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Pregnancy in patients with rheumatic disease: Anti-inflammatory cytokines increase in pregnancy and decrease post partum.

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Abstract

Objective
To investigate changes in the levels of circulating cytokines with a focus on the Th1/Th2 balance during and after pregnancy in patients with rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA) and ankylosing spondylitis (AS).

Methods
Plasma and serum samples of 19 RA, 6 JIA, and 9 AS pregnant patients, of 30 healthy pregnant women, 20 non-pregnant patients and 10 non-pregnant healthy women were analysed for levels of IFNγ, IL-1β, IL-10, IL-1Ra, soluble TNFR (sTNFR), and soluble CD30 (sCD30) by ELISA. Clinical assessment and blood sampling in pregnant women was done once in each trimester and 6, 12 and 24 weeks post-partum. Disease activity in the patients was evaluated by validated clinical instruments and correlated to circulating levels of cytokines.

Results
Low levels of IL-10 were sporadically found whereas IFNγ and IL-1β were below detection level in the samples tested. Significantly higher concentrations of sTNFR and IL-1Ra were measured in pregnant compared to non-pregnant individuals. An increase of IL-1Ra from the second to the third trimester correlated with improvement of disease activity in both RA and AS patients. Compared to non-pregnant patients and to the other pregnant women, RA patients showed markedly elevated levels of sCD30 during pregnancy.

Conclusions
IFN-γ and IL-10, markers of a Th1 and Th2 response respectively, were either low or undetectable in the cohorts analyzed. The increase of cytokine inhibitors IL-1Ra and sTNFR proved to be a pregnancy related phenomenon independent of an underlying disease. An effect of these anti-inflammatory mediators on disease activity appears likely.

Key words: immune response – pregnancy – rheumatic disease - cytokines
Introduction

Several autoimmune, rheumatic diseases show a modulation of disease activity during and after pregnancy. Rheumatoid arthritis (RA) improves in the majority of patients (1), whereas ankylosing spondylitis (AS) remains active and is mitigated only in late pregnancy (2). An aggravation of disease symptoms after delivery is commonly seen in both diseases and occurs in general within the first six months post-partum. Pregnancy induces changes in the maternal immune system in order to protect the fetus from immunologic attack by the mother. Research over the last decade has indicated that no general immunosuppression takes place in the maternal system, rather a shift from a prevailing Th1 response to a type Th2 response (3). CD4+ T cells can be divided in two subsets: one is the T helper 1 type characterized by the production of interferon gamma (IFN\(\gamma\)), interleukin 12 (IL-12), tumor necrosis factor \(\beta\) (TNF\(\beta\)) and interleukin-2 (IL-2) and involved in cell mediated immunity. The other T cell subset consists of Th-2 committed cells which mainly produce interleukin 4 (IL-4), interleukin 10 (IL-10) and interleukin 13 (IL-13), thereby enhancing humoral immunity. The immunological changes taking place during and after pregnancy may modulate disease symptoms according to the underlying pathophysiology of the disease in question. In RA, a Th1 type immune response is predominant whereas a Th0 or Th2 type prevails in AS (4).

IFN-\(\gamma\) is a major contributor to a Th1 immune response upregulating Th1 cell differentiation and inhibiting Th2 cell development. IL-1\(\beta\) and TNF-\(\alpha\) are proinflammatory cytokines that contribute to synovitis and joint destruction in both RA and AS (5-7). IL-10 down-regulates production of pro-inflammatory cytokines by Th1 cells and macrophages. In pregnancy, IL-10 counteracts pregnancy related disorders, such as fetal growth restriction as well as fetal death and preeclampsia (3, 8). The immune modulating activities of cytokines are also regulated by soluble cytokine receptors like TNF receptor (TNFR) which can buffer the biological effects of TNF-\(\alpha\).(9) Another natural inhibitory mechanism involves the blocking of receptor binding by cytokine receptor antagonists like IL-1Ra.(10)

The CD30 receptor belongs to the tumor necrosis factor/nerve growth factor receptor superfamily. CD30 is normally expressed on a subset of activated T cells producing Th2 type cytokines(11). The soluble form of CD30 (sCD30) released upon T cell activation by proteolytic cleavage is regarded as a measure of CD30 turnover. Elevated levels of sCD30 have been detected in Th2-dominated diseases like systemic lupus erythematosus (12), systemic sclerosis (13), ulcerative colitis (14) and allergic disorders (15).

