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Research Article

Activity of the Toll-like receptor ligands in children with high and low socioeconomic backgrounds

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ABSTRACT

Background: Adjuvants are essential in the induction of immunity by vaccines and interact with receptors, including the Toll-like receptors (TLRs). Responsiveness of these receptors differs between and within populations, which impacts vaccine effectiveness.
Objective: Here we examine how the innate cytokine response towards TLR ligands differs between high and low socioeconomic status (SES) school-aged children from Makassar, Indonesia.
Methods: We stimulated whole blood from children, of which 27 attended a high SES school and 27 children a low SES school, with ligands for TLR-2/1, -2/6, -3, -4, -5, -7, -9 and measured pro- (TNF) and anti-inflammatory (IL-10) cytokines released.
Results: In the low SES there is an increased pro-inflammatory response after 24 h stimulation with TLR-2/1 ligand Pam3 and TLR-4 ligand LPS compared to the high SES. Comparison of the response to LPS after 24 h versus 72 h stimulation revealed that the pro-inflammatory response in the low SES after 24 h shifts to an anti-inflammatory response, whereas in the high SES the initial anti-inflammatory response shifts to a strong pro-inflammatory response after 72 h stimulation.
Conclusion: We observed differences in the TLR-mediated innate immune response between children attending low and high SES schools, which can have important implications for vaccine development

1. Introduction

Vaccines are the success stories of modern medicine and immunization prevents the deaths of 2–3 million people every year. Today, more than 20 life-threatening diseases can be prevented by vaccinations and numerous new and improved vaccines against infectious diseases are currently under development (World Health Organization WHO, 2013).

A prerequisite for the development of specific immunity by vaccination is a state of inflammation. Since purified proteins alone are known to lead to a poor immune response, in vaccines these pure antigens are combined with adjuvants, such as microbial products. Adjuvants can promote the induction of specific effector responses by activating the innate immune system through interaction with pattern recognition receptors (PRRs), like Toll-like receptors (TLR). These receptors are expressed on the cell surface or in intracellular compartments and their binding to pathogen associated molecular patterns (PAMPs) initiates a signaling cascade that leads to activation of immune responses (Takeda and Akira, 2005).

Since PRRs play an important role in the activation of the immune response, alterations in responsiveness of these receptors can affect immune response profiles and vaccine effectiveness. For the TLR receptors differences in responsiveness were observed among children from different regions and populations. Previous studies revealed that the TLR-mediated innate cytokine response significantly differed between European and African children (Labuda et al., 2014), as well as between Papua New Guinean and Australian infants (van den Biggelaar et al., 2009). Moreover, it was shown that within the Canadian population innate responsiveness correlates with a child's socioeconomic status (SES), which is known to be a major determinant of environmental exposures such as pathogens (Azad et al., 2012).

Several environmental factors have been linked to the variation in

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List of abbreviations

CpG	CpG2006
Fla	Flagellin
Hklm	Heat-killed Listeria monocytogenes
LPS	Lipopolysaccharide
Pam3	Pam3Cys-ser-(lys)4
PAMP	Pathogen associated molecular pattern
PPR	Pattern recognition receptor
R-848	Resiquimod-848
SES	Socioeconomic status
TLR	Toll-like receptors
WHO	World Health Organization

innate immune responses, including helminth infections. Helminth infections are characterized by Th2 cell expansion and some level of T cell hypo responsiveness, which is accompanied by increased production of anti-inflammatory cytokines such as IL-10. The antigen-specific immunological hypo responsiveness caused by helminth appears to spill over to non-related antigens, resulting in altered immune responses against other pathogens and vaccines (Maizels and Yazdanbakhsh, 2003; van Riet et al., 2007). It was found that responses to several vaccines were lower in helminth-infected subjects (Elias et al., 2008; Nookala et al., 2004; Elias et al., 2001; Sabin et al., 1996) and that Gabonese children infected with *Schistosoma* displayed a differential response to TLR stimulation compared to their uninfected counterparts (Meurs et al., 2011).

