



Cellular gene expression induced by parasite antigens and allergens in neonates from parasite-infected mothers



Peter T. Soboslay^{a,*}, Thorsten Orlikowsky^{b,1}, Xiangsheng Huang^a, Christian Gille^b, Bärbel Spring^b, Lars Kocherscheidt^a, Abram Agossou^c, Meba Banla^c, Michael Bonin^d, Carsten Köhler^a

^a Institute for Tropical Medicine, University of Tübingen (UKT), Wilhelmstr. 27, 72074 Tübingen, Germany

^b Department of Neonatology, University Children's Hospital, UKT, Calwerstr. 7, 72076 Tübingen, Germany

^c Faculté Mixte de Médecine et de Pharmacie, Université de Lomé, B.P. 1515 Lomé, Togo

^d Department of Medical Genetics, University of Tübingen (UKT), Calwerstr. 7, 72076 Tübingen, Germany

ARTICLE INFO

Article history:

Received 31 December 2015

Received in revised form 28 March 2016

Accepted 30 March 2016

Keywords:

Prenatal immune sensitization

Umbilical cord blood cells

Gene expression

Neonates

Helminth and amoeba antigens

Mite allergens

ABSTRACT

Prenatal exposure to parasite antigens or allergens will influence the profile and strength of postnatal immune responses, such contact may tolerize and increase susceptibility to future infections or sensitize to environmental allergens. Exposure *in utero* to parasite antigens will distinctly alter cellular gene expression in newborns. Gene microarrays were applied to study gene expression in umbilical cord blood cell (UCBC) from parasite-exposed (Para-POS) and non-exposed (Para-NEG) neonates. UCBC were activated with antigens of helminth (*Onchocerca volvulus*), amoeba (*Entamoeba histolytica*) or allergens of mite (*Dermatophagoides farinae*). When UCBC from Para-POS and Para-NEG newborns were exposed to helminth antigens or allergens consistent differences occurred in the expression of genes encoding for MHC class I and II alleles, signal transducers of activation and transcription (STATs), cytokines, chemokines, immunoglobulin heavy and light chains, and molecules associated with immune regulation (SOCS, TLR, TGF), inflammation (TNF, CCR) and apoptosis (CASP). Expression of genes associated with innate immune responses were enhanced in Para-NEG, while in Para-POS, the expression of MHC class II and STAT genes was reduced. Within functional gene networks for cellular growth, proliferation and immune responses, Para-NEG neonates presented with significantly higher expression values than Para-POS. In Para-NEG newborns, the gene cluster and pathway analyses suggested that gene expression profiles may predispose for the development of immunological, hematological and dermatological disorders upon postnatal helminth parasite infection or allergen exposure. Thus, prenatal parasite contact will sensitize without generating aberrant inflammatory immune responses, and increased pro-inflammatory but decreased regulatory gene expression profiles will be present in those neonates lacking prenatal parasite antigen encounter.

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1. Introduction

The *in utero* exposure of the fetus to microbial and parasite antigens will influence the profile and strength of postnatal immune responses, and prenatal and early postnatal contact with parasites or bacteria-derived antigens may even prevent the development of allergic disorders (Steel et al., 1994; Holt, 1995; Prescott et al.,

1998; Braun-Fahrlander et al., 2002; Schuijs et al., 2015). The lack of such encounter may facilitate allergic sensitization in children, and the “hygiene hypothesis” is postulated to explain the inverse correlation between the decreasing incidence of infection and the rise of allergic disease (Yazdanbakhsh et al., 2002; Ramsey and Celedon, 2005).

How intrauterine pathogen, antigen or allergen exposure occurs and which type of responses will be generated upon *in utero* encounter is not well understood. The placental barrier is not only a molecular sieve, but actively able to transfer even large molecules and specific maternal IgG or may transfer antigens and allergens across the placenta into the fetus (Ben-Hur et al., 2005). The neonatal immune responses may appear functionally imma-

* Corresponding author.

E-mail address: peter.soboslay@uni-tuebingen.de (P.T. Soboslay).

¹ Current address: Section of Neonatology, Department of Paediatrics, University Hospital of RWTH, Pauwelsstr. 30, 52074 Aachen, Germany.

ture and skewed towards preferential Th2-type cytokine responses (Wegmann et al., 1993; Morein et al., 2002), but cord blood cells will secrete in response to pathogen- and allergen-specific stimulation interleukin 5 (IL-5), IL-10 and interferon (IFN)- γ , and these cytokines are produced in lower quantities by cord blood cells than adult PBMC (Steel et al., 1994). Whether weak neonatal Th1-type cytokine responses are an intrinsic immaturity or based on the cytokine-regulated Th1/Th2 polarization is still not conclusively resolved. Other immune regulators, notably chemokines, may act on the Th1/Th2 polarization as they contribute to cytokine-mediated inflammatory processes, and also to cytokine-driven suppression of inflammation (Luther and Cyster, 2001). Chemokines play an essential role in leukocyte trafficking, tissue infiltration, cell activation, T cell differentiation, angiogenesis and metastatic processes (Tarrant and Patel, 2006; Esche et al., 2005), but relatively little is known about cellular chemokine production in neonates, especially in response to allergens and parasite-derived antigens. In the present study, we analyzed the cellular gene expression in newborns from mothers infected with helminth and protozoan parasites, and observed differentially expressed genes encoding for proteins involved in antigen presentation, immune regulation and inflammatory responses.

2. Methods

2.1. Study participants

The participants of this study were from the Region Central (RC) in Togo/Africa and from the federal state of Baden-Württemberg (BW)/Germany. The study was authorized by the Togolese Ministry of Health and approved by the Ethik Kommission (No.323/2003) at University Clinics of Tübingen/Germany. The RC/Togo is meso- to hyperendemic for malaria, amoebiasis, and intestinal hookworm and filaria infections, whilst in BW/Germany exposure/infection with intestinal and intravascular protozoa and helminths does not occur. All pregnant women from the RC/Togo reported previous infections with intestinal helminth and protozoan parasites, *i.e.* hookworm, amoeba and *Plasmodium* spp., whilst none of the BW/Germany mothers reported such infections. Informed consent was obtained from all mothers after thoroughly explaining to them the procedures, the aims and the risks of this study. Pregnant women from the RC/Togo received anti malaria prophylaxis and anti-parasite treatment according to the national health guidelines of Togo during prenatal consultations and after partition (In the fourth month of pregnancy, all women received anti helminth treatment with albendazole. After partition, they were treated against intestinal protozoan parasites with metronidazole).

2.2. Sample collection and isolation and stimulation of cells

Umbilical cord blood was obtained from the placentas of healthy, full term-infants, after the placentas were delivered and separated from the infants. Umbilical cord mononuclear blood cells (UCBC) were isolated and cultured *in vitro* as previously described (Kirch et al., 2004). Freshly isolated UCBC adjusted to 1×10^7 /ml were cultured in the presence or absence (baseline) of allergens (*Dermatophagoides farinae*), helminth parasite-specific antigens (*Onchocerca volvulus*) or intestinal protozoa-specific antigens (*Entamoeba histolytica*) for 24 h. A total of 6 UCBC samples from helminth and intestinal protozoa infected and exposed (Para-POS) and 6 UCBC from non-exposed neonates (Para-NEG) were collected for analyses.

2.3. Purification of total RNA, generation of cDNA and labeling of cRNA

UCBC were collected from cell cultures and total RNA was purified by RNeasy Mini Kit (Qiagen, Hilden, Germany) and the quality of RNA determined by Agilent Bioanalyser 2100 (Agilent, CA, USA). Double-stranded cDNA was synthesized from the total RNA using a Superscript choice kit (Invitrogen, MA, USA) with a T7-(dT)24 primer incorporating a T7 RNA polymerase promoter (Metabion, Steinkirchen, Germany). cRNA was prepared and biotin labeled by *in vitro* transcription (Enzo Biochemical, NY, USA).

2.4. Oligonucleotide hybridization and microarray

Biotinylated cRNA was fragmented according to Affymetrix (Affymetrix, Santa Clara, CA, USA) and fragmented cRNA (15 μ g) was subjected to oligonucleotide hybridization (Fluidics Station 450, Affymetrix) to the human GeneChip HG U133A (Affymetrix). The quality control of generated cRNA was verified by first hybridization to the GeneChip Test3-Array (Affymetrix) to ensure equal hybridization to 5' and 3' oligonucleotide of housekeeping genes (PDH, GAPDH) before being used for further hybridization with the human GeneChip HG U133A. After hybridization, gene chips were automatically washed and stained with streptavidin-phycoerythrin using a fluidics station. The probe arrays were scanned at 1.4 μ m resolution using a Genechip System 3000 (Affymetrix), and the oligonucleotide hybridization data were exported for gene expression value analysis (GCOS 1.1, Affymetrix and ArrayAssist 3.4 software packages, Stratagene).

2.5. RT-PCR and quantitative real-time PCR

The quantitative Real Time PCR (qRT-PCR) was performed on the LightCycler 480 System (Roche, Basel, Switzerland) in 384 well format. Specific QuantiTect Primer (Qiagen) or Primer designed with the Primer3 Software (<http://bioinfo.ut.ee/primer3-0.4.0/>) spanning an Exon-Exon boundary were applied for amplification. The QuantiTect[®] SYBR[®] Green PCR Kit (Qiagen) with PCR settings according to previously applied protocol (Häbig et al., 2008). The following primers were used to determine the levels of gene expression:

SKALP/P13: FW- CCACTAAGCCTGGCTCCTG, RV – CTCCTCTCACTGGGAAC; SPP1: FW – CCCACAGACCCTTCCAAGTA, RV- GGGGACAACCTGGAGTGAAAA; STAT1: FW – CCCACAGACCCTTCCAAGTA, RV – GGGGACAACCTGGAGTGAAAA; STAT3: FW – GAGGGAACAAGCCCCAAC, RV – AGGGGTCCCACTGTTTCTC; STAT4: FW – AGCCTTGCGAAGTTTCAAGA, RV – ACACCGCATACACACTTGGA; STAT6: FW – GCGGCTCTATGTCGACTTTC, RV – ATGCTCTCAAGGTGCTGAT; FOXP3: FW – TGCTCTCTTCTTCTTCTGA, RV – TTGAGAGCTGGTG-CATGAAA; CCL5: FW – CGCTGTCATCCTCATTGCTA; RV – GGGTGACAAAGAC-GACTGCT; CCL22: FW – GAGGAAGCTGGCTGTGGTAG, RV – CTTGAGC-CCAGGAGTTTGAG; IL10: FW – GAGAACAGCTGCACCCACTT, RV – GGCAAC-CCAGGTAACCTTA; IL10RA: FW – ATCTGTCGCTTCCGAAGTA, RV – GAGCAGGA-CACTGGGTAGCTT; IL12B: FW – TGCTGCTTCACAAAAGGAA, RV – AAGAGCCTCTGCT-GCTTTTG; IL12RB1: FW – CCCTCTCTTCTCTTCTG, RV – CCGAGCCT-GAGTCTGCAT; IL15: FW – GTTACCCCCAGTTGCAAAGT, RV – TTTTCTCTCCAGTTC-CTCACA;

IL16: FW – TCCTGCAGAAAAAGGCAAAG, RV – AAAGCTGCGCA-GAATGAGAT;
 IFNA1: FW – ACCCACAGCCTGGATAACAG, RV – ACTGGTTGCCAT-CAAACCTCC;
 HLA-G: FW – GACITTTCTGTGGGGAGACCA, RV – CCACCTCTCTG-GAACAGGAA.

2.6. Functional classification of gene expression within gene clusters and networks

Gene expression within gene clusters and networks was analyzed through the use of QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, CA, USA). Selected groups of transcripts were integrated into genetic networks available in the Ingenuity database and then ranked by score. The score is defined by the probability that a collection of genes equal to or greater than the number in the respective network could be achieved by chance alone. Therefore, scores of 3 or higher have a 99.9% confidence of not being generated by random chance alone.

2.7. Statistical methods

For analysis, microarray hybridization data were normalized and converted to signal values using ArrayAssist 3.4 (Stratagene). All values (signal strength of expression) were logarithmic transformed to stabilize data variance. In order to correct for the unspecific “mismatch” hybridization background caused by the GC content of probe sets and to stabilize the intra-array variability the log (signal gene expression) values were normalized by GC-normalization; this was performed with the ArrayAssist 3.4 software package.

To equilibrate inter-array variability, from all gene expression data (brut sigLog) the mean “baseline” (sigLog value) of unstimulated resting UCBC (*i.e.* the mean baseline of the Para-POS group, $n = 6$) was subtracted; this standardized inter-individual variation of expression signals and the variation within groups, and resulted in net log signal gene expression values. All statistical analyses were performed with these net log signal gene expression values (*i.e.* standardized means). For analyzing gene expression signals in different groups of neonates, the statistical package JMP 11.0.0 was used. For explorative data analyses, the two sample Wilcoxon test was applied to evaluate differences between groups (p -values are indicated). Data (Sig Logs) from the different groups had a normal distribution. ANOVA tests were performed with statistical significance among the means of more than 2 groups, with the limitation that ANOVA will not make pair wise comparisons. For multiple testing, the application of the Bonferroni Holm adjustment ($n = 22,283$ genes, 2 study groups, 2 antigens, 1 allergen, 1 baseline) resulted in an alpha level of 1.8×10^{-7} . For multiple comparisons, and in order to avoid type I errors, differences of the net log signal gene expression between infection groups were analyzed by the Tukey test.

UCBC samples from 6 Para-Neg and 6 Para-POS newborns were used, and cells were activated with allergens from *D. farinae*, antigens from *O. volvulus*, *E. histolytica* or UCBC remained unstimulated (baseline); a total of 47 micro arrays were analyzed.

