European Journal of Immunology

Immunity to infection

Basic

Short Communication

Chronic schistosomiasis during pregnancy epigenetically reprograms T-cell differentiation in offspring of infected mothers

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Schistosomiasis is a nontransplacental helminth infection. Chronic infection during pregnancy suppresses allergic airway responses in offspring. We addressed the question whether in utero exposure to chronic schistosome infection (Reg phase) in mice affects B-cell and T-cell development. Therefore, we focused our analyses on T-cell differentiation capacity induced by epigenetic changes in promoter regions of signature cytokines in offspring. Here, we show that naïve T cells from offspring of schistosome infected female mice had a strong capacity to differentiate into T_H1 cells, whereas T_H2 differentiation was impaired. In accordance, reduced levels of histone acetylation of the IL-4 promoter regions were observed in naïve T cells. To conclude, our mouse model revealed distinct epigenetic changes within the naïve T-cell compartment affecting T_H2 and T_H1 cell differentiation in offspring of mothers with chronic helminth infection. These findings could eventually help understand how helminths alter T-cell driven immune responses induced by allergens, bacterial or viral infections, as well as vaccines.

Keywords: Epigenetic histone modification · In utero programming · Maternal helminth infection · Schistosomiasis · T-cell differentiation

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Introduction

Over the last century and in strong contrast to developing countries, a continuous rise in allergic diseases, such as asthma, is observed in industrialized countries [1]. It is currently discussed whether helminthic parasites contribute to the low prevalence of allergic diseases in parasite endemic areas [2] due to their well-known ability to suppress bystander immune responses

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(reviewed in [3]). Currently, over 250 million people are infected with schistosomes, the causative agent of the disease called bilharzia. Such parasitic helminths establish long-term immunosuppression that protects the infected host against overwhelming immune responses. For these reasons, the hygiene hypothesis was recently expanded to helminths (reviewed in [4]). Since asthma and allergic diseases affect people of all ages and represent major challenges for the health care system, it is essential to further investigate the underlying mechanisms of the suppressed allergic phenotype in schistosome-infected humans and its impact on the development of allergic diseases in children. Protection against asthma and allergic diseases in adults is not only related to exposure to environmental factors during childhood [5, 6]. Recent findings in different mouse models clearly show that prenatal exposure to different infection-related factors ranging from LPS [7] and Interferon(IFN)-γ [8] to the gram-negative bacterium *Acinetobacter lwoffii* [9] significantly alter the risk of offspring´s susceptibility to allergic airway diseases. Mechanisms thereof involve epigenetic modifications within the T-cell compartment that have been shown to play a crucial role in developing allergic diseases [9, 10]. Adding to these findings, we have recently shown that during murine infection with *Schistosoma mansoni*, mice are protected from the onset of allergic airway inflammation (AAI) partly due to expanding activated regulatory T cells [11, 12]. Furthermore, murine schistosomiasis during pregnancy influences the offspring´s susceptibility to allergic airway inflammation depending on the immunological phase of infection. Distinct immune responses within the host are caused by schistosomiasis starting from an initial T_H1 response, followed by an egg-induced T_H2 phase to a regulatory state of infection (Reg phase) [13]. As such, (noninfected) offspring from schistosome-infected female mice were either protected against allergic airway diseases (infected females mated during the initial T_H1 and long-term immunosuppressive Reg phase of infection) or even displayed aggravated disease symptoms (infected females mated during the intermediate T_H2 phase). In this context, the undelying mechanisms leading to dampened T-cell responses in this Ovalbumin (OVA)-induced model of AAI still remain elusive and prompted us to search for T-cell intrinsic alterations in the offspring. Since the most pronounced protection against AAI was observed in offspring from long-term (Reg phase) *S. mansoni* infected mothers and since this phase most likely represents the majority of human infections prevalent in endemic areas [14], we used our established mouse model to analyzed B and T-cell development and in vitro differentiation of T cells in these offspring. Taken together, we could demonstrate that in vitro differentiated naïve T cells showed a clear bias toward T_H1 rather than T_H2 cell differentiation that correlated with a reduced histone acetylation pattern of T_H2 promotor regions. Our studies emphasize the importance of in utero and early postnatal exposures to schistosome infection-related immune responses and parasitic antigens on epigenetically regulated T-cell differentiation and function in response to unrelated antigens in offspring.

