Cytokine secretion by deciduous lymphocytes in transient hypertension of pregnancy and pre-eclampsia

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BACKGROUND: Transient hypertension (TH) and pre-eclampsia (PE) are believed to have different pathophysiology. However, 15–25% of pregnant women initially diagnosed as having TH develop PE. To clarify the immunopathogenetical connections between the two syndromes, we studied the pattern of T helper cell (Th)1/Th2 cytokine balance disturbances existing inside maternal decidua in normal pregnancy (NP) and pregnancies complicated with TH and PE.

Methods: Third-trimester decidual tissue was obtained by curettage of uterine cavity during elective caesarean sections in NP (n = 11), TH (n = 17) and PE (n = 21) patients. Cell suspensions were prepared by an electromechanical dispersal method and centrifuged using a standard gradient sedimentation technique. Isolated lymphocytes were placed in medium (RPMI 1640, 10% fetal calf serum, L-glutamine, penicillin, streptomycin) and cultured for 72h with or without mitogen phytohaemagglutinin (PHA). The enzyme-linked immunosorbent assay method was used for estimation of interleukin (IL)-2, IL-4, IL-6, IL-10, IL-12 and interferon-γ (IFNγ) in culture supernatant.

Statistical analysis: The Kruskal–Wallis and the Mann–Whitney U tests were used (p < 0.05).

Results: Both spontaneous and PHA-stimulated secretion of Th2-type cytokines IL-6 and IL-10 was decreased in PE patients compared with TH and NP patients. The concentration of Th1-type cytokine IFNγ was increased in patients suffering both from TH and PE.

Conclusion: On the base of decidual cytokine secretion, both PE and TH are syndromes of local Th1/Th2 cytokine balance disturbances as compared with NP, and TH seems to be an intermediate step to PE.

Key words: Lymphocyte, Cytokine, Decidua, Pre-eclampsia, Transient hypertension

Introduction

Although the clinical presentations of pre-eclampsia–eclampsia and transient hypertension of pregnancy at least partially overlap, opinion exists that they are two distinct conditions¹ with different pathophysiology²–⁵ and outcome.⁶ However, epidemiological analysis indicates that approximately 15–25% of pregnant women initially diagnosed as having transient hypertension develop fully symptomatic pre-eclampsia later in pregnancy.⁷ This means that, in some patients, transient hypertension could probably represent an intermediate step in progression to pre-eclampsia.

The involvement of abnormal activation of the innate and acquired immune system in the pathogenesis of pre-eclampsia is well documented.⁸⁹ The elevated serum level of interleukin (IL)-12,¹⁰ fetal monocyte activation⁹ and deficiency of placental IL-10 production¹¹ are responsible for enhanced T helper (Th)1-type cell maturation and hampered immunological tolerance of pre-eclamptic women to the foreign antigens of the fetus. The existence of a superantigen-like effect in a subset of patients¹² supports the idea of the immunopathogenetical background of pre-eclampsia.

A widely accepted hypothesis originally presented by Wegman et al.¹³ underlines the crucial role of Th2-activity cytokines in promoting successful pregnancy. Since that time, existence of the ‘Th2-phenomenon’ during normal human pregnancy has been confirmed by many authors.¹⁴–¹⁸ Pre-eclampsia is accompanied with elevated serum levels of Th1-type cytokines IL-12¹⁰ and tumor necrosis factor-α (TNFα),¹⁹ as well as Th2-type cytokine IL-6.¹⁰ Uproegulation of Th1-activity responses was also shown ‘in vitro’ by increased interferon-γ (IFNγ),¹⁴ IL-2 and TNFα production by peripheral blood lymphocytes. IL-2 and IL-12 stimulate Th1-like cell maturation²¹,²² and TNFα may
contribute for endothelial activation and failure observed in pre-eclampsia.\(^1\)\(^9\) The predominance of Th1-type activity was accompanied by the effect of decreased Th2-type suppressory cytokine IL-10 secretion by peripheral blood lymphocytes ‘in vitro’\(^2\)\(^0\) as well as in placental samples.\(^1\)\(^1\) The investigations performed during pregnancy complicated with pre-eclampsia indicated that disturbances of cytokine Th1/Th2 balance are associated with elevated endothelin-1 levels followed by the presence of hypertension.\(^2\)\(^3\)

