrated a physiological increase in monocytes and neutrophils compared to WT mice, as observed in uninfected mice, but at no time of infection leucocytosis was observed in GATA1$^{-/-}$ mice, while in WT mice leucocytosis was observed at 14 and 63 dpi. At 3 dpi, an increase in lymphocytes and monocytes and a reduction in neutrophils at 14 dpi was observed in GATA1$^{-/-}$ mice compared to WT mice. As expected, no eosinophils were observed in GATA1$^{-/-}$ mice, while in WT mice, there was eosinophilia at 14 and 63 dpi. Thus, larval migration in eosinophil-deficient mice led to an increased liver and lung tissue damage which could be associated with chronic neutrophilic inflammation, which anticipated the peak of systemic leucocytosis in GATA1$^{-/-}$ mice.

Absence of eosinophils reduced the parasite load and increased neutrophil activity in brain tissue during T. canis *infection in mice*

We assessed the parasite load, macrophage and neutrophil activity and haemorrhage score in brain tissue (Fig. 7A–D). The results showed that a reduced number of larvae was recovered from the brain of GATA1$^{-/-}$ mice at 63 dpi compared to WT mice, which may be attributed to the increase in neutrophil activity observed at the same time of infection. No difference in macrophage activity and haemorrhage score was observed between the groups.

Histopathological analysis of the brains of uninfected mice showed a morphological aspect compatible with normality (Fig. 7E). In WT mice at 3 and 14 dpi, foci of haemorrhage and vacuolization and small inflammatory foci were observed. At 63 dpi, there were more areas of vacuolization, multifocal areas of haemorrhage and inflammatory foci. In GATA1$^{-/-}$ mice, the histopathological parameters were minimal and were characterized by haemorrhage, vacuolization and focal inflammation in all infected groups.

Discussion

This study is the first to explain in detail about the influence of eosinophils on liver, lung and brain tissues, evaluating the immunological and pathological aspects during *T. canis* infection in mice. This study found that eosinophils do not promote effective immunity against the parasites. On the contrary, the presence of eosinophils favours the survival and tropism of *T. canis* larvae into the lungs and brain tissues. Eosinophils also mainly trigger the formation of granulomas in toxocariasis which cannot control larval migration, and they mediate a Th17 inflammatory response that generates tissue damage.

T. canis larvae first targets the liver tissue both in dogs being the definitive host and in accidental hosts as well. In experimental models, in the initial stages of infection, the presence of liver damage has been demonstrated, with inflammatory infiltrate with the presence of eosinophils, neutrophils and polymorphonuclear cells, with no significant contribution of lymphocytes (Resende *et al.*, 2015), as observed in the mice in this study, except for the presence of eosinophils in GATA1$^{-/-}$ mice. In the late phase of the infection, granulomatous processes are present in the exudative and productive phases (Fan *et al.*, 2004; Leal-Silva *et al.*, 2021). This study showed that eosinophils are the main triggers for the formation of granulomas in *T. canis* infection. In the eosinophil deficiency, the presence of hepatic granulomas was not observed. Interestingly, we observed that the neutrophil activity was shown to be reduced in GATA1$^{-/-}$ compared to WT mice. Neutrophils and eosinophils have been shown to be involved in adherence to helminths, including *T. canis* larvae (Huwer *et al.*, 1989). Furthermore, high neutrophil counts that followed the migration of larvae through organs and tissues were observed in *T. canis* infection, probably acting as the first line of defense against *T. canis* larvae, as proposed for protozoan parasites (Hermosilla *et al.*, 2014; Resende *et al.*, 2015).