3. Results

3.1. Neonatal gene expression induced by mite allergens and helminth antigens

Gene expression was analyzed in UCBC from Para-POS and Para-NEG neonates. UCBC were activated *in vitro* with mite-specific allergens (*D. farinae*, DF) or helminth (*O. volvulus*, OV) or amoeba (*E. histolytica*, EH) antigens or remained without activation (baseline).

The overall signal strength of gene expression ($n = 22,283$ genes) was higher in Para-NEG (Bonferroni Holm adjusted; $p < 1.8 \times 10^{-7}$) and differed significantly between Para-POS and Para-NEG when their UCBC were activated with DF, EH or OV (Fig. 1). The mean gene expression values as induced by mite allergens and helminth antigens enhanced in Para-NEG but lessened in Para-POS (Fig. 1, Supplementary Table S1).

3.2. Distinct gene expression in UCBC after activation with allergens and helminth and protozoan parasite-derived antigens

Activation of UCBC with mite-derived allergens and helminth- and protozoa-specific antigens induced in Para-POS 31 and in Para-NEG 68 genes commonly expressed in response to the three stimuli (Fig. 2, Supplementary Table S2). Prominently expressed ($SLR > 1.0$) were genes encoding immune regulatory, inflammatory as well as apoptosis inducing molecules, *i.e.* osteopontin (SPP1), cystatin B, tumor necrosis factor alpha-induced proteins (TNFAIP6, TNFAIP3), annexin A5 (ANXA1), caspase 1 (CASP1), dedicator of cytokinesis 4 (DOCKS4), tissue inhibitor of metalloproteinase 1 (TIMP1) and super oxide dismutase 2 (SOD2). Noteworthy, in Para-POS none of the genes was found to be negative regulated following exposure to antigens or allergens. In activated UCBC from Para-NEG and Para-POS similarly regulated was TNF- α inducible protein 6 (TNFAIP6), but differentially expressed in Para-NEG were signal transducer and activator of transcription 1 (STAT1), suppressors of cytokine signaling 1 and 5 (SOCS1, SOCS5). In contrast to Para-POS, in Para-NEG neonates several genes were negative regulated ($SLR < -0.7$) following activation, notably, a depressed expression was observed for activated leukocyte cell adhesion molecule (ALCAM), CD9, CD36, myeloperoxidase (MPO), apoptosis related serine protease caspase 6 (CASP6), protein kinase C alpha (PRKCA), CC-chemokine receptor 2 (CCR2) and defensin alpha 4 (DEFA4, corticostatin). Thus, differing gene expression profiles will be generated in UCBC from Para-NEG and Para-POS neonates following activation with allergen and helminth and protozoa parasite-specific antigens, and the exposure to parasites or allergens *in utero* may account for these changes.

3.3. MHC class I and II alleles

Gene expression of MHC class I and class II alleles differed significantly ($p < 0.0001$) between Para-POS and Para-NEG (Fig. 3). In unstimulated UCBC the expression of MHC class I alleles was lower in Para-NEG than in Para-POS newborns, particularly the HLA-B, -C, F and -G alleles. However, MHC I alleles gene expression was enhanced in Para-NEG in responses to allergen, and amoeba and helminth antigens, while it was strongly diminished in Para-POS newborns after exposed to allergens or helminth (Fig. 3A). In Para-POS newborns, the mean gene expression of MHC II alleles in UCBC was constitutively diminished, and the depression persisted following exposure to allergen (DF) or helminth antigens (OV) (Fig. 3B). *Entamoeba* antigen exposure did not change the MHC I and MHC II expression in Para-POS.

3.4. Immune activation, regulation and apoptosis

A constitutively higher expression of STAT1, STAT4 and STAT5A was found in Para-NEG with higher signals for all STATs irrespective whether UCBC were activated with allergens or helminth antigens (Fig. 4). Stronger expression of STAT1 and STAT4 in Para-NEG infants suggested a preferred development of Th1 immune responses. STAT6 was less expressed in Para-NEG than Para-POS newborns, suggesting a lower Th2-type immune responsiveness in Para-NEG. Amoeba antigens enhanced mean STATs' expression in

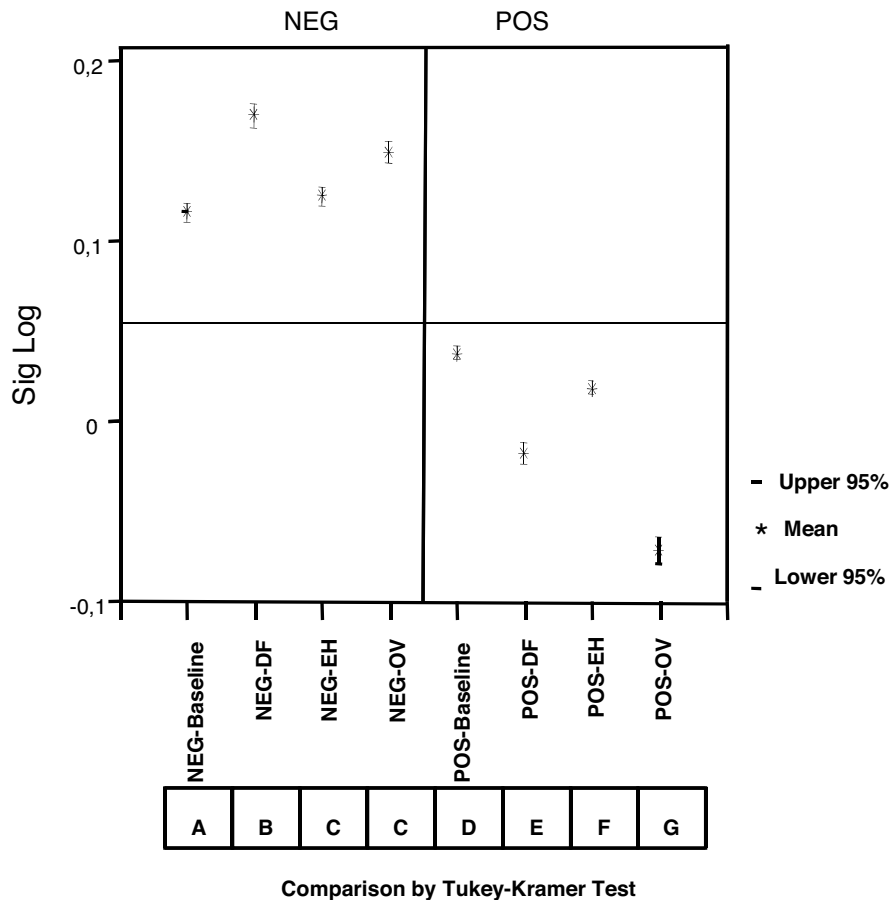


Fig. 1. Gene expression levels (Sig Log means) in parasite exposure/infection positive (Para-POS) and parasite exposure negative (Para-NEG) neonates are shown. Umbilical cord blood cells (UCBC) from Para-POS (n = 6) or Para-NEG (n = 6) were either resting (baseline) or activated with amoeba (*Entamoeba histolytica*, EH) specific antigens, or mite allergens from *Dermatophagoides farinae*, DF) or helminth (*Onchocerca volvulus*, OV) specific antigens for 24 h. Signal strength of gene expression was logarithmic transformed, and to equilibrate inter-array variability, from all gene expression data (brut sigLog) the mean “baseline” values of unstimulated resting UCBC from Para-POS neonates (n = 6) were subtracted; this standardized inter-individual variation of expression signals and the variation within groups, and resulted in standardized means of the log signal gene expression values. The expression of n = 22,283 genes in UCBC was compared between Para-POS and Para-NEG neonate groups after cell activation with helminth (OV) antigen (NEG n = 6, POS n = 5), amoeba (EH) antigen (NEG n = 6, POS n = 6), mite (DF) allergen (NEG n = 6, POS n = 6) and resting (baseline) cell cultures (NEG n = 6, POS n = 6). Statistical analyses were performed by Tukey Test with standardized means of the log signal gene expression values. If levels/groups are not connected with the same letter, differences are significant. The signal strength of gene expression (n = 22,283 genes) differed significantly (Bonferroni Holm adjusted $\alpha = 1.8 \times 10^{-7}$) between Para-POS and Para-NEG neonates being stronger in Para-NEG. Noteworthy, gene expression induced by mite allergens and helminth antigens was strongly enhanced in Para-NEG while becoming depressed in Para-POS.

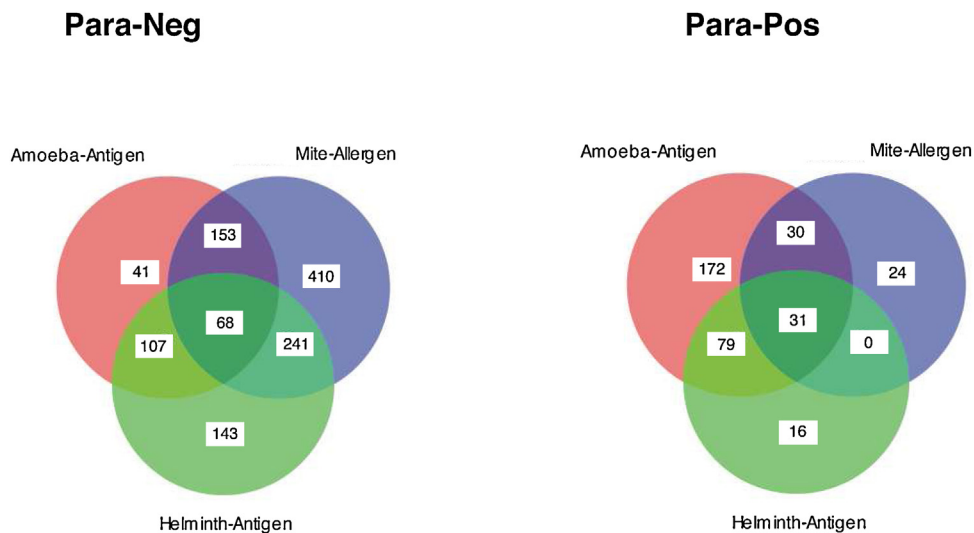
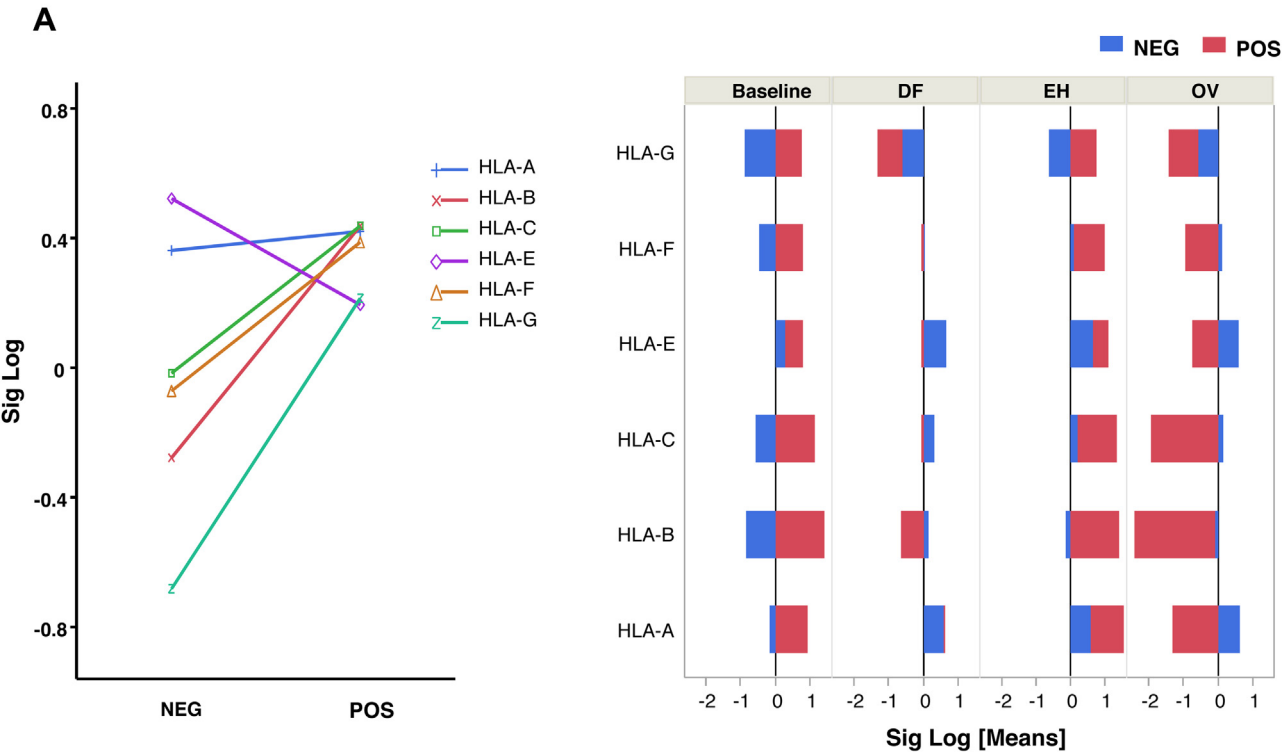


Fig. 2. Identical and differential gene expression in umbilical cord blood cells (UCBC) from newborns of mothers exposed to and infected with helminth and protozoa parasite infections. UCBC were activated with mite allergen (*D. farinae*, DF) and helminth (*O. volvulus*, OV) and protozoa (*E. histolytica*, EH) parasite-specific antigens. UCBC were isolated and cultured for 24 h and gene expression analysis was performed as described in Section 2.

