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IL-10 and pregnancy complications

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Summary

Purpose of investigation: Successful pregnancy depends on the ability of the mother's immune system to undergo a process of immunoregulation in order to tolerate the fetus, and also to create and sustain a nurturing environment during all the stages of pregnancy. Several reports point to interleukin 10 (IL-10) as being vital for normal pregnancy, and low IL-10 levels as being associated with pregnancy complications. This study aimed to compare IL-10 levels in normal and complicated pregnancy conditions. *Material and Methods:* The authors compared levels of IL-10 produced upon stimulation of maternal peripheral blood mononuclear cells (PBMC) from women at different stages of normal gestation with those produced by women with pregnancy complications, such as recurrent spontaneous miscarriage (RSM), preterm delivery (PTD), premature rupture of fetal membranes (PROM), pre-eclampsia, and intrauterine fetal growth retardation (IUGR). *Results:* Median levels of IL-10 are statistically significantly lower in pathological conditions as compared to matching gestational ages of normal pregnancy. *Conclusion:* Healthy pregnancy is associated with higher levels of IL-10, while pathologic pregnancies are associated with lower levels of IL-10.

Key words: IL-10; Pregnancy; Cytokines; Recurrent spontaneous miscarriage; Pre-eclampsia; Preterm labor; Premature rupture of membranes; Intrauterine growth retardation.

Introduction

Considering that the so-called fetal allograft seems to challenge the rules of immunology, pregnancy is often referred to as an "immunological paradox". During pregnancy, the mother's immune system does not seem to be oblivious that her fetus is "foreign", yet the fetus is tolerated and nurtured through gestation. Pregnancy is a delicate balancing act orchestrated by different immune cells, hormones, nutrition, and infection by strict immune regulation [1].

The cellular heterogeneity of the placenta is unique in that it is composed of diverse groups of fetal and maternal lymphoid and non-lymphoid cells. The normal physiological homeostasis appears to be sustained by a cytokine network; dysregulation of this network is associated with several pregnancy complications [2]. The survival and success of the fetal allograft in the face of a potentially hostile maternal immune system has been proposed to be due to pregnancy-conducive immunomodulation at the maternal-fetal interface [3, 4] resulting in a lack of strong maternal cell-mediated anti-fetal reactivity of the T helper 1 (Th1) type [5, 6]. Th1 and Th2 cells represent two polarized forms of Th cells, and as the major functional subsets of Th cells, they marshal different types of effector responses [7]. Th1 cells secrete interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α); these pro-inflammatory cytokines induce several cytotoxic and inflammatory reactions and are responsible for cell-mediated inflammatory reactions, delayed-type hypersensitivity (DTH), and tissue injury in infectious and autoimmune diseases. Th2 cells, on the other hand, produce IL-4, IL-5, IL-6, IL-9, IL-l0, and IL-13 which are associated with help for antibody production by B cells [7].

Successful pregnancy has been shown to occur in a maternal Th2-type milieu, while abnormally elevated concentrations of Th1-type cytokines are associated with spontaneous abortions [2, 6]. Thus cytokines have positive and/or negative effects on pregnancy depending on the types and relative proportions of cytokines secreted.

IL-10 was first described by Mosmann *et al.* as "cytokine synthesis inhibiting factor" and was initially thought to be produced only by Th2 lymphocytes with the ability to inhibit inflammatory Th1-type cells [8]. Subsequently IL-10 was shown to be produced by several cell-types including both immune and non-immune cells [9]. It was soon realized that the roles of this multipotent cytokine was not restricted to cross-regulation of Th1 and Th2 cells but much more. This pleiotropic cytokine works in an autocrine and paracrine manner and plays dual stimulatory and immunosuppressive roles and has been shown to have extensive influences on a variety of immune responses [10]. It was therefore not long before IL-10 was investigated for its possible roles in

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the sustenance of pregnancy.