This study revealed a reduction in the pulmonary parasite load in GATA1$^{-/-}$ mice at 3 dpi and consequently reduced concentrations

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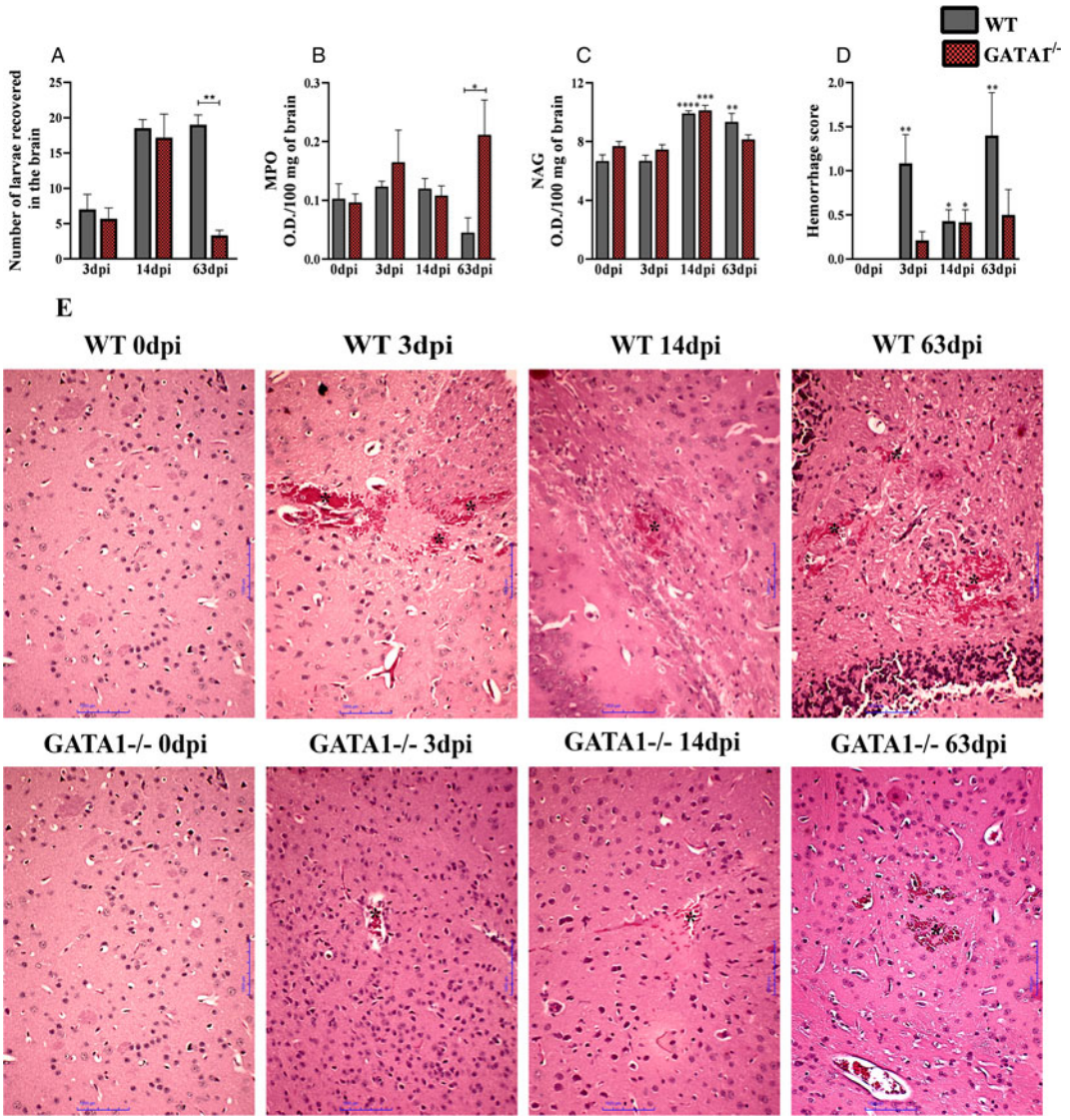

Fig. 7. Parasite burden and inflammation of brain tissue during *T. canis* infection. (A) The number of larvae recovered from the brain; (B) MPO activity in brain tissue; (C) NAG activity in brain tissue; (D) haemorrhage score and (E) representation of haematoxylin and eosin staining of brain sections, haemorrhage area (*), bar = 1000 μm. Statistical comparisons were carried out between each strain with its specific uninfected group (0 dpi) represented by the asterisk without the bar and between the strains at the same time of infection represented by the asterisk with the bar. The results represent the mean ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. One-way ANOVA test and Kruskal–Wallis test followed by Dunn's test were used.

