Human Reproduction vol.15 no.3 pp.713–718, 2000

Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion

R.Raghupathy[1,4], M.Makhseed[2], F.Azizieh[1], A.Omu[2], M.Gupta[3] and R.Farhat[3]

Departments of [1]Microbiology, and [2]Obstetrics & Gynaecology, Faculty of Medicine, Kuwait University, PO Box 24923 and [3]Maternity Hospital, Kuwait

[4]To whom correspondence should be addressed

It has been proposed that successful pregnancy is a T helper 2-type phenomenon, and that T helper (Th)1-type reactivity is deleterious to pregnancy. The objective of this study was to compare the concentrations of Th1 and Th2 cytokines produced by peripheral blood mononuclear cells from women undergoing unexplained recurrent spontaneous abortion (RSA) with those produced during normal pregnancy at a similar gestational stage. The control group consisted of 24 women with a history of successful pregnancies and the abortion group comprised of 23 women with a history of unexplained RSA. Blood from the control group was obtained at the end of the first trimester as gestational age controls for the abortion group from whom blood was collected at the time of abortion. Phytohaemagglutinin-stimulated peripheral blood cell culture supernatants were analysed for concentrations of cytokines. Significantly higher concentrations of Th2 cytokines were produced by the first trimester normal group than by the RSA group, while significantly higher concentrations of Th1 cytokines were produced by the abortion group as compared to first trimester normal pregnancy, indicating a distinct Th2-bias in normal pregnancy and a Th1-bias in unexplained RSA.

Key words: cytokines/recurrent abortions/T helper 1/T helper 2

Introduction

The conceptus appears to be fairly impervious to attack by humoral immunity except for anti-phospholipid antibodies which represent the only well substantiated antibody-mediated cause for pregnancy loss (reviewed by Hill, 1995). On the contrary, cellular immunity, mediated by effector cells and/or cytokines released by them, has significant deleterious effects on the conceptus. Tumour necrosis factor (TNF)-α stimulates the programmed death of human primary villous trophoblast cells and interferon (IFN)-γ augments TNF-mediated killing of trophoblasts (Yui *et al.*, 1994). Both these cytokines inhibit the outgrowth of human trophoblast cells *in vitro* (Berkowitz *et al.*, 1988). Spontaneous abortions in humans have been shown to be associated with increased production of interleukin (IL)-2 and IFN-γ by peripheral blood mononuclear cells (PBMC) and with decreased production of IL-10, as compared to normal pregnancy (Marzi *et al.*, 1996). Studies by Hill and colleagues have shown that trophoblast antigens activate the PBMC of women with a history of unexplained recurrent spontaneous abortion (RSA) to produce the embryotoxic cytokines IFN-γ and TNF-β (Ecker *et al.*, 1993; Hill *et al.*, 1995). The administration of IFN-γ or IL-2 or TNF-α to normal pregnant mice has been shown to cause abortion (Chaouat *et al.*, 1990).

Interestingly, the cytokines that have deleterious effects on the placenta and on pregnancy, i.e. IL-2, IFN-γ and TNF-β, together fit the profile of T helper (Th)1 cells. Th1 and Th2 cells represent two polarized forms of T helper cells, and as the major functional subsets of Th cells they mobilize different types of effector responses (Mosmann and Coffman, 1989; Romagnani, 1994). Th1 cells induce several cell-mediated cytotoxic and inflammatory reactions via IL-2, IFN-γ and TNF-β, while Th2 cells are associated primarily with the provision of help for B cell antibody production via the characteristic Th2-type cytokines, IL-4, IL-5, IL-6 and IL-10 (Mosmann and Sad, 1996). If Th1-type cytokines are indeed deleterious to pregnancy (Chaouat *et al.*, 1990; Hill, 1995; Hill *et al.*, 1995; Marzi *et al.*, 1996), Th2-type cytokines may be conducive to pregnancy, and Th2-type immunity has been proposed to be the normal profile in successful pregnancy (Wegmann *et al.*, 1993; Chaouat *et al.*, 1995; Raghupathy, 1997). Thus, there appears to be a basis for the notion that successful pregnancy is associated with a Th2-bias and that a shift towards Th1-dominance may be associated with unexplained RSA.