The objective of this study was to investigate changes of circulating cytokines and regulatory molecules with a focus on the Th1/Th2 balance during and after pregnancy in patients and healthy controls. By analysing the type of cytokine pattern we sought to determine if the different clinical responses to pregnancy were related to a particular immune response.
Patients and Methods

Patients and samples
The longitudinal prospective study was performed at the Department of Rheumatology and Clinical Immunology/Allergology of the University Hospital of Bern after approval by the institutional review board of Bern. Additional sequential serum samples (collected after centrifugation of venous blood) of ten pregnant patients with RA and 6 patients with juvenile idiopathic arthritis (JIA) and from 8 healthy pregnant women were provided by Professor JL. Nelson, Seattle, USA and by Dr. G. Hebisch, Department of Gynecology at the University Hospital of Zürich, respectively.

A total of 19 pregnant patients with RA, six with JIA, 9 patients with AS (10 pregnancies) and 30 age-matched healthy pregnant women were included in the study. Ten non-pregnant, age matched healthy women were included to get the background measurements for the age group, and nine non-pregnant RA patients and 11 AS patients provided background data of a chronic, inflammatory disease. RA patients fulfilled the American College of Rheumatology (ACR) criteria (16), and AS patients the criteria of the European Spondylarthropathy Study Group (ESSG) (17). Mean age of pregnant patients was 31 (range 21-38), of pregnant healthy women 33 (21-40), of non-pregnant patients 30 (24-37) and of non-pregnant controls 29 years (range 24-35). At the time of blood sampling, none of the participating women had any known infections or was close to labour.

All pregnant women recruited at the University Hospital of Bern were assessed by clinical examination and measurement of CRP at each trimester of pregnancy: gestational week 9-12, gestational week 20-23, gestational week 32-34 and 6, 12 and 24 weeks post-partum. Four RA and four AS patients and three healthy women also had blood sampling 8-12 weeks before the index pregnancy. Clinical evaluation was done by the RA Disease Activity Index as well as the tender and swollen joint count (Radai) (18) in RA patients and by the Bath Ankylosing Spondylitis Activity Index (Basdai) (19) in AS. Overall disease activity was measured by the physician’s global assessment (PGA, a visual analogue scale from 0-100) (20). A remission of the disease during pregnancy was defined as a PGA score of zero, improvement as a decrease of PGA of 20 and active disease by a PGA score above 20. In all pregnant patients recruited by Dr. JL. Nelson, disease activity was expressed along three dimensions: remitted, improved or active. The six JIA patients had polyarticular disease and five were positive for rheumatoid factor. Their serum samples were analyzed together with the RA patients.

Medication during pregnancy: At study entry six patients were on intramuscular gold or sulfasalazine, seven on low-dose prednisone, eight on non-steroidal anti-inflammatory drugs (NSAID). At the third trimester, two were on sulfasalazine, three on prednisone and five on NSAID. At the post partum aggravation, 28 patients received any of the mentioned drugs or anti-TNF therapy.

ELISA
Venous blood samples obtained from patients and controls were drawn into tubes containing 3.8% trisodium citrate as an anticoagulant. Plasma was prepared by centrifugation for 15 min. and aliquots were stored at –80°C. Samples were thawed and assayed for levels of soluble TNF receptor (p75), IL1-Ra and soluble CD30 (sCD30) by solid phase sandwich enzyme-linked immunosorbent assay (ELISA). The minimal detectable concentration of for the sTNFR ELISA (R&D Systems, Minneapolis, USA) and the IL-1Ra ELISA (R&D Systems, Minneapolis, USA) were less than 1.0 pg/ml and 14 pg/ml, respectively. The instant ELISA for sCD30 (Bender MedSystems, Vienna, Austria) had a detection limit of 0.5 Units/ml. ELISA kits were also used to detect levels of IFNγ (Biosource International, Europe, Nivelles, Belgium), IL-1β (R&D Systems, Minneapolis, USA) and IL-10 (Immunotech, Marseille, France) in the plasma. Of the latter assays the detection limits were as follows: <4pg/ml, <1 pg/ml and 5 pg/ml. Intra-assay precision and inter-assay precision of all ELISA applied were below 10%. All cytokine assays were performed according to the manufacturer's instructions.
Statistics
Data were analyzed using the SPSS 11.0 software package. The associations between clinical measurements of disease activity and the TNFR, IL-1Ra, and sCD30 respectively were analyzed using the Spearman’s rank correlation. Correlation was considered strong if the correlation coefficient was $\geq 0.70$, moderate to substantial between 0.30 and 0.70, and weak if $\leq 0.30$. We applied the Mann-Whitney U-test to compare the median levels of the cytokines measured in pregnant individuals with those of the non-pregnant controls. To analyze the longitudinal change of cytokines we used the Wilcoxon test for paired data. Graphical presentation of the summary scores are given in box plots displaying the median and $25^{th}$-$75^{th}$ percentiles and the range of values. $P$-values $<0.05$ were considered statistically significant.
Results