Results and discussion

Maternal schistosomiasis does not alter the composition of offspring´s B-cell and T-cell development

Rising evidence suggests that interindividual variations in immune function development can principally be driven by nonheritable factors acting during the in-utero period such as declining maternal biodiversity [18], maternal stress, and nutrition [19–21] and maternal exposures to environmental pollutants and bacteria [22–24]. In this context, mother-to-child transfer of parasite antigens, either as immune-complexes via the FcyR at the placental-blood barrier or directly via the mother milk by enteric resorption is suggested to affect the developing immune system. This hypothesis is further supported by recent findings showing differences in proportions of immune cells and innate immune responses within peripheral blood mononuclear cells (PBMC) observed between infants born in developed and infants born in developing countries [25], where maternal infection might affect the development of different immune cell subsets. To expand these findings, we analyzed basic T and B-cell development in adult offspring from naïve and Reg-phase infected female mice. However, no effect of the chronic maternal *S. mansoni* infection could be detected on the offspring´s B-cell development within the bone marrow (Fig. 1A) and T-cell development (Fig. 1B) within the thymus with regards to CD4 and CD8 expression. Cell populations were detected according to the gating strategy shown in Fig. 1C, D.

Chronic maternal *S. mansoni* infection shapes in vitro T-cell differentiation

Investigations of immune responses to worm antigens in children born by helminth-infected mothers revealed that maternal helminth infection leads to sensitization against these parasite antigens in cord-blood mononuclear cells [26]. In another trial, hookworm infection during pregnancy led to an increased response to an unrelated vaccine antigen, namely BCG, in children within the first year of life [27]. Both studies exemplify the clear notion that maternal helminth infection exerts an influence on the child´s immune system. The activity and differentiation of naive T lymphocytes is largely dependent on the introduction of antigens by highly specialized antigen-presesenting cells and cytokines in the microenvironment. In addition, it is becoming clear that the preexisting epigenetic landscape eventually influences T-cell lineage differentiation capacity [28]. Thus, we investigated the latter aspect in more detail by culturing sorted naïve T cells from offspring of Reg phase-infected and uninfected mothers under T_H1 or T_H2 conditions followed by analyses of the expression and production of signature cytokines and transcription factors. We found that the T_H2 differentiation capacity of naïve T cells—designated by the induced production of IL-4 (Fig. 2B, C and Supporting Information Fig. 1B) and the expression of the T_H2-related transcription factor GATA-3 (Fig. 2A) as quantified by RT-PCR—was

