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IL-12, IL-6 and IFN-γ production by lymphocytes of pregnant women with rheumatoid arthritis remission during pregnancy

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Background: **Rheumatoid arthritis (RA) is an autoimmune disease with progressive activity. The RA remission was observed in women during pregnancy, but the mechanism responsible for remission is hypothetical only and concerns mechanisms of immune regulation such as lymphocyte subpopulations and interleukin production.**

Aims: **The lymphocyte subpopulations and interleukin production *in vitro* in a group of healthy non-pregnant women, healthy pregnant women and pregnant women suffering from RA may help towards a better understanding of regulation of the immune processes.**

Methods: **The investigations were performed in trimester III – 2 days after delivery and 6 weeks after delivery. Peripheral blood lymphocytes were isolated on Gradisol gradient and analysed immediately or after having been cultured for 72 hours in RPMI medium supplemented with 10% FCS. The cultures were terminated after 72 h, supernatants stored at –72°C for interleukin evaluation. The concentrations of IFN-γ, IL-2, IL-6, IL-12, TNF-α and its soluble receptors R-I, R-II were estimated in non-stimulated and PHA (Sigma, 5 μg/ml) stimulated culture supernatants using ELISA Endogen kits according to the manufacturer's instructions.**

Results: **The general pattern of T cell subpopulation distribution was similar in all analysed groups. Decreased IFN-γ, IL-12 and increased IL-6 production by lymphocytes after PHA stimulation was found in trimester III in pregnant women with RA as compared to healthy pregnant woman.**

Conclusion: **The obtained results suggest that in pregnant women with RA the TH1 cell response predominates, contrary to healthy pregnant women with TH2 type functional response. These phenomena were not observed after delivery.**

Key words: Human pregnancy and RA, IFN-γ, IL-12 and IL-6 production

Introduction

Rheumatoid arthritis (RA) is an autoimmune disorder associated with HLA class II genes responsible for disease progression.[1] Autoreactive $CD4^+CD28^-$ T cells undergo clonal expansion *in vivo* and thus contribute to the pathogenesis of RA.[2] These cells produce high levels of IFN-γ and preferentially express killer cell activating receptors in the absence of inhibitory receptors.[3] Pregnancy influences the disease severity in woman with rheumatoid arthritis, but no specific factor responsible for improvement has been identified.[4] The general view exists that in pregnancy the immune relationship between mother and fetus is suppressed. Suppression in pregnancy depends on hormonal factors such as progesterone depressing the immune response and autoimmune diseases, but is effectively compensated by the innate system avoiding infection. The innate immune system components including phagocytes complement system and acute phase proteins are stimulated in pregnancy and may affect the disease. The RA clinical picture is characterised by widespread variability in a pregnant woman: remission, improvement in parameters of disease activity, nevertheless only 16 per cent of pregnant women with RA are in complete remission.[5] Pregnancy also ameliorates the experimental autoimmune uveitis in mice probably as the effect of selective reduction of Th1 response.[6] The interaction between the maternal immune system in human pregnancy and some autoimmune diseases depends on the type of dominant immune reaction: remission of RA in pregnant women is connected with exacerbation after delivery but exacerbation of systemic

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lupus erythematosus and ankylosing spondylitis exists in pregnant woman.[7] We suggest that RA remission in pregnancy is the effect of diminished IFN-γ production and misbalance in IL-6 and IL-12 production, effects which are almost reversed in the postpartum period.

Materials and methods

Patients

We have investigated the following groups of patients: 14 healthy nulliparous women, volunteers from the hospital staff, 14 healthy pregnant women, and 6 pregnant women suffering from rheumatoid arthritis (Table 1). All patients were 20–30 years old (mean 24.7). Pregnant women were observed from trimester I of pregnancy to 6 weeks after delivery (at that time a few refused). At the time of study and for at least 4 weeks before no patient had clinical signs of active infection. All patients with RA fulfilling the revised American Rheumatology Association (ARA) criteria[8] were included in this study.