Patients characteristics and clinical outcome during pregnancy

All patients and healthy women had uncomplicated pregnancies with delivery of healthy children between 36 and 40 weeks of gestation. Improvement occurred in 17 RA and in five JIA pregnancies, the remaining three patients stayed active during pregnancy. Except for two AS patients with minimal disease activity at study entry, a varying disease course with active disease in the first and second trimester was observed in eight pregnancies. Four AS patients showed improvement in the third trimester. Aggravation of disease symptoms occurred in 22 RA/JIA patients and in eight AS patients between 6-12 weeks after delivery.

Cytokines and Cytokine-receptors

Low levels of IL-10 were sporadically found in five samples whereas IFNγ and IL-1β were below detection levels in the samples tested. For reason of start of anti-TNF therapy, the sTNFR values of two RA patients measured in the post-partum period were excluded.

Circulating levels of sTNFR, IL-1Ra, sCD30 in pregnant women compared to non-pregnant women (Figure 1)

Significantly higher circulating levels of sTNFR (Figure 1a: RA: p<0.001, AS p<0.001, healthy: p=0.001) were measured in the cohorts of pregnant women compared to non-pregnant age-matched women. Levels of IL-1Ra were significantly elevated in pregnant healthy women and pregnant RA patients compared to non-pregnant controls (Figure 1b: healthy: p=0.001, RA: p=0.034), and they exceeded levels measured in pregnant AS patients (RA: p=0.002; healthy: p=0.013). Only RA patients showed significantly higher levels of sCD30 (Figure 1c) in pregnant compared to non-pregnant individuals (p=0.001), and compared to pregnancy levels of AS patients (p<0.001) and healthy women (p=0.001). Among the groups of non-pregnant women, no significant differences could be observed for sTNFR, IL-1Ra and sCD30.

Changes of sTNFR-75, IL-1Ra and sCD30 during and after pregnancy (figures 2-4).

A gradual albeit not significant increase of sTNFR occurred both in healthy women and patients during pregnancy followed by a significant decrease of sTNFR post partum (Figure 2 a,b,c). A moderate negative correlation (r=-0.448, p=0.05) was observed between sTNFR and RADAI. None of the other clinical assessments correlated with levels of sTNFR.

In RA and AS patients, a significant rise of IL-1Ra appeared from the second to the third trimester of pregnancy (Figure 3 a,b). In parallel, disease activity as measured by the physician’s global assessment was at its lowest in the second and third trimester. In healthy women no significant increase of IL-1Ra was found during pregnancy, but a significant decrease of IL-1Ra occurred from 6 to 12 weeks post partum (Figure 3 c). Levels of IL-1Ra in RA and AS patients correlated weakly to moderate with disease activity measured by the physician’s global assessment (r=-0.269, p=0.01), the tender joint count (-0.349, p=0.01) and the swollen joint count (-0.263, p=0.01), but not with RADAI or BASDAI.

sCD30 decreased gradually during pregnancy and showed an increase at six weeks post partum in the three groups of pregnant women (Figure 4a,b,c). In RA and AS patients, levels of sCD30 did not correlate with any of the clinical assessment instruments.
Discussion

Our longitudinal study comparing pregnant patients with rheumatic disease to healthy pregnant women found similar cytokine profiles in both groups with few quantitative differences. This can be expected in patients who enter pregnancy in a state of low disease activity and with little or no organ involvement as did our patients. Thus the physiological alterations induced by pregnancy are not profoundly affected by a chronic inflammatory state, but provide a cytokine milieu that supports pregnancy. In agreement with others studies comparing healthy pregnant women to non-pregnant controls (21 22), we found elevated levels of IL-1Ra and sTNFR in pregnant patients and controls whereas IL-1β, IFNγ and IL-10 were undetectable. Conflicting results exist in regard to circulating levels for IFNγ and IL-1β which have been found either absent or in low levels during pregnancy (23, 24 25). The inability to detect circulating IL-10 in pregnancy corresponds to another longitudinal study investigating IL-10 in plasma of healthy pregnant women at each trimester (22). By contrast, low levels of IL-10 were found in a cross-sectional study of second and third trimester women (26).

