CEOG Clinical and Experimental Obstetrics & Gynecology

Pro-inflammatory and anti-inflammatory cytokine profiles in fetal growth restriction

M. Al-Azemi[1], R. Raghupathy[2], F. Azizieh[3]

[1] Department of Obstetrics & Gynecology, [2] Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait
[3] Department of Mathematics and Natural Sciences, Gulf University for Science and Technology, West Mishref (Kuwait)

Summary

The purpose of this investigation was to measure cytokine production by maternal peripheral blood lymphocytes from women with intrauterine growth restriction (IUGR) and from healthy pregnant women, and to investigate the relationship between cytokine profiles and IUGR. Thirty-six women with IUGR and 22 control healthy pregnant women with normal fetal growth were studied. Levels of pro-inflammatory cytokines (IFNγ, TNFα, IL-8, IL-12, IL-18, IL-23) and anti-inflammatory cytokines (IL-4, IL-10, IL-13) produced by mitogen-stimulated peripheral blood mononuclear cells were measured by ELISA. Levels of the anti-inflammatory cytokine IL-4 were higher in normal pregnancy compared to IUGR, indicating an anti-inflammatory bias. Levels of the pro-inflammatory cytokines IL-6, TNFα, and IL-12 were significantly higher and levels of the anti-inflammatory cytokine IL-10 lower in IUGR with placental insufficiency than in IUGR without placental insufficiency, suggesting a stronger pro-inflammatory bias in IUGR with placental insufficiency. Ratios of pro- to anti-inflammatory cytokines suggest a dominance of pro-inflammatory cytokines. The authors conclude that an increased pro-inflammatory cytokine bias is observed in IUGR compared to normal pregnancy, and an increased pro-inflammatory cytokine dominance is seen in IUGR with placental insufficiency compared to IUGR without placental insufficiency.

Key words: Fetal growth restriction; Pro-inflammatory cytokines; Anti-inflammatory cytokines.

Introduction

Despite our understanding of some of the causes and risk factors of intrauterine growth retardation (IUGR), a definite cause of IUGR remains unidentified in nearly 50% of the cases [1]. While the pathophysiologic mechanisms underlying IUGR are not completely understood, a critical role for the placenta has long been suspected. Research on immunological aspects of other pregnancy complications like recurrent spontaneous miscarriage, pre-eclampsia, and preterm labor have revealed important roles for maternal immunologic factors such as natural killer cells, activated macrophages, and cytokines in these complications. Several cytokines play critical roles in normal pregnancy both in the maintenance of placental growth and in the modulation of maternal immune responses to avoid antagonistic reactivity against the conceptus [2-4]. The maternal immunologic state that is most beneficial to reproductive fitness appears to be nurtured by the local secretion of T helper 2 (Th2) cytokines; as a corollary, recurrent spontaneous miscarriage [5], preterm labour [6, 7], and pre-eclampsia [8] are reported to be associated with a predominance of T helper-1 (Th1) or pro-inflammatory cytokines in the mother [3,4].

Th1 and Th2, the two major subsets of $CD4^+$ T helper cells have different patterns of cytokine production and different roles in immune responses [9]. Th1 cells secrete IFNγ, TNFβ, IL-2, and TNFα; these inflammatory cytokines activate macrophages and cell-mediated immunologic reactions. Th2 cells secrete IL-4, IL-5, IL-10, and IL-13 which are predominantly anti-inflammatory cytokines and augment humoral immunity.

While there are numerous studies on cytokine profiles in pregnancy complications like recurrent miscarriage, preterm delivery, and pre-eclampsia [4-8], immunological studies in IUGR are limited. The demonstration of increased levels of some cytokines and decreased levels of other cytokines in these conditions have led us to consider the possibility of abnormal cytokine profiles in IUGR as well. This study aimed at investigating possible relationships between cytokine production patterns of maternal peripheral blood lymphocytes and IUGR, with and without placental insufficiency.

