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Mediators of Inflammation, 7, 69–72 (1998)

Serum levels of pro- and anti-inflammatory cytokines in non-pregnant women, during pregnancy, labour and abortion

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DISTURBANCE of the cytokine equilibrium has been accused for many pathological disorders. Microbial infections, autoimmune diseases, graft rejection have been correlated to over- or under-production of specific cytokines which are produced as responder molecules to the various immune stimuli. The sole naturally occurring immune reaction in the organism is developed during the gestational period where, despite the presence of a semi-allogeneic graft, maternal immunoreactivity is driven to support fetal growth. The successful embryo development has been attributed to the important intervention of cytokines where some have been characterized as indispensable and others deleterious to fetal growth. However, the physiological levels of many factors during the gestational process have not been determined. Thus, in the present study we have measured and established the values of IL-1α, IL-2, IL-3, IL-4, IL-6, IL-10, IL-12, GM-CSF, TNF-α and IFN-γ during all phases of human pregnancy (first, second and third trimester of pregnancy, labour, abortions of the first trimester) as well as in the non-pregnant control state. This is an attempt to assess serum protein concentrations and present the physiological levels of these cytokines at certain time intervals providing thus a diagnostic advantage in pregnancy cases where the mother cannot immunologically support the fetus. Exploitation of this knowledge and further research may be useful for therapeutic interventions in the future.

Key words: IL-1α, IL-2, IL-3, IL-4, IL-6, IL-10, IL-12, GM-CSF, TNF-α, IFN-γ, Non-pregnant state, Abortions, Trimesters of pregnancy, Labour, Immunological implication in reproduction

Introduction

Despite the unique ability of the immune system to highly specific antigen recognition, its susceptibility to cytokines allows these molecules to dominate all kinds of immune reactions. These proteins, produced in an autocrine or paracrine fashion, bind to specific receptors initiating thus a cascade of reactions on different targets having beneficial or harmful effects since their redundancy and/or pleiotropic nature may account for all possible reactions. With T helper (Th) lymphocytes being the major producers of cytokines, it is believed that the equilibrated balance of Th1 versus Th2 cytokines defines the welfare of the organism.[1,2] The immune system, in order to ensure protection from microbial infections, autoimmune reactions, graft rejection, allergies, etc., leans the balance towards one or the other family of cytokines.

Pregnancy is a natural example of an immune reaction occurring for a determined time period in the organism which opposes the rules of graft rejection. The semi- or allogeneic fetal components growing in the privileged site of uterus, not only escape maternal immune attack but are supported by the maternal immune system. An important role in the maintenance of pregnancy is played by cytokines, where successful fetal outcome is correlated to the production of Th2 cytokines and fetal rejection to Th1 cytokines. Although the protective role of the Th2 cytokine IL-10 during mid-gestation,[3] as well as the harmful effects of Th1 cytokines IL-2,[4] TNF-α[5–7] and IFN-γ[5,8] have been demonstrated in mice, other studies show that each cytokine follows a specific pattern of expression each day of pregnancy in mice.[9] These observations indicate that despite the beneficial or harmful effect of a cytokine during a specific time course of the gestational cycle, a determined up- or downregulation of these factors must be followed, pattern that will decide for the successful or not outcome of pregnancy. It becomes thus mandatory to define the physiological levels of cytokines during

0962-9351/98/020069-04 $9.00

human pregnancy, which may finally have a prognostic character for the outcome of pregnancy.

In the present report, we examined the serum levels of 10 cytokines during the first, second and third trimesters of pregnancy, before pregnancy and labour and compared the values of physiological first trimester of pregnancy with cases of fetal rejection.

Materials and Methods

Patients

After informed consent of all patients, we analysed the serum of 15 non-pregnant-state women, the serum of 19, 17 and 18 first, second and third trimester women respectively, the serum of 15 women in labour and 19 women who underwent spontaneous abortion. All sera, isolated from 5 ml peripheral blood, were provided by the Division of Gynaecology of the University Hospital (Heraklion, Crete).