Responsiveness to TLR ligands has become increasingly important, since research into identification of effective adjuvants for current and new vaccines have been intensified. However, the question whether TLR responsiveness differs between SES groups in low- and middle income countries (LMICs) has not been yet been addressed and is of importance given that SES can differ greatly within a single urban center especially in LMICs facing rapid urbanization. Therefore this study aims to examine how the innate cytokine response towards TLR ligands differs between high and low socioeconomic status in school-aged children from Makassar, Indonesia.

2. Materials and methods

2.1. Study population

Participants originated from two elementary schools in Makassar, the capital city of South Sulawesi Indonesia. These schools were selected based on the socioeconomic background of the children attending them. One school was attended by children from families with low SES (SD Cambaya) and will be henceforth referred to as 'low SES school' whereas the other school was attended by children with a high SES background (SD Mangkura) and will be therefore referred to as 'high SES school'. The schools were about 7 km apart from each other and their level of facilities differed substantially. From each school 27 children agreed to donate blood and collect feces and informed consent was obtained from the legal guardians of each child. The study was approved by the ethics committee of the Faculty of Medicine, Hasanuddin University, Indonesia (ref:0147/H4.8.4.5.31/PP36-KOMETIK/2005).

2.2. Questionnaire and measurements

Information regarding demographics and indicators of socioeconomic status were obtained using a questionnaire. Parental educational level was categorized as 'high' if a parent attended academy or university and 'low' if the parent was illiterate or only completed elementary school or high school. The parental job was categorized as either low-skilled or high-skilled. Body weight and height were measured to calculate the age-standardized z-scores of body mass index (z-BMI).

2.3. Parasitological examination

Stool samples were collected and assessed for the presence of *Ascaris lumbricoides, Trichuris trichuria* and hookworm infections using the Kato Katz methods.

2.4. Whole blood collection and stimulation

Approximately 2 ml of venous blood was collected from the participants into heparinized tubes (BD Bioscience, Franklin Lakes, NJ, USA) and processed within 1 h. Whole blood was diluted 5-fold in RPMI-1640 medium (Invitrogen, Carlsbad, CA, United States) and stimulations were performed in 96-wells round bottom tissue-culture plates (Nunc, VWR International, the Netherlands). To 100 µl of whole blood, 100 µl of medium containing a single TLR ligand (TLR-L) was added to each well. The TLR-L were used in the following final concentrations: Pam3Cys-ser-(lys)4 (Pam3) 100 ng/ml, heat-killed Listeria monocytogenes (Hklm) 1 $\times$ 10^6 bacteria/ml, Poly I:C 50 µg/ml, lipopolysaccharide (LPS) 100 ng/ml, Flagellin (Fla) 100 ng/ml, resiquimod-848 (R-848) 1 µg/ml and CpG2006 (CpG) 5 µM to study TLR-2/1, −2/6, −3, −4, −5, −7 and −9 respectively. As a negative control RPMI-1640 containing medium was used. The culture plates were then incubated for 24 h at 5% CO2 at 37 °C incubator. For LPS, an additional stimulation was performed for 72 h. At the end of the incubation period, the supernatants were harvested, and stored at −20 °C. Supernatants were transported to the Netherlands on dry ice and stored at –80 °C until analysis.

2.5. Cytokine measurement

Since we were interested in the pro- and anti-inflammatory responses, concentrations of IL-10 and TNF were determined in the supernatants by enzyme linked immunosorbent assay (ELISA) using PeliKine Compact™ Human Interleukin commercial kits (Sanquin/CLB, Amsterdam, The Netherlands) following manufacturer's recommendations. Detection limit for IL-10 and TNF was 2.4 pg/ml and 2.8 pg/ml, respectively. To obtain net cytokine responses the level of each cytokine in response to RPMI-1640 medium only was subtracted from the cytokine responses after stimulation with TLR ligands and negative and zeros were subsequently replaced by the lowest positive value.

