

Increased interferon- γ - and interleukin-4-synthesizing subsets of circulating T lymphocytes in pregnant asthmatics

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Summary

Background Pregnancy frequently interferes with the course of bronchial asthma, and asthmatic pregnant women experience less successful pregnancies. T lymphocytes synthesizing IL-4 or IFN- γ are important in allergic mechanisms of the airways as well as in materno-fetal immunity.

Objective We hypothesized that pregnancy (a T helper-2 polarized state) of asthmatics will enhance the number of circulating T2 lymphocytes, but decrease the subset-producing IFN- γ (T1 lymphocytes) and thereby cause a culminating T2 dominance with possible clinical consequences.

Methods IL-4- or IFN- γ -producing T lymphocytes were determined by flow cytometry in healthy ($n = 8$) and asthmatic ($n = 13$) non-pregnant women and healthy ($n = 18$) and asthmatic ($n = 48$) pregnant women of similar chronological and gestational (2nd–3rd trimester) age and asthma severity (Global Initiative for Asthma II–III).

Results In the blood of non-pregnant women – healthy or asthmatic – the numbers of IL-4- and IFN- γ^+ T cells were very low ($< 10/\mu\text{L}$ blood). In contrast, in asthmatic pregnant women, the cell counts were 182 ± 27 and 39 ± 6 for IFN- γ^+ and IL-4 $^+$ T cells/ μL blood, respectively (both $P < 0.05$ vs. respective control values of non-pregnant asthmatics). Within the asthmatic pregnant group, significant negative correlations were revealed between the numbers of IFN- γ^+ or IL-4 $^+$ T cells and maternal peak expiratory flow as well as birth weight of newborns (both $P < 0.05$).

Conclusion These data show a previously unknown immunological interference between asthma and pregnancy. The culminating proliferation of IFN- γ^+ and IL-4 $^+$ T lymphocytes may potentially impair maternal airway symptoms as well as fetal development.

Keywords allergic asthma, birth weight, peak expiratory flow, pregnancy, T lymphocyte subsets

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Introduction

Obstruction of airways in bronchial asthma is initiated by immediate-type hypersensitivity-induced allergic inflammation. Increased synthesis of IL-4 and other T2-type cytokines by T lymphocytes is important in this process [1]. IFN- γ and other T1-type cytokines (e.g. IL-12) can inhibit the development of atopy and asthma at an early stage of disease [2, 3]. Pregnancy also affects the immune response [4]. The fetus is a semi-allograft but under normal circumstances decidual T lymphocytes become polarized towards the T2 phenotype, as they synthesize IL-4, IL-5 and IL-10 [5–12]. The physiological materno-fetal immune response exerts an 'immunotrophic' effect on the conceptus [4, 8, 13]. When the maternal immune system responds to pregnancy with T1-type cytokines (e.g. IFN- γ , TNF- α), women experience spontaneous abortion [8, 11, 13, 14], while such a pregnancy in mice ends in fetal resorption or growth retardation [12, 15].

Not only is the uterus the site of pregnancy-induced changes in immunological mechanisms [5], there are changes, for example, in the course of various systemic immunological diseases, such as systemic lupus erythematosus [16] or rheumatoid arthritis [17]. Symptoms of the latter, a T1 autoimmune disease, become temporarily attenuated, while those of systemic lupus erythematosus, a T2 autoimmune disease, worsen during pregnancy. It is hypothesized that pregnancy (a T2-polarized state) inhibits rheumatoid arthritis but exacerbates systemic lupus erythematosus because of the known antagonism and synergism between the T2 and T1 pattern of immune responses, respectively [18].

The severity of bronchial asthma may change during pregnancy. Although the direction of change is quite unpredictable [19], during the first 3 post-partum months, asthma was reported to revert to its pre-pregnancy severity in 73% of women [20]. There is a concordance between the course of asthma during subsequent pregnancies [20]. The negative influence between pregnancy and asthma may be mutual, as – according to population-based studies including thousands of subjects – even mild and moderate asthma increases the occurrence of fetal growth retardation, pre-term

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labour, pre-eclampsia and other pregnancy complications [21, 22]. Although recent reports suggest that seven out of every 100 pregnant women suffer from asthma [23], interactions between asthma and pregnancy remain largely unstudied.

