Eosinophils are dispensable for the regulation of IgA and Th17 responses in *Giardia muris* infection

Ivet A. Yordanova | Martin Lamatsch | Anja A. Kühl | Susanne Hartmann | Sebastian Rausch

**Abstract**

**Aims:** IgA and Th17 responses are pivotal for the control of *Giardia* infections. Eosinophils support IgA class switching, the survival of intestinal IgA⁺ plasma cells at steady state and can control Th17 activity in the small intestine. To see whether eosinophils regulate adaptive immune responses during giardiasis, we investigated *Giardia muris* infections in wild-type BALB/c and eosinophil-deficient ΔdblGATA-1 mice.

**Methods and results:** Infected ΔdblGATA-1 mice did not differ markedly in parasite control from wild-type mice. Confirming previous studies, naive ΔdblGATA-1 mice displayed diminished IgA⁺ B cell frequencies in Peyer’s patches. However, IgA class switching and intestinal IgA secretion in response to *G. muris* infection were comparable in wild-type BALB/c and ΔdblGATA-1 mice. Both strains displayed similarly low intestinal Th17 responses, accompanied by a mild expansion of type 3 innate lymphoid cells (ILC3).

**Conclusions:** Contrasting previous reports on overt small intestinal Th17 activity in eosinophil-deficient mice, IL-17A production is kept in check in the absence of eosinophils during *G. muris* infection. Suboptimal homeostatic IgA responses in the absence of eosinophils are transiently fostered in infected mice and the maintenance of IgA⁺ plasma cells appears to be restored during persisting *Giardia* infection.

**Keywords**
eosinophil, *Giardia muris*, IgA, ILC3, parasite, protozoa, Th17

**1 | INTRODUCTION**

The small intestinal protozoan parasite *Giardia lamblia* is a major cause of food- and water-borne diarrhoeal disease worldwide. Mice deficient in B cells, IgA, the polymeric immunoglobulin receptor A (pIgR) or IL-17A fail to control infections with *G. lamblia* or the murine species *G. muris*, emphasizing the importance of humoral immunity and Th17 cells for the control of giardiasis. In line with those findings, we have previously observed differential Th17 activity, IgA production and regulatory T-cell responses associated with differences in susceptibility of inbred mouse lines towards *G. muris* infection.

Over the last decade, eosinophils were shown to regulate both IgA production and Th17 activity at steady state and during infection (reviewed in). Eosinophils typically expand during helminth infection and may trap and damage migrating larvae of parasitic...
nematodes. However, the healthy small intestine harbours high numbers of eosinophils, which support Peyer’s patches (PP) development and mucus production and promote homeostatic IgA antibody class switching (CS). Eosinophils also support IgA-producing plasma cells in the small intestinal lamina propria (siLP) at steady state, and during intestinal nematode and Toxoplasma gondii infection. Importantly, eosinophils were also shown to restrict baseline and LPS-induced activity of intestinal Th17 cells and to limit Th1/Th17-induced immunopathology during infections with Helicobacter pylori and Citrobacter rodentium.

Whether eosinophils exert regulatory functions of mucosal immune responses during Giardia infection has not been addressed experimentally before. Investigating G muris infection in BALB/c and eosinophil-deficient ∆dblGATA-1 mice, we hence assessed whether eosinophil deficiency was associated with impaired IgA responses during Giardia infection and whether eosinophils promoted susceptibility in BALB/c mice by controlling Th17 activity.

2 | MATERIALS AND METHODS

2.1 | Mice and G muris infection

Eight-week old female BALB/c mice were purchased from Janvier Labs (Saint-Berthevin, France). Age-matched ∆dblGATA-1 mice (Jackson Laboratory, CA, USA) were bred in house. 10- to 12-week old mice were infected and sacrificed at the indicated time points as previously described. All animal experiments were performed in accordance with the National Animal Protection Guidelines and approved by the Berlin Ethics Committee for the Protection of Animals (G0113/15).

2.2 | Cell isolation

Cells from lymphatic organs and siLP were isolated as described previously. Cell numbers were determined using an automated CASY cell counter (Roche-Innovatis).

2.3 | Flow cytometric analysis

Surface and intracellular stainings were performed as previously described, applying the antibodies listed in Table S1. Samples were analysed on a Canto II flow cytometer (BD Biosciences), and data were analysed using FlowJo software Version 10 (Tree star Inc.).