Maternal cytokine profiles should be flexible enough to maintain an environment that is conducive to pregnancy. It has been suggested that if the cytokine balance shifts significantly towards a pro-inflammatory dominance, pregnancy complications could result [6, 11]. IL-10 is likely to be particularly relevant to pregnancy, considering its anti-inflammatory properties and evidence supporting detrimental effects of pro-inflammatory cytokines on the outcome of pregnancy. Keeping this in mind and given the wide-ranging capabilities of IL-10, the authors compared its production by peripheral mononuclear cells (PBMC) of healthy pregnant women and by women with unexplained pregnancy complications, such as recurrent spontaneous miscarriage (RSM), preterm delivery (PTD), premature rupture of membrane (PROM), pre-eclampsia, and intrauterine fetal growth retardation (IUGR).

Materials and Methods

The study population consisted of the following groups:

Normal delivery (ND) control: this group consisted of 53 women with a history of at least three normal pregnancies, without a history of miscarriage, stillbirth, preterm labor, PROM, or ectopic pregnancy. Blood samples were obtained within the first few hours after normal spontaneous delivery. The mean gestational age of this group was 39.4 ± 1 years. This group is referred to as the ND group.

1st trimester normal pregnancy control: blood samples were obtained from 24 women at the end of the first trimester (mean gestational age 12 ± 2 weeks); this group of women had a history similar to the ND group and went on to deliver normally.

2nd trimester normal pregnancy control: blood samples were obtained from 20 women during the second trimester (17–24 weeks of gestation, mean gestational age 21.5 ± 0.6 weeks). Subjects in this group also had a history similar to the ND group.

3rd trimester normal pregnancy control: blood samples were obtained from 20 women, during the third trimester (mean gestational age 32.4 ± 4.2 weeks). These women also had history similar to the ND group, and went on to deliver normally

RSM: subjects in this group had a history of recurrent spontaneous miscarriage with unexplained etiology. This group comprised of 28 women who were admitted with spontaneous miscarriage for evacuation and (1) had at least two previous unexplained miscarriages, (2) were currently undergoing at least a third miscarriage, and (3) had been fully investigated for possible etiologies of recurrent miscarriage. Investigations included the examination of possible anatomical, endocrinological, infectious, genetic, and immunological causes of miscarriage. Blood samples were obtained from these subjects at the time of miscarriage (mean gestational age 12 ± 3 weeks). This group is referred here as the RSM group.

History of RSM with normal delivery (RSM-ND): this group consisted of 39 women that were initially enrolled into the study with a history similar to the RSM group above, but had their first successful pregnancy and spontaneous vaginal delivery. The mean gestational age of this group, the RSM-ND group, was 39.0 ± 1.1 weeks.

PTD: this group comprised of 30 women admitted with spontaneous preterm labor with intact membranes. These patients were in active labor, with the cervix dilated more than 3 cm, and they delivered prematurely. Intrauterine infection was ruled out in these patients by high vaginal swab culture, urine culture, complete blood count, total and differential WBC count, and estimation of levels of C-reactive protein. This group is referred to as PTD and the mean gestational age of this group was 26.8 + 1.3 weeks.

PROM: this group consisted of 30 pregnant women, admitted to hospital with spontaneous rupture of fetal membranes at term. Intrauterine infection was ruled out using the same protocol as for the PTD groups above. This group is denoted as the PROM group and the mean gestational age of this group was 39 ± 1.1 weeks.

Pregnancy-induced hypertension (PIH) (preeclampsia): this group, termed in this communication as the PIH group, comprised of 32 women with PIH; these women (a) were normotensive before pregnancy and during the first 20 weeks of gestation, (b) developed hypertension (blood pressure > 140/90 mmHg on two or more occasions six hours apart) associated with proteinuria > 300 mg per 24 hours, and (c) established labor either by induction or spontaneous onset. Patients with preterm labor defined as labor before 37 completed weeks of gestation or PROM were excluded. The mean gestational age of this group was 39 ± 1.4 weeks.

