

Parasite-specific antibody and cytokine profiles in newborns from *Plasmodium falciparum* and *Entamoeba histolytica/dispar*-infected mothers

Kirch AK, Agossou A, Banla M, Hoffmann WH, Schulz-Key H, Soboslay PT. Parasite-specific antibody and cytokine profiles in newborns from *Plasmodium falciparum* and *Entamoeba histolytica/dispar*-infected mothers.

Pediatr Allergy Immunol 2004; 15: 133–141. © 2004 Blackwell Munksgaard

Passage of parasites and their antigens across the placenta occurs with metazoan as well as protozoan parasites, and this study addressed to which extent exposure to and infection of mothers with *Plasmodium* spp. and *Entamoeba histolytica/dispar* has sensitized their offspring for parasite-specific immune responses. While at delivery none of the mothers presented with an acute malaria attack, 42% were seropositive for *P. falciparum*. In half of the mothers cysts of *E. histolytica/dispar* were detected in stool specimen, 51% of them were found seropositive for *E. histolytica*, and *E. histolytica*-specific immunoglobulin A (IgA) responses were detected in neonates of seropositive mothers as well. Umbilical cord blood cells (UCBC) from neonates, when activated with the mitogen phytohaemagglutinine (PHA) and bacterial streptolysin O (SL-O), released significantly less interferon (IFN)- γ , interleukin (IL)-10 and tumor necrosis factor (TNF)- α into cell culture supernatants than peripheral blood cells (PBMC) of mothers. In response to *Plasmodium*- and *Entamoeba*-specific antigens UCBC and PBMC produced equal amounts of IL-1 β , TNF- α , IFN- γ and IL-5, but PBMC from mothers secreted significantly more IL-10. Parasite-specific production of inflammatory and Th₁- and Th₂-type cytokines was similar in newborns of *Plasmodium* and *Entamoeba* seropositive and seronegative mothers. In summary, repeated exposure and subclinical infection of mothers with *E. histolytica* or *P. falciparum* will suffice to prime *in utero* their children for inflammatory and both Th₁- and Th₂-type cytokine responses, and such broad and mixed cytokine spectrum may be of advantage upon secondary parasite challenge in later life.

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Key words: newborn; immune responses; cytokines; antibody; *Plasmodium* spp.; *Entamoeba* spp.

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Accepted 1 October 2003

Transplacental passage of parasites occurs with metazoan helminth parasites (1) as well as with protozoa, e.g. *Trypanosoma* (2), *Leishmania* (3), *Plasmodium* (4) and *Toxoplasma* (1) species, which upon replication in the foetus or neonate may induce severe morbidity and significant mortality. Congenital infection and prenatal immune sensitization of the unborn child occurs not solely in women who acquired infection during gestation, but also in children born to semi-immune or subclinically infected mothers

(2, 5). Those women may present no overt clinical signs of infection but are 'carriers' of low pathogen numbers, and placental passage of small amounts of parasite antigens may suffice to prime their offspring *in utero* for parasite-specific immune responses. But there is little information on the maternal–foetal relationship in those situations where maternal infection will sensitize *in utero* without infecting the child. Studies have shown that neonatal T cells are capable of responding to parasite-specific stimulation by producing

cytokines and specific antibodies, i.e. parasite-specific memory responses (2, 6–8). In rural West Africa, pregnant mothers are often chronically infected with intestinal and intravascular helminth and protozoan parasites, one-third were found concurrently infected with more than one parasite, and in up to 20% triple and quadruplicate parasite infections were diagnosed (9, 10).

The present study investigated to which extent exposure to and infection of mothers with *Plasmodium* spp. and *Entamoeba histolytica/dispar* may have sensitized their offspring for parasite-specific immune responsiveness. We found that umbilical cord blood cells (UCBC) from neonates were capable of responding to parasite-specific stimulation by producing several inflammatory and regulatory cytokines without presenting a dominant type 1 or type 2 T-helper cytokine profile.