Based on these considerations, we hypothesized that (1) pregnancy-induced immune alterations may modify allergic mechanisms of asthma and (2) immune responses maintaining asthma may modify fetal development. As T cell synergism or antagonism may develop in pregnant asthmatics, IL-4⁺ and IFN- γ ⁺ subpopulations of circulating T lymphocytes were determined and correlated with maternal lung function, as well as with the birth weight of their babies.

Methods

Subjects

Forty-eight asthmatic pregnant women (2nd trimester: $n = 12$, 3rd trimester: $n = 36$) had routine obstetrical control at the 1st Department of Obstetrics and Gynecology, Semmelweis University. They were referred to the Asthma Ambulance if symptoms, complains or history of asthma were reported. During their visit at the Asthma Ambulance, they were asked about pre-existent atopy, the start and severity of asthmatic symptoms and anti-asthma medications [24]. Pregnancy-related changes in asthma symptoms were also recorded. Venous blood was drawn for routine laboratory tests and also into heparinized test tubes for flow cytometric analysis of lymphocytes, as published earlier [25]. Spirometry, whole-body plethysmography and arterialized capillary blood gas and acid-base were analysed. In pregnant women, skin prick tests, bronchoprovocation and chest X-rays were not performed. Patients with clear evidence of respiratory tract infection (fever, purulent sputum, positive culture of sputum and need for antibiotic treatment) were not included.

Three groups of control subjects were included: healthy pregnant women of similar age and gestational age at pulmonary examination and blood withdrawal ($n = 18$, four women in the 2nd and 14 women in the 3rd trimester), and healthy ($n = 8$) and asthmatic ($n = 13$) non-pregnant women. Attention was paid to the simultaneous parallel recruitment and testing of non-pregnant asthmatic as well as pregnant and non-pregnant healthy controls, whenever asthmatic pregnant women were referred to the Asthma Ambulance. Complete lung function testing was performed only in asthmatics (pregnant and non-pregnant); the other two groups underwent physical exams and their medical history was recorded. Blood gas was analysed in 12 of 18 healthy pregnant.

Asthmatic pregnant and non-pregnant women had been receiving anti-asthma treatment according to Global Initiative for Asthma (GINA) [24]. Some patients required changes of the doses of short- or long-acting β_2 -agonist, or inhaled steroids during the visit. None of them were on oral steroids 6 weeks before the visit or during pregnancy.

The study had the ethical and professional approval of the Institutional Review Board of Semmelweis University.

Analysis of interferon- γ ⁺ and interleukin-4⁺ CD3 lymphocytes

FastImmune Cytokine Assay (BD Biosciences, San Jose, CA, USA) was used for investigation of cytokine production of

peripheral blood lymphocytes. Peripheral blood was collected in sodium heparin Vacutainer tubes. Mononuclear cells were separated via Ficoll-Paque (SIGMA) density-gradient centrifugation. For activation, peripheral blood mononuclear cells were washed and re-suspended at 2×10^6 cells/mL in fresh RPMI-1640 culture medium containing 10% heat-inactivated fetal calf serum. Activation was performed in the presence of Brefeldin A (SIGMA, St Louis, MO, USA), which inhibited the intracellular transport of cytokines. 25 ng/mL phorbol-12-myristate-13-acetate (SIGMA, St Louis, MO, USA) and 1 μ g/mL ionomycin (SIGMA, St Louis, MO, USA) were used for 4 h at 37 °C, 5% CO₂ for activation. Intracellular expression of IL-4 and IFN- γ was stained by fluorescent isotiocyanate- or phycoerythrin-conjugated monoclonal antibodies after labelling of cell surface CD3 molecules, as described earlier [25]. Four percent of paraformaldehyde (SIGMA, St Louis, MO, USA) and 0.1% saponin solution (SIGMA, St Louis, MO, USA) were used for cell permeabilization. Measurements were performed in a FACSCalibur flow cytometer (BD Biosciences), using 3000 CD3⁺ lymphocytes for each measurement. The CellQuest Pro software program was used for analysis.