Materials and Methods

Study center

This prospective study included a total of 36 consecutive women attending the antenatal clinic at Kuwait Maternity Hospital with a diagnosis of IUGR, and 22 healthy control subjects with normal fetal growth. Informed consent was obtained from all women in the study. This study was approved by the Ethics

Revised manuscript accepted for publication September 1, 2015

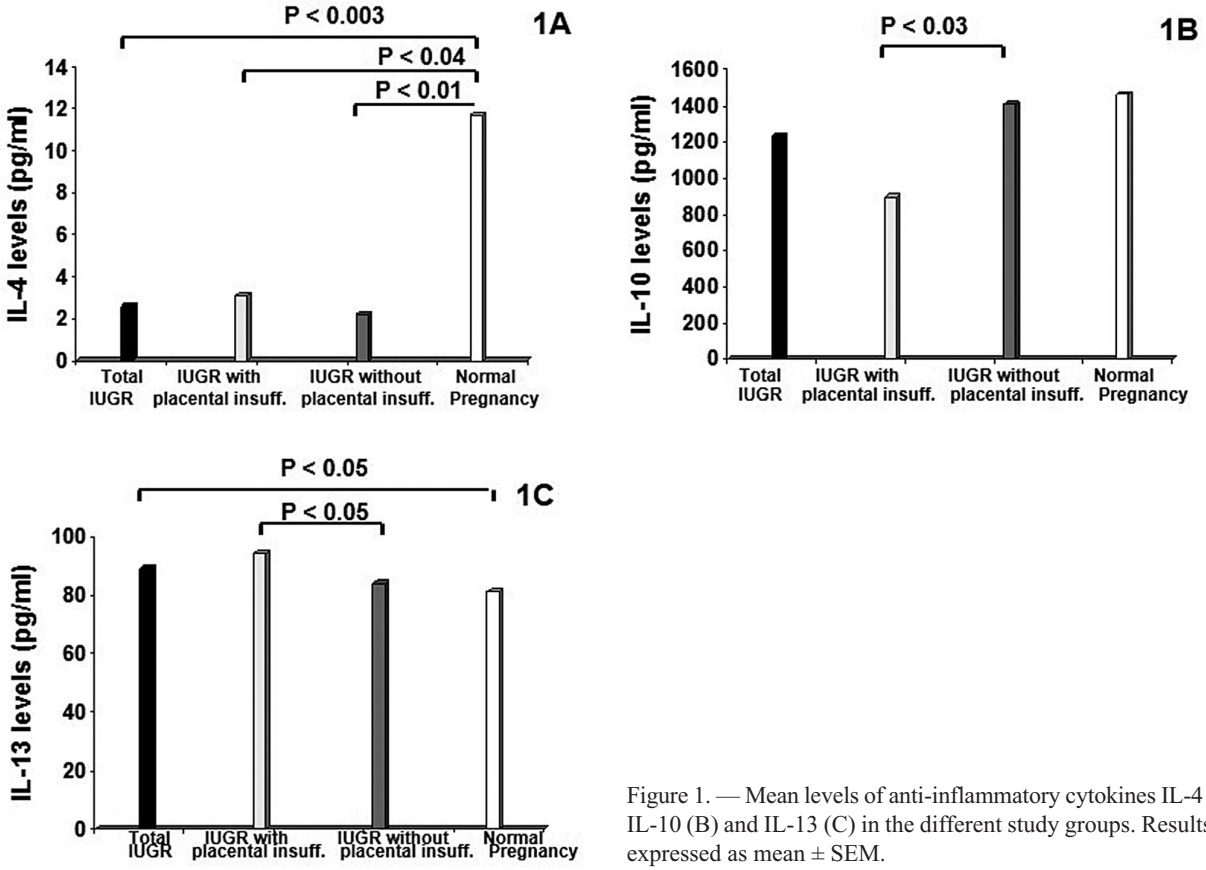

Figure 1. — Mean levels of anti-inflammatory cytokines IL-4 (A), IL-10 (B) and IL-13 (C) in the different study groups. Results are expressed as mean ± SEM.

Committee of the Faculty of Medicine, Kuwait University.

Subjects

Subjects underwent early ultrasound scan to confirm gestational age. IUGR is defined as an estimated birth weight of less than the 10th centile for gestational age by ultrasound scan. Inclusion criteria in this group were fetuses with less than 10th centile abdominal circumference measured by ultrasound scan.

Women in the IUGR group were subdivided into IUGR without placental insufficiency (i.e. symmetrical IUGR; 17 women) and IUGR with placental insufficiency (i.e. asymmetrical IUGR; 19 women) by assessment of fetal anatomy and biometry, amniotic fluid dynamics, uterine, umbilical, and fetal middle cerebral artery by Doppler. Placental insufficiency was diagnosed if pulsatility index in the umbilical artery was raised, with either absent end diastolic flow, or reversed end diastolic flow. Exclusion criteria for the IUGR group included diagnosis of fetal malformations and the presence of co-morbid maternal disease such as pre-eclampsia and infectious diseases.