Material and methods

Location of study and study participants

This study was located in central Togo (West Africa) and authorization and ethical approval for this investigation was obtained by the Ministry of Health of Togo, and the Ethical Commission and Human Investigation Review Board (Ethik Kommission) at University Hospitals of Tübingen, Germany. Informed consent for participation was obtained from mothers during prenatal consultations by the medical staff. Objectives and the procedures how umbilical blood will be collected, and peripheral blood cells (PBMC) from mothers will be obtained was explained in detail, and for better and complete understanding explanations were always given in the local language. Paired cord and maternal blood samples were collected from mothers and their newborns at the Centre Hospitalier Regionale in Sokodé (Togo) by medical staff. Pregnant mothers originated from Sokodé and the surrounding villages. After delivery, UCB samples were collected from the umbilical cord after ligation and severance of the cord. Serum, PBMC, stools and urine samples from mothers were given during the first postnatal consultation, and intestinal helminth or protozoan infections were determined by standard parasitological methodology. Blood samples were examined for *Plasmodium* spp. as well as for microfilariae of *Wuchereria bancrofti* and *Mansonella perstans*. Microfilariae of *M. perstans* were identified after Ficoll–Paque gradient centrifugation of whole blood in the PBMC fraction, and also in the red blood cell (RBC) pellet. Briefly, after Ficoll–Paque sedimentation of whole blood

the RBC pellets were mixed with equal volumes of RPMI cell culture medium (Gibco, Paisley, UK), followed by equal volume of 4.5% dextran in RPMI, and the mixture sedimented for 1.5 h at 1 g at room temperature. Thereafter, the supernatant was collected, centrifuged at 600 g for 15 min, and the pellet examined by microscopy for the presence of microfilariae of *M. perstans*. Isolated PBMC were dispensed in equal volumes into 96-well culture plates, and after overnight incubation plate wells were examined under a microscope for the presence of blood dwelling microfilariae of *M. perstans*. All mothers included in this study were negative for HIV-I and -II as determined by enzyme-linked immunosorbent assay (ELISA; Enzygnost Behring, Marburg, Germany).

Parasite antigen-specific ELISA

Serum samples were obtained from participants and the reactivity of *P. falciparum*, *E. histolytica*, *Ascaris lumbricoides*, *Schistosoma mansoni* and *Onchocerca volvulus* antigen-specific IgA, IgG isotypes and IgE were determined by ELISA. *Plasmodium falciparum* antigen was a kind gift from Dr Birthe Hoegh (Kopenhagen, Denmark), *E. histolytica* (axenic strain HB1) was kindly given by Dr Brigitte Walderich (Tübingen, Germany). Antigen extracts from *O. volvulus* and *A. lumbricoides* were prepared as previously described (9, 10). Microtitration plates (Maxisorb, Nunc, Wiesbaden, Germany) were coated with parasite antigens at 5 µg protein/ml in 0.1 M sodium carbonate buffer (pH 9.6) overnight at 4°C. Plates were blocked with phosphate-buffered saline (PBS) containing 1% bovine serum albumin for 1 h at 37°C, then washed with 0.035 M PBS/0.05% Tween 20 and (final dilution 1:50) was added in duplicates and incubated at 4°C overnight. After washing (as above), anti-human immunoglobuline isotype-specific mouse monoclonal antibody conjugated to alkaline phosphatase (Zymed, San Francisco, CA, USA) was added (1 h at 37°C), and after incubation with *p*-nitrophenyl phosphate (pNPP) for 1 h at room temperature absorbance was read at 405 nm. For determination of seropositivity control sera were used in parallel, and the cut-off was set as the mean optical density (OD) of negative control sera plus five times, the standard deviation (s.d.).

Isolation of umbilical cord blood mononuclear cells and cell culture experiments

Heparinized venous or cord blood was collected from mothers and newborns, and PBMC or

UCBC were isolated by Ficoll–Paque (Pharmacia, Freiburg, Germany) density gradient centrifugation. Cell culture experiments were conducted as previously described (9). Briefly, mononuclear cells were adjusted to 1×10^7 /ml in RPMI (Gibco, Paisley, UK) supplemented with 25 mM HEPES buffer, 100 U/ml penicillin and 100 µg/ml streptomycin, 0.25 µg/ml amphotericin B and immediately used for proliferation assays and to stimulate cytokine secretion. Cells were suspended in RPMI (as above) containing 10% foetal calf serum (FCS), and kept in 5% CO₂ at 37°C and saturated humidity. For purposes of mitogenic stimulation with phytohaemagglutinine (PHA 5 µg/ml; Sigma, Steinheim, Germany), and antigenic stimulation with *P. falciparum*-specific (PfAg), *E. histolytica*-specific (EhAg), and *Streptococcus pyogenes*-specific [Streptolysin-O (SL-O), 1:50, Difco, Augsburg, Germany] antigens were added as indicated in the legends to each Table. Cultures were maintained for 3 and 5 days, respectively. For the last 18 h 1 µCi of ³H-thymidine was added; cells were then harvested on glass fibre filters (Skatron, Freiburg, Germany) and the incorporated radioactivity determined by scintillation spectroscopy (Beta Plate, LKB-Pharmacia, Freiburg, Germany). Data are indicated as mean values of triplicate cultures in counts per minute (cpm) minus baseline stimulation.