Reagents and cytokine determination

Cytokine detection in the serum was achieved by commercial kits purchased from Endogen (Cambridge, MA, USA; IL-1α, IL-2, IL-4, IL-6, IL-10, GM-CSF, TNF-α, IFN-γ) and R&D systems (Oxfordshine, UK; IL-3, IL-12). Serum cytokine measurement was performed using an ELISA method as specified by the suppliers at test and reference wavelengths of 450 and 550 nm respectively. The results are expressed in ng/ml and were compared with standard curves obtained from titrations of the corresponding recombinant factors provided by the suppliers. The Endogen ELISA kits for IL-1α, IL-4, GM-CSF and IFN-γ detect <2 pg/ml of the equivalent protein, whereas the IL-2 kit detects <6 pg/ml, IL-6 <1 pg/ml, IL-10 <3 pg/ml and TNF-α <5 pg/ml. The R&D Systems ELISA kits for IL-3 and IL-12 detect 7.4 and 5 pg/ml of protein respectively. In all cases the above values given for the lower limit of detection is the smallest dose of protein that is not zero with 95% confidence. Both manufacturers guarantee the specificity of each individual kit.

Statistical analysis

Since the abortions studied occurred during the first trimester of pregnancy, the Student's *t*-test was employed in order to compare the significance levels (P) between the abortion values of the different cytokines and those of the actual first trimester of gestation. In this particular group of analysis all P values equal or lower than 0.025 are considered to be non-significant. Degrees of freedom: 18 (n–1).

Results and Discussion

Cytokine levels in the serum seem to reflect the pathologic state of the organism and may, in many cases, have a prognostic character for therapy subscription. In this study, concentrating our interest to human pregnancy where the rates of fetal rejection dangerously increase, we define the physiological levels of 10 different cytokines before, during and after pregnancy.

Assessment of cytokine levels during pregnancy and in the non-pregnant state

The goal of this work was to determine the actual (physiological) levels of diverse cytokines during pregnancy. For this, serum from non-pregnant women as well as during the first, second and third trimester of pregnancy and labour was collected and analysed by ELISA as to the cytokine content. In addition, serum from women who underwent abortion during the first trimester of their pregnancy was also examined and the same cytokines were evaluated. Table 1 thus presents the physiological values of the

Table 1. Physiological cytokine values in human non-pregnant and pregnant state

Cytokine	Cytokine levels (ng/ml±SEM) during the following states				
	Non-pregnant state	1st trimester	2nd trimester	3rd trimester	Labour
IL-1α	1.76 ± 0.40	1.73 ± 0.53	1.55 ± 0.34	1.89 ± 0.42	1.76 ± 0.70
IL-2	3.60 ± 0.30	3.63 ± 0.60	3.56 ± 0.46	3.41 ± 0.61	3.23 ± 0.96
IL-3	3.80 ± 0.40	3.85 ± 0.52	3.36 ± 0.54	3.32 ± 0.55	3.60 ± 0.46
IL-4	0.16 ± 0.01	0.14 ± 0.02	0.16 ± 0.05	0.17 ± 0.04	0.15 ± 0.01
IL-6	2.70 ± 0.30	2.55 ± 0.24	2.91 ± 0.38	2.91 ± 0.34	2.87 ± 0.31
IL-10	0.77 ± 0.02	0.76 ± 0.07	0.74 ± 0.09	0.88 ± 0.10	0.96 ± 0.11
IL-12	0.83 ± 0.11	0.79 ± 0.10	0.85 ± 0.27	0.80 ± 0.08	0.73 ± 0.03
GM-CSF	2.70 ± 0.25	2.78 ± 0.28	3.01 ± 0.32	3.14 ± 0.73	3.52 ± 0.57
TNF-α	0.62 ± 0.04	0.60 ± 0.06	0.57 ± 0.09	0.60 ± 0.07	0.62 ± 0.03
IFN-γ	1.07 ± 0.14	1.06 ± 0.08	1.14 ± 0.11	1.33 ± 0.14	1.35 ± 0.20
n	15	19	17	18	15

cytokines studied. In the course of this analysis we detected statistically significant ($P < 0.05$ to $P < 0.001$) differences between the non-pregnant state and pregnancy for IL-1α (drop in second trimester by 13%), IL-2 (decrease mainly at labour by 11%), IL-3 (decrease at second and third trimester by 14%), IL-10 (increase at labour by 28%), GM-CSF (increase at third trimester and labour by 16% and 30% respectively) and IFN-γ (increase at third trimester and labour by 25% and 26% respectively).