Methods

The venous blood samples were collected between 8–9 am to Vacutainer probes on Heparin 10 U/ml, all tests were performed within 2 h after withdrawal. Peripheral blood lymphocytes were isolated on Gradisol gradient[9] and analysed immediately after withdrawal or after 72 h culture (2×10^6 cells/ml) in RPMI medium supplemented with 10% foetal calf serum, glutamine, and antibiotics. PHA (Sigma) 5 μg/ml lymphocytes (1×10^6/ml) were cultured in CO_2 incubator on 0.2 Nunc plates in RPMI medium supplemented with 10% of fetal calf serum (Hungarpol). The cultures were terminated after 72 h, supernatants stored at –72°C for interleukin evaluation. Measurements of IFN-γ, IL-2, IL-6, IL-12, TNF-α and it soluble receptors R-I, R-II were performed in non-stimulated and PHA stimulated culture supernatants using ELISA Endogen kits according to the manufacturer's instructions.

The lymphocytes submitted to subpopulation analyses were washed and incubated with 10% solution of mouse serum at room temperature. They were then incubated with FITC conjugated anti CD3, CD4, CD8, and CD19 mo Ab respectively, then washed and fixed with 1% paraformaldehyde solution. CD4 RO, RA and CD8 RO, RA antigen expression was evaluated by a double staining procedure and labeled cells were fixed in 1% paraformaldehyde and analyzed using a Becton–Dickinson cytometer. The results are expressed as the percentage of gated double positive cells for antibodies used versus all lymphocytes.

Table 1. Characteristics of the women investigated

Group	*n*	Age (years)	Clinical and laboratory tests
Healthy	14	20–30	No signs of disease. Blood and urine normal
Pregnant	14	19–31	No signs of disease. Blood and urine normal
Pregnant with RA	6	20–30	RF positive in 4 patients, blood and urine normal, sedimentation rate increased, no joint and muscle pain

Statistical assessments

The arithmetic means (X) and standard deviations (SD) were calculated for all parameters. Statistical analyses of differences in all of the data were done using the Student-t test using the software program Statistica. Statistical significance was set at $P \leq 0.05$.

The Institute Ethics Committee approved the experimental protocol. All reported investigations have been performed in accordance with the Declaration of Human Rights (Helsinki 1964). Blood samples were generally taken in the course of routine examinations. Informed consent was obtained from all investigated patients.

Results

The general pattern of T cell subpopulation distribution was similar in all analysed groups, differences in individual results were usually high therefore the statistical significance could not be calculated for most of the analysed results. The significant differences are outlined as follows: CD19 (B lymphocytes) decreased in pregnant women but significantly increased in pregnant women with RA. These differences disappeared 6 weeks after delivery. The percentage of NK cells in healthy pregnant women decreased from 17.4 in trimester III, 18.5 two days after delivery to 7.0 6 weeks after delivery; in pregnant women with RA the respective data are 9.4, 10.4 and 11.5. *In vitro* culture did not reveal any differences in the pattern of CD antigen distribution.

The ability to produce IL-12, IL-6 and IFN-γ by peripheral blood mononuclear cells (PBMC) stimulated with PHA *in vitro* revealed dynamic changes dependent on pregnancy and the clinical status of the investigated women (Fig. 1A,B,C). IL-12 (Fig. 1A) production by stimulated PBMC of pregnant women with RA was diminished in trimester III of pregnancy, significantly increased 2 days after delivery and normalised 6 weeks after delivery. IL-6 (Fig. 1B) production by stimulated PBMC was generally enhanced in pregnant women suffering from RA. IFN-γ (Fig. 1C) production by stimulated PBMC of healthy pregnant woman was enhanced in trimester III and normalised after delivery contrary to PBMC of pregnant woman suffering from RA. Surprisingly, the TNF-α