2.6. Statistical analysis

The distribution of sex and helminth infections between the two schools was tested using Fisher's exact test. Age, z-BMI and infection intensities (calculated in infected children) between two schools were compared using the Mann-Whitney U test. As cytokine concentrations were not normally distributed, the Mann-Whitney U test was used for two independent samples and the Wilcoxon matched-pair signed-rank test for two dependent samples. Data was analysed using IBM Statistical Package for the Social Sciences Statistics version 24 (IBM-SPSS Inc., Chicago, IL, USA) and RStudio. Outcomes of statistical tests were considered significant when p-values were smaller than 0.05.

3. Results

3.1. Characteristics of study participants

A total of 54 school-aged children were included, of which 27 attended the low SES school and 27 attended the high SES school (Table 1). The age, sex distribution and z-BMI did not significantly differ between the two schools. In the high SES school, all children had parents who attended higher education and had a high-skilled job, whereas in the low SES only 44.4% (12/27) of the parents attended higher

Table 1
Characteristics children from low and high SES schools.

	Low SES school $N = 27$	High SES school $N = 27$	p-value
Age (in years, mean, SD)	9.19 (1.60)	9.34 (1.51)	ns[1]
Sex (female%, n/N)	51.9 (14/27)	51.9 (14/27)	0.607[2]
z-BMI (mean, SD)	−0.31 (1.08)	−0.42 (1.25)	ns[1]
Parental education (high%, n/N)	44.4 (12/27)	100 (27/27)	<0.001[2]
Parental job (high-skilled%, n/N)	3.7 (1/27)	100 (27/27)	<0.001[2]
Helminth infection by microscope (%, n/N)			
Any helminth infection	100 (27/27)	44.4 (12/27)	<0.001[2]
A. lumbricoides	96.3 (26/27)	25.9 (7/27)	<0.001[2]
T. trichiura	92.6 (25/27)	33.3 (9/27)	<0.001[2]
Mixed infection	88.9 (24/27)	14.8 (4/27)	0.001[2]
Helminth infection intensity (egp) [geometric mean (95% CI)]			
A. lumbricoides	13,570 (8772 – 20,999)	336 (58 – 1922)	<0.001[1]
T. trichiura	2276 (1417 – 3657)	99 (17 – 580)	<0.001[1]

[1] Mann-Whitney U test.
[2] Fisher's exact test.

education and only one child had parents with a high-skilled job (3.7%). In the low SES school all children were infected with helminths, whereas in the high SES school less than half of the children were infected with helminths (44.4%). The infection intensity was significantly higher in the low SES compared to the high SES for *A. lumbricoides* ($p < 0.001$) and *T. trichuria* ($p < 0.001$). We found no hookworm infection in our study subjects.

3.2. Cytokine response to TLR ligands 24h post-stimulation

The TNF production in the low SES compared to the high SES was significantly higher in response to TLR-2/6 ligand Hklm and TLR-4 ligand LPS (respectively $p = 0.039$ and $p<0.001$), whereas it was significantly lower in response to TLR-9 ligand CpG ($p = 0.021$) (Fig. 1a). The IL-10 production in the low SES school was significantly higher in response to TLR-4 ligand LPS ($p = 0.009$) and there was a trend towards a decrease for the ligand of TLR-9 CpG ($p = 0.088$) (Fig. 1b). When ratios of TNF and IL-10 were calculated as a pro-inflammatory measure, significantly stronger pro-inflammatory responses were observed in low SES in response to TLR-2/1 ligand Pam3 ($p = 0.027$) and TLR-4 ligand LPS ($p = 0.005$) (Fig. 1c).