Determination of cytokine production

Freshly isolated PBMC were suspended at a concentration of 3×10^6 cells/ml in RPMI (as above) supplemented with 10% heat inactivated FCS, in the presence of either *E. histolytica*- and *P. falciparum*-derived antigen, PHA (5 µg/ml, Gibco) or SL-O from *S. pyogenes* (SL-O 1:50; Difco) in 5% CO₂ at 37°C and saturated humidity. Cell culture supernatants were collec-

ted after 48 h and stored at –28°C. Cytokine secretion by stimulated PBMC was quantified by sandwich-ELISA using cytokine-specific monoclonal and polyclonal antibodies for IL-2, IL-5, IL-10 and IFN-γ (Pharmingen, Heidelberg, Germany) as previously described (9). The sensitivity of the cytokine assays for IFN-γ, IL-5, IL-10, IL-12 and IL-13 was <15 pg/ml.

Statistical data analyses

Results are indicated as mean values ± s.e.m. of different groups. For data analyses the statistical package JMP 5 was used. Data were tested for normality and analyses were performed by either the Student's *t*-test or the Mann–Whitney *U*-test.

Results

Parasite infections and serological reactivity to *P. falciparum*- and *E. histolytica*-specific antigen in mothers and their neonates

The mean age of mothers in this study was 26.9 (range: 17–45) and mean number of live births was 1.9 (range: 1–6). In half of the pregnant women cysts of *E. histolytica/dispar* were detected in stool specimen, no trophozoites of *E. histolytica* were found, and also, by serological examination 51% of mothers were found seropositive for *E. histolytica*. Parasite-specific serological reactivity of mothers and their neonates is shown in Fig. 1. While at delivery none of the women presented with an acute malaria attack, 42% were seropositive for *P. falciparum*. In 5% of the mothers, eggs of *Schistosoma* spp. were diagnosed, but one-third (35%) had *S. mansoni*-specific antibody disclosing previous contact with *Schistosoma* spp. (data not shown). Seropositive mothers may have harboured a low level infection with *Plasmodium* spp., *E. histolytica/dispar* or

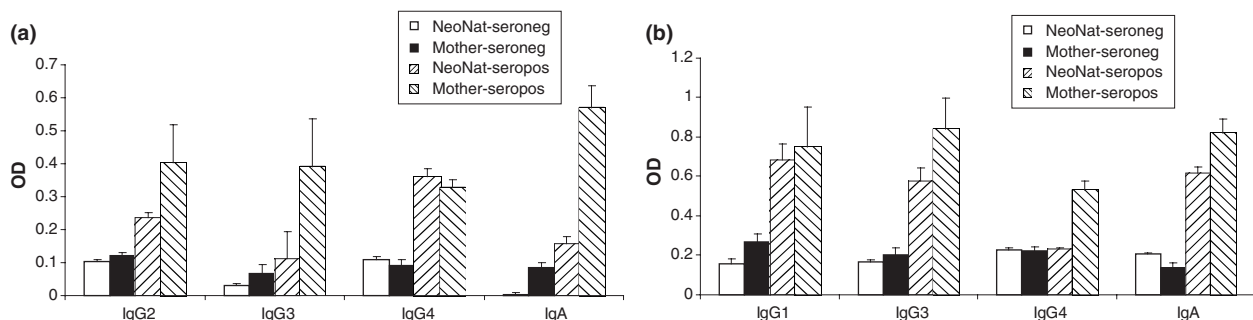


Fig. 1. Reactivity of immunoglobulin (Ig)G2, IgG3, IgG4 and IgA in mothers and their newborn babies to *Plasmodium falciparum*-antigen, and of IgG1, IgG3, IgG4 and IgA to *Entamoeba histolytica*-antigen as determined by enzyme-linked immunosorbent assay (ELISA). Neonates were grouped according to serological reactivity to parasite antigens and the infection status of mothers at the children's birth.