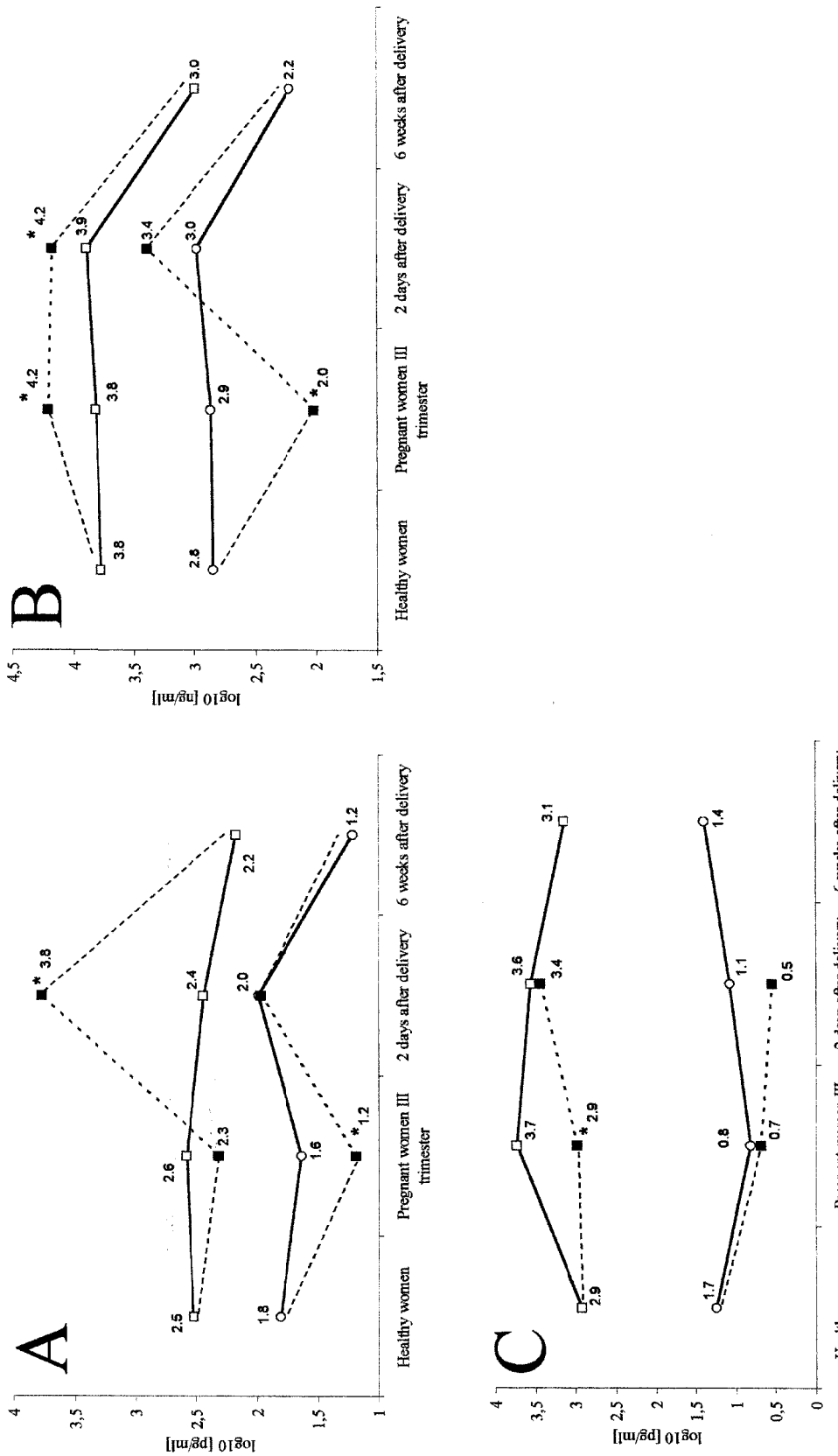

FIG. 1. (A) IL-12, (B) IL-6 and (C) IFN-γ production by PBMC of healthy pregnant (open circles and squares) and pregnant women with RA (closed circles and squares) in 72 h cultures non-stimulated and stimulated by PHA. The results are presented on a logarithmic scale. *Statistical significance $P \leq 0.05$ as compared to healthy pregnant women PBMC.

production by stimulated PBMC and TNF-α receptors (R-I and R-II) shedding generally did not reveal significant differences between analysed groups (not shown). The obtained data have suggested a specific regulatory effect in pregnant women with RA on the ability of PBMC to produce some cytokines that affect clinical outcome.