Controls consisted of women with a history of at least two successful pregnancies with no previous spontaneous miscarriage, pre-eclampsia, preterm labor or IUGR.

Lymphocyte stimulation

Five ml of venous blood samples were taken after spontaneous vaginal delivery at term. Peripheral blood mononuclear cells (PBMC) were separated from blood samples by Ficoll hypaque density gradient centrifugation, suspended in RPMI medium containing 10% fetal calf serum, aliquoted into 96-well tissue culture plates at 10^5 cells per well, and then challenged with the mitogen phytohemagglutinin (PHA) at a concentration of five µg/ml for a period of 96 hours. Culture supernatants were collected after 96 hours of culture and the levels of cytokines estimated by ELISA.

ELISA for cytokines

Samples were tested in triplicate and absorbance values were read using 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.

Statistical analysis

The standard Mann-Whitney-U test was used for non-parametric comparisons of stimulation indices and median cytokine levels, as the data were not normally distributed. Differences were considered significant if the *p*-value was ≤ 0.05.

Results

The mean levels of IL-4 were significantly higher in the normal pregnancy group compared to IUGR subjects as a whole and also compared to IUGR with and without placental insufficiency (Figure 1A).

While the mean levels of IL-10 were not significantly different in the normal control group versus the IUGR group as a whole, the IUGR group with placental insuffi-