Schistosoma spp., which was not detected by the diagnostic methodology used; in the study area malaria is holoendemic and amoebiasis and schistosomiasis may reach mesoendemic levels. In 27% of the mothers no parasite infections were diagnosed in blood and stool samples, and these women tested also ELISA seronegative for *E. histolytica*, *O. volvulus*, *P. falciparum* and *S. mansoni*. In addition, in 18% of women *Giardia lamblia*, in 14% *Trichomonas hominis* infections were diagnosed in stool samples, and microfilariae of *M. perstans* were detected in 9% of the maternal blood samples. Antibody isotype responses to *P. falciparum* and *E. histolytica* were pronounced in seropositive mothers and their neonates disclosing passage of maternal IgG isotypes across the placenta into the foetus (Fig. 1). Remarkably, IgG4 was rather low to *E. histolytica* antigens in neonates of seropositive mothers, but IgA to *E. histolytica* was on similar levels in *E. histolytica*-positive mothers and their children; this indicates *in utero* antigen exposure of the foetus and autonomous production of IgA.

Cellular production of IFN- γ , TNF- α and IL-1 β to mitogen, bacteria and *E. histolytica*- and *P. falciparum*-specific antigens in mothers and their newborn babies

Peripheral blood mononuclear cells from mother and UCBC from neonates were activated with the mitogen PHA, bacterial SL-O, *E. histolytica*- and *P. falciparum*-specific antigens for 48 h and cytokine release into cell culture supernatants was quantified by specific ELISA. UCBC responded vigorously to PHA and bacterial SL-O and secreted IL-1 β , IFN- γ and TNF- α into cell culture supernatants, but IFN- γ and TNF- α release by UCBC was significantly lower than by PBMC of mothers (Table 1). In response to *E. histolytica*- and *P. falciparum*-antigen, UCBC secreted pro-inflammatory cytokines, i.e. IL-1 β , TNF- α and Th₁-type IFN- γ as well (Fig. 2). Low amount of IFN- γ , in the range of 50–85 pg/ml, were produced by neonatal UCBC and maternal PBMC in response to *P. falciparum*-antigen. In response to *E. histolytica*-antigen, substantially more IFN- γ was produced both by UCBC (780 pg/ml) and PBMC (930 pg/ml) (Fig. 3). For TNF- α , UCBC secreted less than PBMC in response to *P. falciparum*- (UCBC 40 pg/ml; PBMC 70 pg/ml) or *E. histolytica*-antigen (UCBC 70 pg/ml; PBMC 100 pg/ml) (Fig. 2). UCBC and PBMC secreted similar amounts of IL-1 β in response to *P. falciparum*- and *E. histolytica*-specific stimulation, and as for TNF- α , highest IL-1 β production was elicited by *E. histolytica*-antigen. Parasite-specific production of IFN- γ , TNF- α and IL-1 β

Table 1. Cellular production of Th₁-type interferon (IFN)- γ , proinflammatory tumour necrosis factor (TNF)- α and interleukin (IL)-1 β as well as Th₂-type IL-5 and IL-10 in response to the mitogen phytohaemagglutinine (PHA) and bacterial streptolysine O (SL-O from *Streptococcus pyogenes*) in mothers and their newborn babies

	PHA		SL-O	
	Mother	Neonate	Mother	Neonate
IFN- γ				
pg/ml	1027.73	426.40**	2254.86	195.06**
s.e.m.	99.79	67.20	781.12	60.91
N	35	56	27	80
IL-10				
pg/ml	895.53	201.66**	832.10	83.97**
s.e.m.	151.62	43.67	204.16	24.53
N	35	47	26	66
IL-1 β				
pg/ml	1653.55	1183.20	106.32	24.47
s.e.m.	191.82	180.17	52.35	10.31
N	33	40	28	65
IL-5				
pg/ml	125.31	168.28	141.29	23.12
s.e.m.	22.00	75.61	115.49	9.05
N	22	46	9	46
TNF- α				
pg/ml	417.26	150.05*	441.29	61.20**
s.e.m.	59.32	33.47	116.96	16.92
N	35	49	27	74

Umbilical cord blood cells (UCBC) from neonates and peripheral mononuclear blood cells (PBMC) from mothers were seeded at 3×10^6 /ml and activated with PHA (5 μ g/ml) or SL-O (1:50). After 48 h cell culture supernatants were harvested and cytokine release was quantified by specific enzyme-linked immunosorbent assay (ELISA). Amounts of cytokines released by UCBC and PBMC are given in pg/ml $\pm$ s.e.m. After Bonferroni correction for multiple testing, the level of significance is $\alpha = 0.01$. Significant differences between mothers and neonates are shown as * $p = 0.05$ or ** $p < 0.01$.

was similar in newborns of *P. falciparum* and *E. histolytica*-positive and negative mothers (Fig. 3).