3.3. Cytokine response to LPS 24 and 72h post-stimulation

In order to study the dynamics of cytokine responses to TLR ligands, responses to TLR-4 ligand LPS were examined 24 h and 72 h post stimulation. After 72 h the TNF production was significantly lower in both low and high SES children than what was seen after 24 h of stimulation (respectively $p<0.001$ and $p<0.001$) (Fig. 2a). The production of IL-10 was also significantly lower in the high SES ($p<0.001$) at 72 h after stimulation compared to 24 h, but it did not change and remained high in the low SES school ($p = 0.124$) (Fig. 2b). The ratio between TNF/IL-10 in the low SES school was significantly lower after 72 h compared to after 24 h post-stimulation ($p<0.001$), indicating a shift from a pro-inflammatory towards an anti-inflammatory response. In the high SES the TNF/IL-10 ratio significantly increased after 72 h stimulation compared to 24 h ($p = 0.002$), reflecting a shift from a predominantly anti-inflammatory response to a strong pro-inflammatory response (Fig. 2c).

3.4. Effect of helminth infections on cytokine responses

Comparison of the cytokine responses between low and high SES of revealed that the response to TLR-4 ligand LPS significantly differs between the SES groups both at 24 h and 72 h (Table 2). To examine the role of helminth infections in these differences, we categorized the children in the high SES as non-infected or infected (Table 2). Of the 27 children, 12 of them were infected (Group B1) and 15 were not infected (Group B2). The results of analysis showed no differences in TNF, IL-10 production or the TNF/IL-10 ratio between helminth-infected and non-infected children, indicating the limited role of having a current helminth infection in the TLR responsiveness in the high SES children.

4. Discussion/Conclusion

This study aimed to examine the cytokine response to an array of TLR ligands in children attending a high or a low SES school in Makassar, Indonesia. The results showed an increased pro-inflammatory response in the low SES compared to the high SES after 24 h stimulation with TLR-ligands Pam3 and LPS. Comparison of cytokine production in response to LPS after 24 h versus 72 h stimulation revealed that the pro-inflammatory response in the low SES after 24 h shifted to an anti-inflammatory response 72 h post-stimulation, whereas in the high SES the anti-inflammatory response at 24 h shifted to a strong pro-inflammatory response after 72 h stimulation.

A varied range of responses was found to TLR ligands, which might reflect the differences in expression of TLRs and the strength of their signaling. Another explanation could be the difference in solubility of the TLR ligands and thus access to receptors. Here we used the whole blood assay which has been regarded to be optimal for testing immunologic property of TLR ligands because it avoids inadvertent activation associated with cellular isolation techniques and maintains the influences of known and unknown soluble factors that can affect experimental condition (Smith et al., 2004; Langezaal et al., 2001). Using ratios of TNF to IL-10, it was possible to get an overall picture of the strength of the pro-inflammatory response induced by a TLR-L with the additional advantage that this parameter would be less affected by the differences in the blood cell counts and by the differential solubility of the ligands.

The higher immune responsiveness in low SES children found in this study was in line with the study of Azad et al. showing that low-SES children had a stronger pro-inflammatory response to 24 h LPS stimulation compared to their high-SES counterparts (Azad et al., 2012). In addition, Miller et al. showed that subjects with low early-life SES produced increased pro-inflammatory cytokines in response to TLR-ligands Poly I:C and Flagellin (Miller et al., 2009). Increased TLR-mediated pro-inflammatory responses have also been demonstrated in children infected with helminths, as shown by Meurs et al. that measured higher TNF and TNF/IL-10 ratios in Schistosoma-infected children compared to those in children without infections (Meurs et al., 2011).