IUGR: This group of subjects included 36 women with a diagnosis of IUGR (mean gestational age 35.1 ± 3.7 weeks). The 36 women in this group were further subdivided into 19 IUGR pregnancies *with* placental insufficiency (i.e., asymmetric IUGR; referred as IUGR-W; mean gestational age 34.6 ± 3.3 weeks) and 17 IUGR pregnancies *without* placental insufficiency (i.e., symmetric IUGR; referred as IUGR-WO; mean gestational age 36.1 ± 4.3 weeks) by assessment of fetal anatomy and biometry, amniotic fluid dynamics, uterine, umbilical, and fetal middle cerebral artery Doppler. Blood velocity waveforms from both uterine arteries, the umbilical artery, and the fetal middle cerebral artery, were measured using duplex pulsed-wave Doppler ultrasound scanner. Pulsatility Index was calculated as (systolic/diastolic)/systolic as described [12]. Placental insufficiency was diagnosed if pulsatility index in the umbilical artery was raised, with either absent or reversed end diastolic flow.

All subjects were enrolled at two high-risk pregnancy clinics at Kuwait Maternity Hospital; informed consent was obtained from all subjects. This study has the approval of the Ethics Committees of the Faculty of Medicine at Kuwait University and of the Maternity Hospital, Kuwait.

Peripheral blood samples from all subjects were obtained by venipuncture at the time of normal or complicated/pathological delivery. Therefore, cytokine profiles reflect the situation existing in the periphery at that point in time. PBMC were separated by Ficoll-paque density gradient centrifugation, suspended in RPMI medium containing 10% fetal calf serum, aliquoted into 96-well tissue culture plates at a density of 10^5 cells per well and then stimulated with the mitogen phytohemagglutinin (PHA) at a concentration of five μg/ml for a period of 96 hours. Supernatants were collected for cytokine estimation on day 4 and stored at -80°C.

Levels of IL-10 were determined by sandwich ELISA. Samples were tested in duplicates and absorbance values measured in an ELISA reader. Accurate sample concentrations of cytokines were determined by comparing their respective absorbencies with those obtained for the reference standards plotted on a standard curve using reference recombinant cytokines. The sensitivity of the assay was 5 pg/ml of IL-10.

Data analysis was performed using the Statistical Package for the Social Sciences, version 22.0. Distribution of data was first

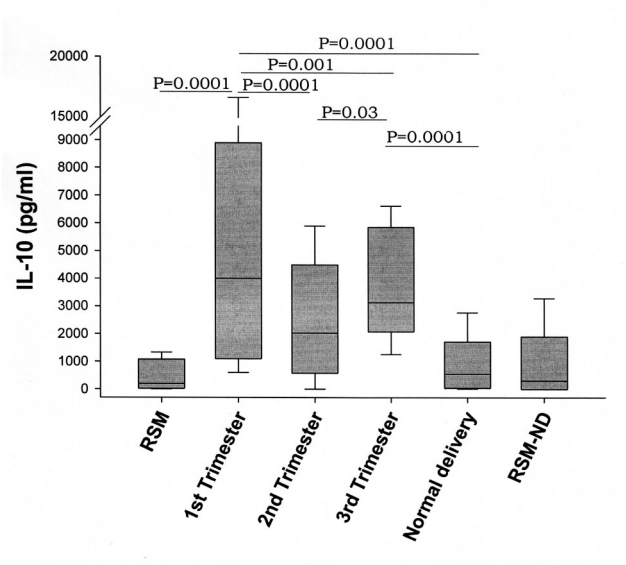

Figure 1. — Median levels of IL-10 produced by mitogen-activated PBMC from healthy pregnant women in their first, second, third trimesters, at ND, women with RSM, and RSM-ND women.