It has previously been demonstrated that upon mitogen stimulation, PBMC obtained at the time of normal delivery from women with a history of successful pregnancy produced significantly higher concentrations of Th2-type cytokines and significantly lower concentrations of Th1-type cytokines as compared to women undergoing recurrent spontaneous abortion (Makhseed *et al.*, 1999). In the present study, the cytokine production profile of women having a history of RSA was compared with the profile seen in normal pregnancy at the end of the first trimester.

Materials and methods

Subjects

Subjects were selected at the Kuwait Maternity Hospital where all patients with two or more previous abortions are routinely referred to the Recurrent Abortion Clinic where they are investigated for possible anatomical, endocrinological, infectious, genetic and immunological causes (i.e. anti-cardiolipin and anti-nuclear antibod-

ies) of abortion. The control group in this study consisted of women who were in labour with a single fetus, who had had at least three previous normal pregnancies, with no history of abortion, ectopic pregnancy, pre-term delivery or stillbirth; blood samples were obtained from 24 women at the end of the first trimester (12 ± 2 weeks); these women went on to have normal delivery. The RSA group of 23 subjects comprised women admitted with spontaneous abortion for evacuation, who had had at least three previous unexplained miscarriages and who had been investigated clinically. Blood was obtained from these subjects at the time of abortion (12 ± 3 weeks). This study had the approval of the Ethics Committees of the Maternity Hospital and of the Faculty of Medicine, Kuwait University; informed consent was obtained before blood samples were collected.

Mitogen-induced stimulation of PBMC

Peripheral blood was obtained by venipuncture and PBMC were separated by Ficoll-paque (Pharmacia Biotech L, Glyfada, Greece) density gradient centrifugation, suspended in Roswell Park Memorial Institute 1640 (RPMI) medium (Gibco BRL, Paisley, Renfrewshire, UK) containing 10% fetal calf serum, aliquotted into 96 well tissue culture plates at a density of 10^5 cells per well and then stimulated with the mitogen phytohaemagglutinin (PHA) (Sigma Chemicals, St Louis, MO, USA) at a concentration of 5 μg/ml for a period of 96 h. Supernatants were harvested at 24 and 96 h; some of the wells were pulsed with [^{3}H]thymidine (Amersham International, Little Chalfont, Bucks, UK; 1 μCi per well) for assessment of mitogen-induced proliferation. Thymidine-pulsed wells were harvested 18 h later and the radioactivity estimated. The stimulation index (SI) was calculated as a ratio of thymidine uptake by PHA-stimulated cells to that by unstimulated cells.

Determination of cytokine concentrations

IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α and IFN-γ were assayed by enzyme-linked immunosorbent assay (ELISA) using kits obtained from Coulter/Immunotech SA (France) and TNF-β by ELISA kits obtained from R & D Systems (Minneapolis, MN, USA). These consisted of sandwich ELISA for which the manufacturers' protocols were followed. Each supernatant sample was tested in triplicate in a blinded fashion. Standard curves were plotted for each of the cytokines using reference recombinant cytokines supplied by the manufacturer and the results read from these curves. The sensitivities of each of the assays were as follows: IL-2, 5 pg/ml; TNF-α, 10 pg/ml; TNF-β, 7 pg/ml; IFN-γ, 3 pg/ml; IL-4, 5 pg/ml; IL-5, 1 pg/ml; IL-6, 3 pg/ml; and IL-10, 5 pg/ml.

Statistical analysis

The standard Mann–Whitney *U*-test was used for non-parametric comparisons of median cytokine concentrations.

Results

Proliferative responses of PBMC

Stimulation indices (SI) of the normal pregnancy first trimester group ranged from 1 to 181 with a median of 29, while the SI of the RSA group ranged from 1 to 204 with a median of 50. No significant differences were apparent in the median SI of the two groups.

Comparison of Th2 cytokine production

PBMC from normal pregnant women and from women with RSA were cultured with PHA and the supernatants tested for the concentrations of Th1 and Th2 cytokines at 24 and 96 h of culture.