of haemoglobin and protein. Interestingly, an inverse relationship was detected between the number of neutrophils present in the airways and the number of larvae recovered from the tissues, suggesting that neutrophils may be necessary for controlling the parasite load. In addition, in WT mice at 3 dpi, even after obtaining an increased number of eosinophils, we still observed a high parasite load, demonstrating that eosinophils were not able to combat the *T. canis* larvae effectively. Unlike other nematodes, in toxocariasis, there seems to be no relation between eosinophils and larval death, and quite possibly, this reflects the evasion capacity of *T. canis* larvae. It was demonstrated *in vitro* that eosinophils from humans with toxocariasis in the presence of infective *T. canis* larvae were able to adhere to the surface of the larvae, form close interdigitations with the cuticular streaks of the larvae, degranulate on the surface of the parasite and the larvae still remained alive, in addition, some larvae have been shown to be able to escape from the areas of dense eosinophil/parasite binding (Fattah *et al.*, 1986). *In vitro* studies have also indicated that neutrophils are not able to kill *T. canis* larvae (Huwer *et al.*, 1989). However, it is known that the *in vivo* and *in vitro* responses are different and that leucocytes are plastic and heterogeneous, being able to adapt to microenvironments, modifying their phenotypic properties and functions (Natoli and Ostuni, 2019; Ng *et al.*, 2019). Thus, we cannot rule out the possibility that neutrophils are active and important immune cells involved in *T. canis* larvae immune response and parasite control.

In addition to a reduction in the formation of hepatic granuloma in infected GATA1$^{-/-}$ mice, a reduction in the formation of pulmonary granulomas was also observed, reaffirming the role of eosinophils in the formation of granulomas. In *T. canis* infection, the larvae trigger the granuloma formation in the lung tissue, which is mainly composed of eosinophils and later on by macrophages. Granulomas are formed due to the chronic persistence of the antigen or parasite in an attempt to contain it, and their formation involves the interaction between the organism that acts as an antigen and the immune cells of the host. The problem is that their presence usually results in scar tissue formation, which ends up reducing the organ's function (Kayes and Adams Oaks, 1978; Sales *et al.*, 2017; Ariyaratne and Finney, 2019). Furthermore, inadequate accumulation and activation of eosinophils can result in direct tissue damage through the release of highly cytotoxic granular proteins (Rankin *et al.*, 2000).