MHC – class I gene expression



MHC – class II gene expression

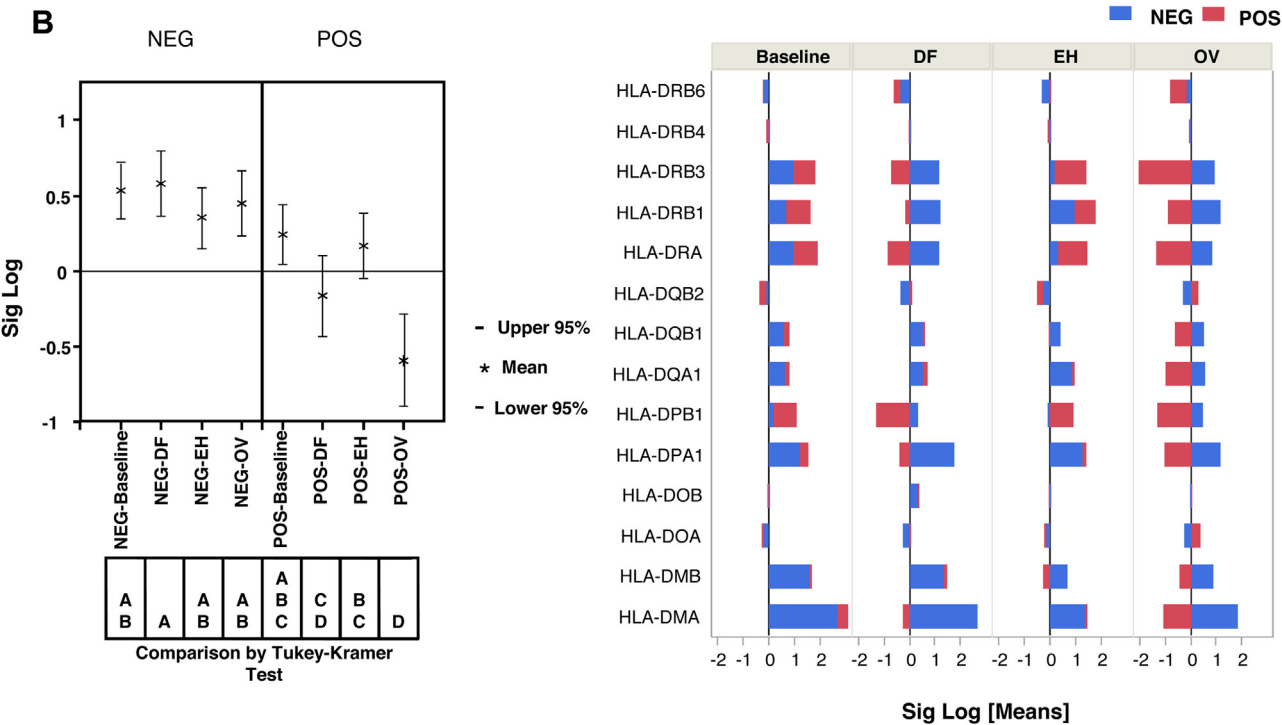


Fig. 3. Gene expression of MHC class I and class II alleles in UCBC from parasite exposure/infection positive (Para-POS) and negative (Para-NEG) neonates. Gene expression levels of MHC class I and II for alleles are shown as signal log means, i.e. for each MHC allele the expression values in UCBC cultured *in vitro* without stimulation (baseline), following activation with mite allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV) or *E. histolytica* (EH). Statistical differences were detected by Tukey Test and if groups are not connected with the same letter differences are significant.

Differential gene expression was evaluated by real time qPCR, and genes' up-regulation or down-regulation (Signal log Ratio) in UCBC of newborn from Para-NEG and Para-POS mothers is shown in Fig. 9. Target genes selection was based on microarray gene expression values and the quantitative rt-PCR confirmed the selectively up-regulated expression of HLA-G, STAT6, SKALP and IL-10 genes in Para-POS, and equally confirmed down-regulation of SP1/Osteopontin, CCL5/RANTES and CCL22/MDC genes in Para-POS neonates. For IFNA1 (type I IFN) we observed its pronounced expression in Para-POS when UCBC were stimulated with mite allergen DF, and amoeba EH and helminth OV antigens. Both allergen and helminth antigens stimulated expression of pro-inflammatory cytokines, stress response and detoxification metabolites; differentially induced were tumor necrosis factor alpha-induced protein 8 (TNFAIP8; NEG>POS; $p<0.0001$), protease inhibitor 3 skin-derived (SKALP; POS>NEG; $p<0.0001$) and

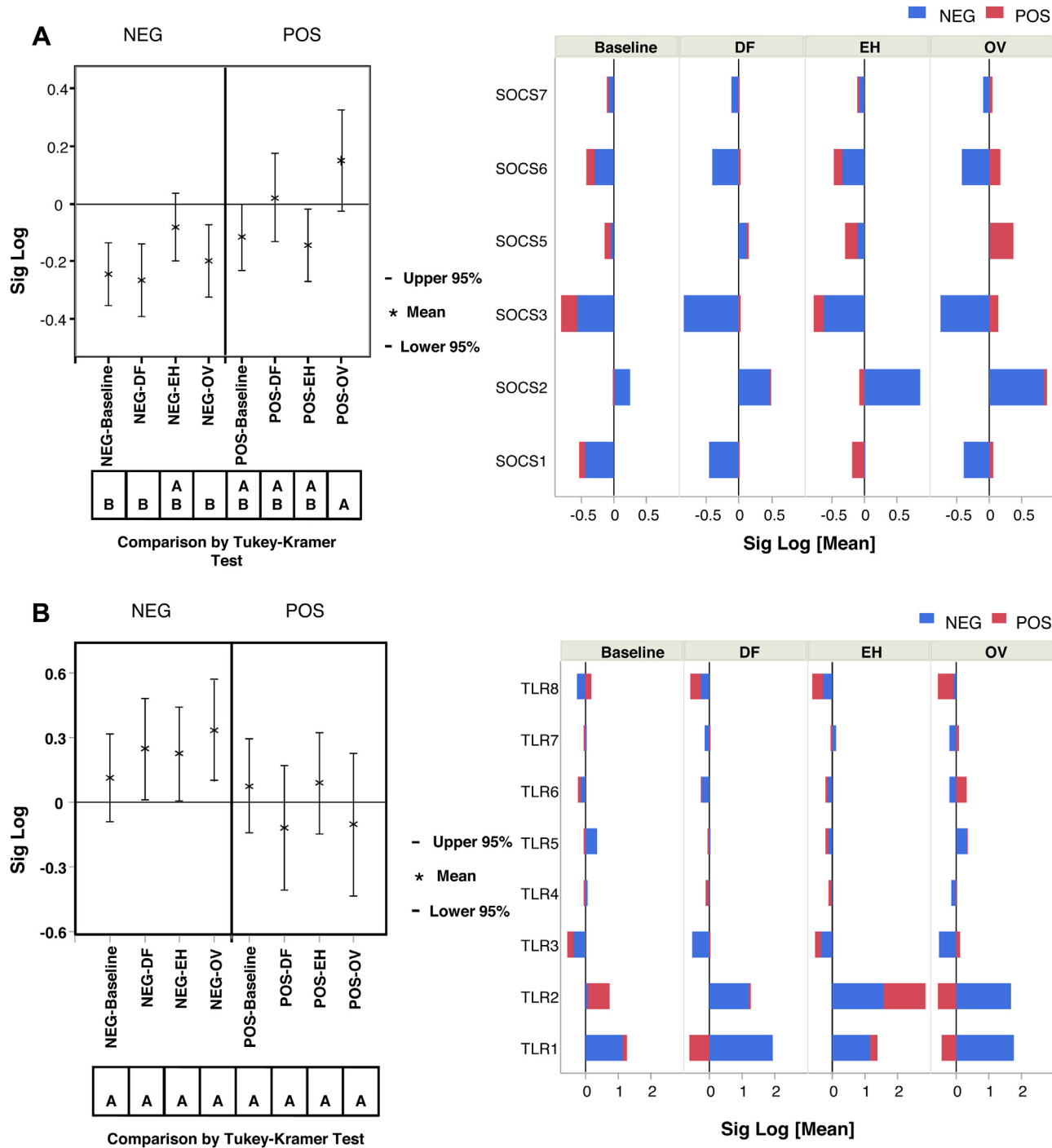


Fig. 5. Gene expression of toll like receptors (TLR) and suppressors of cytokine secretion (SOCS) in parasite infection positive (Para-POS) and negative (Para-NEG) neonates. Gene expression levels are shown (Sig Log) as signal log means, i.e. the expression values in UCBC cultured *in vitro* following activation with allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV), *E. histolytica* (EH) or without stimulation (baseline). Statistical differences were detected by Tukey Test and if groups are not connected with the same letter differences are significant.

interferon alpha genes (IFNA1-21; POS>NEG; $p<0.0001$). Genes grouped within the cluster for Treg (IL-10, TGF β , and FOXP3) were depressed ($p<0.0001$) in Para-NEG newborns (Fig. 9; Supplementary Table S1, Supplementary Fig. 6).

3.8. Functional classification of gene expression within gene clusters and networks

In order to analyze functional and gene network differences between Para-NEG and Para-POS, gene clusters were analyzed as

generated by the ingenuity pathways analysis software (Fig. 10). The selection of gene groups and molecular pathways for analysis was based on differentially ($p<0.05$) expressed genes within a given cluster. Differential expression was evaluated on net values of the signal strength (net SigLog), i.e. the baseline gene expression values (SigLog) of not activated UCBC cultures performed in parallel were subtracted from parasite antigen or mite allergen activated UCBC gene expression values. The major gene clusters (Fig. 10) which evolved with $n=34-35$ genes being significantly and differentially regulated represented “cellular growth and pro-

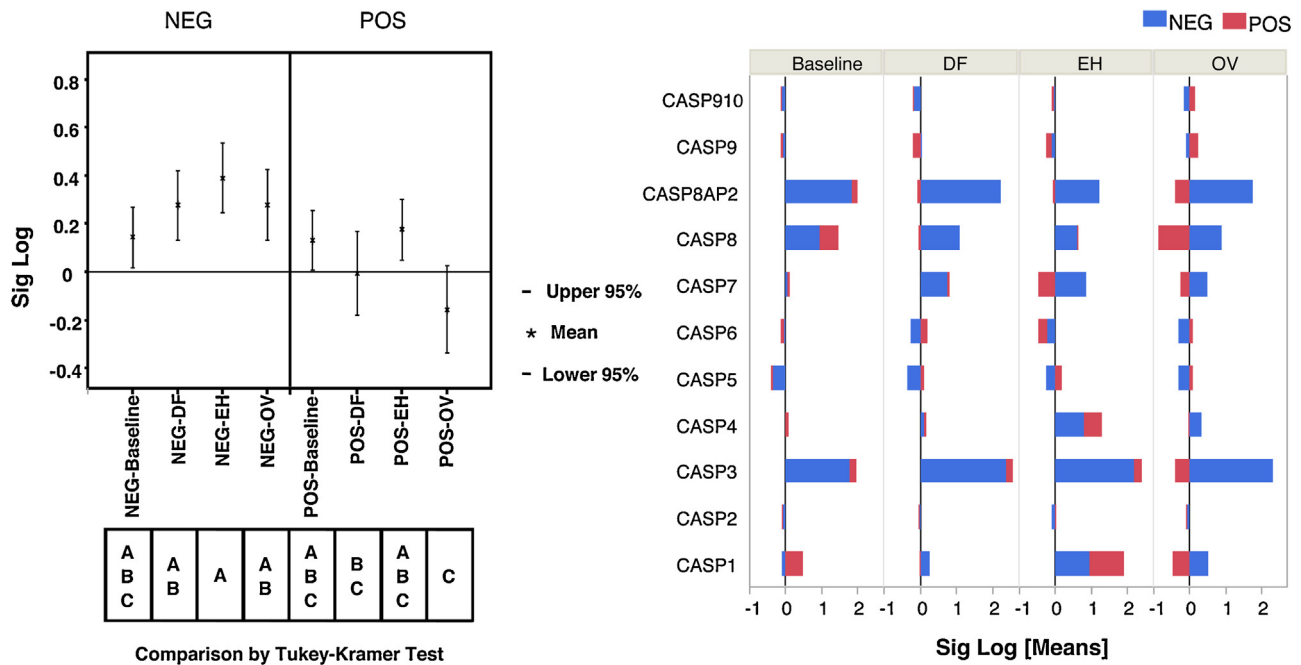


Fig. 6. Gene expression of caspase (CASP) in parasite infection positive (Para-POS) and negative (Para-NEG) neonates. Gene expression levels are shown (Sig Log) as signal log means, i.e. the expression values in UCBC cultured *in vitro* following activation with allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV), *E. histolytica* (EH) or without stimulation (baseline). Statistical differences were detected by Tukey Test and if groups are not connected with the same letter differences are significant.

liferation and hematological system function and development". Within this cluster, the overall expression induced by allergens or antigens differed significantly between Para-POS and –NEG newborns ($p < 0.0001$, Wilcoxon rank sum test). Amongst those being induced by helminth parasite antigens (Fig. 10, Part A) were IL-6, secreted phosphoprotein SPP1, CASP1, proto-oncogene ETS, and suppressed were MAF, MAFB, Fas associated via death domain FADD, CASP6, GATA binding protein GATA3, NALP1 and IL10RA (for all expressed genes see Fig. 10). By mite allergens inducible (Fig. 10, Part B) were interferon alpha receptors 1 and 2 (IFNAR), IL-6, matrix metalloproteinases 8 and 9 (MMPs), serpin peptidase inhibitors (SERPINs), resistin RETN, CD17, CD19, TSL, STAT1, and suppressed became integrin subunit alpha ITGA4, CEBPA, serine/threonine kinase STK11 and myeloperoxidase MPO (for all expressed genes see Fig. 10).

In Para-NEG and Para-POS neonates, the gene expression (log p-values) within functional clusters for cell death, cellular growth and proliferation, immune response expression and strength were compared, and also, predictive estimates for the development of immunological, hematological and dermatological disorders and diseases were calculated. Allergen activation induced several gene clusters in Para-NEG newborns which strongly promoted cellular growth but also cell death and which a gene expression profile in Para-NEG may predispose for the development of dermatological diseases (Fig. 11). In Para-POS newborns, allergens generated gene expression profiles which promoted cell to cell signaling and favored the development of immunological disorders and diseases. Helminth antigen activation of UCBC in Para-NEG induced, in contrast to Para-POS, a stronger expression of gene clusters predicting an increased risk for dermatological diseases and immune disorders. In both groups of neonates and irrespective of the mode of UCBC activation the predictors for respiratory disease remained unaffected (not shown).