At the 24 h time point, three of the four Th2 cytokines tested were produced in significantly higher concentrations by PBMC of normal pregnant women at the first trimester; IL-4, IL-6 and IL-10 were present at significantly greater concentrations, with *P*-values of 0.004, 0.0001 and 0.0001 respectively (Figure 1). At the 96 h time point, IL-6 and IL-10 were secreted at significantly higher concentrations ($P = 0.0001$ in both cases) in the supernatants of PBMC from normal pregnant women as compared to women with RSA. IL-4 and IL-5 at 96 h also showed a trend towards higher concentrations but the differences were not significant. There was therefore an overall tendency for women at the first trimester of normal gestation to secrete higher concentrations of Th2-type cytokines than women undergoing RSA.

Comparison of Th1 cytokine production

All four Th1-type cytokines tested at the 96 h time point were produced at statistically significantly higher concentrations in the RSA group than in the first trimester normal pregnancy group. Concentrations of IL-2, IFN-γ, TNF-α and TNF-β were higher in the RSA group at 96 h, with *P*-values of 0.002, 0.03, 0.04 and 0.05 respectively (Figure 2). Concentrations of IL-2 at the 24 h point were significantly higher ($P = 0.04$) in the RSA group; differences in concentrations of IFN-γ, TNF-α and TNF-β were not significant even though there was a trend towards higher production of these three Th1 cytokines in the RSA group. Overall, in contrast to the higher production of Th2-type cytokines by the normal group at the first trimester, Th1-type cytokines were secreted at higher concentrations by the RSA group as compared to the normal first trimester group.

Ratios of Th1 to Th2 cytokines

The ratios of the various Th1 to Th2 cytokines were calculated by comparing the mean values in different combinations; as can be seen from Table I, the Th1:Th2 cytokine ratios in all the combinations were higher in the RSA group than in the first trimester normal group, suggesting a greater overall bias towards Th1-type reactivity in the RSA group. For example, the IL-2:IL-10 ratio at both the 24 and 96 h points is at least 80-fold higher in the RSA group, than in the normal group, and the IFN:IL-10 ratio at both the time points is about 30 fold higher in the RSA group. Interestingly, although the concentrations of IFN-γ, TNF-α and TNF-β at 24 h were not significantly different, the ratios of these Th1 cytokines to Th2 cytokines were consistently higher in the RSA group (Table I).

Discussion

Chromosomal anomalies, endocrinological problems, anatomical abnormalities and infections form the category of so-called 'explained' causes of recurrent spontaneous abortions (Bennett, 1987; Portnoi *et al.*, 1988; Ashworth, 1992; Hill, 1992, 1995); however, all of these put together account for the aetiology of only 40–60% of couples experiencing RSA (Stray-Pederson and Stray-Pederson, 1984). It has been proposed that cell-mediated immunological mechanisms may