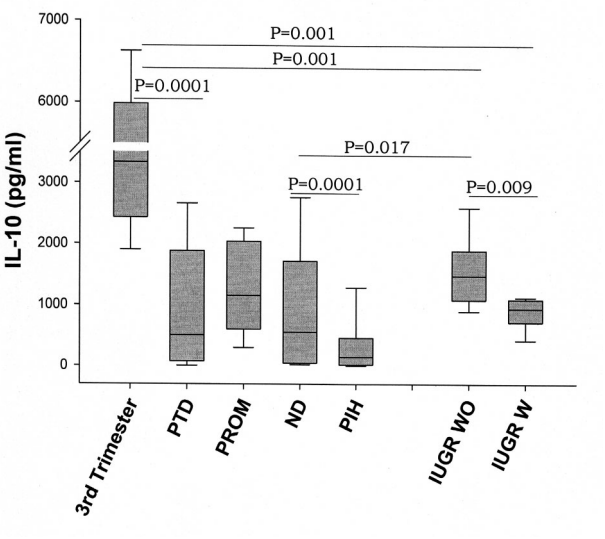

Figure 2. — Median levels of IL-10 produced by mitogen-activated PBMC from healthy pregnant women in their third trimester, PTD, PROM, at ND, preeclampsia (PIH), IUGR WO, and IUGR W.

determined by Kolmogorov-Smirnov test. The non-parametric Mann Whitney *U* test for independent samples was used to assess the statistical significance of difference; data are presented using the box plot (median± 25 and 75 percentiles). A *p*-value of < 0.05 was considered statistically significant.

Results

Figures 1 and 2 present median levels of IL-10 produced by mitogen-activated PBMC from women with normal pregnancy at their first, second, third trimesters, at ND, as well as IL-10 levels produced by PBMC from women with a history of RSM undergoing another miscarriage or term delivery, PTD, PROM, preeclampsia, and women who delivered with IUGR.

In the progression of normal pregnancy from the first trimester up to delivery, median IL-10 levels were statistically significantly the highest at the first trimester, as compared to second trimester ($p = 0.0001$), third trimester ($p = 0.001$), and at ND ($p = 0.0001$). Similarly, statistically significantly higher levels of IL-10 were produced by PBMC obtained in the third trimester as compared to the second trimester ($p = 0.03$), and at ND ($p = 0.0001$) (Figure 1).

PBMC from women with a history of RSM who underwent another abortion produced lower levels of IL-10 levels as compared to the gestational age-matched first trimester normal group ($p = 0.0001$) and to women at normal term delivery ($p = 0.011$) (Figure 1). On the other hand, PBMC from women with a history of RSM and undergoing their first normal term delivery (RSM-N) produced median IL-10 levels comparable to those from women in the ND group ($p = 0.07$), (Figure 1).

Women with PTD produced significantly lower levels of IL-10, as compared to the gestational age-matched third trimester group ($p = 0.0001$), but their levels were comparable to women at ND ($p = 0.18$) (Figure 2). Similarly, women with PROM produced significantly lower levels of IL-10, as compared to normal pregnant women in their third trimester ($p = 0.0001$); IL-10 levels were however comparable to women at ND ($p = 0.72$) (Figure 2).

Women with PIH produced significantly lower median levels of IL-10, as compared with women in the ND group ($p = 0.0001$) (Figure 2).

PBMC from women with IUGR-W or IUGR-WO produced significantly lower median levels of IL-10 compared to the gestational age-matched third trimester group ($p = 0.001$, 0.001 respectively; Figure 2). However, when compared to ND group at parturition, IUGR-W produced comparable IL-10 levels ($p = 0.47$), while IUGR-WO produced higher IL-10 levels ($p = 0.017$) (Figure 2). It is worth noting that IUGR-WO produced significantly higher IL-10 levels as compared to IUGR-W ($p = 0.009$) (Figure 2).

Discussion

During successful pregnancy, the maternal immune system needs to be regulated in such a way that "foreign" antigens in the fetoplacental unit have to be tolerated and the mother has to be defended against infections. It is therefore likely that an array of cytokines is needed, each produced at the right time, and at right levels and location so as to sustain this fine balanced environment. Given their essential roles in gestation, disorders in cytokine networks are associated with several pregnancy complications, including RSM [13-18], PTD [19-21], PROM [22-24], pre-eclampsia [25-27], and IUGR [28-30].