Statistics

Results are given as means $\pm$ SEM, except doses of anti-asthma medications, where medians and smallest–largest values were used. Parametric data of the four groups were analysed by ANOVA, followed by the Dunn–Sidak *post-hoc* test, if overall P was <0.05 . The quantitative relationship between the sizes of various T lymphocyte populations and maternal respiratory function or birth weight was assessed by linear regression. The χ^2 - and the unpaired t -test were used where indicated.

Results

General

The mean age of the various study groups was around 27 years (Table 1). The mean gestational age at pulmonary exam was the 30th week in both healthy and asthmatic pregnant

Table 1. Clinical data of subjects and patients (means $\pm$ SEM)

	Healthy non-pregnant	Healthy pregnant	Asthmatic non-pregnant	Asthmatic pregnant
<i>n</i>	8	18	13	48
Age (year)	27.0 $\pm$ 1.4	27.9 $\pm$ 0.7	27.0 $\pm$ 1.0	26.4 $\pm$ 0.5
Gestational age (week)				
At exam	–	30.3 $\pm$ 3.1	–	30.1 $\pm$ 1.2
At delivery	–	39.8 $\pm$ 0.2	–	38.8 $\pm$ 0.3
Birth weight (g)	–	3326 $\pm$ 102	–	3132 $\pm$ 97*
Number of patients with				
Preeclampsia	–	0	–	3
Gestational diabetes	–	1	–	1
Hypertension	–	0	–	2

* $P < 0.05$ vs. healthy pregnant group. Other data did not show statistically significant difference.

women. The mean gestational age at delivery was also similar in the two groups (Table 1). The ratio of primiparae/multiparae was similar within asthmatic (21/27) and non-asthmatic (seven of 11) pregnant women. The body weight of newborns of asthmatic mothers was significantly smaller than that of healthy controls ($P < 0.05$). Lung function results measured in asthmatic patients are given in Table 2. The peak expiratory flow (PEF) was significantly reduced in both groups of asthmatic patients (non-pregnant and pregnant) relative to predicted control values ($P < 0.05$). Non-pregnant and pregnant asthmatics had airway narrowing of similar severity (Table 2). As in healthy pregnant women pO_2 was 100.6 ± 0.9 mmHg and pCO_2 was 36.7 ± 0.7 mmHg ($n = 12$; both within the reference range of normal pregnancy [26]), hypoxaemia and hypocapnia of non-pregnant and pregnant

asthmatics were of similar severity. According to history, all asthmatic patients (non-pregnant and pregnant) were atopic. The number of current smokers was four (8.3%) among pregnant and three (23.1%) among non-pregnant asthmatics. The daily doses of various anti-asthma medications used are also shown in Table 2. The pregnancy-related change of asthma severity – according to patients' own judgement – was the following: symptoms worsened in 25% (12 patients), improved in 35.4% (17 patients) and remained the same in 39.6% (19 patients).

Healthy and asthmatic pregnant patients had slightly reduced red blood cell count and haemoglobin concentration compared with non-pregnant controls (not shown). These values were all in the reference range of pregnancy, and might have reflected physiologically expanded plasma volume of pregnancy. Both pregnant groups had higher neutrophil counts. Eosinophil number was in the normal range and did not differ among groups.

Table 2. Severity of asthma in non-pregnant and pregnant patients (mean $\pm$ SEM, where appropriate)