4. Discussion

In newborns with prenatal contact to parasites, the gene expression profiles appeared distinctly regulated towards an immune response phenotype which will not evolve with an aberrant or overwhelming inflammatory immune reactivity. The lower expressions of genes coding for proteins which mediate inflammatory responses were observed both in response to parasite antigens as well as allergen-specific cellular activation. This is further support to the hypothesis of a reduced risk and lower incidence of inflammatory and allergic disorders in children from rural and agricultural environments (Braun-Fahrlander et al., 2002; Schuijs et al., 2015). Helminth, amoeba and other protozoa parasite infections are highly prevalent in pregnant women in rural Togo, and the prenatal encounter to parasites has been shown to immune sensitize *in utero* (Kirch et al., 2004; Soboslay et al., 1999; Malhotra et al., 1997). This was considered as favorable by preparing the newborn to respond adequately to postnatal infectious challenge (Malhotra et al., 2006; Ridge et al., 1996; Hermann et al., 2002), but prenatal contact with pathogens may also increase susceptibility to and morbidity of infection (Carlier et al., 2012; Hightower et al., 1993), and in long term, may impede or even distort the development of their immune competence (Steel et al., 1994; Herz et al., 2000; Lewert and Mandlowitz, 1969). Pathogens have evolved with a variety of strategies to escape immune surveillance by their hosts. The intracellular parasite *Toxoplasma gondii* interferes with the MHC class I and class II antigen presentation pathway of murine macrophages (Lüder et al., 1998), and deficient MHC complex dependent antigen presentation has been observed with various infections (De Lerna Barbaro et al., 2005). The broadly reduced expression of the MHC class II alleles in Para-POS newborns suggested that priming of naïve T helper cells is reduced which may limit the generation of T cell dependent immune responses.

Pathogens may also down-regulate MHC class I expression (Schust et al., 1999) thus decreasing the sensitivity to MHC class I restricted cytotoxic T cell mediated lyses. In UCBC from Para-NEG newborns, parasite and allergen antigens activation depressed gene

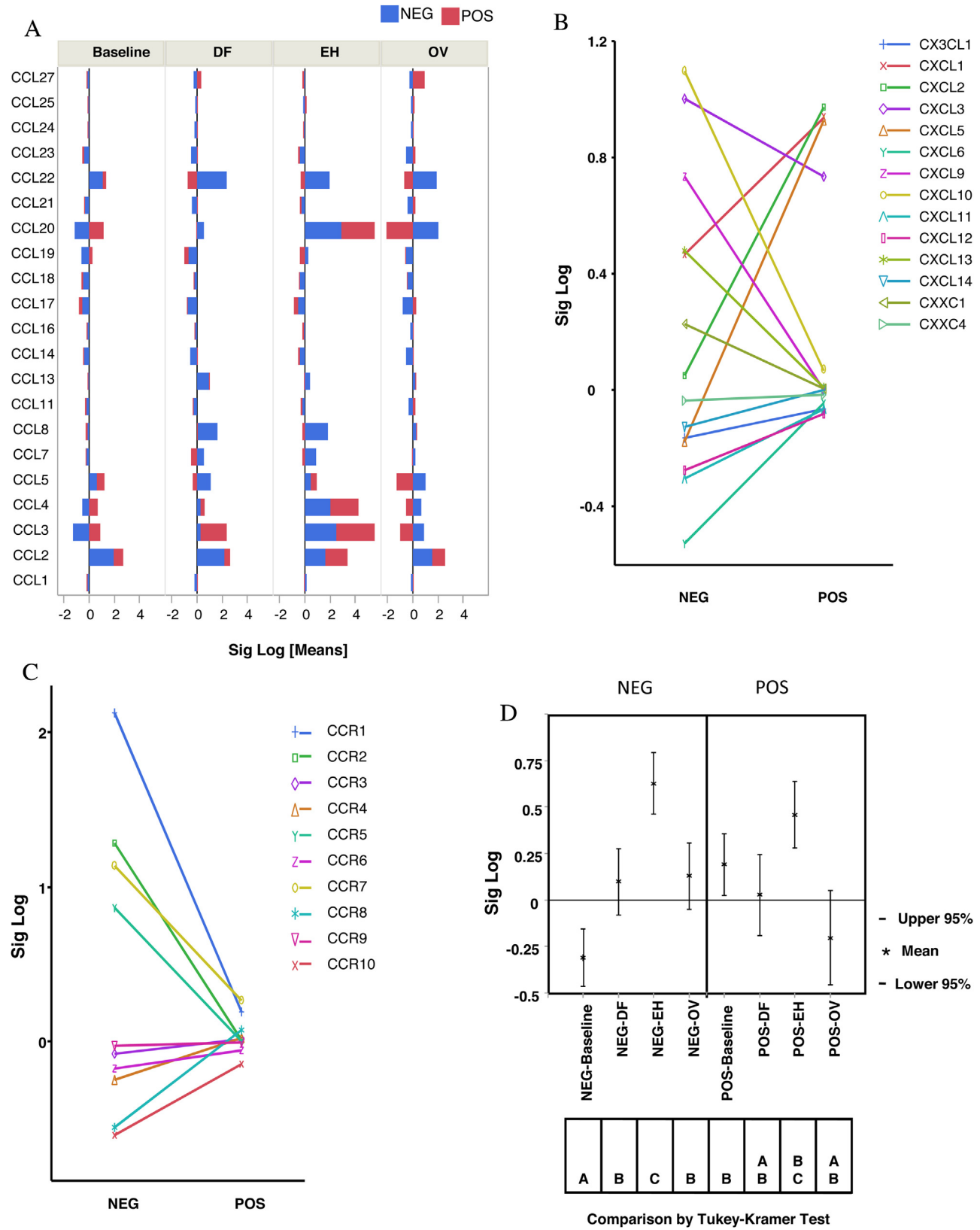


Fig. 7. Gene expression of CC chemokines, CXC chemokines and CC chemokine receptors in parasite infection positive (Para-POS) and negative (Para-NEG) neonates is shown. Gene expression of CC chemokines and CC chemokine receptors are shown (Sig Log) as signal log means, i.e. the expression values in UCBC cultured *in vitro* following activation with allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV), *E. histolytica* (EH) or without stimulation (baseline) are compared. Statistical differences were detected by Tukey Test and if groups are not connected with the same letter differences are significant.

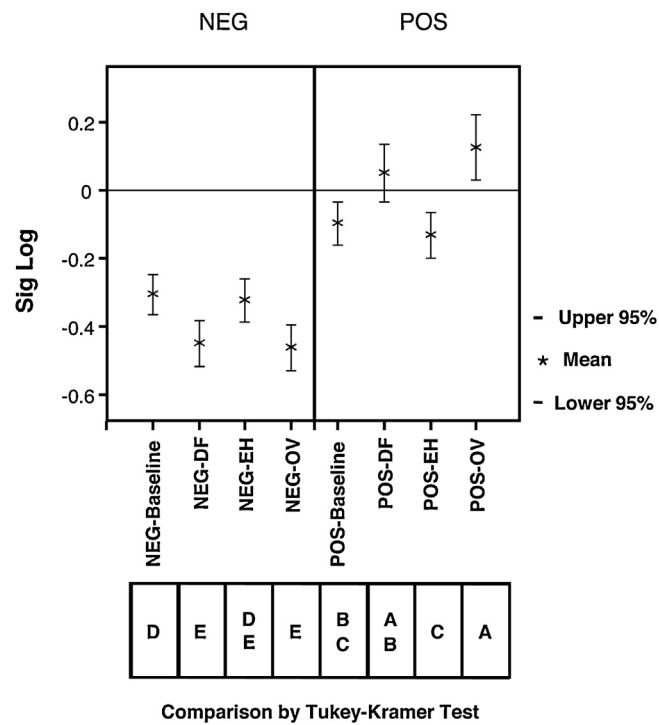


Fig. 8. Gene expression of immunoglobulins (IgG, IgA, IgM, IgD, IgE) heavy and light chains in umbilical cord blood cells from parasite infection negative (Para-NEG) and positive (Para-POS) neonates. Expression levels (Sig Log) are shown as means for individual genes in Para-POS and Para-NEG newborns, i.e. signal log expression values in UCBC from NEG and POS cultured *in vitro* without stimulation (baseline), activated with mite allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV) or *E. histolytica* (EH) are compared between POS and NEG groups. Statistical differences were detected by Tukey Test and if groups are not connected with the same letter differences are significant.

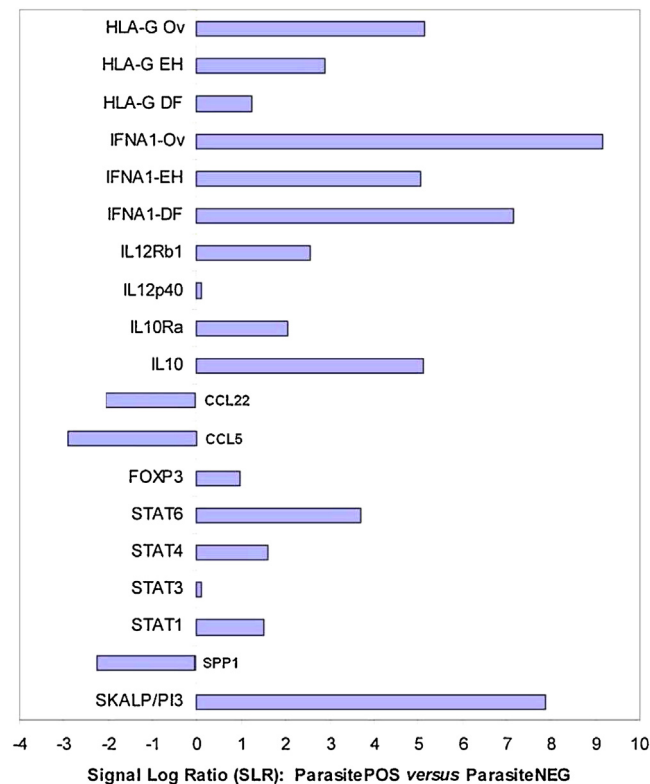


Fig. 9. Target gene up-regulation or down-regulation (Signal log Ratio) as determined by real-time qPCR in umbilical cord blood cells (UCBC) from parasite infection/exposure negative (Para-NEG) and parasite infection positive (Para-POS) neonates. Target genes for real time qPCR were HLA-G, IFN- α , interleukins (IL-10, IL-12) and their receptors, chemokines (CCL5, CCL22), signal transducers of activation and transcription (STAT 1–6), forkhead box protein P3 (FOXP3), osteopontin (SPP1) and skin-derived serine proteinase PI3 (SKALP). UCBC were resting (baseline) or activated with antigens of *O. volvulus* (OV), *E. histolytica* (EH) and mite allergens of *D. farinae* (DF).

