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High capacity for leukotriene biosynthesis in peripheral blood during pregnancy

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ABSTRACT

Pregnancy is accompanied by major immunological changes to maintain both tolerance for the fetus and immune competence. Leukotrienes are powerful 5-lipoxygenase-derived inflammatory mediators and the characteristics of leukotriene-related diseases (e.g., asthma, allergic rhinitis) change during pregnancy. Here, we show that pregnancy affects leukotriene synthesis in human blood and leukocytes. 5-Lipoxygenase product formation in stimulated blood of pregnant women was significantly higher than in non-pregnant females. Although a pregnancy-related increase in neutrophil and monocyte counts may explain these observations, granulocytes of pregnant donors have lower leukotriene-synthetic capacities. On the other hand, granulocytes from non-pregnant woman produced more leukotrienes when resuspended in plasma of pregnant women than of non-pregnant females. Together, we show that leukotriene biosynthesis in maternal blood is increased by the interrelations of higher leukocyte numbers, lower cellular capacity for leukotriene synthesis and stimulatory effects of plasma. This bias may affect leukotriene-related diseases during pregnancy and their pharmacological treatment.

1. Introduction

Pregnancy is associated with major changes of the maternal immune system, which allow tolerance of the semi-allogeneic fetus while protecting from pathogens [1]. Thus, a local down-regulation of adaptive immune responses has been reported in the decidua, which however does not result in a generalized immunosuppression. Clinical differences in the course of autoimmune diseases during pregnancy (e.g., improvement of rheumatoid arthritis [2]) exemplify the consequences of the ongoing changes. Interestingly, the innate immune system is assumed to be activated during pregnancy [1], though some controversial findings were reported [3,4]. In fact, maternal monocytes and granulocytes (also known as polymorphonuclear leukocytes, PMNL) seem to acquire a pro-inflammatory phenotype, which is accompanied by an increase in blood counts of PMNL [5,6]. In contrast, certain functional properties of maternal PMNL analyzed in vitro are reduced (e.g., chemotaxis, adherence [7], and formation of reactive oxygen species (ROS) [4,8]), which may account for higher susceptibility to certain infections during pregnancy.

Leukotrienes (LTs) are important lipid mediators of host defense and innate immunity [9] and have also been suggested to tune adaptive immune responses [10]. The biosynthesis of LTs is initiated by the cytosolic phospholipase A_2, which releases arachidonic acid (AA) from membrane phospholipids. Liberated AA is then delivered to 5-lipoxygenase (5-LO) by a helper protein called 5-LO-activating protein for conversion to 5-hydro(pero)xyeicosatetraenoic acid (5-H(P)ETE) and then to LTA_4. LTA_4 hydrolase and LTC_4 synthases metabolize LTA_4 to LTB_4 and LTC_4, respectively [11]. AA is also used as substrate by other LOs (e.g., 12-LO and 15-LO) and cyclooxygenase (COX) enzymes to form pro-inflammatory mediators. 5-LO is essentially expressed in cells of myeloid origin and the major LT-forming cells in the blood are granulocytes and monocytes [12]. The activity of 5-LO is tightly regulated by several

Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; EDTA, ethylenediaminetetraacetate; ESI, electrospray ionization; fMLP, formyl-methionyl-leucyl-phenylalanine; 12-HHT, 12(S)-hydroxy-5-cis-8,10-trans-heptadecatrienoic acid; 5-H(P)ETE, 5(S)-hydro(pero)xy-6-trans-8,11,14-cis-eicosatetraenoic acid; HPLC, high performance liquid chromatography; LPS, lipopolysaccharide; 5-LO, 5-lipoxygenase; LT, leukotriene; LTRA, leukotriene receptor antagonist; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PG buffer, PBS plus 1 mg/ml glucose; PGC buffer, PBS plus 1 mg/ml glucose plus 1 mM $CaCl_2$; PMNL, polymorphonuclear leukocytes; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEM, standard error of the mean; UPLC-MS/MS, ultra high performance liquid chromatography tandem mass spectrometry.

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mechanisms and co-factors, like Ca^{2+} ions, cellular peroxides and glycerides, interacting proteins (e.g., coactosin-like protein), and post-translational events (e.g., 5-LO phosphorylation by MAPKs or protein kinase A) [11].

5-LO products have been established as mediators of asthma and allergic rhinitis, but may also play roles in sepsis, cardiovascular disease, and cancer [9]. Interestingly, asthma characteristics change during pregnancy [13] and severe asthma tends to get worse during the second and third trimester [14]. Thus, LT receptor antagonists (LTRAs) are considered as controller medications alternative to corticosteroids in asthma therapy during pregnancy [15]. Also, rhinitis is a common condition in pregnant women [16]. Importantly, LTB_4 augments innate immune defenses against the puerperal sepsis pathogen *Streptococcus pyogens*, which represents a leading cause of maternal mortality [17]. On the other hand, a lower production of LTB_4 was reported in PMNL isolated from pregnant donors [18,19], which is accompanied by lower basal levels of LTB_4 in serum [20] and suggests a suppression of the 5-LO pathway during pregnancy. In view of the clinical use of LT modifiers for asthma during pregnancy and the possible role of LO products in the regulation of maternal immune responses, we here performed a comprehensive analysis on whether and how the second and third trimesters of pregnancy affect the biosynthesis of LTs and of other LO products in human blood and leukocytes.