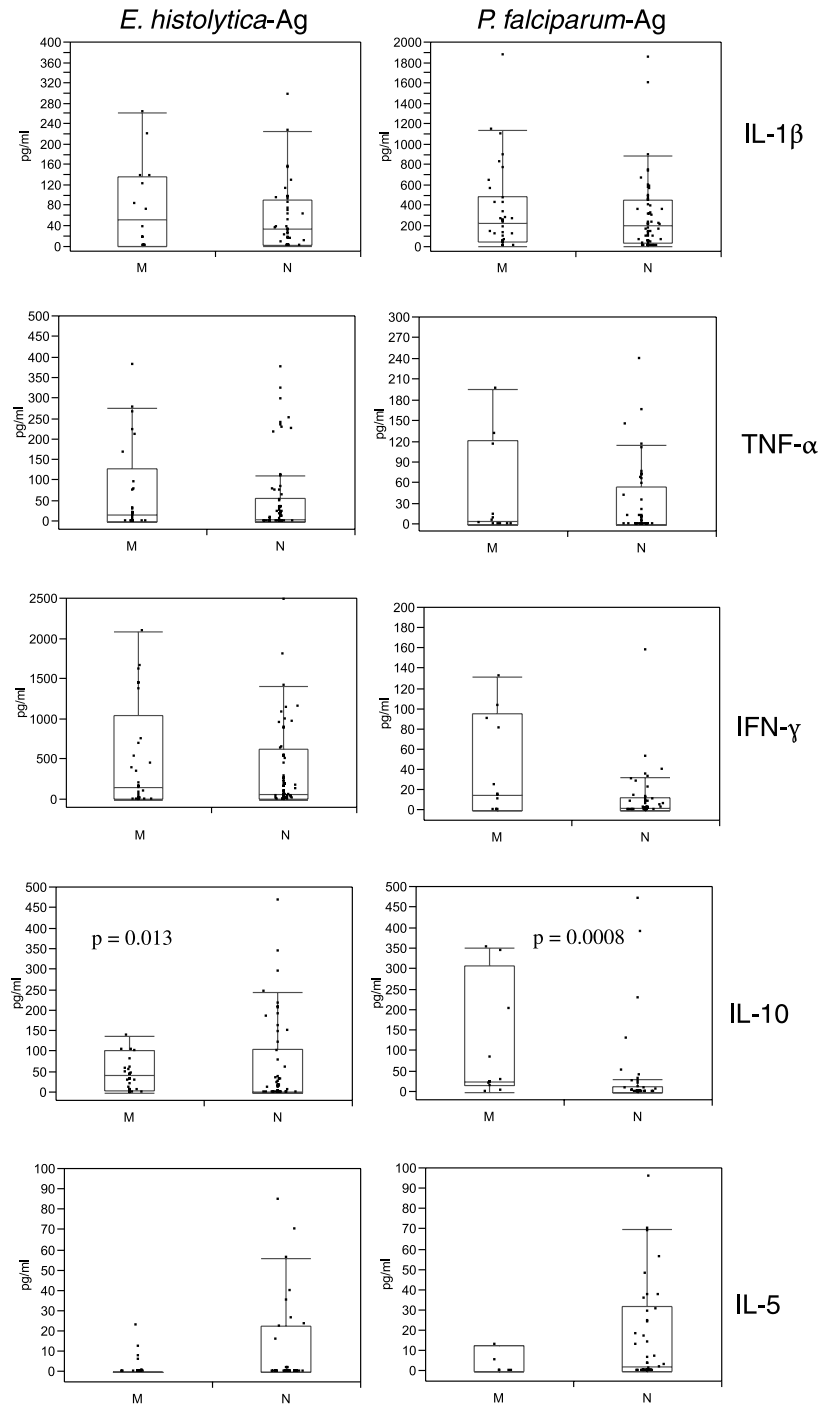
Release of Th₂-type IL-5 and IL-10 in response to *E. histolytica*- and *P. falciparum*-specific antigens in mothers and neonates

Following activation with *P. falciparum*-antigen, PBMC and UCBC released similar amounts of IL-5 (Fig. 2). But PBMC from mothers secreted significantly more IL-10 in response to *P. falciparum*- and *E. histolytica*-antigen than UCBC from neonates (Fig. 2). As observed for inflammatory cytokines, parasite-specific production of IL-5 and IL-10 was similar in neonates from *P. falciparum* and *E. histolytica*-positive and negative mothers (Fig. 3).