IL-10 has been shown to play a pro-pregnancy role in early gestation [10]. During the implantation of the blastocyst and formation of the early placenta, IL-10 may have several beneficial roles; it may inhibit the secretion of pro-inflammatory cytokines such as TNF-α and IFN-γ, which are deleterious to pregnancy [11, 18, 31]. Injection of small doses of these two cytokines individually into pregnant mice results in fetal resorption, while the administration of IL-10 in abortion-prone CBAxDBA pregnant mice, significantly reduces the incidence of spontaneous fetal loss [32]. Women with unexplained recurrent miscarriage were reported to have significantly higher levels of the pro-inflammatory TNF-α and IFN-γ both in their sera and culture supernatants of PBMCs-induced by antigen and mitogen [13, 15]. IL-10 may be particularly relevant in this context; it inhibits cytokine synthesis by Th1 cells and also inhibits IL-2-induced IFN-γ production [33]. IL-10 further inhibits the synthesis of IFN-γ and IL-1α in Th1 cells and $CD8^+$ cells and strongly downregulates the constitutive and interferon-induced MHC class II antigen expression [34]. On the other hand, IL-10, in addition to IL-4 and IL-13, serves to modulate trophoblastic invasion and to maintain an anti-inflammatory milieu [35]. IL-10 gene-deficient mice were reported to over-produce inflammatory cytokines and to develop chronic inflammatory diseases [36]. It is also reported that pregnancy weakens inflammatory reactions of mice to *Leishmania major* infection and causes a decreased production of IFN-γ and increased production of T helper 2 cytokines [37]. IL-10 induces trophoblastic cells to produce vascular endothelial growth factor C (VEGF C) and the aquaporin (AQP1) system, which stimulates placental angiogenesis [38]. Furthermore, IL-10 may act as a mediator of other intrauterine regulators such progesterone, catecholamines, and prostaglandins [39]. Thus, IL-10 may have several potent contributions to the success of the first stage of pregnancy.

During subsequent stages of pregnancy, the milieu appears to be predominantly anti-inflammatory evinced by a local and systemic skew towards Th2 cytokine predominance [40]. During this stage, IL-10, along with other mediators, may contribute to keep deleterious Th1 responses under check. In fact, it has been proposed that an important immuno-regulatory role for IL-10 is the maintenance of Th2 bias and the induction of a shift away from Th1 bias during pregnancy [41]. During the final phase of pregnancy, i.e., parturition, programmed cell death, and activation of catabolic enzymes in a predominant pro-inflammatory milieu lead to delivery. At this point in gestation, IL-10 declines allowing parturition to proceed [40].

In healthy pregnant control subjects, the present authors report statistically significantly higher levels of production of IL-10 by women in the first trimester as compared to second and third trimesters, and at delivery. This supports the vital role of IL-10 during early stages of gestation. Further, IL-10 levels are lower at delivery than in the third trimester of normal pregnancy (Figure 1). A similar situation has been described at the maternal-fetal interface; Hanna *et al.* [39] studied IL-10 expression in human placental tissues and isolated cytotrophoblasts from different gestational ages and reported a significant downregulation at term before the onset of labor. A comparison of placental tissue from elective caesarean (pre-labor) and placental tissue obtained post-labor showed higher IL-10 production in pre-labor tissues. The low levels of IL-10 pre-labor correlated with low levels of prostanglandins E-2 (PGE-2), whereas the opposite was true in the post-labor tissues [42]. This downregulation may serve as one of the signals in the uterine microenvironment to upregulate the pro-inflammatory trigger at parturition. This highlights the key role of IL-10 in orchestrating the changes that are needed at parturition.

The present authors report significantly lower IL-10 levels produced by women with recurrent miscarriage as compared to the gestationally age-matched first trimester group (Figure 1). This is further supported by the work of others who showed decreased IL-10 production from placental and decidual tissues from first trimester missed abortion as compared to first trimester elective termination [43]. On the other hand, the present authors report that women who had a history of unexplained RSM but succeeded to continue their gestation for the first time eventually undergoing normal term delivery (RSM-N) have IL-10 levels comparable to women of the ND group (Figure 1).