By the same token, significant differences within the different pregnancy stages are obtained by IL-1α (minimal production in the second trimester), IL-2 (decrease in third trimester and labour), IL-3 (decrease after the first trimester), IL-6 (increase after the first trimester), IL-10 (increase during labour), GM-CSF and IFN-γ (steady increase after the first trimester).

TNF-α, accused for pregnancy failure,[5–7] presents a stable production profile in all stages. The same is also shown for IL-12, a factor that has been reported as an immunomodulator in various mechanisms during pregnancy.[10–12] Finally, IL-10 and IL-4, named as protective agents during pregnancy,[3,13] show a constant presence at the first two trimesters with IL-10 showing a peak of production during labour (as stated above). These four cytokines show a minimal presence in the serum of pregnant or non-pregnant women at levels ranging from 0.88 to 0.14 ng/ml, implying a possible maintenance role during the gestation period.

Assessment of cytokine levels in the first trimester abortions

The serum from women who aborted during their first trimester was also examined for the same cytokine content and the corresponding values were determined. Table 2 shows not only the cytokine values of aborted women but also a statistical comparison with the actual values (taken from Table 1) of women in their first trimester of continuing pregnancy. It is shown that although there are no differences for IL-4, IL-12 and TNF-α (we consider $P \leq 0.025$ as not significant, as stated in the Methods), the situation with the remaining cytokines is totally different. Whilst only the IL-1α and IL-3 levels drop during abortion (IL-1α not significantly by 8% or $P < 0.3$ but with a considerable 17% decrease in IL-3), on the contrary, IL-2, IL-6, IL-10, GM-CSF and IFN-γ levels increase when abortion occurs with IFN-γ being more obvious in magnitude (53% of increase). Although it is not clear yet whether increase in cytokine production is responsible for the abortion process or it is the abortion itself that modulates cytokine levels, this latter observation gives an insight to and supports the Th1 versus Th2 situation in pregnancy. It is seen here that Th1 cytokine levels are augmented during abortion whereas, Th2 are not significantly different or dropping. Another point that merits attention is the IFN-γ increased levels during abortion. This piece of data confirms previous reports and strengthens the correlation between this agent and fetal rejection.[7]

The results presented here do not differ from the recently published report by Tranchot-Diallo *et al.*[14] where modulation of cytokine gene expression (TNF-α, IFN-γ, GM-CSF, IL-1β, IL-2, IL-4, IL-6 and IL-10) by RT-PCR in pregnancy was evaluated.

Table 2. Cytokine levels during abortions occurred at the first trimester of pregnancy

Cytokine	Cytokine levels (ng/ml±SEM) during abortion in the 1st trimester	Statistical level of significance (*P*) compared with 1st trimester values
IL-1α	1.60 ± 0.46	$P < 0.3$ (NS; decrease 8%)
IL-2	4.05 ± 1.24	$P < 0.005$ (increase 12%)
IL-3	3.28 ± 0.41	$P < 0.001$ (decrease 17%)
IL-4	0.15 ± 0.01	$P < 0.025$ (NS; increase 7%)
IL-6	2.95 ± 0.40	$P < 0.001$ (increase 15%)
IL-10	0.87 ± 0.06	$P < 0.001$ (increase 14%)
IL-12	0.80 ± 0.05	$P < 0.6$ (NS; increase 1%)
GM-CSF	3.40 ± 0.02	$P < 0.001$ (increase 22%)
TNF-α	0.63 ± 0.04	$P < 0.025$ (NS; increase 5%)
IFN-γ	1.62 ± 0.16	$P < 0.001$ (increase 53%)
n	19	19

$P \leq 0.025$ levels are considered to be non-significant (NS).
Degrees of freedom: 18 (*n*–1).
n: Number of cases in each category.

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ACKNOWLEDGMENTS. This work was funded by the Greek Secretariat of Research and Technology under the framework of the EPET II programme (PENED).

Received 7 November 1997;
accepted in revised form 11 November 1997