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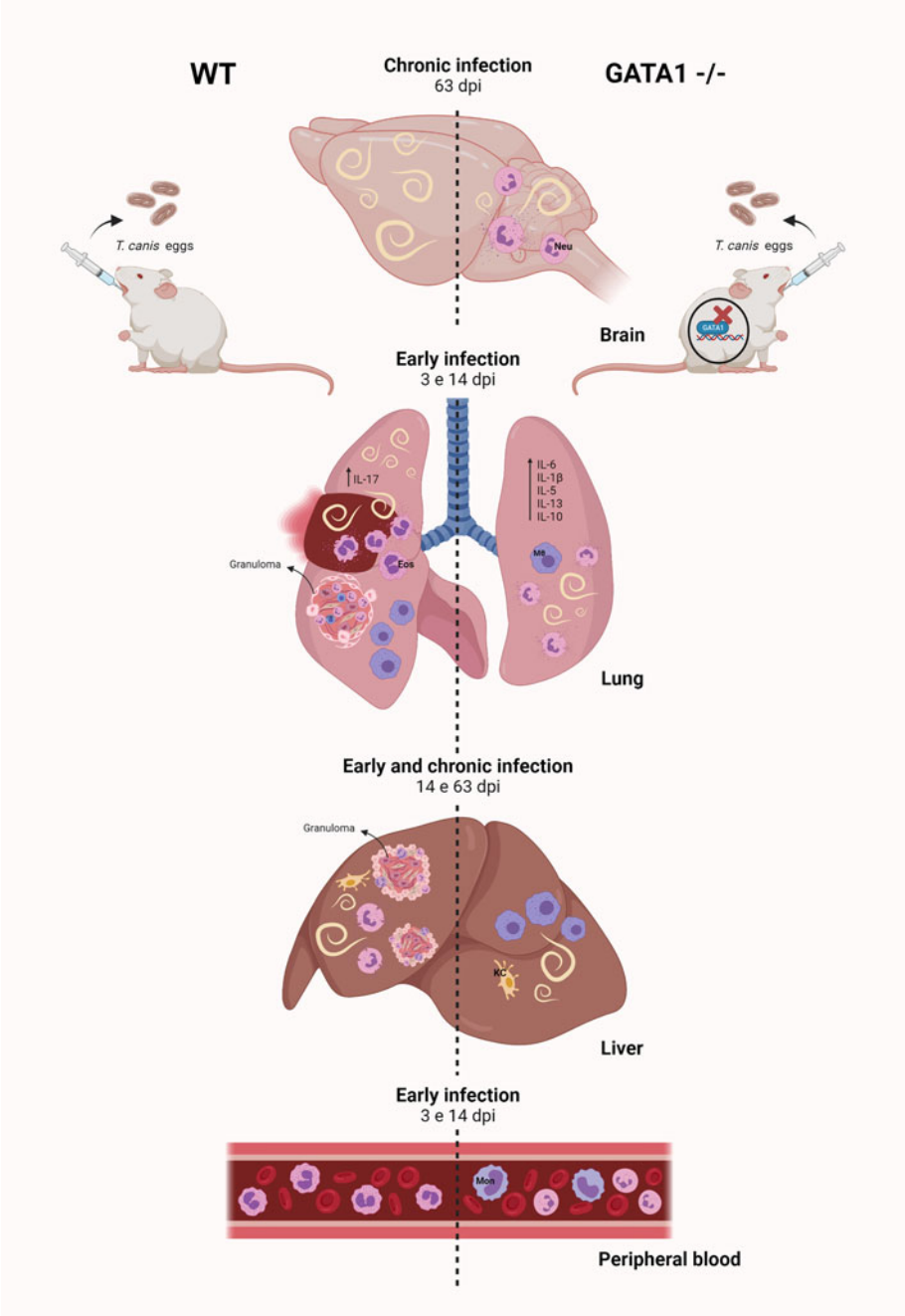

Fig. 8. Influence of transcription factor GATA-1 on *T. canis* infection. The presence of eosinophils during *T. canis* infection contributes to the increase in the pulmonary and cerebral parasite load, to the formation of pulmonary and hepatic granulomas, increases the Th17 response and reduces the number of neutrophils.

Several studies have reported an increase in cytokines Th1, Th2, Th17 and Treg responses in *T. canis* infection, demonstrating that during infection, there is no polarized immune response (Liao *et al.*, 2008; Nagy *et al.*, 2012; Resende *et al.*, 2015; Leal-Silva *et al.*, 2021). This study observed that the absence of eosinophils alters the immune profile during infection, inducing an increase in IL-1β, IL-6, IL-4, IL-5, IL-13 and IL-10. The cytokines IL-4, IL-5 and IL-13 are classified as markers of the Th2 type of immune response and influence the eosinophilic response. IL-5 has been identified as a critical regulator in eosinophil development, migration and activation. At the same time, IL-4 and IL-13 are essential for the recruitment of these cells into lung tissue (Foster *et al.*, 2001), so we believe that the increase in these cytokines in the infected GATA1$^{-/-}$ mice is an immunological mechanism of trying to amplify eosinophilic activity. The cytokine IL-6 in lung tissue is produced from epithelial cells, interstitial fibroblasts, macrophages and other inflammatory cells in response to a variety of stimuli such as environmental particles and inhaled toxic particles, having the function of regulating T cell-mediated responses CD4^{+} which include the production of cytokines such as IL-4, IL-13, IL-17, IL-21 and suppressor activity in Treg cells (Kaur *et al.*, 2020). IL-10 is expressed by many adaptive immune system cells, including Th1, Th2, Th17, Treg, CD8^{+} T, B cells and cells of the innate immune system. Moreover, its primary role appears to be as a feedback regulator of various immune responses, not only Th1 cell responses but also Th2 cell responses to parasites (Saraiva and O'Garra, 2010). Studies have indicated that IL-1β in the presence of type 2 cytokines may also support Th2-mediated immunity (Helmby and Grencis, 2004; Ferguson *et al.*, 2015). In our previous study with mice infected with *T. canis* and genetically deficient for the ST2 receptor, which is a ligand of IL-33 and, therefore, has reduced eosinophils, an increase in IL-1β concentration was also observed in later times of infection (Leal-Silva *et al.*, 2021), suggesting that eosinophils may influence the release of IL-1β. Thus, we observe that the absence of eosinophils increases the