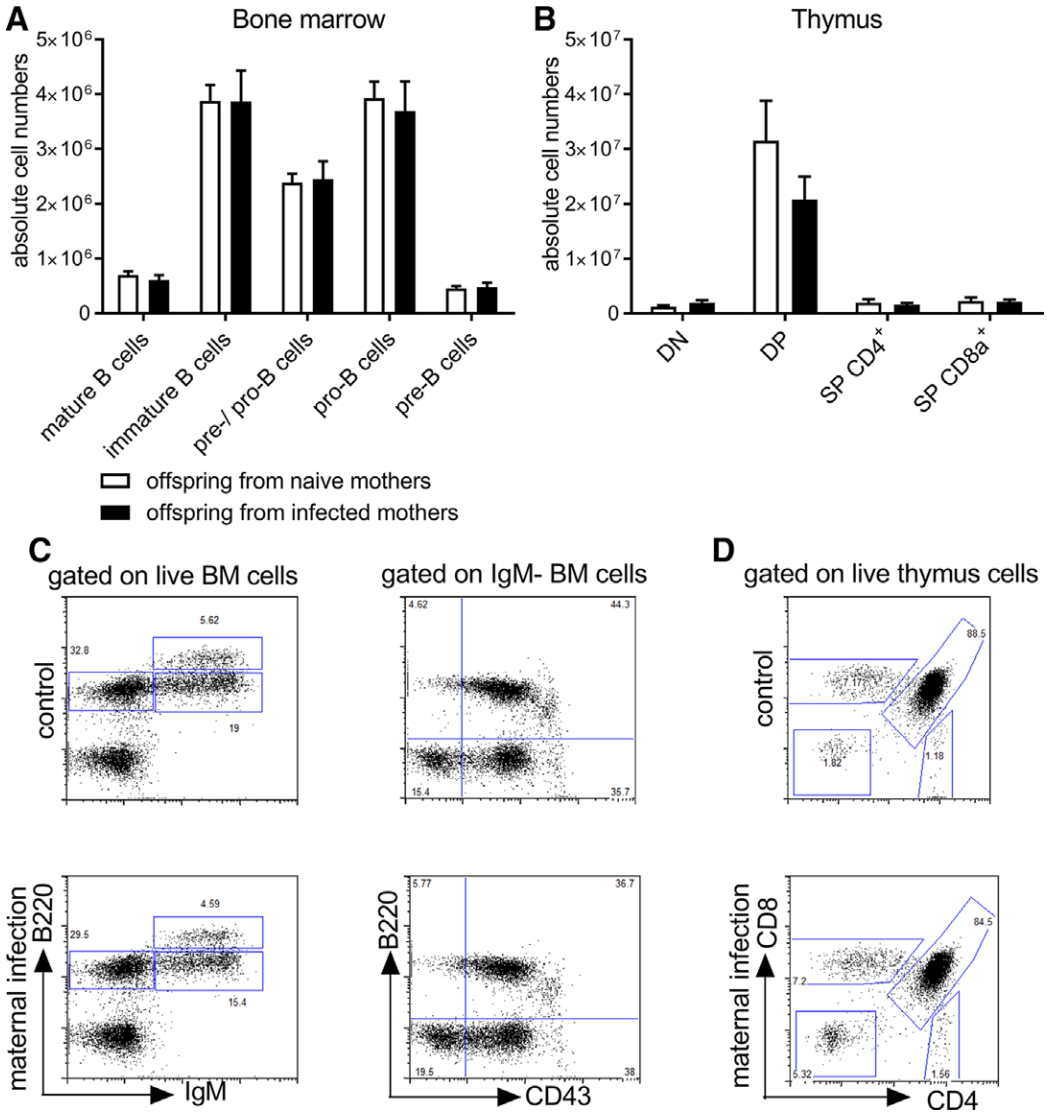

Figure 1. Chronic maternal *S. mansoni* infection does not alter the composition of offspring's B-cell and T-cell compartment. Bone marrow and thymic cells from offsprings of BALB/c mice infected with *S. mansoni* or naïve mothers were analyzed by flow cytometry. B cell subsets in the bone marrow were defined as follows: immature B ($B220^{low}IgM^+$); mature B cells ($B220^{high}IgM^+$); pro- and pre–B cells ($B220^+IgM^-$); pro-B cells ($B220^+CD43^+IgM^-$) and pre-B cells ($B220^+CD43^-IgM^-$) (Fig. 1A, C). T-cell progenitors are defined as DN ($CD4^-CD8^-$) cells; cells in the intermediate developmental stage are defined as DP ($CD4^+CD8^+$); mature cells are defined as SP $CD4^+$ and SP $CD8a^+$ (Fig. 1B, D). Representative results of one of three independent experiments and shown as mean ± SEM with $n \geq 4$ offsprings per experiment (offspring: $n = 15$ from naive versus 19 from infected mothers, mothers: $n = 5$ naïve versus four infected mothers).

significantly reduced in offspring from Reg phase mothers when compared to that of naïve T cells from naïve mice. Intranuclear staining of GATA-3 revealed no significant changes (Fig. 2C). In contrast, T_H1 differentiated naïve T cells revealed a significantly higher expression of the transcription factor t-bet measured by RT-PCR (Fig. 2D) and intranuclear staining (Fig. 2F, Supporting Information Fig. 1A). Moreover, elevated levels of IFN-γ-release (Fig. 2E), measured by ELISA, could be observed. Intracellular IFN-γ levels after PMA/Iono stimulation did not show significant differences (Fig. 2F and Supporting Information Fig. 1A). This might be explained by methodological and technical procedures since cells were short-term (4, 5 h) stimulated with PMA/Iono for FACS analyses and long term (48 h) stimulated with CD3/CD28 for ELISA, measuring IFN-γ that is sequentially produced over 48 hours. Taken together, these results indicate a cell-intrinsic block in T_H2 differentiation within the naïve compartment, rather than an active suppression of T_H2 cell function in vivo. These results are very intriguing since they relate to findings in an Ugandan mother-child cohort in which antihelminthic treatment during pregnancy led to increased incidence of childhood excema—a surrogate for the propensity to develop allergies and asthma later in life—during the first year of life [29].