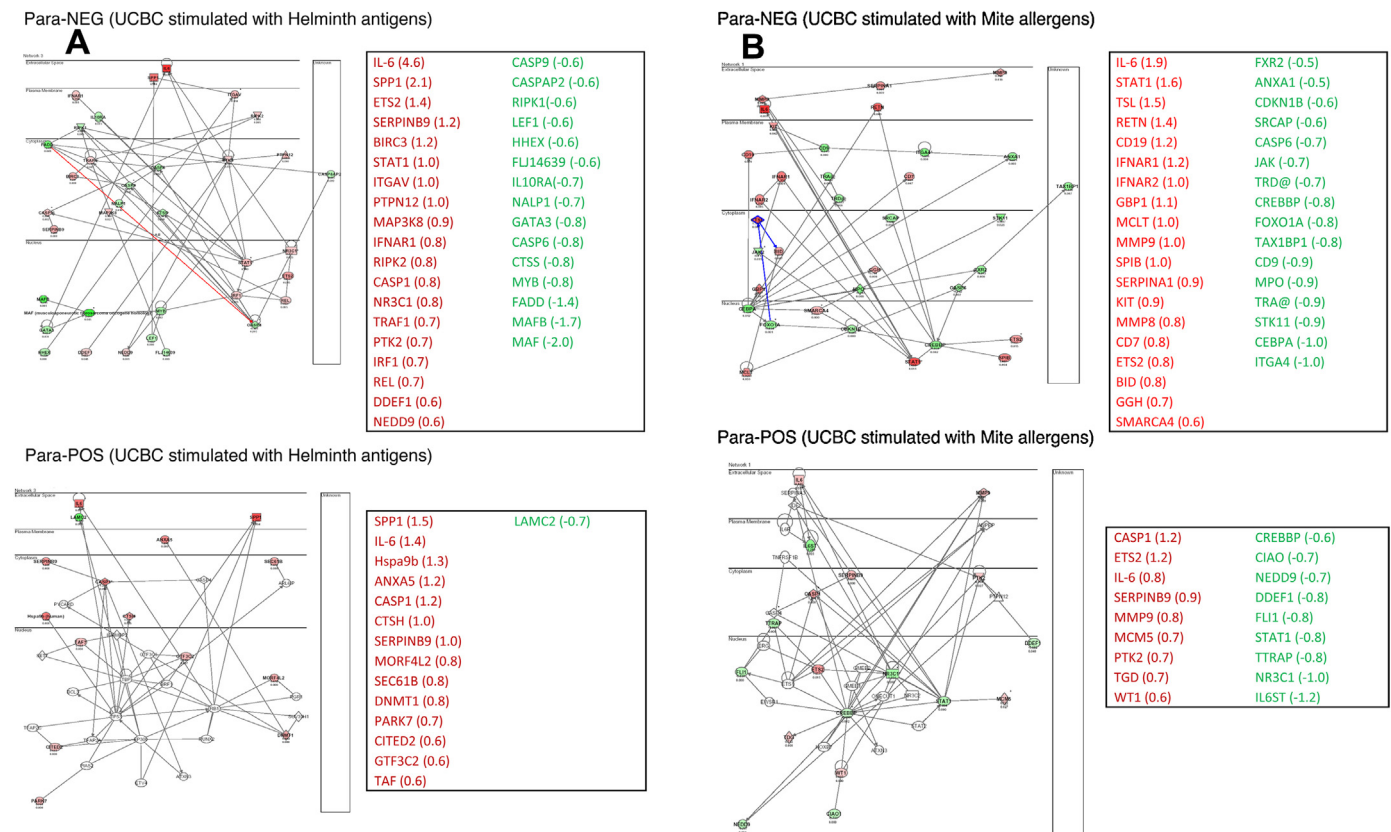


Fig. 10. Genes identified with Ingenuity Pathway Analyses as expressed in umbilical cord blood cells (UCBC) in the networks “Cellular Growth, Proliferation and Hematological System Function and Development” (**PART A**) and “Immunological Diseases and Cancer” (**PART B**) in parasite positive (Para-POS) and parasite-negative (Para-NEG) neonates are shown. Umbilical cord blood cells were stimulated *in vitro* with mite allergens (*Dermatophagoides farinae*, DF) or helminth (*Onchocerca volvulus*, OV) antigens for 24 h. The colored symbols and the color intensity show significant changes and the strength of gene expression (net SigLog). Red symbols are those genes with an increased expression, encircled green are those which decreased, and in white transparent symbols gene expression did not change. Below each symbol the intensity of gene expression (net sigLog) and the p values are given. For the analyses of differential gene expression in UCBC of parasite positive (Para-POS) and parasite-negative (Para-NEG) neonates, net values of the signal strength (netto SigLog) were calculated, i.e. the baseline gene expression values (baseline SigLog) of resting UCBC cultures were subtracted from gene expression values (brut SigLog) as induced by allergen or antigen activation. Differentially expressed genes ($p < 0.05$) within the two networks are listed and values of the signal strength (net SigLog) are given in brackets as increased (in red) or decreased (in green), white transparent symbols with unchanged gene expression are not listed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

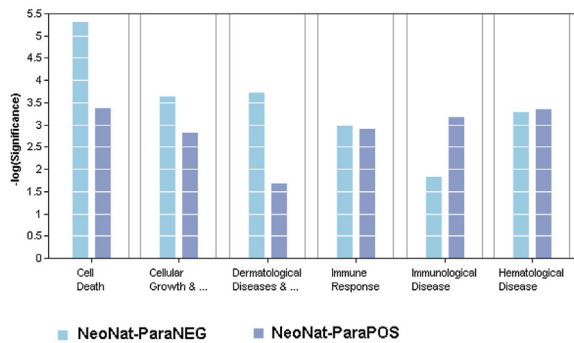
expression for HLA-B, -C, -F and -G alleles, antigen-induced HLA-A expression remained unaffected, whilst HLA-E enhanced (Fig. 3A). Depressed MHC class I expression may favor intracellular parasite development and propagation, and the divergent activation of HLA class I genes suggested that parasite antigens and allergens induce HLA gene transcription through differing pathways. For HLA-E, this may involve IFN- γ and STAT1 via the class II transactivator (CIITA) (Gobin and van den Elsen, 2000). As HLA-G expression is high on placental trophoblasts, the HLA-G-allergen/antigen ligation and its transfer across placental tissues may be a mechanism how the fetus will become exposed; but little is known about the specificities of HLA-G ligands (Munz et al., 1999). Viral and bacterial infection are associated with down regulation or inhibition of HLA-A and HLA-B surface expression, antigen processing, and defective ligation of peptides to MHC class I molecules (Hewitt and Dugan, 2004). Such interferences are considered as important strategies for immune evasion and for intracellular pathogen persistence.

Our finding that genes for STAT 1 and 4 were dominantly expressed, while those for STAT 6, FOXP3 and IL-4 were reduced in Para-NEG newborns, is further indication that exposure *in utero* will direct immune responses in Para-POS towards an anti-inflammatory and regulatory “phenotype”. Also the lower expression of genes within the cluster for regulatory T cells in Para-NEG newborns supported that prenatal parasite and allergen

exposure will sensitize without predisposing for aberrant inflammatory immune responses.

For IFNA1 (type I IFN) we observed its pronounced expression in Para-POS in response to mite allergens DF, amoeba EH and helminth OV antigens (Fig. 9). The Type I IFNs inhibit viral replication and mediate protection against viral infection, but they are also known to mediate anti-proliferative and immunoregulatory properties, and type I IFNs can play a negative role in the control of diverse pathogens with detrimental function on immunity (Stifter and Feng, 2014). Type I IFN induction often associated with microbial evasion mechanisms, e.g. *Listeria monocytogenes* induced type I IFNs can modulate protective mechanisms and impair survival of the infected host. With mycobacterial infection, type I IFNs were shown to increase bacterial burden and tissue pathology by limiting IFN- γ -dependent antimicrobial activity through production of the immunosuppressive cytokine IL-10. Type I IFNs are also shown detrimental for control of New World *Leishmania* spp. by preventing parasite clearance in parasite-infected macrophages, and during severe malaria infection, type I IFNs regulate parasite control by suppressing IFN- γ production. In Para-POS neonates, the elevated IFNA1 gene expression suggests an effective response against viral pathogens, and the higher baseline IL-10, IL-10RA, STAT6 and FOXP3 expression in Para-POS as compared to Para-NEG is further support that prenatal parasite and allergen exposure will

Part 1: Mite-Allergen (DF)



Part 2: Helminth-Antigen (OV)

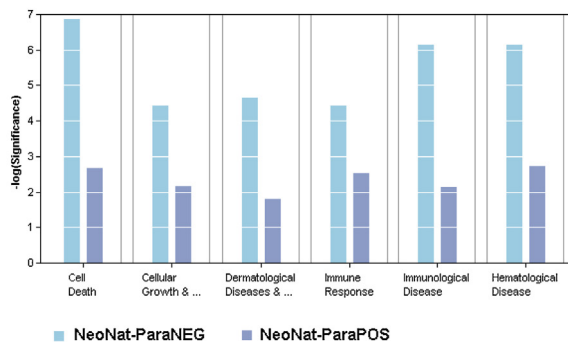


Fig. 11. The comparison of gene expression (log p-values) within functional clusters in Para-NEG and Para-POS neonates following activation of UCBC with mite allergens (*Dermatophagoides farinae*, DF) (Part 1) and helminth antigen (*Onchocerca volvulus*, OV) (Part 2) is shown. Functional gene clusters and networks for cell death, cellular growth and proliferation, immune response expression and strength were compared, and also, predictive estimates for the development of immunological, hematological and dermatological disorders and diseases were calculated. The changes of gene expression (log p-values) were grouped within networks available in databases (Ingenuity) and then ranked by score. The score is defined by the probability that a collection of genes equal to or greater than the number in the respective network could be achieved by chance alone; scores of 3 or higher have a 99.9% confidence of not being generated by random chance alone.

predispose for regulatory and Th2-type immune responses upon infectious challenge.

E. histolytica has been reported to induce the expression of SOCS2 in porcine intestinal cells (Bruel et al., 2010). *E. histolytica* infection drives the T auxiliary response towards a Th17 orientation, which is less suitable to allow full recovery from the infection (Guo et al., 2007). Highly expressed SOCS2 was also observed in UCBC from Para-NEG neonates after *E. histolytica* antigen activation in our study. This could be associated with a subsequent inhibition of the Th1 response consequently helping the parasite to better resist host immune responses. SOCS-3 is predominantly expressed in Th2 cells, and it has been pointed out as an important regulator for the onset and maintenance of Th2 mediated allergic disease. Chen et al. (2006) reported that SOCS3 plays an important role in Th cell differentiation by limiting the development of Th17 cells; the enhanced SOCS3 levels in Para-POS neonates following antigen stimulation suggested dominant Th2 type responses upon parasite exposure.

TLR2 in association with TLR1 or TLR6 can recognize a broadest range of ligands (Ozinsky et al., 2000; Jin et al., 2007). The recognition of PAMPs by TLR2 complexes with TLR1 or TLR6 leads to the recruitment of myeloid differentiation factor 88 (MyD88) adaptor (Akira, 2006). TLR2 interaction with MyD88 is indirect and is medi-

ated by the (MAL)/TIR domain-containing adaptor protein (TIRAP) (O'Neill and Bowie, 2007), and TLR2 binds microbial glycolipids and lipopeptides independent of the presence of TLR6 or TLR1. Thus, the activation of TLR2/TLR6 and TLR2/TLR1 could be downregulated by competing for ligand binding with the TLR2 transmembrane receptor (Jiménez-Dalmaroni et al., 2015). TLR-2 expression was highly inducible in Para-NEG neonates while TLR-1 and TLR-6 at similar levels as the baseline suggesting that TLR2 expression was independent from the presence of allergens or parasite antigens. Moreover, SOCS-1 mediates degradation of TIRAP, which may negatively regulate of the TLR pathways (Liew et al., 2005), and in this study, the low SOCS-1 expression in Para-NEG newborns may favor the induction of TLR-2.

The activation of caspases is mediated through their recruitment to inflammasomes (Martinon et al., 2002). In response to specific internal or external cellular attacks, the activation of TLRs or NOD-like receptor proteins that can detect PAMPs derived from bacteria or viruses leads to formation of distinct inflammasome complexes (Lamkanfi and Dixit, 2014). Caspase-1 is activated by recruitment to NLRP3/NALP3/cryopyrin inflammasome which is activated by a wide range of PAMPs as well as whole pathogens (Martinon et al., 2002, 2004). This step is essential for the processing and maturation of the pro-inflammatory cytokines IL-1 β and IL-18 facilitating their secretion and the promotion of innate immune responses (Thornberry et al., 1992; Ghayur et al., 1997). Activation of caspase-1 by *Schistosoma mansoni* soluble egg antigens induced the release of bioactive IL-1 β in an NLRP3 inflammasome-dependent manner and manipulated the outcome of adaptive immune responses and immunopathology (Ritter et al., 2010). High expression of IL-1 β as induced by helminth antigens or allergens, and elevated caspase-1 expression levels following *E. histolytica* antigen stimulation in our study, indicated the development and the expansion of inflammatory responses. Mice deficient for caspase-1 are protected from experimental mucosal inflammation (Siegmund, 2002). Previous studies have reported that caspase-8 will inhibit extensive inflammation *in vivo* (Ben Moshe et al., 2007; Kovalenko et al., 2009), and the low expression levels of caspase-8 after antigen stimulation in neonates may suggest further expanded inflammatory responses.

Parasite antigens and allergens induced the gene expression of several CC as well as CXC chemokines which play an essential role in leukocyte trafficking, cell tissue infiltration and inflammation (Oliveira and Lukacs, 2003). They are important mediators between innate and adaptive immunity by regulating T cell differentiation, apoptosis, angiogenesis and metastatic processes, are functionally involved in regulation of Th1 or Th2 polarization, and monocyte and macrophage activation (Luther and Cyster, 2001; Tarrant and Patel, 2006; Esche et al., 2005). The pronounced expression in Para-NEG of CCL2/MCP1, CCL5/RANTES, CCL7/MCP3, CCL8/MCP2, CCL13/MCP4 genes corresponded with the expression of their receptors CCR1 and CCR2. The Th1-type and pro-inflammatory chemokines CXCL9/MIG and CXCL10/IP10 were stronger expressed in Para-NEG, whilst CCL11/Eotaxin, CCL17/TARC, CCL24/Eotaxin-2, CCL27/CTACK and CXCL12/SDF-1 were higher in Para-POS. The promiscuous nature of the chemokine and chemokine receptor interaction (Charo and Ransohoff, 2006) did not permit to draw a stringently polarized immune expression profile for chemokines and their receptors, but noteworthy, the major actors for directing Th2 lymphocytes, i.e. IL-4, IL-5, TARC and Eotaxin, dominated in Para-POS neonates. Considering the gene expression of MHC class II alleles, STATs, Th2 and Treg cytokines and chemokines, inflammatory immune processes were stronger present in Para-NEG, and these newborns were not exposed *in utero* to parasite antigens. Such overall enhanced gene expression to allergens and parasite antigens in Para-NEG may generate an inflammatory phenotype associated with more severe disease upon postnatal helminth parasite infection or allergen exposure.

In Para-POS neonates, an enhanced expression of immunoglobulin isotype genes was observed when compared to Para-NEG, and this is further evidence that parasite antigen encounter *in utero* will selectively expand naïve B cells which then may switch immunoglobulin classes. Immunoglobulin class switch is regulated by IL-4 which was stronger expressed in Para-POS. Furthermore, Ig epsilon chain expression was observed in Para-POS and this supports fetal IgE production in response to helminth antigen or allergen stimulation. Sensitization may start *in utero* under the influence of the mothers' exposure to infection, and parasite-specific IgE was detected in offspring from filaria- (Weil et al., 1983) or *Plasmodium falciparum*-infected (Desowitz et al., 1993) mothers, and also in neonates (Pfefferle et al., 2008) sensitized to environmental allergens prenatally.

In order to determine whether Para-NEG neonates are prone for an increased risk of developing inflammatory disorders, differentially expressed genes within functional networks and profiles were analyzed using the Ingenuity Pathways Analysis software. This identified differentially expressed genes within a functional pathway, allowed to compare functional gene clusters, and to estimate the predisposition for immune function related disorders and diseases. Within differentially regulated gene clusters (Figs. 9 and 10) Para-NEG neonates presented significantly higher expression signals than Para-POS newborns. Such heightened response profiles to allergens and parasite antigens may associate with severe disease, as it has been described before for neonatal and adult macrophages infected with avian influenza virus (Zhou et al., 2006), experimental cerebral malaria (Delahaye et al., 2006), lymphatic filariasis (Babu et al., 2005) and the skin lesions of atopic dermatitis and psoriasis (Nomura et al., 2003). These findings support our single gene expression analyses that prenatal exposure to parasite antigens will impact on strength and width of the postnatal immune responsiveness.