	Asthmatic non-pregnant (13)	asthmatic Pregnant (48)
GINA (II/III)	9/2	37/11 ^{ns}
PEF (% of predicted)	78 $\pm$ 6*	71 $\pm$ 2*
FEV ₁ (% of predicted)	85 $\pm$ 3	93 $\pm$ 2
R_{aw} (kPa $\times$ s/L)	0.30 $\pm$ 0.02†	0.31 $\pm$ 0.02†
pO_2 (mmHg)	88 $\pm$ 2*	96 $\pm$ 1‡
pCO_2 (mmHg)	34 $\pm$ 1*	30 $\pm$ 0.4‡
pH	7.45 $\pm$ 0.01	7.46 $\pm$ 0.00
Smoking when examined	3	4 ^{ns}
Daily dose of inhaled short-acting β_2 -agonist (puffs)	2 (0, 8)§	2 (0, 8)§
Daily dose of inhaled long-acting β_2 -agonist (puffs)	0 (0, 4)§	0 (0, 4)§
Daily dose of inhaled corticosteroid (μ g)§	400 (0, 1600)§	400 (0, 1600)§

GINA, Global Initiative for Asthma; PEF, peak expiratory flow; R_{aw} , airway resistance; pO_2 , partial pressure of oxygen in arterialized capillary blood; pCO_2 , partial pressure of carbon-dioxide in arterialized capillary blood; FEV₁, forced expiratory volume in 1 s; ^{ns} non-significant vs. non-pregnants (χ^2 test).

*Less than the healthy age-matched population mean minus 2 $\times$ SD;

†More than the healthy age-matched population mean plus 2 $\times$ SD;

‡Less than the healthy age-matched pregnant population mean minus 2 $\times$ SD;

§Median, smallest, largest.

Lymphocyte subsets

Healthy pregnant women had slightly reduced total and T lymphocyte counts relative to healthy non-pregnant controls (Figs 1a and b). At the same time, within T cells, those subpopulations that synthesized IFN- γ and – to a lesser extent – IL-4, became larger in healthy pregnant than in non-pregnant women (Figs 2a and b). In healthy pregnant women, the IFN- γ^+ subset increased 15 times ($P < 0.05$), and the IL-4⁺ subset five times above non-pregnant values. Asthmatic non-pregnant women showed no change in T cell count or the two T subsets vs. the healthy non-pregnant values. Asthmatic pregnant women, however, evidenced T lymphocytosis (Figs 1a and b). About 50% of the increase was encountered by the IFN- γ^+ subpopulation (Fig. 2a): their number was $182 \pm 27/\mu$ L blood, a density four times higher than that in healthy pregnant women ($45 \pm 8/\mu$ L), or 18 times higher than in asthmatic non-pregnant women (10 ± 4 , both $P < 0.05$). To a lesser extent, but also the IL-4 synthesizing population became larger in asthmatic pregnant women (Fig. 2b): $39 \pm 6/\mu$ L blood as compared with 10 ± 2 and 2 ± 0.4 in healthy pregnant or asthmatic non-pregnant women, respectively (both $P < 0.05$).

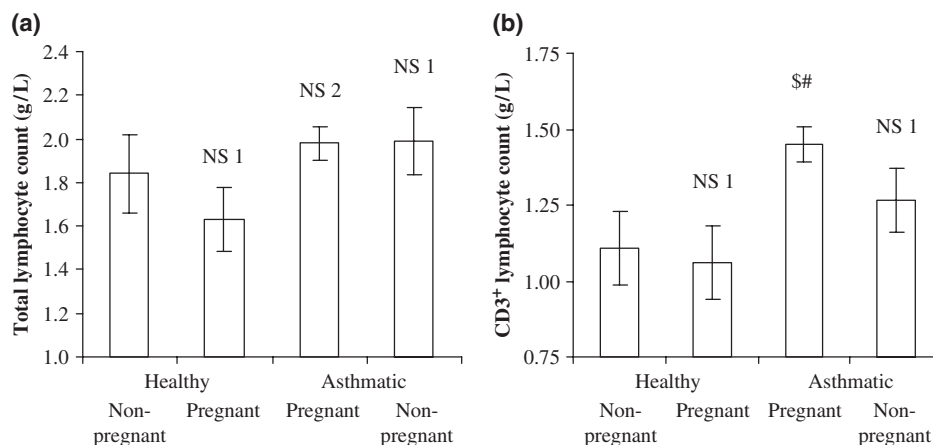


Fig. 1. Total (a) and CD3⁺ (b) peripheral blood lymphocyte counts of patients and controls (means $\pm$ SEM). Healthy non-pregnant ($n = 8$); healthy pregnant ($n = 18$); asthmatic pregnant ($n = 48$); asthmatic non-pregnant ($n = 13$); ^{\$} $P < 0.05$ vs. healthy pregnant; [#] $P < 0.05$ vs. asthmatic non-pregnant; ^{NS 1} non-significant vs. healthy non-pregnant; ^{NS 2} non-significant vs. healthy pregnant.