Considerable evidence exists to support a role for pro-inflammatory cytokines in the sequence of events leading to PTD and PROM, regardless of the initiating signal (infection or otherwise). It is proposed that inflammatory cytokines activate matrix metalloproteinases that degrade the membrane matrix, predisposing them to rupture of fetal membranes either by apoptosis [44, 45] or by increased production of prostaglandin E2, and increased uterine activity which could lead to PTD or PROM [46]. The present data supports this notion; the authors report significantly lower production of IL-10 levels by women with unexplained PTD and PROM as compared to the third trimester control subjects. Lower IL-10 levels suggest a higher pro-inflammatory bias in these two conditions as compared to

the third trimester of normal pregnancy. Interestingly, IL-10 levels in PTD and PROM were not different from those seen at ND in control subjects; this can be attributed to the increased pro-inflammatory cytokine profile that occurs around parturition [40] (Figure 2).

Subjects in the pre-eclampsia group produced significantly lower median levels of IL-10, as compared to women in the healthy control ND group (Figure 2). Several researchers suggest that excessive inflammatory cytokine production may lead to the immunological dysfunction in this syndrome [47, 48]. The present results are in line with other reports of decreased placental production of IL-10 in pre-eclamptic placentas along with increase in inflammatory cytokines [49]. The pathophysiology of pre-eclampsia has been described to be due to defective trophoblast invasion and deficient spiral artery remodeling leading to placental ischemia/hypoxia which eventually results in the production of inflammatory molecules and a state of systemic inflammation [50].

Women with IUGR also show interesting differences in IL-10 production. IUGR is generally categorized into two types, IUGR-W and IUGR-WO. IUGR-WO is believed to be mainly due to genetic and chromosomal abnormalities with no obvious pathological changes in the placenta [51]. However, IUGR-W involves significant placental pathological findings and is believed to be due to a reduction in uteroplacental blood flow [52], a feature that may be caused by inflammatory cytokines such as TNF-α.

In the present study, both IUGR subgroups produced lower IL-10 levels as compared to the third trimester normal pregnant women. Furthermore, the authors found statistically significant higher IL-10 levels produced by the IUGR-WO compared to IUGR-W (Figure 2). While the cause of IUGR-WO is mainly genetic, higher IL-10 may be produced to compensate for the condition; which is not the case in IUGR-W, as the failure is more due to cytokine imbalance [53]. The lower levels of IL-10 in IUGR-W may be suggestive of a higher proinflammatory bias in this subgroup *vs*. IUGR-WO subgroup. IL-10 is reported to inhibit Th1 cytokine release [54] and its levels are decreased in placentas of IUGR pregnancies, therefore suggested to contribute in the pathology [53]. The present data suggests that the tilt toward proinflammatory cytokines in IUGR-W may be due, at least in part, to lower IL-10 levels. This is also supported by the significantly lower IL-10 levels produced by normal pregnant women at parturition as compared to IUGR-WO, but comparable levels to IUGR-W (Figure 2).

Limitations to this study include the use of peripheral blood cells and the use of a mitogen to elicit cytokine production. Measuring the production of cytokines by PBMC *in vitro* only partly reflects the much more complex *in vivo* scenario at the feto-maternal interface, where trophoblast cells and a multitude of immune and other cells, cytokines, and factors interact with each other in intricate ways. Phytohaemagglutinin, the mitogen used in this study, causes non-specific stimulation of almost all T cells in culture. Stimulating PBMC with trophoblast-derived antigens and the investigation of cytokine production in decidual lymphocytes might yield more pertinent results. However, studies on decidual tissue from women with recurrent abortion will obviously be based on samples obtained not before miscarriage, but only after.

Conclusions

IL-10 appears to play crucial roles in the success of pregnancy, and the absence or paucity of this cytokine, along with other cytokines, may contribute to complications such as RSM, PTD, PROM, pre-eclampsia, and IUGR. It is of great scientific and therapeutic interest to explore modalities for upregulating IL-10 in these conditions, to induce a maternal cytokine profile that is conducive to successful pregnancy.

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