Discussion

Human pregnancy is compared with tissue transplantation but clearly is not the same. Fetal allograft does not require surgical trauma, it takes place in a unique specialized organ i.e. uterus, grows progressively as the placenta produces hormones and cytokines responsible for suppression of specific immune reactions, occurring during establishment and maintenance of pregnancy.[5,10] Successful implantation of pregnancy is thought to require the establishment of Th2 cytokines profile of CD4 cells. Progesterone and interleukins 4 and 10 suppress Th1 cytokine responses and this is predicted to be responsible for remission of rheumatoid arthritis during pregnancy.[10] The central role in the adaptation of suppressed maternal immune system in pregnancy to avoid infection is activation of the innate immune reaction such a complement system, phagocytosis, oxygen burst, IL-12 and chemokines production which is in some way comparable to sepsis.[11] The increased IFN-γ production in human uterine endometrium also plays an important role in modulating immune responses to infectious challenge. IFN-γ makes the lymphocytes and neutrophils functionally hyperactive and can exert an autocrine stimulatory effect.[11] We have observed the significantly enhanced IFN-γ production by PBL of pregnant woman after PHA stimulation *in vitro*. PBL of pregnant women with RA in the same conditions do not produce an enhanced amount of IFN-γ. Dendritic cells derived from non-obese diabetic mice treated *in vitro* with IFN-γ could protect from diabetes when transferred into susceptible mice.[12] Hypothetically, the same mechanisms responsible for INF-γ production in normal human pregnancy can effectively protect from autoimmune diseases. Diminished IFN-γ production in pregnant women with RA may be responsible for disease exacerbation.

IL-6 is required for the development of autoimmune arthritis; IL-6 deficient mice are resistant to the induction of autoimmune disease.[13] It has been found that IL-6 is required for disease development in the rodent autoimmune model. IL-6 pretreated dendritic cells can process autoantigen, present cryptic determinants to T cells and contribute to the autoimmunity.[13] Slightly diminished IL-6 production by non-stimulated lymphocytes from pregnant woman with RA and enhanced IL-6 production after PHA stimulation has proved the possibility that IL-6 can play a key role in the development of arthritis in these patients. Reduced severity of RA was observed in IL-6 deficient mice.[14] The rational explanation of these differences is difficult but could suggest the existence of specific regulatory mechanisms involved in RA during pregnancy. Anti IL-12 and anti TNF-antibodies suppress the progression of murine experimental arthritis.[15] Blocking the functional IL-12 secretion by dendritic cells inhibits IFN-γ production.[16] The reduced amounts of IL-12 production *in vitro* by stimulated PBL of pregnant women with RA may result in the generation of Th1 cells. Exogenous IL-12 modified the development of Th cells primed *in vitro* by dendritic cells, which resulted in the generation of Th1-like cells from naive precursor. However, *in vivo* dendritic cells are effective inducers of both Th1- and Th2-type cytokines. Our results show that diminished IL-12 generation by lymphocytes of pregnant women with RA in trimester III correlated with enhanced IL-6 production and lowered IFN-γ secretion which may result in a Th1 cell-biasing function contrary to the observed down-regulation of Th1 response during normal pregnancy.[5] McRae *et al.*,[16] using purified cell lines proved that IL-12 promotes differentiation of CD4 T cells to produce IFN-γ. Diminished IL-12 production in trimester III of pregnancy may also result in inhibition of IFN-γ production but IL-12 overproduction after delivery does not confirm such a hypothesis and suggests that the mechanism responsible for RA exacerbation in the postpartum period is more complicated. An immunomodulatory effect of non-inherited HLA antigens cannot be excluded.[5]

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