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secretion of pulmonary cytokines from the innate and the Th2 immune responses.

During *T. canis* infection, an increase in blood leucocytes is often observed, with an increase in neutrophils, followed by eosinophilia. In mice, the peak eosinophilia is usually observed between 14 and 18 dpi, but it can persist to the chronic phase at 45 dpi (Pecinali *et al.*, 2005; Margareth *et al.*, 2014; Resende *et al.*, 2015). In this study, eosinophilia was observed from 14 dpi, persisting up to 63 dpi in WT mice. On the contrary, in the infected GATA1$^{-/-}$ mice, leucocytosis was not observed at any time of infection, probably due to the absence of eosinophils.

In neuro-toxocariasis, haemorrhage has been shown to be the main histopathological finding, extending for long periods after infection. The presence of activated microglia and focal accumulation of gitter cells has also been related (Janecek *et al.*, 2014), as observed in this study. Interestingly, in addition to lung tissue, the parasite load in brain tissue was also reduced in GATA1$^{-/-}$ mice at 63 dpi, and again we observed an increased neutrophil activity. In the central nervous system, neutrophils are rarely found in the brain parenchyma due to a blood–brain barrier. However, under pathological conditions such as infections, trauma and haemorrhage, a more significant number of neutrophils enter the brain tissue. These cells can cause degranulation, phagocytosis and release the extracellular neutrophil trap, which is composed of histones, enzymes and granules that help eliminate pathogens (Joice *et al.*, 2009; Liu *et al.*, 2018). However, this process can lead to tissue damage, so neutrophils are often phagocytosed or inhibited by macrophages or lymphocytes after pathogen digestion to minimize tissue damage (Liu *et al.*, 2018). Thus, we believe that neutrophils are important cells in combating *T. canis* larvae, but further studies are needed regarding the function and mechanism of action of cells, especially in the brain tissue, where the damage caused by these cells can lead to significant morbidity.

In conclusion, this work described the influence of eosinophils on acute and chronic toxocariasis (Fig. 8). The presence of these cells induces susceptibility to infection, alters the pulmonary immune response, cannot reduce the pulmonary and cerebral parasite load and decreases the neutrophilic activity in tissues, which seem to play an important role in the control of *T. canis* infection. We also observed that eosinophils are the primary triggers in the formation of pulmonary and hepatic granulomas that can lead to damage and loss of tissue function. Thus, we believe that future research investigating the escape mechanisms of *T. canis* larvae from the immune system is essential to expand therapeutic measures for the disease and dissecting the role of neutrophils in the context of *T. canis* infection may contribute to understanding the anti-parasite role of this granulocyte subtype.

Data. All data generated or analysed during this study are included in this published article. The datasets used and/or analysed during the present study are available from the corresponding author upon reasonable request.

Acknowledgements. We thank Michele Silva de Matos and Vanessa Gomes Fraga for their technical assistance and support during the experiments. Figures were prepared using biorender.com. This work was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Author contributions. T. L.-S., C. A. L., F. V.-S., F. M. S. O., L. K., L. L. S. P., C. C. O. A., J. L. N. S., F. S. B. and M. A. R.: carried out sampling and laboratory analyses. T. L.-S., R. C. R., R. T. F. and L. L. B.: conceived the study. All authors contributed to data analysis and interpretation, and preparation of the manuscript. All authors read and approved the final manuscript.