Inflammatory rheumatic diseases are associated with a proinflammatory cytokine profile systemically and locally not only at times of active symptoms, but also during quiescent disease (8,9,27, 28). Upregulation of the inflammatory cytokine IL-1β has been reported in RA and AS, sometimes accompanied by a IL-1Ra deficiency (29,30,31). Treatment with the recombinant TNFR:Fc fusion protein and with recombinant IL-1Ra have shown good efficacy in RA and AS (32 33 34 35). Thus, increased levels of IL-1Ra in the third trimester might overcome a deficiency in pregnant RA and AS patients and lead to improvement of the disease. The negative correlation between IL-1Ra and disease activity supports this notion. Likewise, elevated sTNFR levels in pregnant RA and AS patients could contribute to an improvement of the disease in late pregnancy.

It has been suggested that the remission, so often observed in RA during pregnancy, may relate to the increased gestational secretion of IL-10 resulting in the induction of tolerance (36). In our study, IL-10 was either below detection levels or present in few RA patients with active disease post partum. Even in the absence of elevated circulating levels of IL-10, T cells of peripheral blood could still increase their IL-10 secretion as shown recently in four pregnant RA patients (37). However, the role of IL-10 in joint inflammation is ambiguous. Experiments on joint tissues have shown suppression of inflammation by IL10 (38). Furthermore, Th2 committed T cells and regulatory T cells which counteract inflammation and autoimmunity produce high levels of IL-10 (39,14). On the other hand, a study of IL-10 polymorphism in RA found an association between high production of IL-10 and joint destruction (10). This may in part be due to the effect of IL-10 on monocyte maturation rendering them into tissue infiltrating cells (41) or to the stimulation of autoantibody production and enhancement of Fc gamma receptor expression on monocytes (42). Also in patients with active spondylarthropathy elevated levels of circulating IL-10 have been found (29).

sCD30 is an indirect marker for a Th2 immune response and can be induced by progesterone together with IL-4 production (43). A rise of sCD30 during pregnancy would therefore be expected. Interestingly, this was observed exclusively in our pregnant RA patients who had markedly higher gestational levels of sCD30 compared to the other groups of pregnant women and to non-pregnant patient cohorts. Due to the few pre-pregnancy samples available, no statement can be made whether sCD30 levels increased during the first trimester in RA patients and then decreased again. A counter-regulatory role of CD30+ T cells providing an attempt to control inflammation in a Th1-driven disease like RA has been discussed (44), and higher circulating levels of sCD30 have been found in non-pregnant RA patients compared to healthy controls (45). The latter finding was not confirmed by our results. In our longitudinal study, concentrations of sCD30 in patients and healthy controls decreased progressively during pregnancy, possibly as a result of the increasing plasma volume. The same dynamics were previously shown by others investigating sCD30 in healthy pregnant women (46). However, by analyzing the ratio of sCD26, a Th1-related molecule, and sCD30, a predominance of the Th2 marker was demonstrated throughout pregnancy. Circulating sCD30 levels in our cohort of
pregnant RA patients did not correlate with disease activity measurements. This is at variance with another study showing an inverse correlation between sCD30 and CRP (47). Lack of correlation in our study was probably due to the low range of disease activity with mostly normal CRP levels among our RA patients.

Our study has several limitations, like the limited number of patients included and the few women with pre-pregnancy measurements. The gestational rise of a marker is sometimes only reflected by a significant drop after delivery and the significant difference of circulating levels in pregnant and non-pregnant individuals. Taking into account the dilution effect of the increasing plasma volume during pregnancy, underscores that indeed an increase of the measured protein has taken place. A cytokine increase might also be hidden in the large inter-individual variation that arises from different levels of disease activity at study entry, from therapy, and from genetic variation. However, the limited number of patients does not allow a separate analysis for subgroups.