2.4 | Quantitative real-time PCR analysis

Tissue sampling, RNA isolation, cDNA preparation and quantitative real-time PCR (qRT-PCR) using 10 ng of cDNA and FastStart Universal SYBR Green Master Mix (Roche) were performed as described previously. Efficiencies for each primer pair were determined by generating a standard curve, and mRNA expression was normalized to the housekeeping gene β-glucuronidase (GUSB). Primers pairs are listed in Table S2.

2.5 | Faecal IgA ELISA

Snap-frozen faecal pellets were weighed and homogenized in 1 mL sterile PBS per 100 mg faecal material. After vigorous vortexing and centrifugation at 20 000 xg (10 minutes, 4°C), supernatants were used for quantification of total faecal IgA via ELISA.

2.6 | Histopathological scoring and immunohistology

Formalin-fixed, paraffin-embedded 1-2 μm sections of duodenum, jejunum and ileum were subjected to immunohistology and inflammation scoring as described previously.

2.7 | Statistical analysis

Statistical analysis was performed using GraphPad Prism software (La Jolla, CA, USA). Results are displayed as mean ± SD, and significance is displayed as *P < .05, **P < .01, ***P < .001. Results were tested for normal distribution using the Shapiro-Wilk normality tests, followed by ANOVA or Kruskal-Wallis combined with Tukey’s or Dunn’s multiple comparison testing.

3 | RESULTS

3.1 | Control of G muris infection is not impaired in the absence of eosinophils

Monitoring cyst shedding of wild-type (WT) BALB/c and eosinophil-deficient ∆dblGATA-1 mice over 6 weeks after primary G muris infection revealed similar dynamics in parasite control between the two strains (Figure 1). Nevertheless, on average, ∆dblGATA-1 mice shed mildly elevated cyst numbers over the 6-week infection period, with cumulative cyst counts confirming the trend of overall higher cyst shedding in ∆dblGATA-1 mice (Figure 1B). Independent of eosinophil deficiency, histopathological scoring of the small intestine confirmed the lack of overt intestinal pathology caused by G muris (Figure 1C). In accordance with stable eosinophil frequencies in siLP, PP, mLN and spleen (Figure 1D,E; Figure S1A), we determined stable gene expression of GM-CSF, IL-5 and IL-33 as well as CCL11, CCL24, CCL26 and CCL28 known to expand and attract eosinophils, respectively (Figure S1B,C). Hence, ∆dblGATA-1 mice displayed a similar course of chronic G muris infection as WT mice, with no indication of overt immunopathology.
Therefore, eosinophils appear dispensable for sustained IgA secretion in naïve controls (Figure 2E). Taken together, these data indicate that frequencies of total and IgA⁺B220⁺PNA⁺ germinal centre (GC) B cells detected in chronically infected ΔdblGATA-1 mice, compared with naïve and G muris-infected BALB/c mice, did not differ significantly depending on the host genotype or in -
sence of eosinophils, 13,18

Previous work has demonstrated that efficient control of
Th17 responses to
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Defective IgA production recovers in G muris-infected ΔdblGATA-1 mice

Confirming previous reports on poor IgA class switching in the absence of eosinophils, 13,18 naïve ΔdblGATA-1 mice harboured lower frequencies of total and IgA⁺B220⁺PNA⁺ germinal centre (GC) B cells in PP than naïve BALB/c mice (Figure 2A-C). However, infected ΔdblGATA-1 mice displayed a transient increase in IgA⁺ GC B cells in PP (Figure 2B,C). While the frequencies of IgA⁺B220⁺ plasma cells did not differ significantly depending on the host genotype or infection status (Figure 2D), a significant increase in faecal IgA was detected in chronically infected ΔdblGATA-1 mice, compared with naïve controls (Figure 2E). Taken together, these data indicate that poor homeostatic IgA class switching seen in PP of eosinophil-deficient mice is transiently upregulated during Giardia infection. Therefore, eosinophils appear dispensable for sustained IgA secretion in Giardia-infected mice.