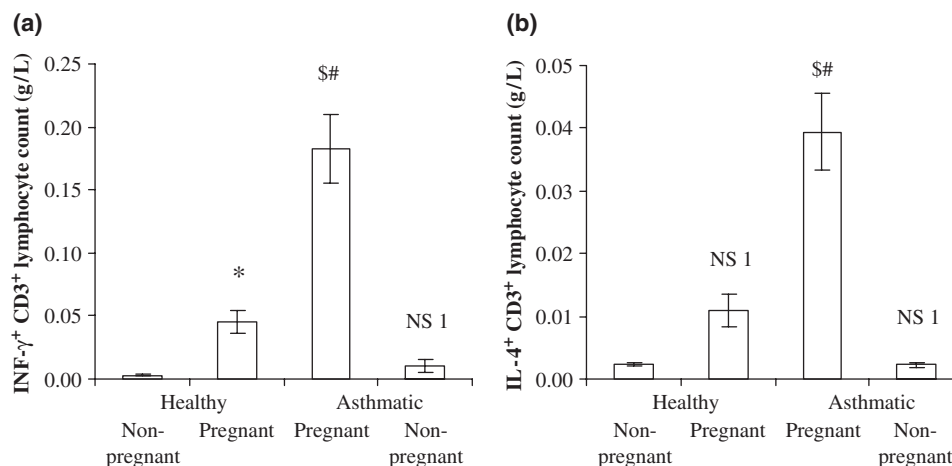


Fig. 2. IFN- γ^+ CD3 $^+$ (a), and IL-4 $^+$ CD3 $^+$ (b) peripheral blood lymphocyte counts of patients and controls (means $\pm$ SEM). Healthy non-pregnant ($n = 8$); healthy pregnant ($n = 18$); asthmatic pregnant ($n = 48$); asthmatic non-pregnant ($n = 13$); * $P < 0.05$ vs. healthy non-pregnant; § $P < 0.05$ vs. healthy pregnant; # $P < 0.05$ vs. asthmatic non-pregnant; NS ^{1} non-significant vs. healthy non-pregnant.

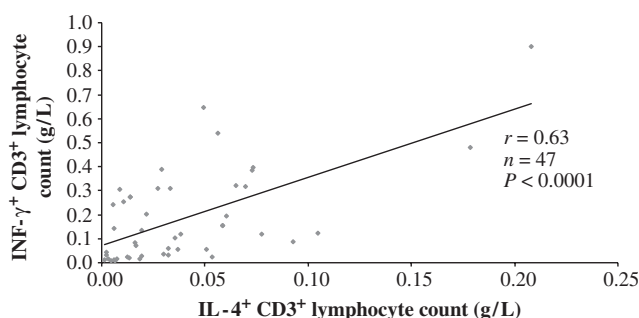


Fig. 3. Positive correlation between IL-4-producing and IFN- γ -producing T cell counts in asthmatic pregnant patients ($n = 47$).

A significant positive correlation was revealed between the sizes of the IFN- γ^+ and the IL-4 $^+$ T-subsets in circulating blood of asthmatic pregnant patients (Fig. 3). No such relationship was found within the healthy pregnant ($r = 0.111$, $n = 18$, $P > 0.05$) or non-pregnant groups.