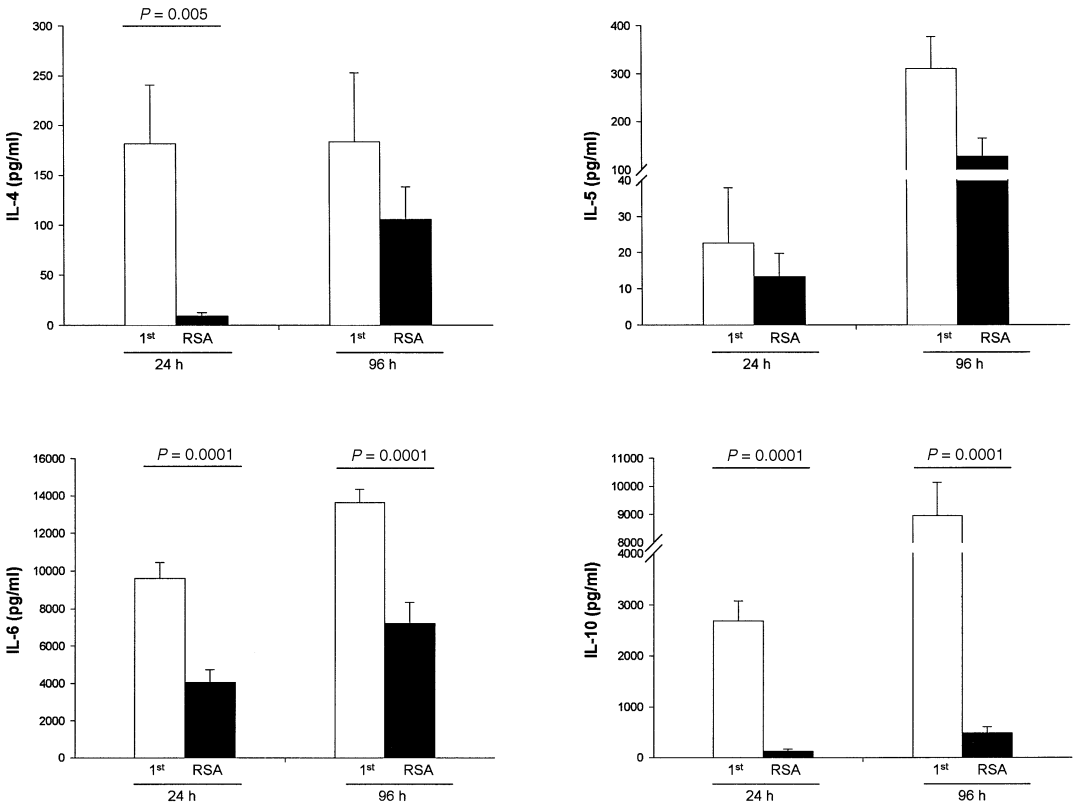

Figure 1. Concentrations of T helper (Th)2-type cytokines produced by mitogen-stimulated peripheral blood mononuclear cells (PBMC) from women at the end of the first trimester in normal pregnancy (1st) and women undergoing recurrent spontaneous abortion (RSA) after 24 and 96 h of culture. Concentrations that were statistically significantly different are indicated in the graphs. IL = interleukin.

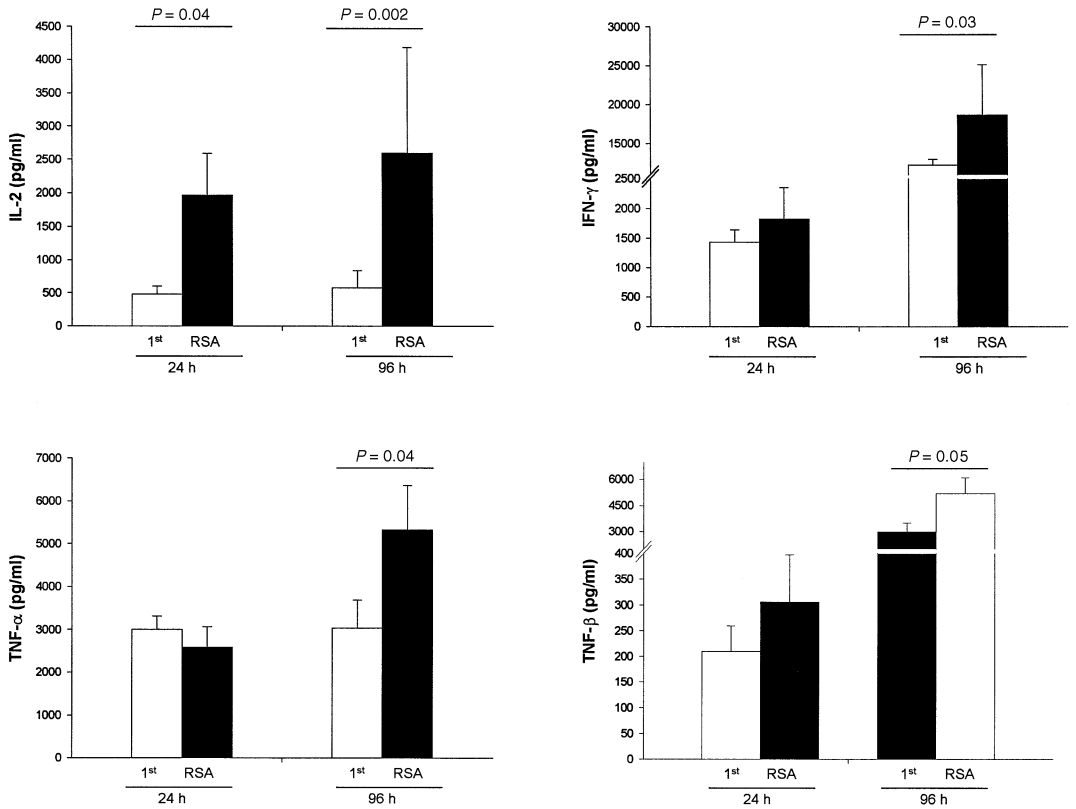

Figure 2. Concentrations of Th1-type cytokines produced by mitogen-stimulated PBMC from women at the end of the first trimester in normal pregnancy (1st) and women undergoing RSA after 24 and 96 h of culture. Concentrations that are statistically significantly different are indicated in the graphs. IFN-γ = interferon-γ; TNF = tumour necrosis factor.