Eosinophil-deficient mice display transient Th17 responses to G muris

Previous work has demonstrated that efficient control of Giardia infection relies on Th17 cells and IL-17A production. 5,6 As eosinophils were reported to regulate Th17 cells and IL-17A production, 16,17 we assessed whether eosinophil deficiency was associated with deregulated IL-17A production in ΔdblGATA-1 mice at steady state or upon G muris infection. We could show that naïve and infected WT mice harboured similar frequencies of Th17 cells producing IL-17A in response to in vitro stimulation at steady state and during Giardia infection (Figure 3A-C). Minor differences were detected in ΔdblGATA-1 mice, namely a transient increase of Th17 cells at day 18 post-infection and slightly lower frequencies of Th17 cells at day 40 compared with BALB/c mice (Figure 3A-C). Innate lymphoid cells (ILC) have previously been suggested as a potential alternative source of IL-17A during giardiasis. 5 Here, ΔdblGATA-1 mice harboured lower frequencies of ILC3 at steady state and poorly sustained ILC3 during G muris infection (Figure 3D). While ILC3 expanded slightly in G muris-infected WT mice, IL-17A production was barely detectable for ILC3 from either mouse strain (Figure 3E). The modest differences in mucosal Th17 cells and ILC3 responses between Giardia-infected WT and eosinophil-deficient mice did not translate to changes in small intestinal IL-17A gene expression (Figure 3F). Hence, eosinophils appear dispensable for the regulation of Th17 and ILC3-derived IL-17A responses during G muris infection.

DISCUSSION

Several studies have previously demonstrated that small intestinal eosinophils support IgA class switching and the maintenance of intestinal IgA secreting plasma cells in the small intestine. 13,15,23 As IgA is important for the control of both the human-pathogenic G lamblia and the rodent G muris infection, 3,4 we investigated whether
Ionomycin stimulation. The numbers in italics report the frequencies of IL-17A+RORγt+IL-17A expression by CD3ε+CD4+ γε+CD4+ T cells in siLP of naïve and G muris-infected BALB/c and ΔdblGATA-1 mice. A, Representative FACS plots and (C) frequencies of IgA+ B220+PNA+ GC B cells in PP. B, Frequencies of IgA+ B220+ cells in siLP of naïve and G muris-infected BALB/c and ΔdblGATA-1 mice. E, Faecal IgA levels. Data are pooled from three (A-D) and two (E) independent experiments with n = 3-5 mice per group. Statistical analysis was done using Kruskal-Wallis test combined with Dunn’s or Tukey’s multiple comparison test. *P < .05, **P < .01, ***P < .001

**FIGURE 2** Intestinal IgA responses to Giardia muris infection are independent of eosinophil support. A, Frequencies of B220+PNA+ germinal centre (GC) B cells in PP of naïve and G muris-infected BALB/c and ΔdblGATA-1 mice. B, Representative FACS plots and (C) frequencies of IgA+ B220+PNA+ GC B cells in PP. D, Frequencies of IgA+ B220+ cells in siLP of naïve and G muris-infected BALB/c and ΔdblGATA-1 mice. E, Faecal IgA levels. Data are pooled from three (A-D) and two (E) independent experiments with n = 3-5 mice per group. Statistical analysis was done using Kruskal-Wallis test combined with Dunn’s or Tukey’s multiple comparison test. *P < .05, **P < .01, ***P < .001

Eosinophil-deficient mice display a transient Th17 response to Giardia muris infection. A, Representative FACS plots of RORγt+ and IL-17A expression by CD3ε+CD4+ T cells in siLP of naïve and G muris-infected BALB/c and ΔdblGATA-1 mice following ex vivo PMA/Ionomycin stimulation. The numbers in italics report the frequencies of IL-17A+RORγt+ Th17 cells. Frequencies of (B) RORγt+ Th17 cells and (C) IL-17A+RORγt+ Th17 in siLP. Frequencies of (D) CD3ε+RORγt+ type 3 innate lymphoid cells (ILC3) and (E) IL-17A-producing ILC3s in siLP (F) IL-17A gene expression relative to the housekeeping gene β-glucoronidase (GUSB), determined in proximal small intestinal tissue samples (duodenum). Data are pooled from three (A-E) and two (F) independent experiments with n = 3-5 mice per group. Statistical analysis was done using Kruskal-Wallis test combined with Dunn’s or Tukey’s multiple comparison test. *P < .05, **P < .01