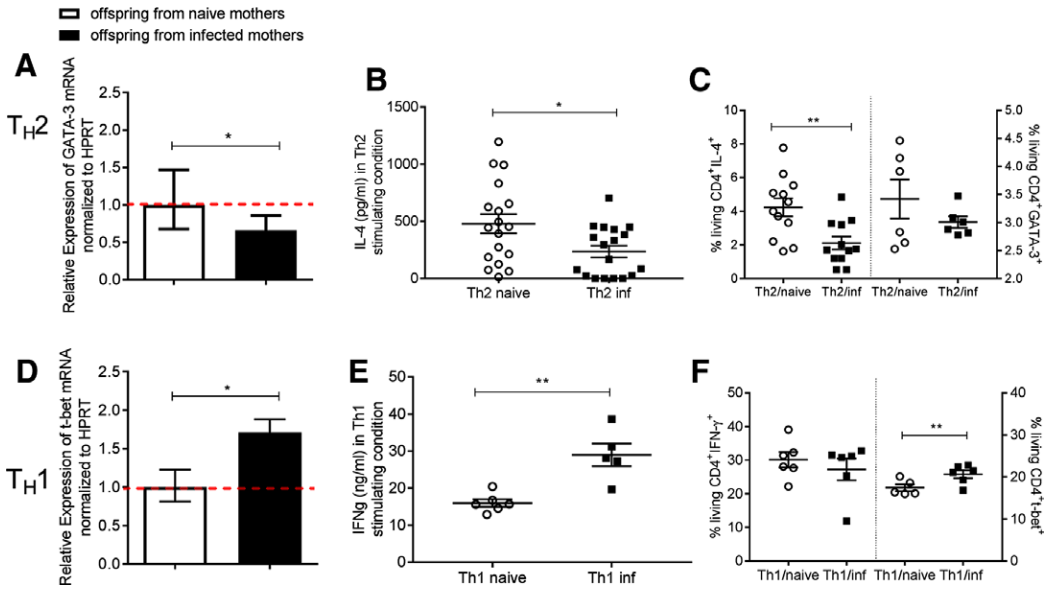

Figure 2. Maternal *S. mansoni* infection leads to stronger T_H1 and impaired T_H2 cell differentiation of $CD4^+CD62L^+$ T cells. MACS-sorted naïve $CD4^+CD62L^+$ cells from spleen of offsprings from naïve and chronic schistosome infected mothers were differentiated into T_H1 and T_H2 cells. Expression and protein levels of cytokines and transcription factors were measured by RT-PCR, ELISA, intranuclear, and intracellular staining (ICS). Shown is the gene expression of transcription factor t-bet (T_H1) and GATA-3 (T_H2) relative to the naïve offspring. Statistical differences were obtained after using REST software (Fig. 2A, D). Cytokine levels of IFN-γ and IL-4 under Th1 and Th2 differentiating conditions are detected by ELISA and shown after substracting the corresponding cytokine levels of undifferentiated T cells (Fig. 2B, E). Frequencies of $CD4^+$ effector cells were analyzed by ICS for IFN-γ, t-bet (T_H1), IL-4, and GATA-3 (T_H2) expression levels (Fig. 2C, F). Asterisks show statistical differences indicated by brackets. ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). Statistical differences were obtained after using the parametric Student's t test in case of normal distribution and the Mann–Whitney test when data are not normally distributed. Data shown as mean ± SEM depicting one of two representative experiments (Fig. 2A, E, F) or pooled data from two experiments with similar outcomes (Fig. 2B, C, D). Each experiment was conducted with offspring: $n \geq 5$, mothers: $n \geq 3$ per goup.

Impact of maternal *S. mansoni* infection on histone acetylation within naïve T-cell compartment

As a potential underlying mechanism for the observed differences in T-cell differentiation, the epigenetic signature of naïve T cells was assessed by the analysis of histone acetylation of specific promoter regions. ChIP-based histone acetylation of sorted splenic $CD4^+CD62L^+$ T cells before differentiation revealed that offspring from chronically infected mothers showed decreased H4 acetylation at IL-4 (Fig. 3A) and IL-5 (Fig. 3B) promotor regions compared to those from naïve offspring. This could imply that decreased histone acetylation leads to repressed chromatin and less active transcription at IL-4 and IL-5 promoter regions resulting in reduced protein levels as observed (Fig. 2). No differences in H3 and H4 acetylaction at IFN-γ (Fig. 3C) promoter regions were observed between the groups. However, other epigenetic modifications such as DNA methylation are currently considered the main epigenetic mechanisms that control T_H1 responses and could therefore contribute to the enhanced T_H1 differentiation levels observed in the Reg-phase offspring [30]. Overall, our findings of epigenetically modified T-cell differentiation toward reduced T_H2 cells is highly coincident with and could