Table I. Ratios of T helper (Th)1 and Th2 cytokines in normal pregnant women at the first trimester and in women undergoing unexplained recurrent spontaneous abortion (RSA). Ratios were calculated from the mean values depicted in Figures 1 and 2.

Cytokine ratio	Normal first trimester	RSA
24 h culture		
IL-2:IL-4	3	218
IL-2:IL-5	22	151
IL-2:IL-6	0.05	0.5
IL-2:IL-10	0.2	16
IFN:IL-4	8	203
IFN:IL-5	65	140
IFN:IL-6	0.2	0.5
IFN:IL-10	0.5	15
TNFα:IL-4	17	286
TNFα:IL-5	136	198
TNFα:IL-6	0.3	0.6
TNFα:IL-10	1	21
TNFβ:IL-4	1	34
TNFβ:IL-5	9	24
TNFβ:IL-6	0.02	0.08
TNFβ:IL-10	0.08	3
96 h culture		
IL-2:IL-4	3	25
IL-2:IL-5	2	20
IL-2:IL-6	0.04	0.4
IL-2:IL-10	0.06	5
IFN:IL-4	67	178
IFN:IL-5	39	147
IFN:IL-6	0.9	3
IFN:IL-10	1	38
TNFα:IL-4	17	51
TNFα:IL-5	10	42
TNFα:IL-6	0.2	0.7
TNFα:IL-10	0.3	11
TNFβ:IL-4	17	51
TNFβ:IL-5	10	42
TNFβ:IL-6	0.2	0.7
TNFβ:IL-10	0.3	11

IL = interleukin; IFN = interferon; TNF = tumour necrosis factor.

account for a proportion of the cases of 'unexplained' RSA and it is likely that Th1-mediated maternal reactivity may well turn out to be an important cause of recurrent pregnancy loss (Hill, 1992, 1995; Wegmann *et al.*, 1993; Hill *et al.*, 1995; Marzi *et al.*, 1996; Raghupathy, 1997).

Studies on human pregnancy failure conducted in four laboratories, support the concept that successful pregnancy occurs in a Th2-biased situation, while Th1-type immunity may lead to pregnancy failure. Studies in Hill's laboratory (Hill *et al.*, 1995) have shown that the PBMC of women with a history of RSA when stimulated with a trophoblast antigen extract produce higher concentrations of the Th1 cytokines IFN-γ and TNF as compared to normal pregnancy. It has been demonstrated that the stimulation of maternal PBMC with autologous placental cells *in vitro* results in a Th1-biased production of cytokines in women undergoing unexplained RSA (Raghupathy *et al.*, 1999). That this is mirrored by the situation at the maternal–fetal interface was shown by Piccinni *et al.* (Piccinni *et al.*, 1998) when they demonstrated significantly higher concentrations of IL-4- and IL-10-producing T cell clones from the decidua of women with normal pregnancy than from recurrent aborters. One other study on 40 women with normal pregnancy and five with spontaneous abortions showed increased production of IL-4 and IL-10 and decreased production of IFN-γ and IL-2 by PBMC obtained from normal pregnant women as opposed to PBMC from spontaneous aborters (Marzi *et al.*, 1996). In a larger study consisting of 54 women with normal pregnancy and 23 women with unexplained RSA, a similar Th1–Th2 dichotomy was shown when normal pregnant women were compared at delivery with women undergoing RSA (Makhseed *et al.*, 1999). However, a much more appropriate gestational age control for women undergoing RSA is the end of the first trimester in normal pregnancy. In the present communication, such a comparison is reported between 24 normal pregnant women and 23 women with unexplained RSA, which is a larger sample size than has been reported previously and also with a wider range of Th1 and Th2 cytokines.