**FIGURE 3** Eosinophil-deficient mice display a transient Th17 response to Giardia muris infection. A, Representative FACS plots of RORγt+ and IL-17A expression by CD3ε+CD4+ T cells in siLP of naïve and G muris-infected BALB/c and ΔdblGATA-1 mice following ex vivo PMA/Ionomycin stimulation. The numbers in italics report the frequencies of IL-17A+RORγt+ Th17 cells. Frequencies of (B) RORγt+ Th17 cells and (C) IL-17A+RORγt+ Th17 in siLP. Frequencies of (D) CD3ε+RORγt+ type 3 innate lymphoid cells (ILC3) and (E) IL-17A-producing ILC3s in siLP (F) IL-17A gene expression relative to the housekeeping gene β-glucoronidase (GUSB), determined in proximal small intestinal tissue samples (duodenum). Data are pooled from three (A-E) and two (F) independent experiments with n = 3-5 mice per group. Statistical analysis was done using Kruskal-Wallis test combined with Dunn’s or Tukey’s multiple comparison test. *P < .05, **P < .01

Eosinophil deficiency was associated with poor control of G muris infection and whether this was linked to impaired IgA production. While earlier work determined proportional cyst shedding and trophozoite loads in the murine small intestine, trophozoite counts are considered a more reliable measure of parasite load. Facing technical limitations in co-assessing trophozoite counts and small intestinal immune cells, here we relied on cyst counts as a measure of infection intensity. The kinetics of cyst excretion in G muris-infected wild-type and eosinophil-deficient mice were similar over a 6 week infection period. The moderate elevation in cyst shedding by ΔdblGATA-1 mice reached statistical significance only when calculating average and cumulative cyst counts, indicating that parasite control was largely unaffected in the absence of eosinophils.

These differences in cyst shedding were reflected in minor differences regarding IgA and Th17 responses between the two mouse lines. Confirming previous findings, ΔdblGATA-1 mice harboured lower frequencies of GC B cells and IgA+ GC B cells in PP at steady
However, IgA class switching was upregulated in PP of G muris-infected ΔdblGATA-1 mice and IgA⁺ cell frequencies in the small intestine were comparable to WT mice. Similarly, elevated faecal IgA levels detected in chronically infected ΔdblGATA-1 mice suggest that IgA responses to Giardia infection were not defective in the absence of eosinophils. The late rise of intestinal IgA in infected ΔdblGATA-1 mice may have been driven by continuously high parasite loads, indicating that Giardia possibly profitted from other defects in eosinophil-deficient mice.

As small intestinal eosinophil numbers were reported to inversely correlate with IL-17A production,16 we asked whether eosinophils were responsible for the low Th17 activity previously determined for G muris-infected BALB/c mice by our group.8 In line with the absence of intestinal immunopathology, we saw no evidence for deregulated Th17 or ILC3 activity in infected ΔdblGATA-1 mice. Th17 cell frequencies and IL-17A production were rather low in eosinophil-deficient mice, just transiently reaching levels similar to those seen in WT mice. Hence, the rise in intestinal IgA levels determined in chronically infected ΔdblGATA-1 mice may have resulted from poor Th17-mediated parasite control.

Our data show that murine giardiasis contrasts other models, where eosinophils were reported to attenuate Th17-mediated inflammation, such as Th17 responses to LPS challenge16 or Th1/Th17-driven immunopathology in bacterial infections.17 Along that line, eosinophils were reported to contribute to, rather than limit, Th17-driven inflammation during fungal infection.26,27 Furthermore, our group and work from others showed that eosinophils promote small intestinal IgA responses during H polygonus24 and T gondii15 infections, but restrain IgA responses in the large intestine during infections with the murine whipworm Trichuris muris.23

In summary, our study therefore demonstrates that eosinophils are dispensable for intestinal IgA responses and the regulation of Th17 responses during G muris infection. Together with previous reports, these findings highlight the contextual nature of eosinophils as regulators of mucosal immune responses during parasitic infections.

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CONFLICT OF INTEREST
The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS
IAY and ML carried out the experiments, and IAY and SR analysed the data. AAK performed the histological analysis of tissue samples. SH and AR provided valuable input into the study design, data analysis, critically reviewed and edited the manuscript. SH and SR originally designed the study, and IAY and SR wrote the manuscript.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1111/pim.12791.

DATA AVAILABILITY STATEMENT
The data presented here are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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