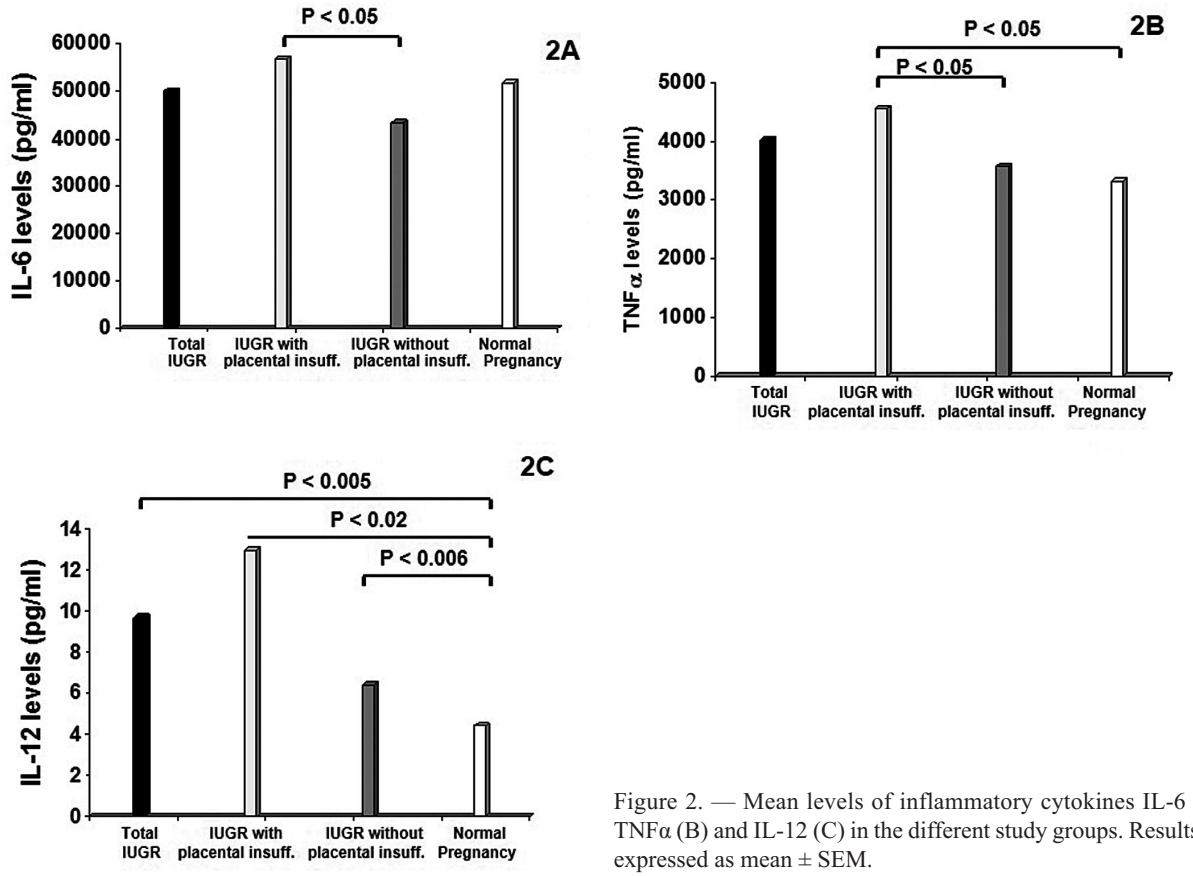

Figure 2. — Mean levels of inflammatory cytokines IL-6 (A), TNFα (B) and IL-12 (C) in the different study groups. Results are expressed as mean ± SEM.

ciency had significantly lower levels of IL-10 compared to IUGR without placental insufficiency (Figure 1B).

Interestingly, the Th2 cytokine IL-13 was significantly higher in the IUGR group as a whole when compared to the normal pregnancy group. The IUGR group with placental insufficiency had higher levels of IL-13 than the IUGR group without placental insufficiency (Figure 1C).

Mean levels of IL-6 were significantly higher in IUGR with placental insufficiency compared to IUGR without placental insufficiency; higher levels of IL-6 in IUGR with placental insufficiency might suggest a possible connection between this inflammatory cytokine and IUGR with placental insufficiency. IL-6 levels were not significantly different when the IUGR group as a whole was compared to the normal pregnancy control group (Figure 2A).

Levels of the pro-inflammatory Th1 cytokine TNFα were higher in IUGR with placental insufficiency compared to IUGR without placental insufficiency and also compared to normal pregnancy (Figure 2B), suggesting that IUGR with placental insufficiency is associated with higher levels of this inflammatory cytokine.

IL-12 is a Th1-inducing inflammatory cytokine and is thus responsible for influencing the Th1/Th2 patterns of T cell reactivity. Compared to normal pregnancy, mean levels of IL-12 were significantly higher in the IUGR group, as well in IUGR with and without placental insufficiency (Figure 2C). Higher levels of IL-12 might indicate a propensity towards a Th1 profile in IUGR.

Mean levels of IFNγ (Figure 3A) and IL-8 (Figure 3B) were not significantly different in the four groups studied. Given that cytokines are part of a large network of cytokines, receptors and antagonists, cytokine levels by themselves may not provide sufficient information on the *overall* immune reactivity; the *relative* levels of cytokines may provide a better picture of possible biases in cytokine profiles and thus immune reactivity. Keeping this in mind, the authors calculated the means of ratios of inflammatory to non-inflammatory cytokines in different permutations.

In most of the combinations, ratios involving IL-4 were higher in IUGR compared to controls (Table 1). For example, the TNFα/IL-4, IFNγ/IL-4, IL6/IL-4, IL-8/IL-4, and IL-12/IL-4 ratios were significantly higher in the IUGR group compared to normal pregnancy controls. These ratios were also significantly higher in the IUGR with placental insufficiency and IUGR without placental insufficiency as compared to normal controls. This is suggestive of a stronger inflammatory bias in the IUGR groups compared to the normal control group.