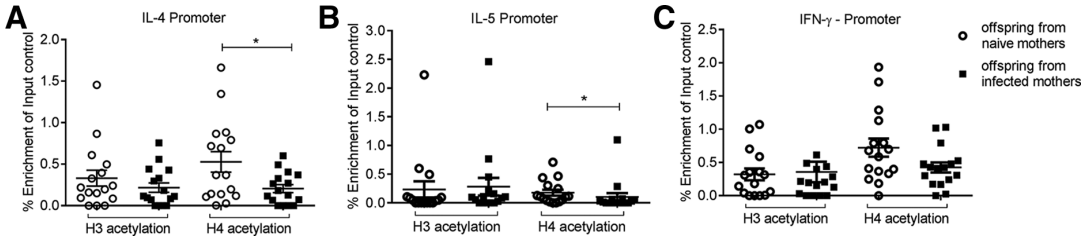

Figure 3. Maternal *S. mansoni* infection influences the offspring's histone acetylation within the naïve T-cell compartment. $CD4^+CD62L^+$ MACS or MoFlow sorted splenocytes from offspring with maternal infection and naive mothers were analyzed by ChIP assay. H3 and H4 acetylation and RT-PCR was performed to assess the percent enrichment at IL-4, IL-5, and IFN-γ, promoter regions (Fig. 3A–C). Results were pooled from three independent experiments and depicted as mean ± SEM. Statistical significances between the indicated groups (offspring: $n = 16$ from naive versus 16 from infected mothers, mothers: $n = 9$ naïve versus six infected mothers) are obtained after using the parametric Student's t test in case of normal distribution and the Mann–Whitney test when data are not normally distributed (IL-5) ($^*p < 0.05$).

therefore explain our previous findings of asthma protection in offspring from chronically schistosome infected mothers [13]. In the light of different helminth species, one has to mention that we have learned in the last years that the immunomodulatory effects of helminths clearly show differences [31, 32]. In the future, it will be extremely interesting to investigate the impact of maternal infection due to other helminths such as filariasis and ultimately, the influence of schistosome infection in pregnant women on their children's T-cell development.

Concluding remarks

Taken together, maternal helminth infection leads to distinct epigenetic modifications within the naïve T-cell compartment possibly explaining the differences in T_H1 and T_H2 differentiation capacity. Besides the reduced reactivity to allergens, these changes could have further implications for other T-cell driven immune responses such as autoimmue diseases, bacterial and viral infections or vaccination.

Materials and methods

Animals

BALB/c mice were purchased from Harlan Winkelmann GmbH (Germany). In vivo experiments were conducted in accordance with local government authorities Bezirksregierung Oberbayern (license number AZ. 55.2.1.54-2532-147-08). Animals were maintained under specific pathogen-free conditions at MIH in accordance with national and EU guidelines 86/809.

Schistosome infection and mating

Female BALB/c mice (6–8 weeks) were infected with *S. mansoni* for 16 weeks as previously described [15, 16]. Infected and age matched naïve female mice stemming from the same batch were mated with uninfected BALB/c males. After weaning, parasite burden of infected mothers was determined as described previously [16].