When cytokine responses to LPS were compared between 24 h and 72 h post stimulation, in the low SES the production of IL-10 was maintained at a high level, which resulted in a change from a pro- to an anti-inflammatory cytokine profile at 24 h versus 72 h. In contrast, the

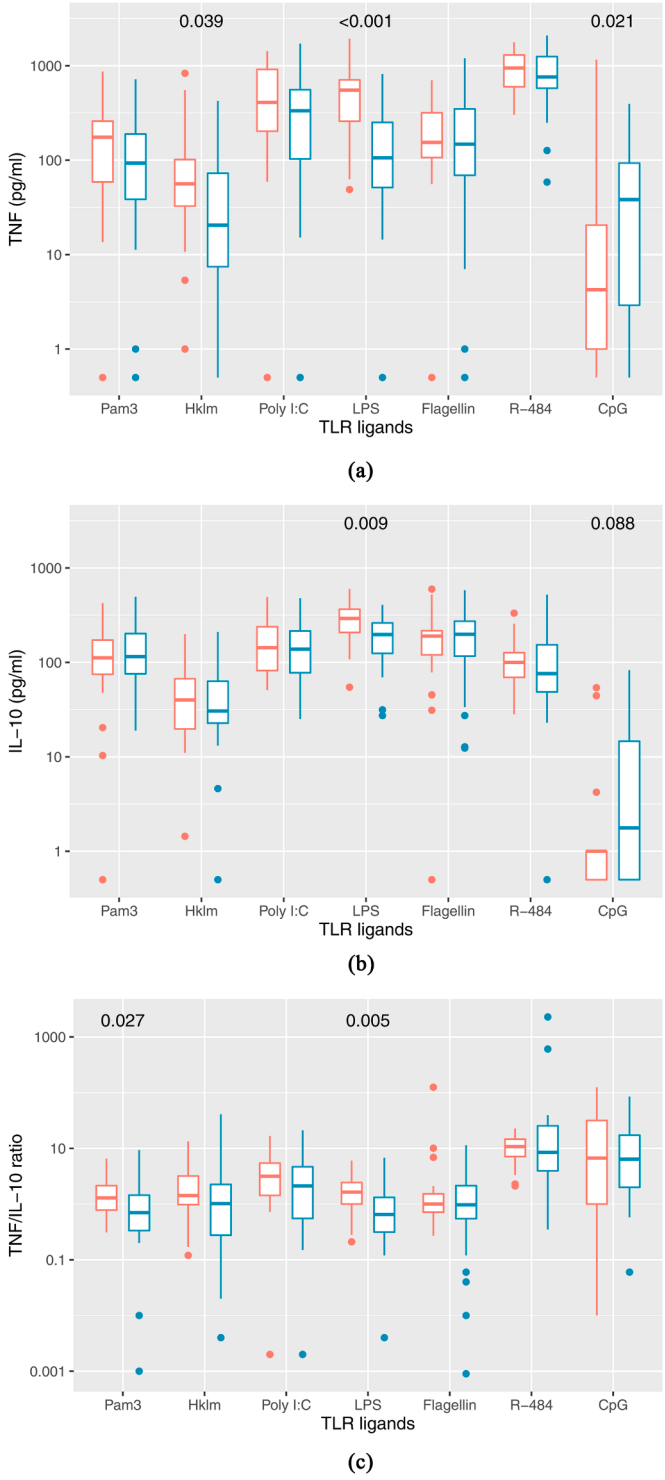

Fig. 1. A. TNF production, B. IL-10 production and C. TNF/IL-10 ratio in response to different toll-like receptor ligands in the low SES (red) and high SES school (blue). Boxplot represents the geometric mean, the 25th and 75th percentiles and the 95th and 5th percentile (whiskers) per school. Mann-Whitney U test. Only significant p-values < 0.10 are shown.