Financial support. This investigation received partial support from Fundação de Amparo a Pesquisa do Estado de Minas Gerais/FAPEMIG, Brazil (grant no. CBB APQ-00766-18), the Brazilian National Research Council (CNPq) (grant nos. 421392/2018-5 and 302491/2017-1) and Pró-Reitoria de Pesquisa of Universidade Federal de Minas Gerais to cover research inputs. T. L.-S. is grateful for the Ph.D. fellowship provided by the Brazilian National Research Council (CNPq), Post-graduation Program in Infectology and Tropical Medicine/Universidade Federal de Minas Gerais. M. A. R., R. C. R., R. T. F. and L. L. B. are Research Fellows from the Brazilian National Research Council (CNPq). The funders had no role in study design, data collection, analysis, decision to publish, publication fees or manuscript preparation.

Conflict of interest. The authors declare there are no conflicts of interest.

Ethical standards. The maintenance and use of mice were carried out following the Brazilian College of Animal Experimentation (COBEA) recommendations, and all efforts were made to minimize animal suffering. The present study was submitted and approved by the Ethics Committee for Animal Experimentation (CEUA) of the Federal University of Minas Gerais, Brazil, through protocol no. 56/2018.

References

Ariyaratne A and Finney CAM (2019) Eosinophils and macrophages within the Th2-induced granuloma: balancing killing and healing in a tight space. *Infection and Immunity* **87**, e00127-19.

Barcelos LS, Talvani A, Teixeira AS, Vieira LQ, Cassali GD, Andrade SP and Teixeira MM (2005) Impaired inflammatory angiogenesis, but not leukocyte influx, in mice lacking TNFR1. *Journal of Leukocyte Biology* **78**, 352–358.

Fabre V, Beiting DP, Bliss SK, Gebreselassie NG, Gagliardo LF, Lee NA, Lee JJ and Appleton JA (2009) Eosinophil deficiency compromises parasite survival in chronic nematode infection. *The Journal of Immunology* **182**, 1577–1583.

Fan C, Lin Y, Hung C, Chang S and Su K (2004) Enhanced inducible nitric oxide synthase expression and nitrotyrosine accumulation in experimental granulomatous hepatitis caused by *Toxocara canis* in mice. *Parasite Immunology* **26**, 273–281.

Fattah DI, Maizels RM, McLaren DJ and Spry CJF (1986) *Toxocara canis*: interaction of human blood eosinophils with the infective larvae. *Experimental Parasitology* **61**, 421–431.

Ferguson BJ, Newland SA, Gibbs SE, Tourlomousis P, Fernandes dos Santos P, Patel MN, Hall SW, Walczak H, Schramm G, Haas H, Dunne DW, Cooke A and Zaccone P (2015) The *Schistosoma mansoni* T2 ribonuclease omega-1 modulates inflammasome-dependent IL-1β secretion in macrophages. *International Journal for Parasitology* **45**, 809–813.

Foster PS, Mould AW, Yang M, Mackenzie J, Mattes J, Hogan SP, Mahalingam S, Mckenzie ANJ, Rothenberg ME, Young IG, Matthaei KI and Webb DC (2001) Elemental signals regulating eosinophil accumulation in the lung. *Immunological Reviews* **179**, 173–181.

Gazzinelli-Guimarães AC, Gazzinelli-Guimarães PH, Nogueira DS, Oliveira FMS, Barbosa FS, Amorim CCO, Cardoso MS, Kraemer L, Caliari MV, Akamatsu MA, Ho PL, Jones KM, Weatherhead J, Bottazzi ME, Hotez PJ, Zhan B, Bartholomeu DC, Russo RC, Bueno LL and Fujiwara RT (2018) IgG induced by vaccination with *Ascaris suum* extracts is protective against infection. *Frontiers in Immunology* **9**, 1–15.

Gebreselassie NG, Moorhead AR, Fabre V, Gagliardo LF, Lee NA, Lee JJ and Appleton JA (2012) Eosinophils preserve parasitic nematode larvae by regulating local immunity. *The Journal of Immunology* **188**, 417–425.

Hagan P, Wilkins HA, Blumenthal UJ, Hayes RJ and Greenwood BM (1985) Eosinophilia and resistance to *Schistosoma haematobium* in man. *Parasite Immunology* **6**, 625–632.