None of the aforementioned studies referred to the autocrine pattern of Th1/Th2 cytokine balance inside maternal decidua in pre-eclampsia and transient hypertension, and their potential role in understanding the local regulatory mechanisms seen in those two syndromes. To clarify this subject, we have focused our attention on comparison between spontaneous and mitogen-stimulated production of IL-2, IL-4, IL-6, IL-10, IL-12 and IFN\(_\gamma\) in cultured lymphocytes isolated from third trimester decidua of healthy pregnant women, pre-eclamptics and those pregnant with transient hypertension. The obtained results have suggested that disturbances in autocrine regulation may be decisive for disease outcome.

**Materials and methods**

**Patients**

The study group was chosen from pregnant women who were hospitalized between September 1998 and January 2001 in three departments: the Department of Materno-Fetal Medicine, the Department of Perinatology and the Department of the Obstetrics of Polish Mother’s Health Center Research Institute, Lodz, Poland.

Inclusion criteria were normal pregnancy, and pregnancy complicated with transient hypertension or pre-eclampsia. Pre-eclampsia was characterized by an increase in systolic pressure of 30 mmHg or of diastolic pressure of 15 mmHg compared with blood pressure measurements obtained before 20 gestational weeks (or, if these pressure levels were not known, a blood pressure of 140/90 mmHg or greater obtained in two consecutive measurements 6 h apart after 20 gestational weeks), with concurrent proteinuria greater than 0.3 g per 24 h or greater than 30 mg/dl in a specimen. Transient hypertension was characterized by the same blood pressure measurements but without proteinuria or with proteinuria less than 0.3 g per 24 h.\(^1\)

Exclusion criteria were diabetes mellitus, gestational diabetes mellitus, renal diseases, chronic hypertension that predated pregnancy, infectious diseases recognized in the course of pregnancy, presence of premature or full-term uterine contractions, premature rupture of membranes or clinical chorioamnionitis. The pregnant women were not treated with corticosteroids before inclusion for the study. Establishment of such a wide range of exclusion criteria allowed us to eliminate possible clinical situations that could have a strong impact either on the composition of decidual lymphocytes or on the pattern of cytokine secretion.

Finally, 49 pregnant women were chosen for the study and, in all cases, the selection was made before decidual material was collected. All of them had been qualified for elective caesarean sections. In this group, 11 pregnant women had normal pregnancy (controls), 17 presented with transient hypertension of pregnancy and 21 with pre-eclampsia. Some of the data characterizing each group are presented in Table 1. In the group of controls, the indications for elective caesarean sections were retinal degeneration changes, serious cardiac defects, pelvic deformations, cerebral vessels malformations, multifetal pregnancy and breech position in women with high-risk pregnancy. In the group of transiently hypertensive patients the most common indication

| Parameter                     | Normal pregnancy (\(n = 11\)) | Transient hypertension (\(n = 17\)) | Pre-eclampsia (\(n = 21\)) |
|-------------------------------|-------------------------------|-------------------------------------|-----------------------------|
| Patient age (years)           | 29.5 ± 5.7                    | 28.6 ± 5.3                          | 28.7 ± 6.1                  |
| Gestational age (weeks)       | 36.5 ± 4.9                    | 35.1 ± 3.5                          | 33.3 ± 2.7                  |
| Primigravidae (\(n\))         | 8                             | 13                                  | 16                          |
| Mean systolic pressure (mmHg) | 127.0 ± 7.8                   | 171.9 ± 15.8                        | 178.5 ± 21.9                |
| Mean diastolic pressure (mmHg)| 78.7 ± 7.7                    | 107.1 ± 10.8                        | 111.6 ± 12.3                |
| Proteinuria (mg/dl)           | –                             | 28.3 ± 2.9                          | 509.9 ± 851.7               |
| Proteinuria/24 h (g/24 h)     | –                             | –                                   | 4.9 ± 6.1                   |
| Serum uric acid (mg/dl)       | 3.8 ± 1.3                     | 5.7 ± 2.0                           | 7.0 ± 1.9                   |
| Platelet count (G/l)          | 235.7 ± 43.8                  | 208.8 ± 40.9                        | 204.7 ± 55.6                |
| Serum asparagine aminotransferase (IU/l) | 27.9 ± 4.1                  | 40.7 ± 19.8                        | 54.5 ± 30.8                 |
| Serum alanin aminotransferase (IU/l) | 17.4 ± 15.2                  | 27.6 ± 13.2                        | 62.4 ± 55.2                 |