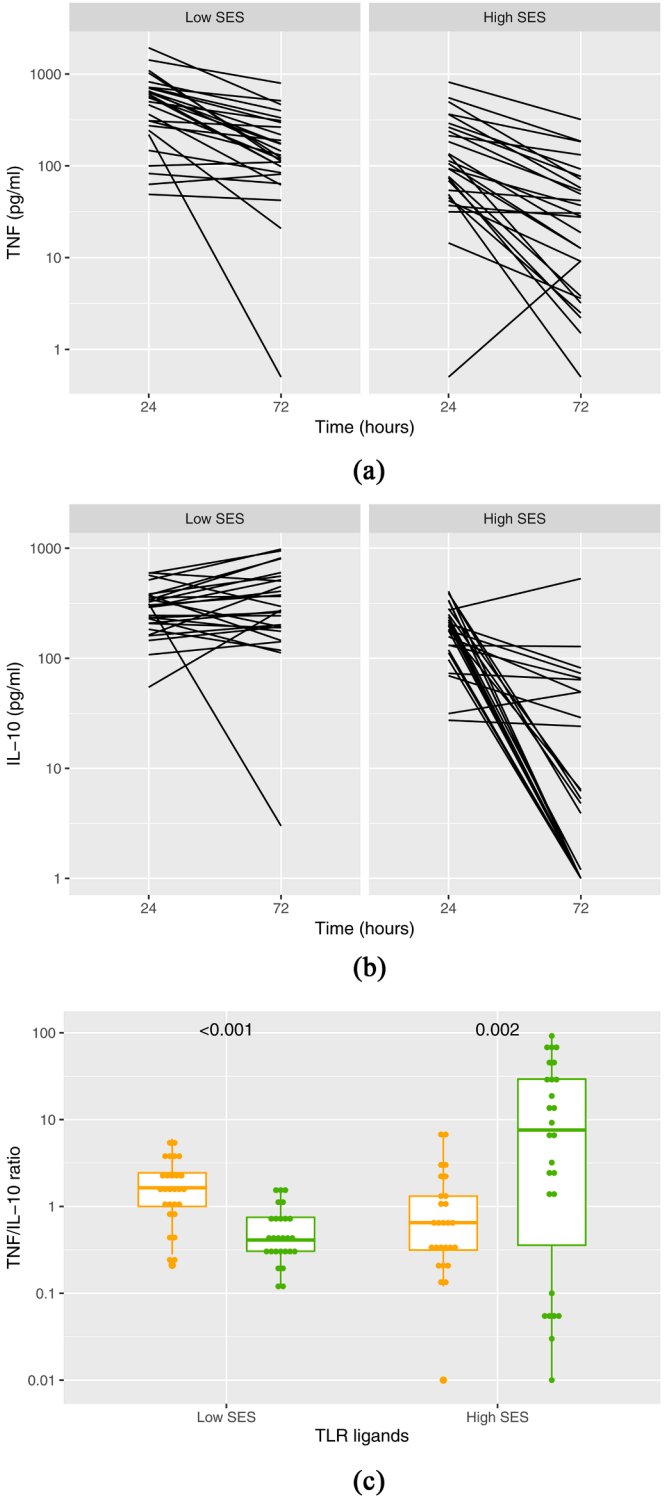

Fig. 2. A. TNF production, B. IL-10 production and C. TNF/IL-10 ratio in response to LPS after 24 h (orange) and 72 h (green) post-stimulation in children attending the high SES or low SES school. Fig 2A and B, one line represents one individual. Fig 2C represents the geometric mean, the 25th and 75th percentiles and the 95th and 5th percentile (whiskers) per school. Wilcoxon matched-pair signed-rank test.

IL-10 production steeply declined in the high SES, resulting in a strong pro-inflammatory cytokine profile 72 h post-stimulation. Two possibilities could account for this observation. First, in children from the low SES, cells other than those involved in an early burst of response to TLR ligands, start to produce IL-10 at later time points during culture and such cells would not be present in children from the high SES. Second,

IL-10 consumption might be different between children attending the low or high SES school, which might be caused by a variation in the expression of IL-10 receptors in these children. Differential gene expression profiles of genes involved in immune activation and

Table 2
Response to LPS after 24 and 72 h stimulation with LPS in helminth-infected and non-infected children from the high SES.