T-cell differentiation assays

For T_H0, T_H1, and T_H2 in vitro differentiation, $CD4^+CD62L^+$ MACS-sorted splenocytes (T cell isolation kit II, mouse, Miltenyi Biotec) from 8 weeks old offspring were incubated with 10 ng/mL IL-2 in a 48-well plate. For T_H1 conditions, 2×10^6 splenocytes (FACS and RT-PCR/ELISA) were incubated with 10 μg/mL α-IL-4, 20 ng/mL IL-12, and 20 ng/mL IFN-γ for 3 days. For the differentiation control a T_H0/undiff condition was performed with the identical amount of cells and 10 μg/mL α-IFN-γ and 10 μg/mL α-IL-4 for 3 days, respectively. For T_H2 conditions, 10 μg/mL α-IFN-γ, 10 μg/mL α-IL-12, and 25 ng/mL IL-4 were added to 3×10^6 splenocytes (FACS–analysis) or 1.5×10^6 (RT-PCR/ELISA) for 6 days and again for the differentiation control a T_H0/undiff condition was performed with 10 μg/mL α-IFN-γ and 10 μg/mL α-IL-4 for 6 days, respectively. To determine cytokines in the supernatant and transcription factor mRNA expression levels, differentiated T cells were stimulated with αCD3/αCD28 T-cell beads (ratio 1:2) on day 3 (T_H1/T_H0) or day 6 (T_H2/T_H0) for 6 h. To determine intracellular cytokines and transcription factor expression, differentiated cells were stimulated with 750 ng/mL ionomycin, 50 ng/mL phorbol 12-myristate 13-acetate in the presence of 10 μg/mL Brefeldin A for 4.5 h. For surface, intracellular and intranuclear staining the following antibodies were used: FITC-anti-CD4, PE-anti-IFN-γ, APC-anti-t-bet, APC-anti-IL-4, PE-anti-GATA-3 (eBioscience). In the analysis, dead cells (positive for ethidium monoazide bromide) were excluded. Cell acquisition was performed with CyAn ADP Lx P8 or Calibur II Cytometers (BD Biosciences). Primers and probes for RT-PCR of t-bet and GATA-3 were designed using Roche Universal Probe Library software. PCR (hot start: 95°C, 1 cycle; amplifikation: 95°–60°–72°C, 45 cycles; cooling: 45°, 1 cycle) was performed using LightCycler®480 (Roche). Levels of IFN-γ and IL-4 were measured using the Ready-Set-Go!® ELISA Kit (eBioscience).

Flow cytometry analysis of bone-marrow cells and thymocytes

Bone marrow cells from 8 weeks old offspring were flushed from femurs and tibias with HF2+ buffer (Hank's balanced salt solution, supplemented with 2% FCS, 10 mM HEPES buffer, and antibiotics) and single cell supensions of thymuses prepared via 70 μm nylon strainers. Cells were stained with antibodies in HF2+ buffer for 15 min on ice in the dark. Hematopoietic populations were separated with the following antibodies: PB-anti-B220, FITC-anti-CD43, PE-anti-IgM, PECy5-anti-CD4, PECy7-anti-CD8a (eBioscience, Affymetrix). FACS analyses were performed on a CyAn ADP Lx P8 (Coulter-Cytomation Cytometer) and data were analyzed with FlowJo software (TreeStar Inc).

Histone acetylation studies

$CD4^+CD62L+$T cells were isolated from splenocytes of 8 weeks old offspring by using beads and columns of the T-cell isolation kit II, as recommended by the manufacturer (Miltenyi Biotec). DNA-protein interactions in isolated cells were cross-linked in the presence of 1% formaldehyde for 10 min at room temperature. Subsequently, chromatin immunoprecipitation (ChIP) was performed, as described previously [17], with antibodies against acetylated histone H4 (H4ac; Milipore, Germany) and acetylated histone H3 (H3ac; Millipore, Germany). Immunoprecipitated and eluted DNA was purified with QIAquick columns (Qiagen) and amplified by means of quantitative PCR. For analysis, the

percent enrichment of the negative control (IgG) was subtracted from this value and then normalized to the RPL32 housekeeping gene (HKG). The normalization was done according to the following formula: Normalized enrichment to desired gene = % Enrichment to desired gene/% Enrichment to HKG. Primer sequences are shown in Table 1 Supporting Information. All amplifications were performed in duplicate by using 2 μL of DNA per reaction.

Statistical analysis

Using GraphPad Prism software 5 (San Diego, CA, USA) statistical differences were assessed by one-way ANOVA or Student's *t*-test when data were normally distributed or otherwise using Mann–Whitney. Normal distribution was assessed by d'Agostino and Pearson omnibus normality test.

Acknowledgments: This work was supported by the Deutsche Forschungsgemeinschaft (CO 457/8-1, SFB TR22, A23) and the Else Kröner Fresenius Stiftung (2010/A47). We thank Marija Ram and Sabine Paul for excellent technical assistance and Ulla Henn and Stephanie Fetzer for schistosome life-cycle maintenance.

Conflict of interest: The authors declare no commercial or financial conflict of interest.

References

1 **Bach, P. B., Cramer, L. D., Schrag, D., Downey, R. J., Gelfand, S. E. and Begg, C. B.**, The influence of hospital volume on survival after resection for lung cancer. *N. Engl. J. Med.* 2001. **345**: 181–188.