In summary, our data disclose that *in utero* encounter with parasites and environmental allergens will impact on cellular gene expression in neonates. Such prenatal contact will sensitize without generating aberrant inflammatory immune responses, and inappropriately increased or decreased gene expression profiles, will be present in neonates lacking such parasite antigen exposure.

Acknowledgements

We thank the laboratory staff members at the Centre Hospitalier Régionale (CHR), Sokodé, Togo. For expert help and advice we thank Mr. Sven Poths from the Department of Medical Genetics, University Clinics Tübingen (UKT). For their friendly cooperation we are grateful to the medical staff at Children's Hospital and Neonatology at UKT. This work was supported by the Baden-Württemberg Stiftung (grant No. P-LS-AL2/06), the Bundesministerium für Bildung und Forschung (BMBF-Project Nr. 01AK1008), and the Commission of the European Community FP7 Project (Grant Acronym E-PIAF #242131). We acknowledge the support by Deutsche Forschungsgemeinschaft and Open Access Publishing Fund of Tübingen University. Authors disclose no financial or other conflict of interests relevant to the research.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.molimm.2016.03.015>.

References

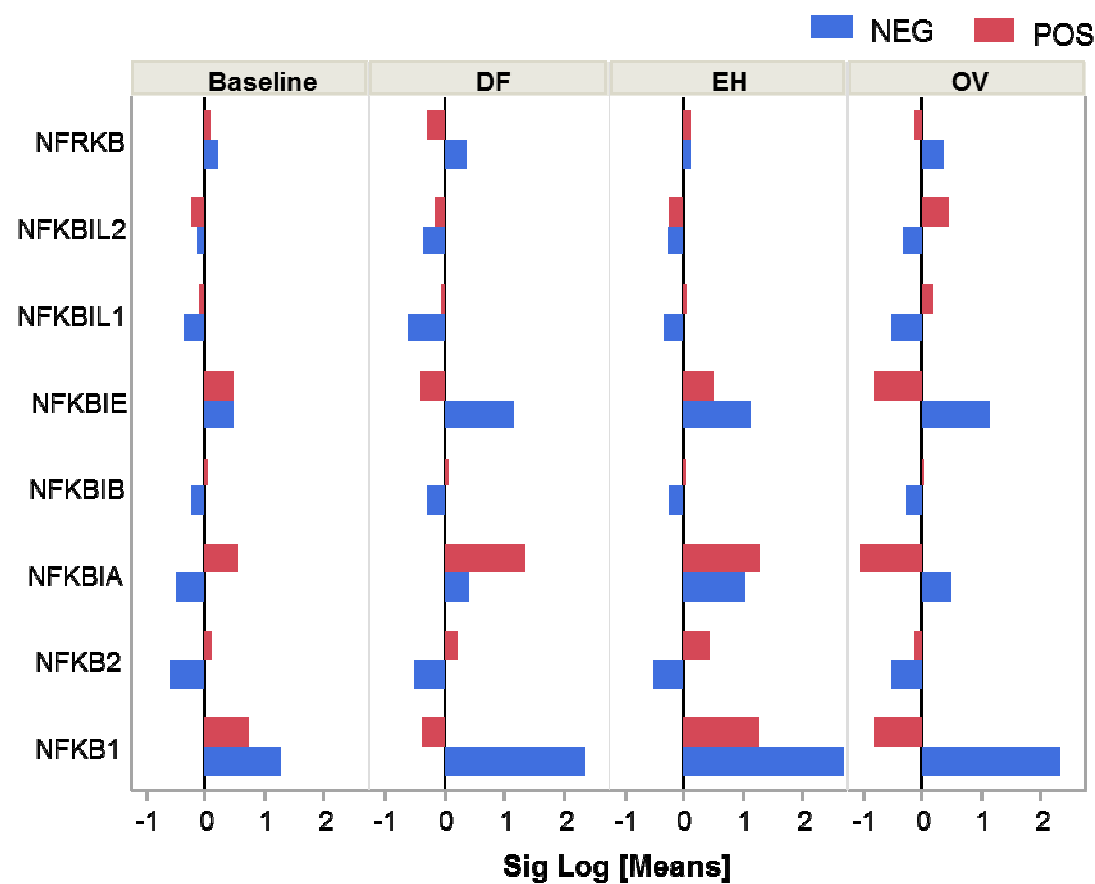
Akira, S., 2006. TLR signaling. *Curr. Top. Microbiol. Immunol.* 311, 1–16.

- Babu, S., Kumaraswami, V., Nutman, T.B., 2005. Transcriptional control of impaired Th1 responses in patent lymphatic filariasis by T-box expressed in T cells and suppressor of cytokine signaling genes. *Infect. Immun.* 73, 3394–3401.
- Ben Moshe, T., et al., 2007. Role of caspase-8 in hepatocyte response to infection and injury in mice. *Hepatology* 45, 1014–1024.
- Ben-Hur, H., et al., 2005. Transport of maternal immunoglobulins through the human placental barrier in normal pregnancy and during inflammation. *Int. J. Mol. Med.* 16, 401–407.
- Braun-Fahrlander, C., et al., 2002. Allergy and Endotoxin Study Team: environmental exposure to endotoxin and its relation to asthma in school-age children. *N. Engl. J. Med.* 347, 869–877.
- Bruel, T., et al., 2010. Epithelial induction of porcine suppressor of cytokine signaling 2 (SOCS2) gene expression in response to *Entamoeba histolytica*. *Dev. Comp. Immunol.* 34 (5), 562–571.
- Carlier, Y., et al., 2012. Congenital parasitic infections: a review. *Acta Trop.* 121, 55–70.
- Charo, I.F., Ransohoff, R.M., 2006. The many roles of chemokines and chemokine receptors in inflammation. *N. Engl. J. Med.* 354, 610–621.
- Chen, Z., et al., 2006. Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. *Proc. Natl. Acad. Sci. U. S. A.* 103, 8137–8142.
- De Lema Barbaro, A., et al., 2005. MHC immunoevasins: protecting the pathogen reservoir in infection. *Tissue Antigens* 66, 2–8.
- Delahaye, N.F., et al., 2006. Gene-expression profiling discriminates between cerebral malaria (CM)-susceptible mice and CM-resistant mice. *J. Infect. Dis.* 206, 312–321.
- Desowitz, R.S., Elm, J., Alpers, M.P., 1993. *Plasmodium falciparum*-specific immunoglobulin G (IgG), IgM, and IgE antibodies in paired maternal-cord sera from east spik province Papua New Guinea. *Infect. Immun.* 61, 988–993.
- Esche, C., Stellato, C., Beck, L.A., 2005. Chemokines: key players in innate and adaptive immunity. *J. Invest. Dermatol.* 125, 615–628.
- Ghayur, T., et al., 1997. Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. *Nature* 386, 619–623.
- Gobin, S.J., van den Elsen, P.J., 2000. Transcriptional regulation of the MHC class II genes HLA-e HLA-F, and HLA-G. *Hum. Immunol.* 61, 1102–1107.
- Guo, X., Stroup, S.E., Houpt, E.R., 2007. Persistence of *Entamoeba histolytica* infection in CBA mice owes to intestinal IL-4 production and inhibition of protective IFN-gamma. *Mucosal Immunol.* 1 (2), 139–146.
- Häbig, K., et al., 2008. RNA interference of LRRK2-microarray expression analysis of a Parkinson's disease key player. *Neurogenetics* 9, 83–94.
- Hermann, E., et al., 2002. Human fetuses are able to mount an adultlike CD8 T-cell response. *Blood* 100, 2153–2158.
- Herz, U., et al., 2000. Impact of in utero Th2 immunity on T cell deviation and subsequent immediate-type hypersensitivity in the neonate. *Eur. J. Immunol.* 30, 714–718.
- Hewitt, E.W., Dugan, G.E., 2004. Virus subversion of protective immunity. *Curr. Allergy Asthma Rep.* 4, 365–370.
- Hightower, A.W., Lammie, P.J., Eberhard, M.L., 1993. Maternal filarial infection – a persistent risk factor for microfilaremia in offspring? *Parasitol. Today* 9, 418–421.
- Holt, P.G., 1995. Environmental factors and primary T-cell sensitisation to inhaled allergens in infancy: reappraisal of the role of infections and air pollution. *Pediatr. Allergy Immunol.* 6, 1–10.
- Jiménez-Dalmaroni, M.J., et al., 2015. Soluble human TLR2 ectodomain binds diacylglycerol from microbial lipopeptides and glycolipids. *Innate Immun.* 21, 175–193.
- Jin, M.S., et al., 2007. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. *Cell* 130, 1071–1082.
- Kirch, A.K., et al., 2004. Parasite-specific antibody and cytokine profiles in newborns from *Plasmodium falciparum* and *Entamoeba histolytica*/dispar-infected mothers. *Pediatr. Allergy Immunol.* 15, 133–141.
- Kovalenko, A., et al., 2009. Caspase-8 deficiency in epidermal keratinocytes triggers an inflammatory skin disease. *J. Exp. Med.* 206, 2161–2177.
- Lüder, C.G., et al., 1998. Down-regulation of MHC class II molecules and inability to up-regulate class I molecules in murine macrophages after infection with *Toxoplasma gondii*. *Clin. Exp. Immunol.* 112, 308–316.
- Lamkanfi, M., Dixit, V.M., 2014. Mechanisms and functions of inflammasomes. *Cell* 157, 1013–1022.
- Lewert, R.M., Mandlowitz, S., 1969. Schistosomiasis: prenatal induction of tolerance to antigens. *Nature* 224, 1029–1030.
- Liew, F.Y., et al., 2005. Negative regulation of toll-like receptor-mediated immune responses. *Nat. Immunol.* 2, 102–107.
- Malhotra, I., et al., 1997. In utero exposure to helminth and mycobacterial antigens generates cytokine responses similar to that observed in adults. *J. Clin. Invest.* 99, 1759–1766.
- Malhotra, I., et al., 2006. Prenatal T cell immunity to *Wuchereria bancrofti* and its effect on filarial immunity and infection susceptibility during childhood. *J. Infect. Dis.* 193, 1005–1013.
- Martinon, F., Burns, K., Tschopp, J., 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol. Cell* 10, 417–426.
- Martinon, F., et al., 2004. Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. *Curr. Biol.* 14, 1929–1934.
- Morein, B., Abusugra, I., Blomqvist, G., 2002. Immunity in neonates. *Vet. Immunol. Immunopathol.* 87, 207–213.

- Munz, C., Stevanovic, S., Rammensee, H.G., 1999. Peptide presentation and NK inhibition by HLA-G. *J. Reprod. Immunol.* 43, 139–155.
- Nomura, I., et al., 2003. Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J. Allergy Clin. Immunol.* 112, 1195–1202.
- O'Neill, L.A., Bowie, A.G., 2007. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat. Rev. Immunol.* 7, 353–364.
- Oliveira, S.H., Lukacs, N.W., 2003. The role of chemokines and chemokine receptors in eosinophil activation during inflammatory allergic reactions. *Braz. J. Med. Biol. Res.* 36, 1455–1463.
- Ozinsky, A., et al., 2000. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13766–13771.
- Pfefferle, P.I., et al., 2008. Cord blood allergen specific IgE is associated with reduced IFN- γ production by cord blood cells: the Protection against Allergy-study in Rural Environments (PASTURE) Study. *J. Allergy Clin. Immunol.* 122, 717–728.
- Prescott, S.L., et al., 1998. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J. Immunol.* 160, 4730–4737.
- Ramsey, C.D., Celedon, J.C., 2005. The hygiene hypothesis and asthma. *Curr. Opin. Pulm. Med.* 11, 14–20.
- Ridge, J.P., Fuchs, E.J., Matzinger, P., 1996. Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. *Science* 271, 1723–1726.
- Ritter, M., et al., 2010. *Schistosoma mansoni* triggers Dectin-2: which activates the Nlrp3 inflammasome and alters adaptive immune responses. *Proc. Natl. Acad. Sci. U. S. A.* 107, 20459–20464.
- Schuijs, M.J., et al., 2015. Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. *Science* 349, 1106–1110.
- Schust, D.J., Tortorella, D., Ploegh, H.L., 1999. HLA-g and HLA-c at the feto-maternal interface: lessons learned from pathogenic viruses. *Semin. Cancer Biol.* 9, 37–46.
- Siegmund, B., 2002. Interleukin-1 β converting enzyme (caspase-1) in intestinal inflammation. *Biochem. Pharmacol.* 64, 1–8.
- Soboslay, P.T., et al., 1999. Prenatal immune priming in onchocerciasis-onchocerca volvulus-specific cellular responsiveness and cytokine production in newborns from infected mothers. *Clin. Exp. Immunol.* 117, 130–137.
- Steel, C., et al., 1994. Long-term effect of prenatal exposure to maternal microfilaraemia on immune responsiveness to filarial parasite antigens. *Lancet* 343, 890–893.
- Stifter, S.A., Feng, C.G., 2014. Interfering with immunity: detrimental role of type I IFNs during infection. *J. Immunol.* 194, 2455–2465.
- Tarrant, T.K., Patel, D.D., 2006. Chemokines and leukocyte trafficking in rheumatoid arthritis. *Pathophysiology* 13, 1–14.
- Thornberry, N.A., et al., 1992. A novel heterodimeric cysteine protease is required for interleukin-1 β processing in monocytes. *Nature* 356, 768–774.
- Wegmann, T.G., et al., 1993. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol. Today* 14, 353–356.
- Weil, G.J., et al., 1983. Prenatal allergic sensitization to helminth antigens in offspring of parasite-infected mothers. *J. Clin. Invest.* 71, 1124–1129.
- Yazdanbakhsh, M., Kremsner, P.G., van Ree, R., 2002. Allergy, parasites, and the hygiene hypothesis. *Science* 296, 490–494.
- Zhou, J., et al., 2006. Differential expression of chemokines and their receptors in adult and neonatal macrophages infected with human or avian influenza viruses. *J. Infect. Dis.* 194, 61–70.