		TNF-a (pg/ml) GM (95%CI)		IL-10 (pg/ml) GM (95%CI)		TNF-a/IL-10 ratio GM (95% CI)	
Group	n	24 hr	72 hr	24 hr	72 hr	24 hr	72 hr
A (low SES school)	27	401.7 (277.3–581.8)	123.3 (71.80–211.7)	267.1 (215.9–330.2)	268.4 (173.8–414.6)	1.50 (1.07–2.11)	0.46 (0.35–0.61)
B (high SES school)	27	98.45 (55.68–174.2)	20.21 (10.50–38.90)	167.6 (127.4–220.5)	6.60 (2.98–14.63)	0.59 (0.32–1.06)	3.06 (0.78–9.60)
p-value A and B		< 0.001	< 0.001	0.009	< 0.001	0.005	0.003
B1 (helminth-infected)	12	109.2 (58.87–202.6)	24.69 (8.48–71.88)	174.7 (113.2–269.5)	10.60 (2.99–37.60)	0.63 (0.29–1.36)	2.33 (0.43–12.57)
B2 (non-infected)	15	90.61 (34.21–240.1)	17.21 (6.81–43.53)	162.2 (108.7–242.2)	4.52 (1.49–13.76)	0.56 (0.22–1.43)	3.81 (0.67–21.68)
p-value B1 and B2		0.932	0.712	0.216	0.932	0.345	0.842

Mann-Whitney U test.

regulation, including IL-10 and TLRs, has also been observed between low SES children compared to their high SES counterparts in an urban area in Ghana (Amoah et al., 2014). Both of these options need to be substantiated by flow cytometry. Finally, these findings highlight the dynamics of cytokine production and the importance of the time of measurement. Previous studies that reported enhanced pro-inflammatory cytokine responses in low SES (Azad et al., 2012) or helminth infected children (Labuda et al., 2014; Meurs et al., 2011) compared to their counterparts, only measured cytokine responses after 24 h stimulation. However, the results found in this study, indicate that cytokine response to LPS are highly dynamic and that cytokine profiles may change after prolonged stimulation. It is important to assess which time points of in vitro testing best reflect the response to adjuvants in vaccines and to realize that this could be different depending on the population studied.

Comparison of the cytokine responses between helminth-infected and non-infected children in the high SES showed no difference between these groups. However, due to the small sample size we were unable to properly examine the interaction between helminth infections and SES. To unravel the effect of SES and helminth infections and their effect on TLR-mediated immune responses, future studies should include larger number of subjects. Another limitation of the current study is that the cell counts were not measured and controlled for, while these are known to vary between individuals and can change over time and therefore might be responsible for the observed differences in cytokine production in response to the TLR ligands. However, by calculating ratios between TNF and IL-10, this limitation can be overcome to some extent, allowing us to compare the TLR responsiveness over time and between individuals. Moreover, whole blood with its varied composition between individuals might represent the varied response seen across individuals better. In addition, it is known that the repertoire of pro- and anti-inflammatory responses goes beyond TNF and IL-10. However, in the current study TNF and IL-10 were measured as a proxy for the pro- and inflammatory cytokines response, respectively, and further studies including an increased number of pro- and inflammatory biomarkers would be of interest.

The results of the current study have important implications for vaccine development and improvement of current vaccines on the market. TLRs are a well-studied class of PPRs and are often used in vaccines to trigger the innate immune response and thus increase immunogenicity and efficacy. Reduced vaccine efficacy has been reported for a number of established vaccines in LMICs, where vaccines are needed the most (Jiang et al., 2010; Fine, 1995). Strategies to increase the efficacy of established and to be developed vaccines across populations could benefit from better adjuvants. This requires the careful examination of responses to adjuvants in populations that show poor response to vaccines. The finding here that variations in responsiveness exists between individuals with high or low SES highlights the need to work on understanding the mechanisms behind in order to help improved vaccines

To conclude, we observed significant differences in the TLR-mediated innate immune response between children attending a low SES school compared to their counterparts attending a high SES school. Since adjuvants, such as TLR ligands are essential for the induction of immunity by vaccines, these results can be relevant for the development of new and improved vaccines and should be taken along while studying their effectiveness.