Table 1. — *Ratios of pro- to anti-inflammatory cytokines.*

Cytokine ratio	Total IUGR (I)	IUGR with placental insufficiency (W)	IUGR without placental insufficiency (WO)	Normal pregnancy control (C)	Significant differences
TNF/IL-4	1543	1469	1621	283	I *vs.* C = $p < 0.002$; WO *vs.* C = P < 0.02; W *vs.* C = $p < 0.005$
IFN/IL-4	3668	3251	4176	873	I *vs.* C = $p < 0.01$; WO *vs.* C = P < 0.05; W *vs.* C = $p < 0.05$
IL-6/IL-4	19270	18254	19701	4429	I *vs.* C = $p < 0.007$; WO *vs.* C = P < 0.04; W *vs.* C = $p < 0.04$
IL-8/IL-4	516	466	571	124	I *vs.* C = $p < 0.006$
IL-12/IL-4	3.7	4.2	2.9	0.38	I *vs.* C = $p < 0.0001$; WO *vs.* C = $p < 0.002$; W *vs.* C = $p < 0.01$
TNF/IL-10	3.3	5.1	2.5	2.3	W *vs.* S = $p < 0.03$
IFN/IL-10	7.8	11.2	6.5	7	W *vs.* S = $p < 0.008$
IL-6/IL-10	41	63	31	35	W *vs.* S = $p < 0.03$
IL-8/IL-10	1.1	1.6	0.9	1	WO *vs.* C = $p < 0.0001$; W *vs.* C = $p < 0.0001$; W *vs.* S = $p < 0.03$
IL-12/IL-10	0.008	0.02	0.005	0.003	W *vs.* C = $p < 0.012$
TNF/IL-13	45	49	43	41	
IFN/IL-13	108	107	109	126	
IL-6/IL-13	563	605	516	640	
IL-8/IL-13	15	16	15	15	
IL-12/IL-13	0.1	0.1	0.08	0.05	

All IUGR samples = I; IUGR with placental insufficiency = W; IUGR without placental insufficiency = WO; Control = C.

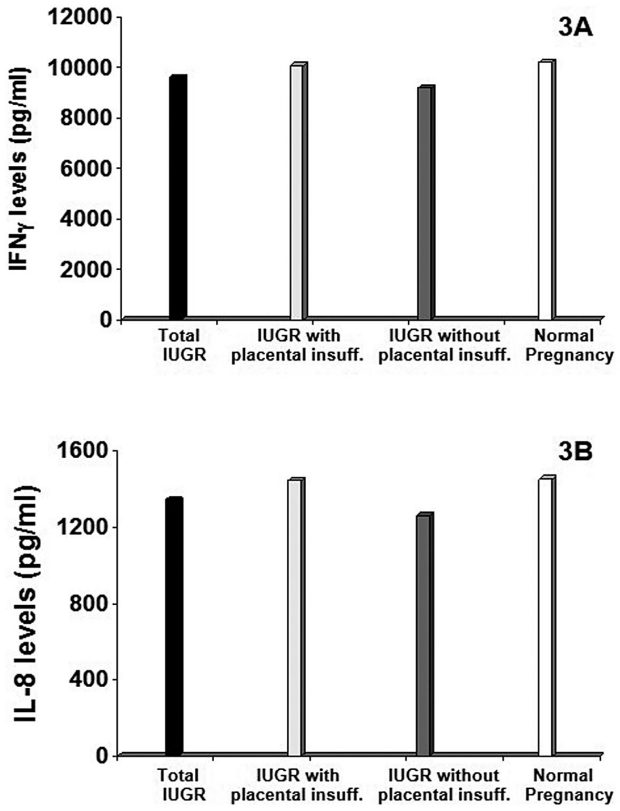

Figure 3. — Mean levels of inflammatory cytokines IFNg (A) and IL-8 (B) in the different study groups. Results are expressed as mean ± SEM.

Ratios involving IL-10 revealed an interesting pattern. TNFα/IL-10, IFNγ/IL10, IL-6/1L-10, and IL-8/IL-10 were all higher in IUGR with placental insufficiency compared to IUGR without placental insufficiency. IL-10 is a characteristic Th2 cytokine that antagonizes the generation of Th1 responses. Higher ratios involving IL-10 can be interpreted as a stronger Th1 bias in IUGR with placental insufficiency as compared to IUGR without placental insufficiency.

Discussion

A few previous studies have estimated some cytokines in the serum and amniotic fluid of IUGR pregnancies, but none have focused on cytokine production by maternal lymphocytes. In this report, maternal peripheral blood mononuclear cells were stimulated with a mitogen after which cytokine production profiles were assessed in three groups of subjects, IUGR with placental insufficiency, IUGR without placental insufficiency, and normal pregnant women.

IL-4 levels are significantly lower in IUGR with placental insufficiency, IUGR without placental insufficiency, and the IUGR group as a whole, when compared to normal pregnancy controls. This suggests the possibility of a decreased Th2 profile in IUGR and therefore a stronger Th1 bias in IUGR, which is supported by the significantly higher TNFα/IL-4, IFNγ/IL-4, IL-6/IL-4, IL-8/IL-4, and IL-12/1L-4 ratios in IUGR. IL-4 is the hallmark Th2 cytokine that directs Th2 development [10]. Because of its strong Th2-stimulating properties, high levels of IL-4 would eventually result in preferential stimulation of Th2 (i.e. anti-inflammatory) activity.