Correlations between lymphocyte counts, respiratory function and birth weight of fetuses

Pregnancy-related change of asthma severity (documented according to patients' own judgements) – whether worsening, improving or staying stable – was not associated with differing counts of any T cell subsets measured in peripheral blood. Lung function test other than PEF or blood gas values showed no quantitative relationship with either cell subpopulations. However, significant negative correlations were revealed between the IFN- γ^+ T cell counts and PEF rates (Fig. 4a), as well as between IL-4 $^+$ counts and PEF rates (Fig. 4b). Furthermore, when asthmatic pregnant women were grouped according to their daily use of inhaled budesonide, patients on higher doses of maintenance therapy (800–1600 μ g/day inhaled budesonide; $n = 18$) had significantly higher numbers of IFN- γ^+ (0.325 ± 0.056 g/L) and IL-4 $^+$ (0.060 ± 0.013 g/L) T cells than others ($n = 30$) with daily inhaled budesonide usage of 400 μ g (IFN- γ : 0.107 ± 0.019 ; IL-4: 0.030 ± 0.005 g/L, both $P < 0.05$). In

addition, a significant negative correlation was observed between maternal IFN- γ^+ T cell counts and birth weights of newborns in the asthmatic group (Fig. 5), but no relationship could be documented between PEF, forced expiratory volume in 1 s (FEV $_1$), raw or arterialized capillary pO $_2$ and birth weights. Maternal blood pH was shown to be directly related to birth weight. Within the healthy pregnant group, birth weights were not related to the number of IFN- γ^+ ($r = 0.33$, $n = 18$, $P > 0.05$) or IL-4 $^+$ ($r = 0.08$, $n = 18$, $P > 0.05$) T cells.

Three pregnant asthmatics suffered from pre-eclampsia. These patients had significantly higher numbers of circulating IFN- γ^+ and IL-4 $^+$ T cells, lower birth weight and somewhat reduced PEF rate than the values of asthmatic pregnant patients without pre-eclampsia (Table 3).

Discussion

Schatz et al. [20] followed 366 pregnancies of asthmatic women, and in 35% of them, asthma worsened during pregnancy, but in most of the patients symptoms reverted

Table 3. Blood pressure values, platelet counts, PEF rates and CD3 $^+$ lymphocyte counts of asthmatic pregnant with and without preeclampsia (means $\pm$ SEM, where appropriate)

	Asthmatic pregnant without preeclampsia	Asthmatic pregnant with preeclampsia
<i>n</i>	45	3
Blood pressure:		
Systolic	116 $\pm$ 1.4	133 $\pm$ 3.3*
Diastolic	79 $\pm$ 1.1	87 $\pm$ 3.3 ^{ns}
Proteinuria	No	From + to + + + +
Platelets ($\times 10^9$ /L)	234.5 $\pm$ 12.4	292.0 $\pm$ 42.0 ^{ns}
PEF (% of predicted)	72 $\pm$ 2.4%	58 $\pm$ 4.9% ^{ns}
IFN- γ^+ CD3 $^+$ lymphocyte count (g/L)	0.16 $\pm$ 0.02	0.51 $\pm$ 0.20*
IL-4 $^+$ CD3 $^+$ lymphocyte count (g/L)	0.04 $\pm$ 0.01	0.09 $\pm$ 0.06*
Birth weight (g)	3235 $\pm$ 84	1900 $\pm$ 50*

PEF, peak expiratory flow; INF, interferon; IL, interleukin; ^{ns}non-significant vs. asthmatic pregnant without preeclampsia;

* $P < 0.05$ vs. asthmatic pregnant without preeclampsia (unpaired *t*-test).

