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

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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.

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-Fahrländer 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-

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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 activiation, 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 metronidozole).

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 synthesize 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 μg) 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 μm 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 – CTCCCTCTCACTGGGGAAC; SPP1: FW – CCCACAGACCCTTCCAAGTA, RV-GGGGACAACTGGAGTGAAAA; STAT1: FW – CCCACAGACCCTTCCAAGTA, RV – GGGGACAACTGGAGTGAAAA; STAT3: FW – GAGGGAACAAGCCCCAAC, RV – AGGGGTCCCAACTGTTTCTC; STAT4: FW – AGCCTTGCGAAGTTTCAAGA, RV – ACACCGCATACACACTTGGA;
STAT6: FW – GCGGCTCTATGTCGACTTTC, RV – ATGCTCTCAAGGGTGCTGAT;
FOXP3: FW – TGCCTCCTCTTCTTCCTTGA, RV – TTGAGAGCTGGTGCATGAAA;
CCL5: FW – CGCTGTCATCCTCATTGCTA; RV – GGGTGACAAAGACGACTGCT;
CCL22: FW – GAGGAAGCTGGCTGTGGTAG, RV – CTTGAGCCCAGGAGTTTGAG;
IL10: FW – GAGAACAGCTGCACCCACTT, RV – GGCAACCCAGGTAACCCTTA;
IL10RA: FW – ATCTGTCGCTTCCCGAAGTA, RV – GAGCAGGACACTGGGTAGCTT;
IL12B: FW – TGCTGCTTCACAAAAAGGAA, RV – AAGAGCCTCTGCTGCTTTTG;
IL12RB1: FW – CCCTCCTCTTCCTCTTCCTG, RV – CCGAGCCTGAGTCTGCAT;
IL15: FW – GTTCACCCCAGTTGCAAAGT, RV – TTTTTCCTCCAGTTCCTCACA;