The present authors found higher levels of IL-10 in IUGR without placental insufficiency compared to IUGR with placental insufficiency. Most of the ratios of inflammatory cytokines to IL-10 are higher in IUGR with placental insufficiency compared to IUGR with placental insuffi-

ciency, suggestive of a stronger pro-inflammatory bias in IUGR with placental insufficiency compared to IUGR without placental insufficiency. IL-10 is a Th2-type immunosuppressive cytokine which antagonizes the generation of Th1 cells and exerts strong anti-inflammatory functions [10, 11]. Amu *et al*. reported a significant decrease in serum and decidual IL-10 in IUGR pregnancies compared to normal pregnancies and propose that low levels of IL-10 in the placenta may be associated with the pathogenesis of IUGR [12].

IL-13 also makes significant contributions to Th2 responses [10, 13]. However, in contrast to the trends suggested by IL-4 and IL-10 levels and ratios, IL-13 levels produced in mitogen-stimulated cultures were actually higher in IUGR than in normal pregnancy. On the other hand, the IL-13-related ratios were not significantly different between the different groups.

This study also found significantly elevated production of the pro-inflammatory cytokine IL-6 in IUGR with placental insufficiency compared to IUGR without placental insufficiency, as well as higher IL-6/IL-4 and IL-6/IL-10 ratios, suggesting a stronger pro-inflammatory bias in IUGR with placental insufficiency. IL-6, along with other inflammatory cytokines such as IL-1, TNFα, and IL-12, plays very significant roles in the development of acute and chronic inflammatory responses [14]. The present authors did not observe significantly higher production of IFNγ in the IUGR groups but found higher IFNγ/IL-4 and IFNγ/IL-10 ratios in IUGR with placental insufficiency compared to IUGR without placental insufficiency. This could be significant considering that the relative levels of IL-10 and IFNγ may influence the balance between immunopathologic reactions and weak cell-mediated responses [15]. IFNγ is a classical Th1/inflammatory cytokine and is responsible for triggering Th1 responses and inhibiting Th2 responses [10, 16]. Interestingly, increased cord blood levels of IFNγ were reported in small-for-gestational age pregnancies compared to normal babies; these authors suggest that IFNγ levels at birth are related to fetal growth restriction [17].

TNFα levels are higher in IUGR with placental insufficiency compared to IUGR without placental insufficiency and to normal control pregnancy. The ratios of TNFα/IL-4 and TNFα/IL-10 are also higher in IUGR than in normal pregnancy, indicative of a stronger pro-inflammatory bias in IUGR versus normal pregnancy and in IUGR with placental insufficiency versus IUGR without placental insufficiency. Known to be a strong mediator of inflammatory reactions, TNFα is a cytotoxic cytokine which also promotes thrombotic processes. Along with IL-1 and IL-6, TNFα induces many of the localized and systemic changes observed in the acute inflammatory responses, such as increased vascular permeability, chemokine induction, increased expression of adhesion molecules on vascular endothelium, and coagulation. TNFα also plays a central role in chronic inflammation [18]. Interestingly, Johnson *et al*. did not find an increase in serum levels of the pro-inflammatory cytokines IL-6, IL-8, and TNFα in fetal growth restriction [19]. On the other hand, Bartha *et al*. showed that women with IUGR due to placental insufficiency had significantly higher serum levels of TNFα and a higher rate of detectable TNFα than did normal pregnant women [20]. These researchers suggest that elevations of TNFα could be a specific phenomenon of certain subsets of IUGR, identifying cases with placental dysfunction. Amarilyo *et al*. (2011) found increased cord blood concentrations of IL-6 and TNFα in small-for-gestational age infants when compared with controls [21].

While IL-8 levels by themselves were not significantly different in the different groups, the IL-8/IL-4 ratio was higher in IUGR compared to normal pregnancy. Similarly, the IL-8/IL-10 ratio was higher in IUGR with placental insufficiency. IL-8 is a powerful chemokine that functions primarily as a chemoattractant and activator of neutrophils and plays important roles in inflammatory responses. The authors also found higher levels of IL-12 in the IUGR group as a whole and in both IUGR subgroups when compared to normal pregnancy. IL-12/IL-4 and IL-12/IL-10 ratios are also higher in IUGR. IL-12 is a potent regulator of Th1 differentiation and also has potent pro-inflammatory capabilities [22]. In the cord blood of small-for-gestational age preterm infants, Lindner *et al.* recently reported elevated levels of IL-12 [23].