Data presented as mean ± standard deviation.
was severe hypertension complicated with cardio-
tocographic signs of fetal distress, while in pre-
eclamptic patients the indications were symptoms of
imminent eclampsia and/or cardio-tocographic signs
of threaten fetal asphyxia. All patients gave written
and informed consent for participation in clinical
research and the agreement of the Polish Mother's
Health Center Research Institute Ethical Committee
for performing the study was obtained.

Cell isolation
Third-trimester maternal decidual tissue was obtained
by curettage of uterine cavity during caesarean
sections. The blood clots and fragments of the fetal
membranes were removed macroscopically using
sterile pincers. Then the samples were placed into
bottles containing sterile phosphate-buffered saline
(PBS) (WSS, Lublin, Poland). Some of the collected
samples after initial washing in PBS were randomly
submitted to histopathological examination, which
revealed that trophoblastic villi were present in less
than 5% of the specimen volume, giving the certainty
that samples contained almost pure decidua. Inside
laminar flow cabinet decidual slices were rinsed
several times in PBS to remove residual blood and
then mechanically disaggregated to fragments of
approximately 3–10 mm³ volume. Subsequent decid-
ual cell suspensions were prepared by an electro-
mechanical dispersal method using Medimachine
(Dako, Copenhagen, Denmark). Tissue fragments
with 1.5 ml PBS were placed in the Medicon dis-
aggregator chamber (Dako) with 50 μm of separa-
tor mesh. The optimal time of disaggregation was 20 sec.
Cell suspensions were filtered using disposable sterile
Filcon (Dako) with a 50–70 μm pore size range and
washed twice to eliminate cell debris. To isolate
lymphocytes, cell suspensions were centrifugated using the standard gradient sedimentation technique
on Gradiisol G (Polfa, Kutno, Poland). Isolated lympho-
cytes were washed and suspended in PBS in a final
density of 1.0 × 10⁵ cells/ml. The viability of the cells
in suspension was about 98%, as tested with the
trypan-blue exclusion method.

Lymphocyte subset analysis
Monoclonal antibodies labeled with fluorescein iso-
thiocyanate (FITC) or phycoerytrin (PE) were used in the study. One-color or two-color immunofluores-
cence staining was performed with the use of the following antibodies (Becton-Dickinson, San Jose, CA,
USA): anti-CD3 (against mature T lymphocytes), anti-
CD19 (against B lymphocytes), anti-CD4/CD8 (against
helper/inducer and suppressor/cytotoxic lympho-
cytes), and anti-CD56/CD16 (against natural killer
(NK) cells). Flow cytometry was performed on
FACSCalibur (Beckton-Dickinson) with a 488 nm argon
laser. Optimal scatter gates were set using LeucoGATE
(anti-CD45 FITC and anti-CD14 PE; Becton-Dickinson)
so that analysis gate for cells included more than 96% of
lymphocytes and less than 3% of monocytes/granulo-
cytes. For background control, immunoglobulin
(IgG1 FITC and IgG2a PE (Becton-Dickinson) were
used. No less than 1 × 10⁴ cells were measured in each
analysis. The results are presented as the percentage
of positive cells in the tested sample.