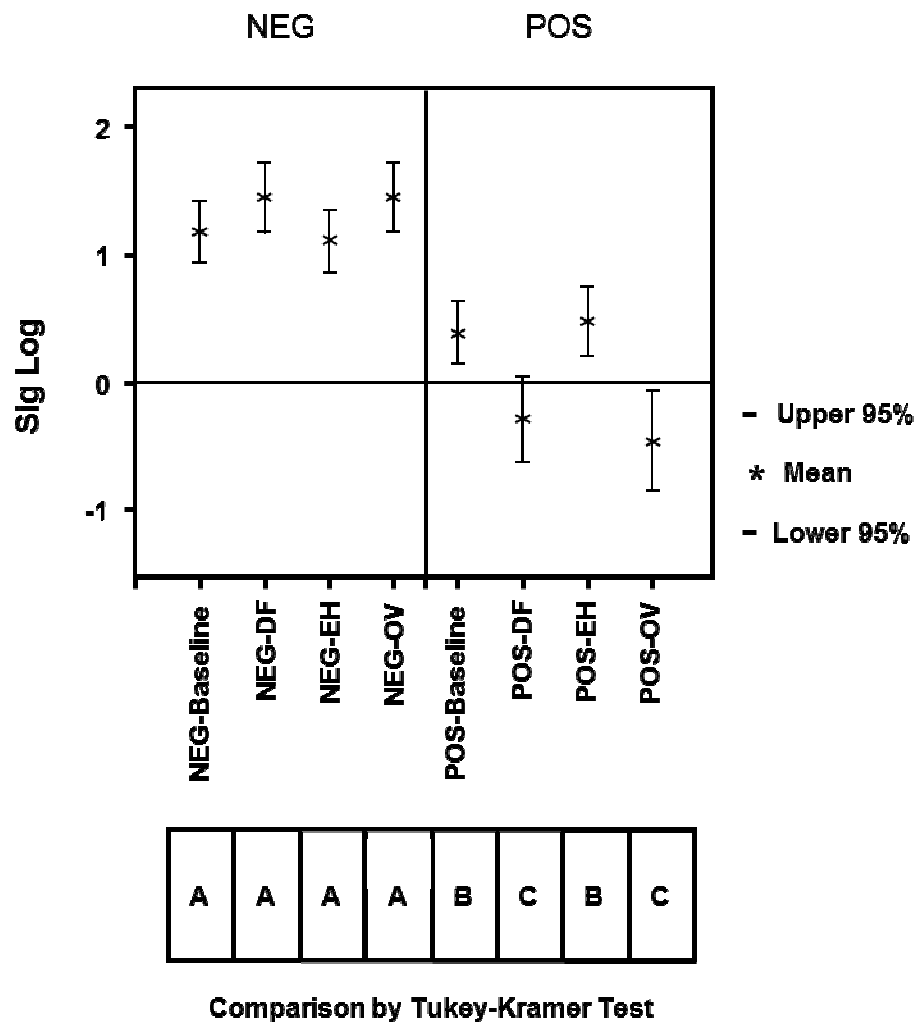
Supplementary Fig. 1

Gene expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB) in parasite infection positive (Para-POS) and negative (Para-NEG) neonates. Gene expression levels are shown (Sig Log) as signal log means, i.e. the expression values in UCBC cultured in vitro following activation with allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV), *E. histolytica* (EH) or without stimulation (baseline) are compared.



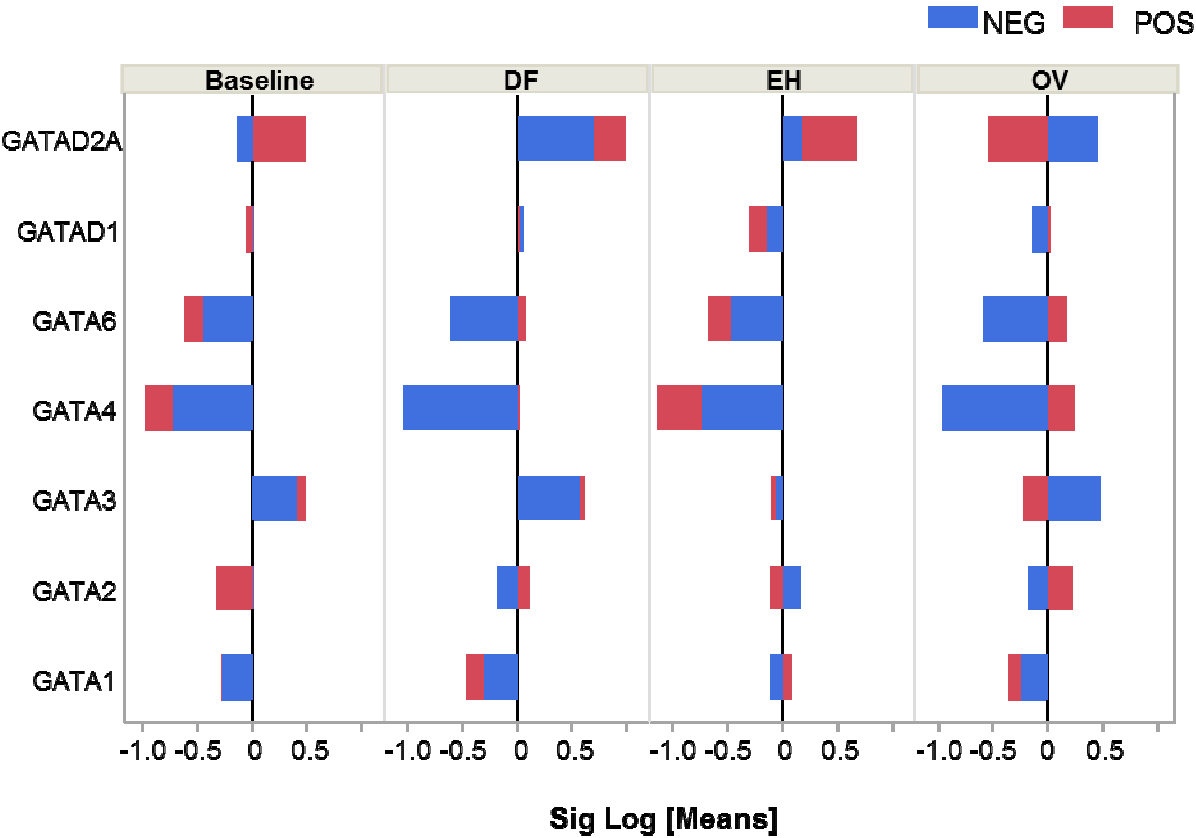
Supplementary Fig. 2

Gene expression of Annexins (ANXA) in parasite infection positive (Para-POS) and negative (Para-NEG) neonates is shown. Gene expression levels are shown (Sig Log) as signal log means, i.e. the expression values in UCBC cultured in vitro following activation with allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV), *E. histolytica* (EH) or without stimulation (baseline) are compared. Statistical differences were detected by Tukey Test and if groups are not connected with the same letter differences are significant.



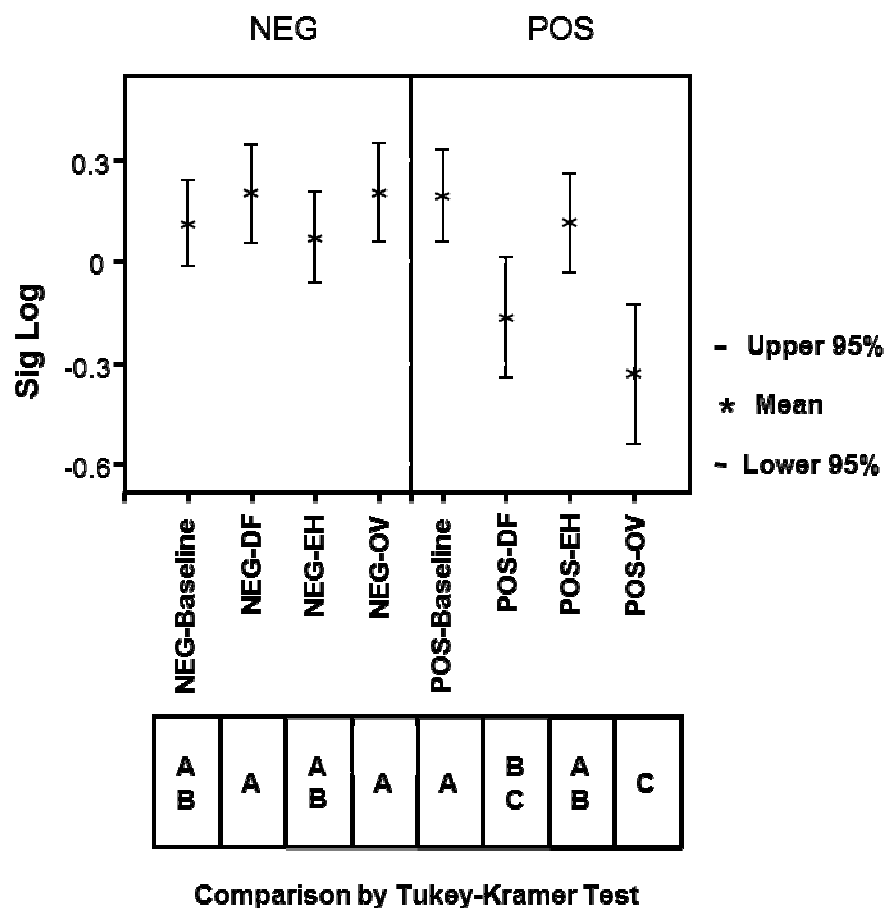
Supplementary Fig. 3

Gene expression of GATA transcription factors in parasite infection positive (Para-POS) and negative (Para-NEG) neonates. Gene expression levels are shown (Sig Log) as signal log means, i.e. the expression values in UCBC cultured in vitro following activation with allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV), *E. histolytica* (EH) or without stimulation (baseline) are compared.



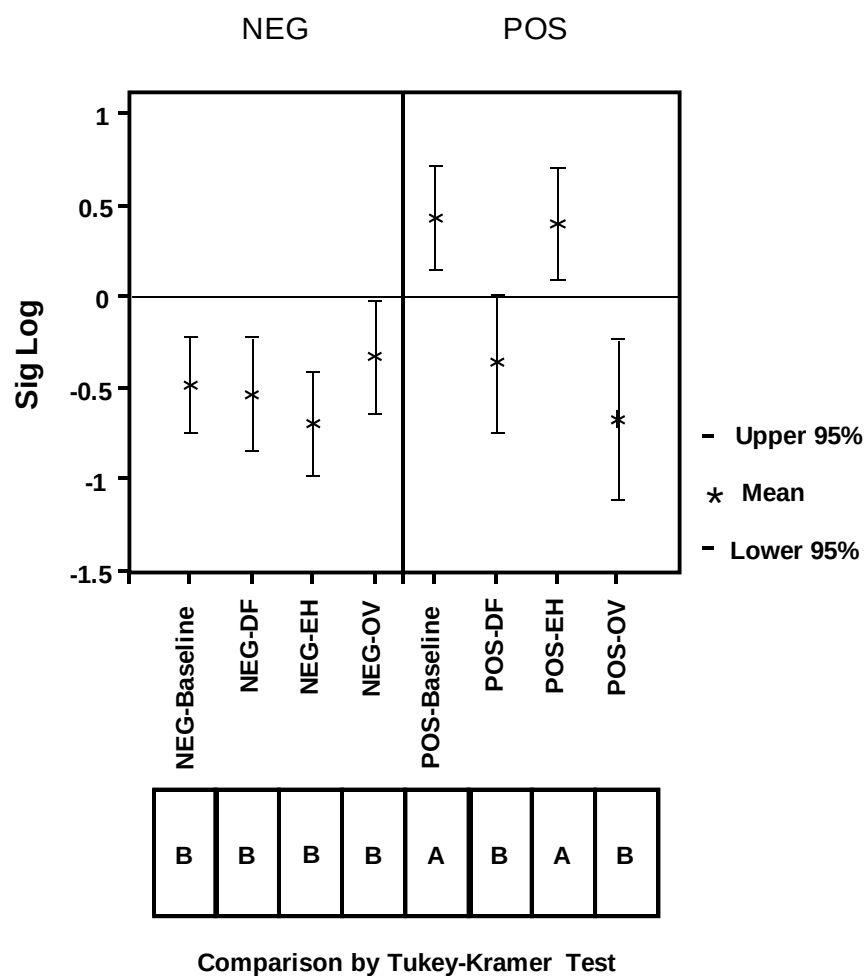
Supplementary Fig. 4

Gene expression of CD antigens in parasite infection positive (Para-POS) and negative (Para-NEG) neonates. Gene expression levels are shown (Sig Log) as signal log means, i.e. the expression values in UCBC cultured in vitro following activation with allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV), *E. histolytica* (EH) or without stimulation (baseline) are compared. Statistical differences were detected by Tukey Test and if groups are not connected with the same letter differences are significant.