2 **Asher, M. I., Montefort, S., Björkstén, B., Lai, C. K. W., Strachan, D. P., Weiland, S. K. and Williams, H.**, Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* **368**: 733–743.

3 **Maizels, R. M. and Yazdanbakhsh, M.**, Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat. Rev. Immunol.* 2003. **3**: 733–744.

4 **Yazdanbakhsh, M., Kremsner, P. G. and van Ree, R.**, Allergy, parasites, and the hygiene hypothesis. *Science* 2002. **296**: 490–494.

5 **Braun-Fahrländer, C., Riedler, J., Herz, U., Eder, W., Waser, M., Grize, L., Maisch, S. et al.**, Environmental Exposure to Endotoxin and Its Relation to Asthma in School-Age Children. *New England Journal of Medicine* 2002. **347**: 869–877.

6 **Ege, M. J., Mayer, M., Normand, A. C., Genuneit, J., Cookson, W. O. C. M., Braun-Fahrländer, C., Heederik, D. et al.**, Exposure to environmental microorganisms and childhood asthma. *N. Engl. J. Med.* 2011. **364**: 701–709.

7 **Blümer, N., Herz, U., Wegmann, M. and Renz, H.**, Prenatal lipopolysaccharide-exposure prevents allergic sensitization and airway inflammation, but not airway responsiveness in a murine model of experimental asthma. *Clin. Exp. Allergy* 2005. **35**: 397–402.

8 **Lima, C., Souza, V. M. O., Faquim-Mauro, E. L., Hoshida, M. S., Bevilacqua, E., Macedo, M. S., Tavares-de-Lima, W. and Vargaftig, B. B.**, Modulation of the induction of lung and airway allergy in the offspring of IFN-γ-treated mother mice. *J. Immunol.* 2005. **175**: 3554–3559.

9 **Brand, S., Teich, R., Dicke, T., Harb, H., Yildirim, A. Ö., Tost, J., Schneider-Stock, R. et al.**, Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. *J. Allergy Clin. Immunol.* 2011. **128**: 618–625.e617.

10 **Bégin, P. and Nadeau, K. C.**, Epigenetic regulation of asthma and allergic disease. *Allergy Asthma Clin. Immunol.* 2014. **10**: 27–27.

11 **Layland, L. E., Straubinger, K., Ritter, M., Loffredo-Verde, E., Garn, H., Sparwasser, T. and Prazeres da Costa, C.**, Schistosoma mansoni-mediated suppression of allergic airway inflammation requires patency and foxp3 treg cells. *PLoS Negl. Trop. Dis.* 2013. **7**: e2379.

12 **Smits, H. H. and Akdis, C. A.**, In utero priming by worms protects against respiratory allergies. *J. Allergy Clin. Immunol.* 2014. **134**: 1280–1281.

13 **Straubinger, K., Paul, S., Prazeres da Costa, O., Ritter, M., Buch, T., Busch, D. H., Layland, L. E. and Prazeres da Costa, C. U.**, Maternal immune response to helminth infection during pregnancy determines offspring susceptibility to allergic airway inflammation. *J. Allergy Clin. Immunol.* 2014. **134**: 1271–1279.e1210.

14 **2013. World Health Organization**. *Schistosomiasis Progress Report (2001–2011) and Strategic Plan* (2012–2020). World Health Organization Press; Geneva, Switzerland, 2013.

15 **Layland, L. E., Mages, J., Loddenkemper, C., Hoerauf, A., Wagner, H., Lang, R. and Prazeres da Costa, C. U.**, Pronounced phenotype in activated regulatory T cells during a chronic helminth infection. *J. Immunol.* 2010. **184**: 713–724.

16 **Layland, L. E., Rad, R., Wagner, H. and da Costa, C. U.**, Immunopathology in schistosomiasis is controlled by antigen-specific regulatory T cells primed in the presence of TLR2. *Eur. J. Immunol.* 2007. **37**: 2174–2184.

17 **Harb, H., A. M., Ashley, S., Tulic, K. M., Pfefferle, P. I., Potaczek, D. P., Martino, D. et al.**, Epigenetic regulation in early childhood: a miniaturized and validated method to assess histone acetylation. *Int. Arch. Allergy Immunol.* 2015. **168**: 173–181.

18 **Khan, T. K., Palmer, D. J. and Prescott, S. L.**, In-utero exposures and the evolving epidemiology of paediatric allergy. *Curr. Opin. Allergy Clin. Immunol.* 2015. **15**: 402–408.