Overall the present results suggest that peripheral blood mononuclear cells from women with IUGR show a pro-inflammatory bias. The observation of increased levels of the inflammatory cytokines IL-6 and TNFα in IUGR with placental insufficiency than in IUGR without placental insufficiency, and higher levels of the anti-inflammatory cytokine IL-10 IUGR without placental insufficiency point to the possibility of a stronger inflammatory bias in IUGR with placental insufficiency when compared to IUGR without placental insufficiency. This is supported by higher TNFα/IL-10 and IFNγ/IL-10 ratios in IUGR with placental insufficiency.

In a recent companion study, the present authors found interesting differences in the levels of cytokines produced by maternal PBMC upon antigenic stimulation with trophoblast antigens. IL-8 was produced at higher levels by blood cells of the IUGR group than in normal pregnant women, while IL-13 was produced at lower levels. IL-8, IFNγ, and TNFα were higher in IUGR with placental insufficiency than in normal pregnancy. IL-12 levels were higher and IL-10 levels were lower in IUGR with placental insufficiency than in IUGR without placental insufficiency [24].

If the association between cytokine profiles and IUGR is confirmed in studies with larger sample sizes, how might cytokines bring about adverse effects? TNFα inhibits the growth of the trophoblast [25], interferes with placental development and invasion of the spiral arteries, is directly toxic to the endothelium, and may damage the decidual vas-

culature [26]. TNFα interferes with the anticoagulant system and may induce placental thrombosis. In fact, massive chronic intervillositis and thrombotic vasculopathy [27] are known to cause IUGR and it is suggested that inflammatory cytokines like TNFα may contribute to these pathologies [25]. Inflammatory cytokines may also cause trophoblast dysregulation at a subcellular level and the loss of functional mass of the villous trophoblast via cell death pathways [28]. It is also proposed that increased TNFα in the vicinity of the placenta may lead to IUGR by preventing nutrient uptake by the fetus. A pro-Th1 bias would result in higher levels of TNFα, hence the possible association between Th1 reactivity and IUGR is of interest.

Investigations on the associations and the roles of inflammatory mediators like cytokines, will contribute not only to the elucidation of the pathophysiology of this disease, but also to the identification of markers that can predict neonatal complications, as well as fetal maturity and weight.

Acknowledgment

The study was supported by Kuwait University Research Grant No. MI02/05.

References

[1] Creasy R.D., Resnick R: "Intrauterine growth restriction". *In:* Creasy R.K., Resnik R., Iams J.D., Lockwood C.J., Moore T.R., Greene M.F. (eds). *Creasy and Resnik's Maternal Fetal Medicine: Principles and Practice.* 7th ed. Philadelphia: Saunders, 2013, 743.

[2] Wegmann T.G., Lin H., Guilbert L., Mosmann T.R.: "Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon?" *Immunol. Today* 1993, *14,* 353.

[3] Challis J.R., Lockwood C.J., Myatt L., Norman J.E., Strauss III J.F., Petraglia F.: "Inflammation and pregnancy". *Reprod. Sci*., 2009, *16*, 206.

[4] Saito S., Nakashima A., Shima T., Ito M.: "Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy". *Am. J. Reprod. Immunol*., 2010, *63*, 307.

[5] Polgar K., Hill J.A.: "Identification of the white blood cell populations responsible for Th1 immunity to trophoblast and the timing of the response in women with recurrent pregnancy loss". *Gynecol. Obstet. Invest*., 2002, *53*, 59.

[6] Makhseed M., Raghupathy R., EI-Shazly S., Azizieh S., Al-Harmi J., Al-Azemi M.: "Proinflammatory maternal cytokine profile in preterm delivery". *Am. J. Reprod. Immunol*., 2002, *49,* 1.

[7] Sykes L., MacIntyre D.A., Yap X.J., Teoh T.G., Bennett PR.: "The Th1:Th2 dichotomy of pregnancy and preterm labour". *Med. Inflamm*., 2012, *2012*, 967629.