CRediT authorship contribution statement

Sitti Wahyuni: Formal analysis, Investigation, Data curation, Writing – original draft, Validation. **Marloes M.A.R. van Dorst:** Data curation, Writing – original draft, Validation. **John Tuyp:** Investigation, Data curation, Validation. **Franca Hartgers:** Formal analysis, Validation. **Erliyani Sartono:** Writing – review & editing, Validation. **Maria Yazdanbakhsh:** Formal analysis, Writing – review & editing, Validation.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Informed consent was obtained from the legal guardians of each child and the study was approved by the ethics committee of the Faculty of Medicine, Hasanuddin University, Indonesia (ref:0147/H4.8.4.5.31/PP36-KOMETIK/2005).

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References

Amoah, A.S., Obeng, B.B., May, L., et al., 2014. Urban-rural differences in the gene expression profiles of ghanaian children. Genes Immun.

Azad, M.B., Lissitsyn, Y., Miller, G.E., et al., 2012. Influence of socioeconomic status trajectories on innate immune responsiveness in children. PLoS ONE.
Elias, D., Britton, S., Aseffa, A., et al., 2008. Poor immunogenicity of bcg in helminth infected population is associated with increased in vitro TGF-β production. Vaccine.
Elias, D., Wolday, D., Akuffo, H., et al., 2001. Effect of deworming on human t cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille calmette-guérin (BCG) vaccination. Clin. Exp. Immunol.
Fine, P.E.M., 1995. Variation in protection by BCG: implications of and for heterologous immunity. The Lancet.
Jiang, V., Jiang, B., Tate, J., Parashar, D.U., et al., 2010. Performance of rotavirus vaccines in developed and developing countries. Human Vaccines.
Labuda, L.A., De Jong, S.E., Meurs, L., et al., 2014. Differences in innate cytokine responses between european and african children. PLoS ONE.
Langezaal, I., Coecke, S., Hartung, T., 2001. Whole blood cytokine response as a measure of immunotoxicity. Toxicol. Vitr.
Maizels, R.M., Yazdanbakhsh, M., 2003. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat. Rev. Immunol.
Meurs, L., Labuda, L., Amoah, A.S., et al., 2011. Enhanced pro-inflammatory cytokine responses following toll-like-receptor ligation in schistosoma haematobium-infected schoolchildren from rural gabon. PLoS ONE.
Miller, G.E., Chen, E., Fok, A.K., et al., 2009. Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. Proc Natl Acad Sci U S A.
Nookala, S., Srinivasan, S., Kaliraj, P., et al., 2004. Impairment of tetanus-specific cellular and humoral responses following tetanus vaccination in human lymphatic filariasis. Infect. Immun.
Sabin, E.A., Araujo, M.I., Carvalho, E.M., et al., 1996. Impairment of tetanus toxoid-specific Th1-like immune responses in humans infected with schistosoma mansoni. J. Infect. Dis.
Smith, R.L., Chong, T.W., Hughes, M.G., et al., 2004. Impact of immunomodulatory oligodeoxynucleotides on cytokine production in the lipopolysaccharide-stimulated human whole blood model. Surgery.
Takeda, K., Akira, S., 2005. Toll-like receptors in innate immunity. Int. Immunol.
van den Biggelaar, A.H.J., Prescott, S.L., Roponen, M., et al., 2009. Neonatal innate cytokine responses to bcg controlling T-cell development vary between populations. J. Allergy Clin. Immunol.
van Riet, E., Hartgers, F.C., Yazdanbakhsh, M., 2007. Chronic helminth infections induce immunomodulation: consequences and mechanisms. Immunobiology.
World Health Organization WHO, 2013. Global vaccine action plan. Vaccine.