Cytokine production
Isolated decidual lymphocytes (1.0 × 10⁵ cells/ml)
were placed in culture medium: RPMI 1640 (Flow)
enriched with 10% fetal calf serum (Sigma, St Louis,
MO, USA), 2 mM L-glutamine (Serva, Heidelberg,
Germany), and antibiotics (100 U/ml of penicillin,
10 μg/ml of streptomycin). To stimulate lymphocyte
proliferation, phytohaemaglutinin (PHA) (Sigma) in a
concentration of 5 μg/ml of culture medium was used.
Cultures were conducted in flat-bottomed microplates
(Nunc, Kamstrupvij, Denmark) using 10³ cells/100 μl
of medium, for 72 h in humidified air with 5% CO₂
(Asab, Stockholm, Sweden), and each experiment
carried out in triplicate. Control triplicates had no
mitogen added. At the end of the culture, microplates
were centrifugated at 2000 × g for 10 min. Then the
culture supernatant was collected and frozen at −80°C.
A standard immunoenzymatic enzyme-linked immuno-
sorbent assay method was used for estimation of IL-2,
IL-6, IL-10, IL-12, IFNγ (ENDOGEN, Minneapolis, MN,
USA) and IL-4 (Hybridomus; Cytotech, Copenhagen,
Denmark) concentrations. The results are presented as
picograms per milliliter. The minimal detection limits
for cytokines were as follows: < 6 pg/ml for IL-2,
1.1 pg/ml for IL-4, < 1 pg/ml for IL-6, < 3 pg/ml for IL-
10, < 5 pg/ml for IL-12, and < 2 pg/ml for IFNγ. Intra-
assay and inter-assay coefficients of variation for all
studied cytokines were < 10%.

Statistical analysis
Results are reported as the group median with cut-off
points of 25% and 75% of results. Analysis of
differences between three groups was initially carried
out using the Kruskal–Wallis test. Than the Mann–
Whitney U test (p < 0.05) was performed as
appropriate to test for the statistical significance of
differences between each pair of groups. We used
licensed Statistica 5.0 for Windows.

Results
The examples of the main maternal lymphocyte
subsets obtained during isolation from third-trimester
decidual tissue are presented in Table 2. Pre-eclamptic
patients, compared with controls and those pregnant
and with transient hypertension, were characterized
with significantly increased percentage of classical
NK CD3^+CD56^+CD16^+ cells and decreased percentage of mature CD3^+ T and CD19^+ B lymphocytes.

The concentrations of cytokines secreted spontaneously to supernatant of cultured decidual lymphocytes are compared in Table 3 and presented graphically in Figs 1, 3, 5, and 7. Analysis indicated significantly decreased levels of IL-6 and IL-10 in pre-eclamptic patients as well as increased level of IFN\(\gamma\) in patients suffering from both transient hypertension and pre-eclampsia.

The concentrations of cytokines secreted on mitogen stimulation of cultured decidual lymphocytes are presented in Table 4 and presented graphically in Figs 2, 4, 6, and 8. Lymphocytes of pre-eclamptic women secreted significantly decreased amounts of IL-6, IL-10 and IL-12 but extremely increased amounts of IFN\(\gamma\).
Our study, in concordance with previous investigations performed on cultured peripheral blood mononuclear cells (PBMCs), revealed that also lymphocytes isolated from decidua of pre-eclamptic patients are capable of producing, spontaneously and after PHA stimulation, large amounts of IFN\(\gamma\). IFN\(\gamma\) belongs to cytokines of strong Th1-like activity and is mainly produced by T lymphocytes and activated decidual NK cells. Because PHA is believed to exert its mitogenic effects mainly on T lymphocytes, it is possible that increased levels of IFN\(\gamma\) origin from NK cells activated vigorously by intercellular regulatory signals sent by T cells. Increased concentrations of this cytokine are harmful for pregnancy, causing fetal resorptions in the mice model of recurrent spontaneous abortions, and could also be responsible for induction of trophoblast apoptosis.

Decreased IFN\(\gamma\) production by PBMCs was noted in pregnant women in remission of rheumatoid arthritis. Of all investigated cytokines, only IFN\(\gamma\) is significantly increased when patients with transient hypertension are compared with normotensive pregnant controls. Despite the lack of changes concerning the rest of the investigated cytokines, this result indicates the presence of a prominent local Th1 shift in transient hypertension similar to that existing in pre-eclampsia. The diminished production of IL-6 and IL-10 observed in our study indicates downregulation of Th2-type activity in decidual tissue, which is supplementary to that observed in peripheral blood and placenta during pre-eclamptic pregnancy, and may result in IFN\(\gamma\) overproduction. Deficiency in IL-6 secretion observed during pre-eclampsia lowers trophoblastic hCG production and causes defective placental development. Lowered IL-10 production in pre-eclampsia could be associated with heightened maternal anti-foetal immunity and inadequate placental development. IL-12 promotes the potential of CD4 T cells to produce IFN\(\gamma\); however, decreased IL-12 production does not result in inhibition of IFN\(\gamma\) secretion, which is confirmed by our results.