In this study, highly significantly increased concentrations of three Th2-type cytokines tested, i.e. IL-4, IL-6 and IL-10 were found at the 12 week point in normal pregnancy compared to the same stage in women with a history of unexplained RSA. On the other hand, statistically significantly increased concentrations of all four Th1 cytokines tested in women with RSA were found as compared to women with normal pregnancy. Keeping in mind the point that the absolute concentrations of cytokines secreted *in vitro* may not in themselves be indicative of a Th1- or Th2-bias, the ratios of Th1–Th2 cytokines were compared in the various permutations in the two groups. It is clear from Table I that in every combination of Th1:Th2 cytokines, the ratios were higher in the RSA group as compared to the normal pregnancy group, indicating a greater Th1-bias in RSA and/or a greater Th2-bias in normal pregnancy. Given that Th1 and Th2 cytokines are mutually inhibitory for each other (Mosmann and Coffman, 1994; Romagnani, 1994), it is likely that a shift towards Th1 bias may tend to down-regulate further Th2 reactivity.

The data presented here may be viewed with three caveats. Firstly, chromosomal abnormalities are responsible for a proportion of spontaneous abortions and while the recurrent aborters in this study and their husbands were karyotyped, the abortuses were not. Thus, it was not possible to rule out the possibility of aneuploidy in the fetuses. Secondly, the data presented reflect events related to maternal blood cells in the periphery and not to the placenta itself, as events at the maternal–fetal interface were not investigated. It would be of great interest to analyse events at the interface and at the placental bed. Piccini *et al.* (Piccini *et al.*, 1998) examined T cell clones generated from T cells infiltrating the decidua and found significantly increased concentrations of Th2 cytokine production in normal women as compared to recurrent aborters. Thirdly, the current study, like many of the studies on human pregnancy failure, has not addressed a direct cause-and-effect relationship between Th1-type reactivity and pregnancy loss; for example, an inflammatory maternal response to a fetus that has died due to non-immunological factors, could well manifest a Th1 profile. However, given the association between Th1 cytokines and pregnancy failure and the demonstrated deleterious effects of Th1 cytokines on the conceptus and on pregnancy, it is suggested that Th1-mediated effects may have been the cause of pregnancy failure in at least a proportion of the cases

in the study. TNF-α and IFN-γ inhibit the proliferation of human trophoblast cells *in vitro* (Berkowitz *et al.*, 1988) and are cytotoxic to human trophoblast cells (Yui *et al.*, 1994). The administration of TNF-α, IFN-γ or IL-2 to normal pregnant mice causes abortion (Chaouat *et al.*, 1990). Concentrations of TNF have been shown to be high in other complications of pregnancy, such as pre-eclampsia in which elevated TNF concentrations appear to be a feature of the disease (reviewed by Kilpatrick *et al.*, 1999). Uterine resorption sites in a murine model of recurrent abortion are infiltrated by natural killer (NK) cells (Gendron and Baines, 1988); given the fact that the activation of NK cells has been shown to be detrimental to murine pregnancy (Kinsky *et al.*, 1990) and that NK cells are activated by the Th1 cytokine IFN-γ, the relevance of these data to pregnancy failure is obvious. Concentrations of NK cells have been shown to be abnormally high in women with unexplained recurrent abortion (reviewed by Somigliana *et al.*, 1999) Activated macrophages are present in the decidua of resorbing murine embryos (Baines *et al.*, 1997) and embryo loss in mice has been shown to be associated with local production of nitric oxide (Haddad *et al.*, 1995). Strong Th1-dominant responses against pathogens compromise pregnancy; for example, infection by *Leishmania major* results in resorptions, with a concurrent increase in concentrations of IFN-γ in the placenta (Krishnan *et al.*, 1996). In a murine model of immunological abortion, Clark and colleagues have demonstrated that the abortion is mediated by Th1 cytokine-triggered thrombotic and inflammatory processes (Clark *et al.*, 1998). Thus, a considerable amount of evidence suggests that Th1 cytokines might well be implicated in adversely affecting pregnancy, directly by interfering with trophoblast survival and function, and indirectly by activating cell-mediated immune effectors.