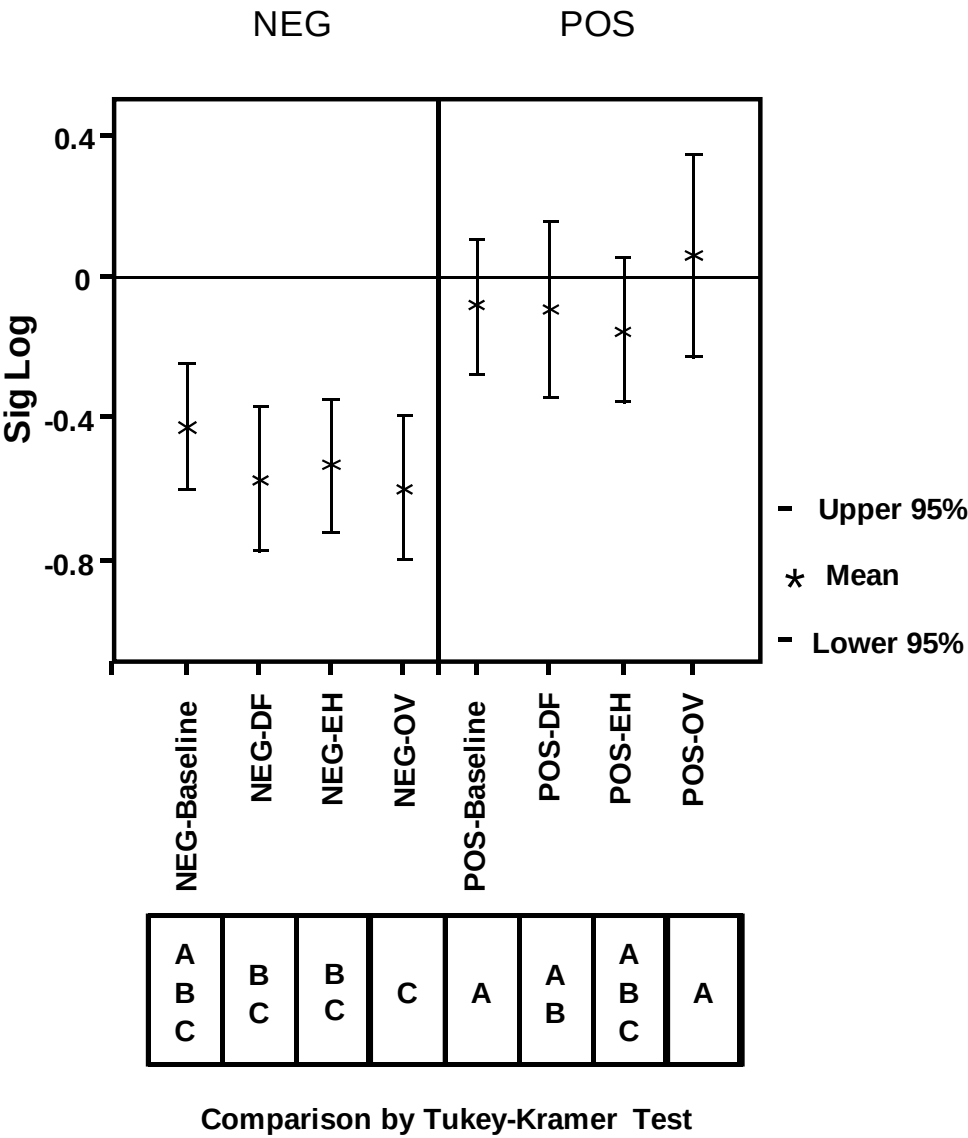
Supplementary Fig. 5

Gene expression of immunoglobulin receptors (FcR) in parasite infection positive (Para-POS) and negative (Para-NEG) neonates. Gene expression levels are shown (Sig Log) as signal log means, i.e. the expression values in UCBC cultured in vitro following activation with allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV), *E. histolytica* (EH) or without stimulation (baseline) are compared. Statistical differences were detected by Tukey Test and if groups are not connected with the same letter differences are significant.



Supplementary Fig. 6

Gene expression of transforming growth factor beta (TGF- β) in parasite infection positive (Para-POS) and negative (Para-NEG) neonates. Gene expression levels are shown (Sig Log) as signal log means, i.e. the expression values in UCBC cultured in vitro following activation with allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV), *E. histolytica* (EH) or without stimulation (baseline) are compared. Statistical differences were detected by Tukey Test and if groups are not connected with the same letter differences are significant.



Supplementary Table S1

Signal strength of gene expression (mean Sig log) in umbilical cord blood cells (UCBC) from parasite exposure/infection negative (Para-NEG) and positive (Para-POS) neonates is shown. UCBC were activated with with mite allergens of *D. farinae* (DF), antigens of *O. volvulus* (OV) or *E. histolytica* (EH) or cultured in vitro without stimulation (baseline). Mean signal strength of gene expression to antigens or allergens is shown for n=22.283 genes (Overall Mean Sig Log). Mean expression values between groups were compared by Tukey Test and if groups are not connected with the same letter differences are significant.

Supplementary Table S1

	NEG Baseline	NEG DF	NEG EH	NEG OV	POS Baseline	POS DF	POS EH	POS OV
Sig log (n=22.283 genes)	0,117 C	0,170 A	0,126 C	0,150 B	0,038 D	-0,016 E	0,019 F	-0,070 G
MHC- I	-0,456 C	0,132 B	0,073 B C	0,065 BC	0,864 A	-0,249 B C	0,869 A	-1,30 D
MHC- II	0,647 A B	0,731 A	0,495 A B	0,618 A B	0,325 A B C	-0,197 C D	0,255 B C	-0,736 D
STAT	0,216 B C D	0,722 A B	1,091 A	0,692 A B	0,313 B C D	-0,325 C D	0,366 B C	-0,480 D
SOCS	-0,244 B	-0,263 B	-0,081 A B	-0,199 B	-0,115 A B	0,021 A B	-0,143 A B	0,149 A
Interleukin	-0,456 D	-0,305 A B C	0,189 A	-0,197 B C D	0,090 A B	0,031 A B C	0,167 A	-0,182 A B C D
CC-Chemokine	-0,159 C	0,228 A B C	0,454 A	0,142 A B C	0,130 A B C	0,038 A B C	0,293 A B	-0,124 B C
CXC-Chemokine	-0,508 D	-0,069 C D	0,851 A	0,113 B C	0,268 A B C	0,013 B C D	0,672 A B	-0,306 C D
CCR	0,723 A	0,735 A	0,254 A B	0,678 AB	0,066 B	0,050 A B	0,084 B	-0,073 B
IgG,IgM,IgA, IgE	-0,304 D	-0,448 E	-0,321 D E	-0,461 E	-0,094 B C	0,051 A B	-0,129 C	0,126 A
Ig E chain	-0,393 A B	-0,478 B	-0,361 A B	-0,459 B	-0,069 A	0,015 A	-0,092 A B	0,063 A
ANXA	1.179 A	1.443 A	1.107 A	1.449 A	0.385 B	-0.288 C	0.477 B	-0.462 C
CD	0.110 AB	0.199 A	0.069 AB	0.203 A	0.192 A	-0.166 BC	0.112 AB	-0.334 C
CASP	0.146 ABC	0.279 AB	0.392 A	0.279 AB	0.133 ABC	-0.003 BC	0.177 ABC	-0.154 C
TLR	0.114 A	0.249 A	0.226 A	0.335 A	0.076 A	-0.116 A	0.091 A	-0.102 A
SOD	-0.118 C	0.775 ABC	1.243 A	0.768 ABC	0.446 ABC	0.774 ABC	1.137 AB	-0.256 BC
GATA	-0.019 A	0.008 A	-0.094 A	-0.069 A	-0.070 A	0.053 A	-0.077 A	-0.013 A
NFkB	-0.091 B	0.142 AB	0.188 AB	0.134 AB	0.176 AB	0.050 AB	0.363 A	-0.238 B
TGFbeta	-0.422 ABC	-0.570 BC	-0.531 BC	-0.594 C	-0.082 A	-0.089 AB	-0.152 ABC	0.058 A
FcR	-0.480 B	-0.531 B	-0.687 B	-0.324 B	0.431 A	-0.365 B	0.396 A	-0.670 B

Supplementary Table S2

GenBank	SLR	p values	Gene Title	Gene Symbol	Function
Identical gene expression in response to protozoa, helminth antigens and mite allergens in					
Parasite-negative (Para-NEG) neonates					
AW188198	2,74	0,007	tumor necrosis factor, alpha-induced protein 6	TNFAIP6	Inflammatory Response
NM_005569	2,08	0,002	LIM domain kinase 2	LIMK2	Actin polymerisation
BC002666	2,00	<0,001	guanylate binding protein 1, interferon-inducible, 67kDa	GBP1	Signal Transduction
BC002704	1,89	0,003	signal transducer and activator of transcription 1	STAT1	Signal Transduction
NM_000295	1,59	0,003	serine (or cysteine) proteinase inhibitor, clade A	SERPINA1	Cell growth, proliferation
NM_007203	1,52	<0,001	paralemmin 2	PALM2	Membrane formation, neuronal growth
BE966236	1,36	<0,001	ribonucleotide reductase M2 polypeptide	RRM2	DNA synthesis and repair.
AL136896	1,34	0,011	suppressor of cytokine signaling 5	SOCS5	Immune regulation
AB029156	1,32	0,007	hepatoma-derived growth factor, related protein 3	HDGFRP3	Special function in DNA in neuronal cells
AA873600	1,32	0,047	sphingomyelin phosphodiesterase, acid-like 3A	SMPDL3A	
AF119873	1,31	0,001	Homo sapiens PRO2275 mRNA, complete cds	---	
NM_012177	1,31	<0,001	F-box only protein 5	FBXO5	
NM_018109	1,27	<0,001	hypothetical protein FLJ10486	FLJ10486	Promotes production of TNF α and IL-2 with T cell activation
AB005043	1,24	<0,001	suppressor of cytokine signaling 1	SOCS1	Immune regulation
-/-	-/-	-/-	-/-	-/-	-/-
NM_000647	-2,18	0,049	chemokine (C-C motif) receptor 2	CCR2	Chemokine receptor
NM_006682	-2,41	0,018	fibrinogen-like 2	FGL2	Pro-inflammatory activation of thrombin
U19970	-2,48	0,036	cathelicidin antimicrobial peptide	CAMP	Innate Immune response
NM_005615	-2,49	0,020	ribonuclease, RNase A family, k6	RNASE6	antiviral agent
NM_002933	-2,83	0,002	ribonuclease, RNase A family, 1 (pancreatic)	RNASE1	antiviral agent
AK026737	-2,89	0,025	fibronectin 1	FN1	Possible acute phase protein, inflammation
NM_000147	-2,99	<0,001	fucosidase, alpha-L- 1, tissue	FUCA1	Degradation of fucose
NM_002003	-3,09	0,031	ficolin (collagen/fibrinogen domain containing) 1	FCN1	Plasma protein with elastin-binding activity
X02761	-3,28	0,014	fibronectin 1	FN1	Possible acute phase

					protein, inflammation
NM_001925	-3,35	<0,001	defensin, alpha 4, corticostatin	DEFA4	antibacterial peptides
BC005858	-3,38	0,012	fibronectin 1 /// fibronectin 1	FN1	Possible acute phase protein, inflammation

Identical gene expression in response to protozoa, helminth antigens and mite allergens in

Parasite-positive (Para-POS) neonates

M83248	2,53	0,011	secreted phosphoprotein 1 (osteopontin)	SPP1	macrophage function and differentiation
AW188198	1,90	0,045	tumor necrosis factor, alpha-induced protein 6	TNFAIP6	Inflammatory Response
NM_002356	1,65	0,041	myristoylated alanine-rich protein kinase C substrate	MARCKS	Cell motility, phagocytosis, membrane trafficking
NM_005502	1,63	0,037	ATP-binding cassette, sub-family A, member 1	ABCA1	Efflux pump in cellular lipid removal pathway
NM_000161	1,43	0,008	GTP cyclohydrolase 1	GCH1	Tetrahydrobiopterin (BH4) biosynthesis
NM_001154	1,17	0,002	annexin A5	ANXA5	Apoptosis
U13699	1,16	0,014	caspase 1, apoptosis-related cysteine protease	CASP1	Apoptosis
NM_014705	1,15	0,048	dedicator of cytokinesis 4	DOCK4	Regulation of adherens junctions between cells
NM_000100	1,13	<0,001	cystatin B (stefin B)	CSTB	Cell Differentiation
AA890010	1,10	0,011	SEC22 vesicle trafficking protein-like 1	SEC22L1	
NM_006290	1,06	0,039	tumor necrosis factor, alpha-induced protein 3	TNFAIP3	Inflammatory Response
NM_003254	1,01	0,049	tissue inhibitor of metalloproteinase 1	TIMP1	Growth factor, inhibitor of metalloproteinases
BF575213	1,01	0,013	superoxide dismutase 2, mitochondrial	SOD2	Detoxification of metabolites
BC004372	1,01	0,013	CD44 antigen (homing function)	CD44	Adhesion, cell interaction

Supplementary Table S3

The correlations between chemokines and their receptors

Receptor	Ligands
CCR1	CCL4, CCL5, CCL6, CCL14, CCL15, CCL16, CCL23
CCR2	CCL2, CCL8, CCL16
CCR3	CCL5, CCL7, CCL11, CCL13, CCL15, CCL18, CCL24, CCL26, CCL28
CCR4	CCL3, CCL5, CCL17, CCL22
CCR5	CCL3, CCL4, CCL5, CCL8, CCL11, CCL13, CCL14, CCL16
CCR6	CCL20
CCR7	CCL19, CCL21
CCR8	CCL1, CCL16
CCR9	CCL25
CCR10	CCL27, CCL28
CCR11	CCL19, CCL21, CCL25
CXCR1	CXCL6, CXCL8
CXCR2	CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7
CXCR3	CXCL4, CXCL9, CXCL10, CXCL11
CXCR4	CXCL12
CXCR5	CXCL13
CXCR6	CXCL16
CXCR7	CXCL11, CXCL12
XCR1	XCL1, XCL2
CX3CR1	CX3CL1