Lymphocyte subset analysis indicates that the decidua of pre-eclamptic patients contains increased numbers of classical NK cells. The co-existence of a lowered CD3\(^+\) T subset percentage together with NK population shift could be responsible for the observed cytokine disbalance.

Very low representation of decidual CD19\(^+\) B lymphocytes in pre-eclampsia argues against thesis of their possible role in pathogenesis of this syndrome. The classical NK CD3\(^-\)CD56\(^+\) cells are more abundant in the decidua of healthy pregnant subjects than in pregnant women with transient hypertension. The candidate for local immunoregulatory mechanism inhibiting NK proliferation is antigen HLA-G, whose expression on the trophoblastic surface in the cases of transient hypertension is higher compared with pre-eclampsia.

Taking into consideration the disturbances of local cytokine production by decidual lymphocytes ‘in vitro’, we conclude that transient hypertension
FIG. 1. Comparison of IL-6 spontaneous secretion in healthy pregnant women (controls) \((n = 11)\), and pregnant women with transient hypertension (TH) \((n = 17)\) and pre-eclampsia (PE) \((n = 21)\). Statistically significant are the differences between controls and PE \((p < 0.002)\) and between TH and PE \((p < 0.0008)\).

FIG. 2. Comparison of IL-6 PHA-stimulated secretion in healthy pregnant women (controls) \((n = 11)\), and pregnant women with transient hypertension (TH) \((n = 17)\) and pre-eclampsia (PE) \((n = 21)\). Statistically significant are the differences between controls and PE \((p < 0.02)\) and between TH and PE \((p < 0.002)\).

FIG. 3. Comparison of IL-10 spontaneous secretion in healthy pregnant women (controls) \((n = 11)\), and pregnant women with transient hypertension (TH) \((n = 17)\) and pre-eclampsia (PE) \((n = 21)\). Statistically significant are the differences between controls and PE \((p < 0.05)\).

FIG. 4. Comparison of IL-10 PHA-stimulated secretion in healthy pregnant women (controls) \((n = 11)\), and pregnant women with transient hypertension (TH) \((n = 17)\) and pre-eclampsia (PE) \((n = 21)\). Statistically significant are the differences between controls and PE \((p < 0.002)\) and between TH and PE \((p < 0.008)\).

FIG. 5. Comparison of IL-12 spontaneous secretion in healthy pregnant women (controls) \((n = 11)\), and pregnant women with transient hypertension (TH) \((n = 17)\) and pre-eclampsia (PE) \((n = 21)\). The differences between all groups studied are not statistically significant.

FIG. 6. Comparison of IL-12 PHA-stimulated secretion in healthy pregnant women (controls) \((n = 11)\), and pregnant women with transient hypertension (TH) \((n = 17)\) and pre-eclampsia (PE) \((n = 21)\). Statistically significant are the differences between controls and PE \((p < 0.05)\).

FIG. 7. Comparison of IFN\(\gamma\) spontaneous secretion in healthy pregnant women (controls) \((n = 11)\), and pregnant women with transient hypertension (TH) \((n = 17)\) and pre-eclampsia (PE) \((n = 21)\). Statistically significant are controls and TH \((p < 0.03)\).

FIG. 8. Comparison of IFN\(\gamma\) PHA-stimulated secretion in healthy pregnant women (controls) \((n = 11)\), and pregnant women with transient hypertension (TH) \((n = 17)\) and pre-eclampsia (PE) \((n = 21)\). Statistically significant are the differences between controls and PE \((p < 0.0002)\) and between TH and PE \((p < 0.02)\).
constitutes an intermediate step in the etiology of pre-eclampsia and is like pre-eclampsia characterized by a disturbed Th1/Th2 balance, although intensity of these disturbances is different in each syndrome.

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