Parasite-specific cellular proliferation in neonates

Cellular proliferation of UCBC in response to mitogen and bacterial antigen stimulation was vigorous ranging from 3000 to 150,000 cpm, while

Fig. 2. Production of interferon (IFN)- γ , tumour necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-5 and IL-10 following stimulation with *Plasmodium falciparum* (PfAg, 31 μ g/ml) and *Entamoeba histolytica* (axenic atrain HB1, EhAg, 25 μ g/ml) antigens in mothers (M) and their neonates (N). Umbilical cord blood cells (UCBC) from neonates and peripheral mononuclear blood cells (PBMC) from mothers were seeded at 3×10^6 /ml and cultured in the presence of parasite antigens. After 48 h cell culture supernatants were harvested and cytokine release was quantified by specific enzyme-linked immunosorbent assay (ELISA). Amounts of cytokines released by UCBC and PBMC are given in pg/ml $\pm$ s.e.m. For significant differences between groups p-values are shown. Box plots show median with the 25% and 75% quartiles and 1.5 $\times$ of the interquartile range.



responses to *P. falciparum*- and *E. histolytica*-antigen were lower ranging from 200 to 8000 cpm. *Entamoeba histolytica*-antigen elicited the higher proliferation (5000–8000 cpm) than *P. falciparum*-antigen which stimulated UCBC for responses in the range of 2000–4500 cpm. Cellular proliferation to mitogen and parasite antigens did not differ between neonates of *P. falciparum* or *E. histolytica* seropositive and seronegative mothers.

Discussion

Umbilical cord blood cells of neonates secreted, following activation with *P. falciparum* and *E. histolytica* antigens, inflammatory and also Th₁- and Th₂-type cytokines. In addition, newborns of *E. histolytica*-positive mothers were sensitized *in utero* to produce parasite-specific IgA.