This study also allows us to compare the status of T helper reactivity vis-à-vis Th1 and Th2 activity at the first trimester with that at the time of delivery described by us previously (Makhseed *et al.*, 1999). At the 24 h time point, statistically significant decreases were observed in the concentrations of three of the four Th2 cytokines at delivery as compared to the first trimester. At the 96 h point, this holds true for IL-10. As for the Th1 cytokines, the concentrations of IFN-γ and TNF-α were significantly reduced at delivery as compared to the first trimester while IL-2 concentrations were significantly increased at delivery. Thus, we do not observe a clear and consistent picture of significant changes in Th1 and Th2 cytokines produced by PBMC obtained at the first trimester versus at delivery, except for decreases in concentrations of the three Th2 cytokines and two Th1 cytokines in the 24 h culture supernatants. However, a comparison of the ratios of mean concentrations of Th1 to Th2 cytokines at the first trimester (Table I) and at delivery (Makhseed *et al.*, 1999), shows an increase in Th1:Th2 cytokine ratios from the first trimester to delivery in many cases, decreased ratios in fewer cases and no changes in several cases. A larger number of the Th1:Th2 ratios appear to be increased and this might point to a probable shift towards increased Th1-bias with gestation. The inference of a shift towards increased Th1-bias at delivery as compared to the first trimester is also supported by the significant decrease in IL-5, IL-6 and IL-10 in the supernatants and the significant increase in IL-2 concentrations. In a study comprising normally pregnant women of whom 16 were tested by mitogen-stimulation at the first trimester and 12 in the third trimester, it was concluded (Marzi *et al.*, 1996) that a Th1 to Th2 shift characterizes the third trimester. One possible explanation for this apparent discrepancy is that Marzi *et al.* obtained blood samples at 34 ± 3 weeks of gestation, whereas our samples were collected from women at the time of delivery and our respective data may reflect the situation prevailing at the corresponding stages of gestation.

In summary, the data presented here point to an increased Th1-bias in women undergoing unexplained recurrent spontaneous abortion as compared to normal pregnant women at similar stages of gestation. It would be of great significance to demonstrate a cause-and-effect relationship between robust Th1-reactivity and pregnancy loss and to develop preventive and therapeutic methods for this problem.

Acknowledgements

This study was supported by a grant from the Kuwait Foundation for the Advancement of Sciences (Project KFAS 95–07–10).

References

Ashworth, F. (1992) Septic abortion. In Stabile, I., Grudzinskas, G. and Chard, T. (eds), *Spontaneous Abortion: Diagnosis and Treatment.* Springer-Verlag, London, p. 119.

Baines, M.G., Duclos, A.J., Antecka, E. and Haddad, E.K. (1997) Decidual infiltration and activation of macrophages leads to early embryo loss. *Am. J. Reprod. Immunol.*, **37**, 471–479.

Bennett, M.J. (1987) Congenital abnormalities of the fundus. In Bennett, M.J. and Edmonds, D.K. (eds), *Spontaneous and Recurrent Abortion.* Blackwell, Oxford, p. 109.

Berkowitz, R., Hill, J.A., Kurtz, C.B. and Anderson, D.J. (1988) Effects of products of activated leukocytes (lymphokines and monokines) on the growth of malignant trophoblast cells *in vitro. Am. J. Obstet. Gynecol.*, **151**, 199–203.

Chaouat, G., Menu, E., Clark, D.A. *et al.* (1990) Control of fetal survival in CBAxDBA/2 mice by lymphokine therapy. *J. Reprod. Fertil.*, **89**, 447–458.

Chaouat, G., Meliani, A.A., Martal, J. *et al.* (1995) IL-10 prevents naturally occurring fetal loss in the CBAxDBA/2 mating combination, and local defect in IL-10 production in this abortion-prone combination is corrected by *in vitro* injection of IFN-τ. *J. Immunol.*, **154**, 4261–4268.

Clark, D.A., Chaouat, G., Arck, P.C. *et al.* (1998) Cytokine-dependent abortions in CBAxDBA/2 mice is mediated by the procoagulin fg12 pathway. *J. Immunol.*, **160**, 545–554.

Ecker, J.L., Laufer, M.R. and Hill, J.A. (1993) Measurement of embryotoxic factors is predictive of pregnancy outcome in women with history of recurrent abortion. *Obstet. Gynecol.*, **81**, 84–87.