Helmby H and Grencis RK (2004) Interleukin-1 plays a major role in the development of Th2-mediated immunity. *European Journal of Immunology* **34**, 3674–3681.

Hermosilla C, Caro TM, Silva LMR, Ruiz A and Taubert A (2014) The intriguing host innate immune response: novel anti-parasitic defence by neutrophil extracellular traps. *Parasitology* **141**, 1489–1498.

Huang L and Appleton JA (2016) Eosinophils in helminth infection: defenders and dupes. *Trends in Parasitology* **32**, 798–807.

Huang L, Gebreselassie NG, Gagliardo LF, Ruyechan MC, Luber LK, Lee NA, Lee JJ and Appleton JA (2015) Eosinophils mediate protective immunity against secondary nematode infection. *Bone* **23**, 1–7.

Huwer M, Sanft S and Ahmed JS (1989) Enhancement of neutrophil adherence to *Toxocara canis* larvae by the C3 component of complement and IgG

https://doi.org/10.1017/S0031182022000075 Published online by Cambridge University Press

antibodies. *Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene – Series A* **270**, 418–423.

Janecek E, Beineke A, Schnieder T and Strube C (2014) Neurotoxocarosis: marked preference of *Toxocara canis* for the cerebrum and *T. cati* for the cerebellum in the paratenic model host mouse. *Parasites & Vectors* **7**, 1–13.

Joice SL, Mydeen F, Couraud PO, Weksler BB, Romero IA, Fraser PA and Easton AS (2009) Modulation of blood–brain barrier permeability by neutrophils: *in vitro* and *in vivo* studies. *Brain Research* **1298**, 13–23.

Kaur S, Bansal Y, Kumar R and Bansal G (2020) A panoramic review of IL-6: structure, pathophysiological roles and inhibitors. *Bioorganic and Medicinal Chemistry* **28**, 115327.

Kayes SG and Adams Oaks J (1978) Development of the granulomatous response in murine toxocariasis. *American Journal of Pathology* **93**, 277–294.

Leal-Silva T, Vieira-Santos F, Oliveira FMS, Padrão LLS, Kraemer L, da Paixão Matias PH, de Almeida Lopes C, Loiola Ruas AC, de Azevedo IC, Nogueira DS, Rachid MA, Caliari MV, de Castro Russo R, Fujiwara RT and Bueno LL (2021) Detrimental role of IL-33/ST2 pathway sustaining a chronic eosinophil-dependent Th2 inflammatory response, tissue damage and parasite burden during *Toxocara canis* infection in mice. *PLoS Neglected Tropical Diseases* **15**, 1–25.

Liao C-W, Fan C-K, Kao T-C, Ji D-D, Su K-E, Lin Y-H and Cho W-L (2008) Brain injury-associated biomarkers of TGF-beta1, S100B, GFAP, NF-L, tTG, AbetaPP, and tau were concomitantly enhanced and the UPS was impaired during acute brain injury caused by *Toxocara canis* in mice. *BMC Infectious Diseases* **8**, 1–15.

Liu YW, Li S and Dai SS (2018) Neutrophils in traumatic brain injury (TBI): friend or foe? *Journal of Neuroinflammation* **15**, 1–18.

Nagy D, Bede O, Danka J, Szénási Z and Sipka S (2012) Analysis of serum cytokine levels in children with chronic cough associated with *Toxocara canis* infection. *Parasite Immunology* **34**, 581–588.

Natoli G and Ostuni R (2019) Adaptation and memory in immune responses. *Nature Immunology* **20**, 783–792.

Ng LG, Ostuni R and Hidalgo A (2019) Heterogeneity of neutrophils. *Nature Reviews Immunology* **19**, 255–265.

Nogueira DS, Gazzinelli-Guimarães PH, Barbosa FS, Resende NM, Silva CC, de Oliveira LM, Amorim CCO, Oliveira FMS, Mattos MS, Kraemer LR, Caliari MV, Gaze S, Bueno LL, Russo RC and Fujiwara RT (2016) Multiple exposures to *Ascaris suum* induce tissue injury and mixed Th2/Th17 immune response in mice. *PLoS Neglected Tropical Diseases* **10**, 1–19.