19 **Veru, F., Laplante, D. P., Luheshi, G. and King, S.**, Prenatal maternal stress exposure and immune function in the offspring. *Stress* 2014. **17**: 133–148.

20 **Marques, A. H., Bjorke-Monsen, A. L., Teixeira, A. L. and Silverman, M. N.**, Maternal stress, nutrition and physical activity: impact on immune function, CNS development and psychopathology. *Brain Res.* 2015. **1617**: 28–46.

21 **Velazquez, M. A.**, Impact of maternal malnutrition during the periconceptional period on mammalian preimplantation embryo development. *Domest. Anim. Endocrinol.* 2015. **51**: 27–45.

22 **Prescott, S. L. and Clifton, V.**, Asthma and pregnancy: emerging evidence of epigenetic interactions in utero. *Curr. Opin. Allergy Clin. Immunol.* 2009. **9**: 417–426.

23 **Sugiyama, M., Arakawa, H., Ozawa, K., Mizuno, T., Mochizuki, H., Tokuyama, K. and Morikawa, A.**, Early-life risk factors for occurrence of atopic dermatitis during the first year. *Pediatrics* 2007. **119**: e716–e723.

24 **Conrad, M. L.**, **Ferstl, R.**, **Teich, R.**, **Brand, S.**, **Blumer, N.**, **Yildirim, A. O.**, **Patrascan, C. C. et al.**, Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe *Acinetobacter lwoffii* F78. *J. Exp. Med.* 2009. **206**: 2869–2877.

25 **van der Kleij, D.**, **van den Biggelaar, A. H.**, **Kruize, Y. C.**, **Retra, K.**, **Fillie, Y.**, **Schmitz, M.**, **Kremsner, P. G. et al.**, Responses to Toll-like receptor ligands in children living in areas where schistosome infections are endemic. *J. Infect Dis.* 2004. **189**: 1044–1051.

26 **Malhotra, I.**, **Ouma, J.**, **Wamachi, A.**, **Kioko, J.**, **Mungai, P.**, **Omollo, A.**, **Elson, L. et al.**, In utero exposure to helminth and mycobacterial antigens generates cytokine responses similar to that observed in adults. *J. Clin. Invest.* 1997. **99**: 1759–1766.

27 **Elliott, A. M.**, **Namujju, P. B.**, **Mawa, P. A.**, **Quigley, M. A.**, **Nampijja, M.**, **Nkurunziza, P. M.**, **Belisle, J. T. et al.**, Mother, and t. Baby study. A randomised controlled trial of the effects of albendazole in pregnancy on maternal responses to mycobacterial antigens and infant responses to Bacille Calmette-Guerin (BCG) immunisation [ISRCTN32849447]. *BMC Infect Dis.* 2005. **5**: 115.

28 **Wang, C.**, **Collins, M. and Kuchroo, V. K.**, Effector T cell differentiation: are master regulators of effector T cells still the masters? *Curr. Opin. Immunol.* 2015. **37**: 6–10.

29 **Ndibazza, J.**, **Mpairwe, H.**, **Webb, E. L.**, **Mawa, P. A.**, **Nampijja, M.**, **Muhangi, L.**, **Kihembo, M. et al.**, Impact of anthelminthic treatment in pregnancy and childhood on immunisations, infections and eczema in childhood: a randomised controlled trial. *PLoS One* 2012. **7**: e50325.

30 **Harb, H. and Renz, H.**, Update on epigenetics in allergic disease. *J. Allergy Clin. Immunol.* 2015. **135**: 15–24.

31 **Blackwell, A. D.**, **Tamayo, M. A.**, **Beheim, B.**, **Trumble, B. C.**, **Stieglitz, J.**, **Hooper, P. L.**, **Martin, M. et al.**, Helminth infection, fecundity, and age of first pregnancy in women. *Science* 2015. **350**: 970–972.

32 **Cooper, P. J.**, **Chico, M. E.**, **Amorim, L. D.**, **Sandoval, C.**, **Vaca, M.**, **Strina, A.**, **Campos, A. C. et al.**, Effects of maternal geohelminth infections on allergy in early childhood. *J. Allergy Clin. Immunol.* 2016. **137**: 899–906.

Abbreviations: **AAI**: allergic airway inflammation · **Reg**: regulatory

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Received: 29/11/2016
Revised: 3/2/2017
Accepted: 1/3/2017
Accepted article online: 8/3/2017