Gendron, R.L. and Baines, M. (1988) Immunohistological analyses of decidual natural killer cells during spontaneous abortions in mice. *Cell. Immunol.*, **113**, 261–267.

Haddad, E.K., Duclos, A.J. and Baines, M.G. (1995) Early embryo loss is associated with local production of nitric oxide by decidual mononuclear cells. *J. Exp. Med.*, **185**, 1143–1151.

Hill, J.A. (1992) Immunologic mechanisms of pregnancy maintenance and failure: a critique of theories and therapy. *Am. J. Reprod. Immunol.*, **22**, 33–42.

Hill, J.A. (1995) Immunologic factors in recurrent spontaneous abortions. In Kurpisz, M. and Fernandez, N. (eds), *Immunology of Human Reproduction.* BIOS, Oxford, pp. 401–424.

Hill, J.A., Anderson, D.J. and Polgar, K. (1995) T helper 1-type cellular immunity to trophoblast in women with recurrent spontaneous abortions. *J. Am. Med. Assoc.*, **273**, 1933–1958.

Kilpatrick, L. (1999) Influence of human leukocyte antigens and tumor necrosis factor genes in the development of pre-eclampsia. *Hum. Reprod. Update*, **5**, 94–102.

Kinsky, R., Delage, G., Rosin, M. *et al.* (1990) A murine model of NK cell mediated resorption. *Am. J. Reprod. Immunol.*, **23**, 73–77.

Krishnan, L., Guilbert, L.J., Wegmann, T.G. *et al.* (1996) T helper 1 response against *Leishmania major* in pregnant C57BL6 mice increases implantation failure and fetal resorptions. *J. Immunol.*, **156**, 653–662.

Makhseed, M., Raghupathy, R., Azizieh, F. *et al.* (1999) Mitogen-induced cytokine responses of maternal peripheral blood lymphocytes indicate a differential Th-bias in normal pregnancy and pregnancy failure. *Am. J. Reprod. Immunol.*, **42**, 273–281.

Marzi, M., Vigano, A., Trabattoni, D. *et al.* (1996) Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. *Clin. Exp. Immunol.*, **106**, 127–133.

Mosmann, T.R. and Coffman, R.L. (1989) Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.*, **7**, 145–173.

Mosmann, T.R. and Sad, S. (1996) The expanding universe of T-cell subsets. *Immunol. Today*, **17**, 138–46.

Piccinni, M.-P., Beloni, L., Livi, C. *et al.* (1998) Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. *Nature Med.*, **4**, 1020–1024.

Portnoi, M.F., Joye, N., Vanden Akker, J. *et al.* (1988) Karyotypes of 1142 couples with recurrent abortion. *Obstet. Gynecol.*, **72**, 31–42.

Raghupathy, R. (1997) Th1-type immunity is incompatible with successful pregnancy. *Immunol. Today*, **18**, 478–482.

Raghupathy, R., Makhseed, M., Azizieh, F. *et al.* (1999) Maternal Th1- and Th2-type reactivity to placental antigens in normal human pregnancy and unexplained recurrent spontaneous abortions. *Cell. Immunol.*, **196**, 122–130.

Romagnani, S. (1994) Lymphokine production by human T cells in disease states. *Annu. Rev. Immunol.*, **12**, 227–257.

Somigliana, E., Vigano, P. and Vignali, M. (1999) Endometriosis and unexplained recurrent abortion: pathological states resulting from aberrant modulation of natural killer function? *Hum. Reprod. Update*, **5**, 40–51.

Stray-Pederson, B. and Stray-Pederson, S. (1984) Etiologic factors and subsequent reproductive performance in 195 couples with a prior history of habitual abortion. *Am. J. Obstet. Gynecol.*, **148**, 140–151.

Wegmann, T.G., Lin, H., Guilbert, L. and Mosmann, T.R. (1993) Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol. Today*, **14**, 353–356.

Yui, J., Garcia-Lloret, M., Wegmann, T.G. and Guilbert, L.J. (1994) Cytotoxicity of tumor necrosis factor-α (TNF-α) and gamma-interferon (IFN-γ) against primary human placental trophoblasts. *Placenta*, **15**, 819–826.

Received on June 30, 1999; accepted on November 29, 1999