[8] Saito S., Shiozaki A., Nakashima A., Sakai M., Sasaki Y.: "The role of the immune system in preeclampsia". *Molec. Aspects Med.*, 2007, *28*, 192.

[9] Romagnani S.: "Regulation of the T cell response". *Clin. Exp. Aller*., 2006, *36*, 1357.

[10] Amsen D., Spilianakis C.G., Flavell R.A.: "How are TH1 and TH2 effector cells made?" *Curr. Opin. Immunol*., 2009, *21*, 153.

[11] Sabat R., Grutz G., Warszawska K., Kirsch S., Witte E., Wolk K., Geginat J.: "Biology of interleukin-10". *Cytokine Growth Factor Rev*., 2010, *21*, 331.

[12] Amu S., Han-Zoric M., Malik A., Ashraf R., Zaman S., Kjellmer I., *et al.*: "Cytokines in the placenta of Pakistani newborns with and without Intrauterine growth retardation" *Pediatr. Res*., 2006, *59*, 254.

[13] Strober W.: "Immunology: the expanding Th2 universe". *Nature*., 2010, 463, 434.

[14] Rincon M.: "Interleukin-6: from an inflammatory marker to a target for inflammatory diseases." *Trends Immunol*., 2012, *33*, 571.

[15] Trinchieri G.: "IL-10 production by effector T cells: Th1 cells show self control". *J. Exp Med* 2007, *204*, 239.

[16] Cope A., Le Friec G., Cardone J., Kemper C.: "The Th1 life cycle: molecular control of IFN-gamma to IL-10 switching". *Trends Immunol*., 2011, *32*, 278.

[17] Neta G.I., von Ehrenstein O.S., Goldman L.R., Lum K., Sundaram R., Andrews W., Zhang J.: "Umbilical cord serum cytokine levels and risks of small-for-gestational-age and preterm birth". *Am. J. Epidemiol*., 2010, *171*, 859.

[18] Bradley JR.: "TNF-mediated inflammatory disease". *J. Pathol*., 2008, *214*, 149.

[19] Johnson M.R., Anim-Nyame N., Johnson P., Sooranna D.R., Steer P.J.: "Does endothelial cell activation occur with intrauterine growth restriction?" *BJOG*, 2002, *109*, 836.

[20] Bartha J.L., Romero-Carmona R., Comino-Delgado R.: "Inflammatory cytokines in intrauterine growth retardation". *Acta Obstet. Gynecol. Scand*., 2003, *82*, 1099.

[21] Amarilyo G., Oren A., Mimouni F.B., Ochshorn Y., Deutsch V., Mandel D.: "Increased cord serum inflammatory markers in small-for-gestational-age neonates". *J. Perinatol*., 2011, *31*, 30.

[22] Athie-Morales V., Smits H.H., Cantrell D.A., Hilkens C.M.: "Sustained IL-12 signaling is required for Th1 development". *J. Immunol*., 2004, *172*, 61.

[23] Lindner U., Tutdibi E., Binot S., Monz D., Hilgendorff A., Gortner L.: "Levels of cytokines in umbilical cord blood in small for gestational age preterm infants". *Klinische Pediatrie*, 2013, *225,* 70.

[24] Raghupathy R., Al-Azemi M., Azizieh F.: "Intrauterine growth restriction: cytokine profiles of trophoblast antigen-stimulated maternal lymphocytes". *Clin. Develop. Immunol*., 2012, *2012*, 734865.

[25] Haider S., Knofler M.: "Human tumour necrosis factor: physiological and pathological roles in placenta and endometrium". *Placenta*, 2009, *30*, 111.

[26] Leonard S., Murrant C., Tayade C., van den Heuvel M., Watering R., Croy B.A.: "Mechanisms regulating immune cell contributions to spiral artery modification – facts and hypotheses – a review". *Placenta*, 2006, *27*, 40.

[27] Faye-Petersen O.M.: "The placenta in preterm birth". *J. Clin. Path*., 2008, *61*, 1261.

[28] Scifres C.M., Nelson M.D.: "Intrauterine growth restriction, human placental development and trophoblast cell death".| *J. Physiol*., 2009, *587*, 3453.

Corresponding Author:
R. RAGHUPATHY, PhD, FRCPath
Department of Microbiology
Faculty of Medicine, Kuwait University
PO Box 24923, Safat 13110 (Kuwait)
e-mail: raj@hsc.edu.kw