2. Materials and methods

2.1. Materials

Monovettes were from Sarstedt (Nümbrecht, Germany). RPMI 1640 medium, L-glutamin, penicillin/streptomycin were from PAA Laboratories GmbH (Pasching, Austria). Lipopolysaccharide (LPS), *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), Ca^{2+}-ionophore A23187, ionomycin, AA, 2′,7′-dichlorofluorescein-diacetate and all other chemicals were from Sigma-Aldrich (Deisenhofen, Germany), unless stated otherwise. Mouse anti-5-LO primary monoclonal antibody was generous gift by Dr. Dieter Steinhilber (Goethe University, Frankfurt am Main, Germany). Rabbit anti-β-actin antibody was from Cell Signaling Technology, Inc. (Danvers, MA). High performance liquid chromatography (HPLC) solvents were from VWR (Darmstadt, Germany).

2.2. Description of the study

Blood samples were pairwise collected from healthy pregnant and non-pregnant donors in Tuebingen (November 2008–July 2010; approval numbers: 338/2006V and 148/2010BO2) and Jena (December 2010–November 2011; approval number: 3197-07/11), with consent. All subjects had no apparent inflammatory conditions at the time of blood withdrawal and had not taken any drug for at least ten days prior to blood collection. Out of 30 pregnant donors, 27 pregnant were finally included in the study (Table 1). The different pairs were analyzed on different experimental days except one case, when two pregnant women were analyzed at the same day and one non-pregnant donor was used as control. Additionally, two pregnant donors were analyzed twice (once during the second and once during the third trimester). In total, 3 pregnant women were excluded from the study for the following reasons: one donor experienced preeclampsia thereafter, one donor reported pregnancy rhinitis during the two previous pregnancies, and one pregnant female was excluded since the corresponding non-pregnant control had hyperandrogenism (high total plasma testosterone levels, namely 5.3 nM) [21]. As shown in Table 1, the gestational age of the analyzed healthy pregnant women was between weeks 15th to 36th. The pregnant donors were distributed almost equally in the second (week 13th to 24th) and the third (week 25th to 36th) trimesters (14 and 13 donors, respectively). Withdrawn blood was immediately analyzed or used for experiments.

Table 1
Characteristics of the donors [a].

	Pregnant	Non-pregnant
Subjects (*n*)	27	26
Age (years)	29 (24–36)	25 (20–30)
Gestational week	24 (15–36)	–
Cycle day	–	13 (3–32)

[a] Data are presented as median and range.

2.3. Preparation of whole blood and plasma, isolation of granulocytes and monocytes, quantification of blood cells and sex hormones

Peripheral venous blood (30 to 40 ml) was collected in heparinized tubes (16 I.E. heparin/ml blood) or ethylenediaminetetraacetate (EDTA) tubes (1.6 mg EDTA-K_3/ml) by venipuncture. The complete blood count was performed by flow cytometry at the central laboratories of the University Hospital Tuebingen and Jena. For isolation of plasma, heparinized blood was centrifuged at $600 \times g$/10 min/4 °C. The supernatant was centrifuged again ($800 \times g$/10 min/4 °C), and the resulting supernatant was analyzed to confirm the absence of cellular contaminations. Total plasma estradiol, progesterone and testosterone were immediately analyzed by an automated chemiluminescence immunoassay system (ADVIA Centaur, Siemens Healthcare, Munich, Germany), according to the manufacturer's instructions. Plasma was stored at −80 °C until use for experiments with partially purified 5-LO and resuspended cells (see below, Section 2.5.). Remaining cell pellets were directly used for the isolation of granulocytes and peripheral blood mononuclear cells (PBMC) and applied to dextran sedimentation. For isolation of granulocytes and PBMC from buffy coats, venous blood was subjected to centrifugation ($4000 \times g$/20 min/20 °C) for preparation of leukocyte concentrates. Granulocytes and PBMC were promptly isolated at 4 °C by dextran sedimentation, centrifugation on Nycoprep cushions (PAA Laboratories, Pasching, Austria), and hypotonic lysis of erythrocytes as described [21,22]. Where indicated, monocytes were further isolated from PBMC by adherence to culture flasks (Greiner, Nuertingen, Germany, cell density was 2×10^7 cells/ml RPMI 1640 medium containing 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin), for 1.5 h at 37 °C. The cells were finally resuspended in ice-cold phosphate buffered saline (PBS) plus 1 mg/ml glucose (PG buffer) or in PG buffer supplemented with 1 mM $CaCl_2$ (PGC buffer).

2.4. Expression and purification of 5-LO

5-LO was expressed in *Escherichia coli* Bl21 (DE3) cells, transformed with pT3–5LO, and purification of 5-LO was performed as described previously [23]. In brief, *E. coli* were harvested by centrifugation ($7700 \times g$ for 15 min) and lysed in 50 mM triethanolamine/HCl, pH 8.0, 5 mM EDTA, 60 μg/ml soybean trypsin inhibitor, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol and 1 mg/ml lysozyme, homogenized by sonication (3×15 s) and centrifuged at $10{,}000 \times g$ for 15 min followed by centrifugation at $40{,}000 \times g$ for 70 min at 4 °C. The supernatant was then applied to an ATP-agarose column (Sigma-Aldrich; Deisenhofen, Germany) for purification. Partially purified 5-LO was immediately used for activity assays.