To summarize, among the cytokines and regulatory molecules tested for, an elevation of the anti-inflammatory cytokines IL-1 Ra and sTNFR was found during pregnancy. IFN-γ and IL-10, markers of a Th1 and Th2 response respectively, were either low or undetectable. It cannot be excluded that at a local level a more pronounced change in the Th1/Th2 balance during and after pregnancy could have been measured. In addition, upregulation of IL-1Ra and sTNFR as well as sCD30 in RA patients during pregnancy may be indirect signs of a Th2 immune response since Th2 cytokines have been shown to induce the production of both sTNFR and IL-1Ra (48 49). On the other hand, evidence accumulates that pregnancy promotes not only a Th2 response, but rather an immunomodulation that is complex and dependent on the stage of pregnancy (50). Since most of the disease activity markers did not correlate to the changes of circulating cytokines, the extent to which they contribute to pregnancy related improvement of disease activity in the third trimester remains open.
The study was supported by grant 31-59979 from the Swiss National Research Fund, and by grants from the Kurt and Senta Hermann-Stiftung and from the Swiss League against Rheumatism.
Legend, figure 1:

Levels of sTNFR (a), IL-1Ra (b) and sCD 30 (c) in patients with rheumatoid arthritis (shaded bars), ankylosing spondylitis (dark grey bars) as well as in healthy women (white bars). Values presented in the group of non-pregnant (no pre-pregnancy or post-partum data included) and pregnant (pooled data of 1., 2. and 3. trimester) individuals. Horizontal bar within the box marks the median, the boxes represent the range of ± 25% around the median (interquartile range). Vertical bars indicate 95% confidence interval.

+ = significant difference compared to the non-pregnant control group (p<0.05 by Mann-Whitney U-test)
‡ = significant difference compared to pregnant ankylosing spondylitis patients (p<0.05 by Mann-Whitney U-test)
† = significant difference compared to healthy pregnant women (p<0.05 by Mann-Whitney U-test)

Legend, figure 2:

Levels of sTNFR before, during (1., 2., 3. trimester) and after pregnancy (6, 12 and 24 weeks post-partum) in patients with rheumatoid arthritis (a), ankylosing spondylitis (b) and healthy controls (c). Horizontal bar within the box marks the median, the boxes represent the range of ± 25% around the median (interquartile range). Vertical bars indicate 95% confidence interval. Significant changes indicated by p-values (Wilcoxon-test for paired data).

Legend, figure 3:

Levels of IL-1Ra before, during (1., 2., 3. trimester) and after pregnancy (6, 12 and 24 weeks post-partum) in patients with rheumatoid arthritis (a), ankylosing spondylitis (b) and healthy controls (c). Horizontal bar within the box marks the median, the boxes represent the range of ± 25% around the median (interquartile range). Vertical bars indicate 95% confidence interval. Significant changes indicated by p-values (Wilcoxon-test for paired data).

Legend, figure 4:

Levels of sCD 30 before, during (1., 2., 3. trimester) and after pregnancy (6, 12 and 24 weeks post-partum) in patients with rheumatoid arthritis (a), ankylosing spondylitis (b) and healthy controls (c). Horizontal bar within the box marks the median, the boxes represent the range of ± 25% around the median (interquartile range). Vertical bars indicate 95% confidence interval. Significant changes indicated by p-values (Wilcoxon-test for paired data).
Statement
There are no competing interests for any of the authors.
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Figure 1

a) soluble TNFR
b) IL-1Ra
c) soluble CD30
Figure 2

a) rheumatoid arthritis

b) ankylosing spondylitis

c) healthy controls

soluble TNFR (pg/ml)

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p = 0.036
p = 0.036
p < 0.001
p = 0.033
p = 0.033
p = 0.028
p = 0.028
p = 0.028
Figure 3

a) rheumatoid arthritis

b) ankylosing spondylitis

c) healthy controls

IL-1Ra (pg/ml)
Figure 4

a) rheumatoid arthritis

b) ankylosing spondylitis

c) healthy controls

soluble CD 30 (U/ml)

N = 4 13 21 26 25 16 10

N = 4 7 8 9 8 8 5

N = 2 24 27 26 21 21 13
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