Amoebiasis and malaria are meso- and holoendemic in central Togo, and therefore,

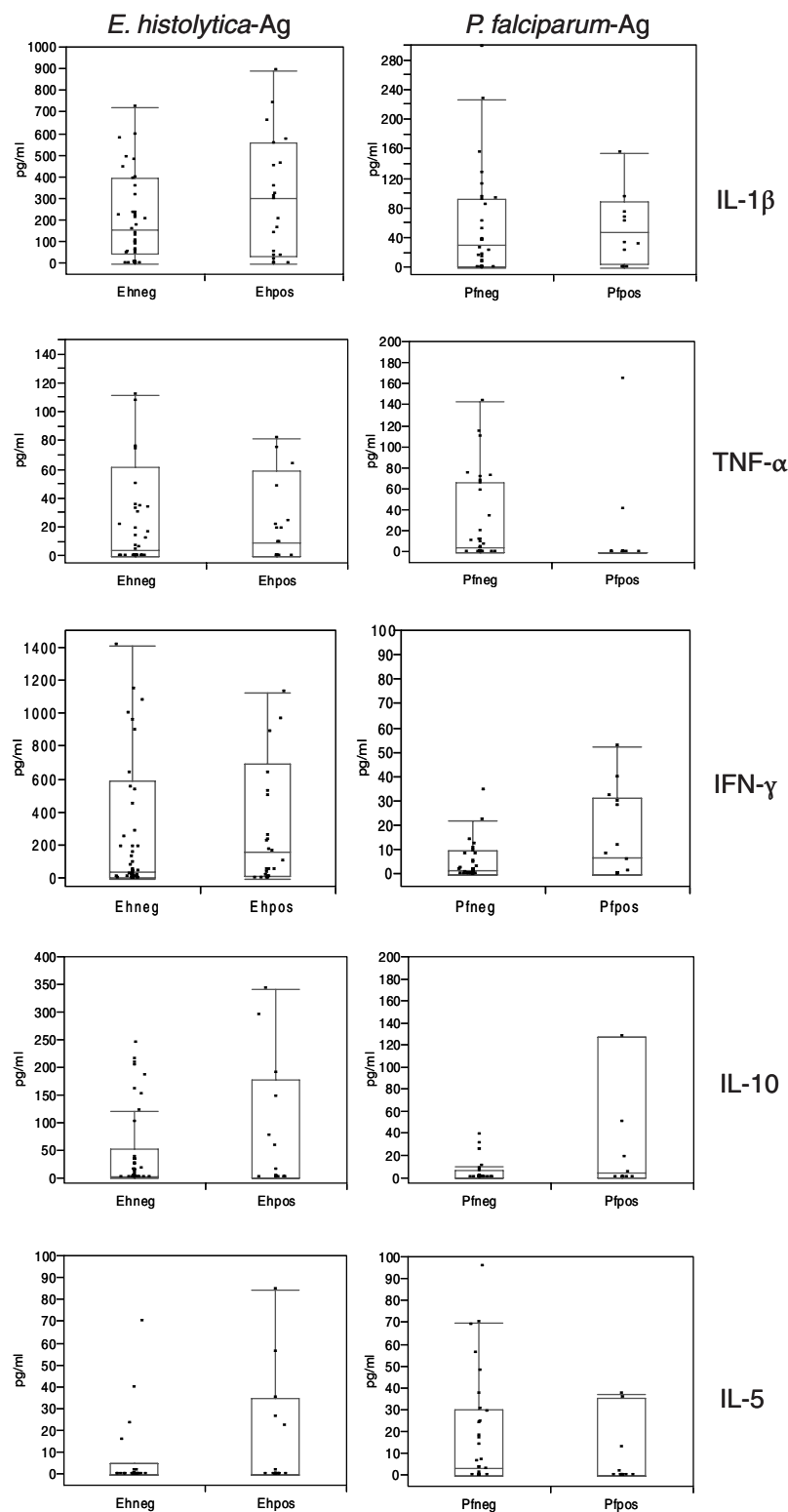


Fig. 3. Cellular production of interferon (IFN)- γ , tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-5 and IL-10 in response to *Plasmodium falciparum*-specific (PfAg, 31 μ g/ml) and *Entamoeba histolytica*-specific (axenic atrain HB1, EhAg 25 μ g/ml) antigens in neonates seropositive (pos) or negative (neg) to PfAg and EhAg. Umbilical cord blood cells (UCBC) from neonates were seeded at 3×10^6 /ml and cultured in the presence of parasite-specific antigens and after 48 h cell culture supernatants were harvested and cytokine release was quantified by specific enzyme-linked immunosorbent assay (ELISA). Amounts of cytokines released by UCBC are given in pg/ml $\pm$ s.e.m. Box plots show median with the 25% and 75% quartiles and 1.5 times of the interquartile range.

constantly and repeatedly mothers will become exposed to *Plasmodium* spp. and *Entamoeba* spp. Such continuous parasite contacts have generated memory immune responses and some degree of acquired immunity, which will contribute to

control parasite densities and prevent severe clinical malaria (11) and amoebiasis (12). At delivery, none of the mothers presented with an acute malaria attack or invasive amoebiasis. Their neonates and infants, however, are at high

risk for severe clinical attacks following infection with protozoan parasites, and this is due to their not yet fully developed immune competence (13, 14). In this study, mitogen and bacterial superantigen-induced cellular production of IL-10, IFN- γ and TNF- α was statistically higher in mothers, but cytokine responses to parasite antigens were greatly similar in mothers and their newborns; only parasite-specific *in vitro* IL-10 production was significantly lower in neonates. This is evidence for a not yet fully developed cellular immune competence in newborns; however, responses to parasite antigens were evolved to similar levels as observed in their mothers. Therefore, prenatal sensitization may have prepared offspring for postnatal parasite challenge without biasing or depressing children's cellular responsiveness. Furthermore, during the first months of life, babies will to some extent be protected from clinical malaria attacks by maternal antibodies, and clinical immunity to *Plasmodium* spp. and *Entamoeba* spp. will develop only gradually in children – this requires repeated exposure and infectious episodes before being able to control the parasite and symptoms of infection (15, 16). Acquired immunity to *Plasmodium* spp. is directed against a broad range of parasite antigens, which act additive or synergistic, to protect against clinical disease (17). The induction of cytokines by *P. falciparum* require T cell-derived IFN- γ which will upregulate macrophage TNF- α production to levels which cause fever (18). In our study, newborns were primed for Th₁-type IFN- γ and inflammatory TNF- α responses and both cytokines are considered as contributors to the development of clinical disease, but they may also contribute to protection against the asexual blood stages of *Plasmodium* spp. (19). Protection is thought to depend on antibodies and proinflammatory TNF- α and IFN- γ which act to induce nitric oxide (19). Therefore, children exposed to parasite antigens and immune sensitized *in utero* may generate early cytokine responses, those being of advantage to infants when exposed in later life. Infants who have not previously been exposed will not have primed T cells and may therefore release low levels of proinflammatory as well as regulatory cytokines, possibly too low for an early and appropriate antiparasite response. Furthermore, intraerythrocyte stages of *P. falciparum* selectively accumulate in placental tissue at much higher concentrations than found in peripheral blood (20), meaning while presenting no clinical malaria during pregnancy and child birth, *P. falciparum* will sequester in placental tissues in many cases. Such sequestration will facilitate

congenital parasite antigen transfer, and immune sensitization of the foetus. The newborns of this study approached adult-like parasite-specific cytokine responses, with only IL-10 being lower in newborns than in their mothers, and this suggested that antigen exposure primed T cells for inflammatory as well as regulatory cytokine responses and activated B cells for *E. histolytica*-specific IgA production.