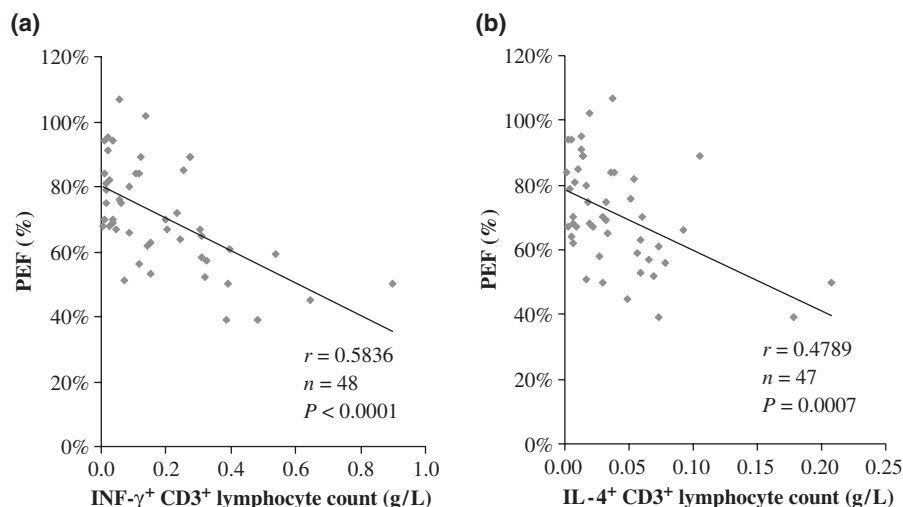


Fig. 4. Negative correlations between IFN- γ (a, $n = 48$)- and IL-4 (b, $n = 47$)-producing T cell counts and peak expiratory flow (PEF) rates in asthmatic pregnant patients.

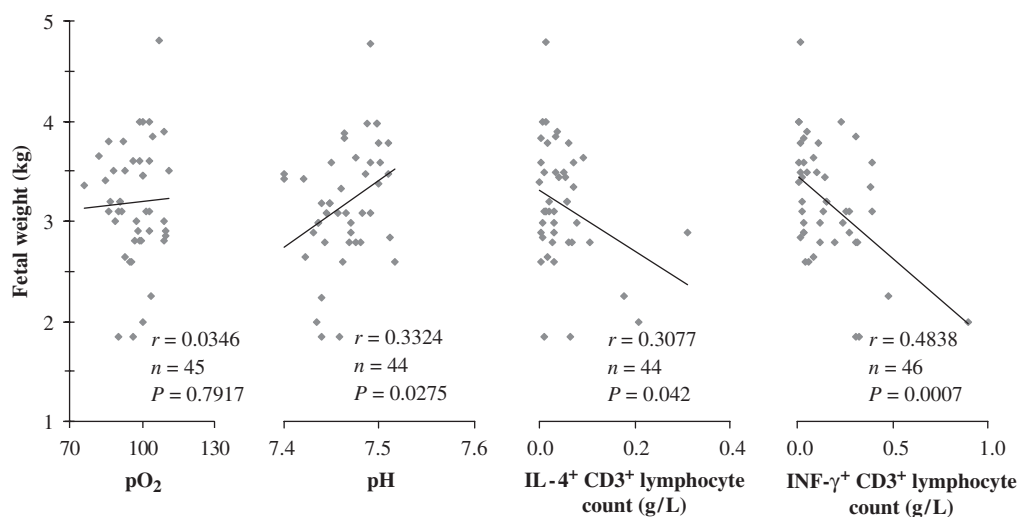


Fig. 5. Correlations between arterialized capillary blood pO_2 ($n = 45$), pH ($n = 44$) and peripheral blood IL-4 ($n = 44$)- and IFN- γ ($n = 46$)-producing T cell counts and fetal birth weights in asthmatic pregnant patients.

within 3 months after delivery. These epidemiological observations indicate that pregnancy may exaggerate asthma. On the other hand, asthma is known to cause fetal growth retardation and pre-term delivery [21, 27]. Kramer et al. [28] screened 241 mothers with idiopathic pre-term and 311 women with term delivery, and found that the signs and symptoms of asthma and doctor-diagnosed asthma were about three times more frequent among the former than the latter. Although the negative impact of bronchial asthma on pregnancy may be because of generalized smooth muscle (bronchial, uterine, uterine vascular) irritability or perhaps insufficient fetal oxygen supply [19, 20, 27], an immunodystrophic effect mediated by T cells and various cytokines (e.g. IFN- γ) is also possible. Pathologically increased IFN- γ production is toxic to trophoblasts [29], promotes spontaneous abortion and retardation of fetal growth. Wegman et al. [4], Lin et al. [7], Raghupathy et al. [12] and Palmer et al. [18] defined pregnancy as immunodystrophic, if the latter complications developed together with or as a consequence of a T1-type materno-fetal immune response.