Obata-Ninomiya K, Domeier PP and Ziegler SF (2020) Basophils and eosinophils in nematode infections. *Frontiers in Immunology* **11**, 1–12.

Oliveira FMS, Matias PHP, Kraemer L, Gazzinelli-Guimarães AC, Santos FV, Amorim CCO, Nogueira DS, Freitas CS, Caliari MV, Bartholomeu DC, Bueno LL, Russo RC and Fujiwara RT (2019) Comorbidity associated to *Ascaris suum* infection during pulmonary fibrosis exacerbates chronic lung and liver inflammation and dysfunction but not affect the parasite cycle in mice. *PLoS Neglected Tropical Diseases* **13**, 1–30.

Pecinali NR, Gomes RN, Amendoeira FC, Bastos ACMP, Martins MJQA, Pegado CS, Bastos OMP, Bozza PT and Castro-Faria-Neto HC (2005) Influence of murine *Toxocara canis* infection on plasma and bronchoalveolar lavage fluid eosinophil numbers and its correlation with cytokine levels. *Veterinary Parasitology* **134**, 121–130.

Rankin SM, Conroy DM and Williams TJ (2000) Eotaxin and eosinophil recruitment: implications for human disease. *Molecular Medicine Today* **6**, 20–27.

Resende NM, Gazzinelli-Guimarães PH, Barbosa FS, Oliveira LM, Nogueira DS, Gazzinelli-Guimarães AC, Gonçalves MTP, Amorim CCO, Oliveira FMS, Caliari MV, Rachid MA, Volpato GT, Bueno LL, Geiger SM and Fujiwara RT (2015) New insights into the immunopathology of early *Toxocara canis* infection in mice. *Parasites & Vectors* **8**, 354.

Rodolpho JMA, Oliveira SRP, Souza LC, Dejani NN, Neris DM, Correia RO, Zaia MG, Faccioli LH, Melo DG and Anibal FF (2014) Expression of the costimulatory molecules CD80, C86 and MHC II in eosinophil, during the peak of eosinophilia in the syndrome larvas migrans. *Bio Technology* **66**, 20216–20221.

Rosenberg HF, Dyer KD and Foster PS (2013) Eosinophils: changing perspectives in health and disease. *Nature Reviews Immunology* **13**, 9–22.

Rostami A, Riahi SM, Holland CV, Taghipour A, Khalili-Fomeshi M, Fakhri Y, Omrani VF, Hotez PJ and Gasser RB (2019) Seroprevalence estimates for toxocariasis in people worldwide: a systematic review and meta-analysis. *PLoS Neglected Tropical Diseases* **13**, e0007809.

Rothenberg ME and Hogan SP (2006) The eosinophil. *Annual Review of Immunology* **24**, 147–174.

Sales F, Tristão M, Rocha FA, Carlos D, Ketelut-carneiro N, Oliveira C, Souza S, Milanezi CM, Silva JS and Hube B (2017) Th17-inducing cytokines IL-6 and IL-23 are crucial for granuloma formation during experimental paracoccidioidomycosis, **8**, 949.

Saraiva M and O'Garra A (2010) The regulation of IL-10 production by immune cells. *Nature Reviews Immunology* **10**, 170–181.

Swartz JM, Dyer KD, Cheever AW, Ramalingam T, Pesnicak L, Domachowske JB, Lee JJ, Lee NA, Foster PS, Wynn TA and Rosenberg HF (2006) *Schistosoma mansoni* infection in eosinophil lineage-ablated mice. *Blood* **108**, 2420–2427.

Weller PF and Spencer LA (2017) Functions of tissue-resident eosinophils. *Nature Reviews Immunology* **17**, 746–760.

Yu C, Cantor AB, Yang H, Browne C, Wells RA, Fujiwara Y and Orkin SH (2002) Targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter leads to selective loss of the eosinophil lineage *in vivo*. *Journal of Experimental Medicine* **195**, 1387–1395.

https://doi.org/10.1017/S0031182022000075 Published online by Cambridge University Press