Half of the mothers from this study were seropositive for *E. histolytica*, but only few *E. histolytica*-infected individuals will show amoebic colitis or amoebic liver abscesses (12). While amoebiasis is common in children of the developing world (12), most *E. histolytica* carriers will never develop the disease and eventually clear their infection. Naturally acquired immunity to *E. histolytica* can be seen in humans, it is linked to a mucosal immune response mediated by IgA against a major virulence factor of the parasite, a Gal/GalNAc lectin, but immunity appeared to be short lived, not completely effective, and will not develop in all individuals infected with *E. histolytica* (21, 22). The neonates from *E. histolytica*-positive mothers were primed *in utero* for IgA production, and B-cell activation and parasite-specific IgA release may protect against early postnatal infection, prevent *E. histolytica* tissue invasion or favour asymptomatic colonization. Furthermore, UCBC secreted, when stimulated with *E. histolytica*-antigens, inflammatory TNF- α , Th₁-type IFN- γ as well as the regulatory cytokine IL-10, and such broad responsiveness may contribute to disease limitation and parasite localization (23, 24). In humans, *in utero* sensitization to *P. falciparum* (25), *Trypanosoma cruzi* (2), and helminth parasites (7, 9, 10) has been reported, and such prenatal immune responses did not favour a strict Th₂ cytokine profile. Also, despite the harmful sequel that Th₁-cytokine responses may impose for a successful pregnancy (14, 26, 27), PBMC from mothers were prominent producers of Th₁-type IFN- γ and inflammatory TNF- α . Recent studies in schistosomiasis have shown that strictly polarized cytokine profiles of either Th₁- or Th₂-type will favour infection-associated pathogenesis (28), and therefore, appropriately balanced cytokine responses may prevent unrestricted parasite persistence and pathogenesis. Immune competence in neonates has been considered as immature and intrinsically deficient upon antigen encounter, but recent studies have shown that neonatal T cells, like those in adults, can be appropriately activated or switched to Th₁- or Th₂-responses by parameters as the dosage of antigen, type of antigen presentation, and the mode of immunization (29–31).

Early exposure to parasite and bacterial antigens, and potentially allergens as well, may generate a broad and unbiased spectrum of immune responsiveness being of advantage upon secondary challenge (32–34). In the present study, we found in neonates significantly lower IL-10, IFN- γ and TNF- α responses to polyclonal mitogen and bacteria antigen stimulation than in their mothers, indicating a not yet fully developed immune competence, but following activation with *E. histolytica*- or *P. falciparum*-antigens only IL-10 was lower in newborns. While inflammatory cytokine responses to *E. histolytica*- or *P. falciparum*-antigens appeared similar in neonates and their mothers, the immune regulatory network involving IL-10 appeared not yet fully developed in newborns. Such an IL-10 deficiency in newborns and infants may contribute to an exacerbated and uncontrolled inflammatory response following parasite exposure, and indeed, in malaria, parasite-specific *in vitro* IL-10 production and antibody responses predicted accelerated parasite clearance in children (35). Infectious challenge during the neonatal and early postnatal period comprises a major threat for children, and therefore early expression of adult-like but also balanced neonatal immune responses, specific for *E. histolytica* or *P. falciparum*, may facilitate the development of immunity against unrestricted parasite multiplication and clinical disease.

In conclusion, our results indicate that children sensitized *in utero* by maternal *E. histolytica*- or *P. falciparum*-infection will develop a mixed Th₁ and Th₂ cytokine profile, with neonatal B cell producing *E. histolytica*-specific IgA and potentially other immunoglobulin subclasses as well. While neonatal parasite-specific cytokine responses appeared not to be broadly compromised; only IL-10 appeared deficient in newborns, it remains to follow in children the course, expression and longevity of parasite-specific primary immune responses. Since in malaria and amoebiasis, immunity against infection is incomplete and short lived, there is an urgent need for the generation of robust secondary responses in infants through appropriate boosting or immunization.

Acknowledgments

This investigation was supported by the Deutsche Forschungsgesellschaft (DFG So 3671-2), the DFG-Graduiertenkolleg Lebensstile, soziale Differenzen und Gesundheitsförderung (A.K.K.) and the fortune Programme at University Clinics of Tübingen (Grant 1040-0-0).

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