In a healthy 'immunotrophic' pregnancy, T2 cytokines are highly expressed within the feto-maternal interface as well as in circulating lymphocytes [6, 7, 9, 10], although IFN- γ may also play some role in normal decidualization [30–32]. Our hypothesis was that normal pregnancy would synergize with the T2 cytokine pattern of atopic asthma. A large IL-4 $^{+}$ and a low IFN- γ^{+} T subset were expected. Our overall approach proved to be useful but actual measurements yielded quite unexpected data. Peripheral blood of healthy pregnant controls contained measurably increased IL-4 $^{+}$ and IFN- γ^{+} subpopulations of CD3 cells. This finding indicated proliferation (activation?) of some T subsets during 'immunotrophic', normal pregnancy. Our asthmatic pregnant patients, however, had much higher T cell counts, which did support the original idea of a culminating T cell response in this state. In contrast to expectations, however, IFN- γ -synthesizing T cells were found in greatly increased numbers, although the IL-4 $^{+}$ fraction was also increased. These women had about 20-fold higher number of IFN- γ producing T cells than non-pregnant women suffering from the same disease of compar-

able severity. The IL-4 subset also increased proportionally with that of the IFN- γ^+ subset, but in a smaller magnitude. Therefore, pregnancy in asthma seems to lead to a culminating cytokine response, which is of mixed phenotype, but, considering absolute numbers – dominated by IFN- γ .

Although at its induction atopic asthma is governed by T2 cytokines like IL-4 [1], the current finding of a simultaneous increase of IFN- γ^+ and IL-4 $^+$ T cells in adult atopic asthmatics is not surprising. In adults, symptoms of asthma have been reported to be related to a mixed cytokine phenotype (IL-4, IL-5, IFN- γ , TNF- α) [33, 34]. Moreover, a significant negative correlation was observed between the serum level of IFN- γ and FEV $_1$, but not between IL-4 or IL-5 and FEV $_1$ [35]. Other authors have shown that allergen-specific T cell clones isolated from adult asthmatics were of the T helper-0 phenotype [36, 37]. In more recent studies of adult asthmatics [38–40] as well as 7–8-years-old-children [41], IFN- γ was observed to be the dominating cytokine over IL-4 and IL-5 in the bronchoalveolar lavage [41], blood [38, 39] or lung tissue [40]. Especially during pregnancy, when cellular immunity may be weaker [42], exacerbations could frequently be triggered by viral or bacterial infections that induce T1 type of lymphocyte activation [43].

Which mechanisms were responsible for the pregnancy-induced peaking of T cell subsets in the peripheral blood? These subsets of T cells have been known to occur only locally, in the airway mucosa of moderate asthmatics but not the circulating blood in the non-pregnant state [44]. During pregnancy, certain subsets of maternal lymphocytes recognize the trophoblast cells [6, 9, 10]. Perhaps, this priming of lymphocytes or adjuvant-like effect of pregnancy [45] may trigger a stronger T cell reaction to allergens or infection responsible for asthmatic symptoms.

Our measurements did not provide further information on the involved subsets of T cells, as to whether they were from the CD8 [46, 47], the γ/δ T [48], T $_{reg}$ (CD25 $^+$ CD4 $^+$) or natural killer T [47] subsets. These subsets are suspected, as all of these are capable of simultaneous synthesis of IFN- γ and IL-4, and are increased by both pregnancy and asthma.

Considering that pregnant asthmatics had high pro-inflammatory cytokine production, it was surprising that their symptoms were not more severe than the symptoms of non-pregnant asthmatics. It is speculated that maternal cortisol, progesterone and prostaglandin-E $_2$ might have counterbalanced [18] the effects of high IFN- γ and IL-4 synthesis, except during those three pregnancies that ended up in pre-eclampsia. T1 cytokines and T lymphocytes have been speculated [47] to have a role in the pathogenesis of pre-eclampsia, and our data suggest that exceedingly high IFN- γ^+ T cell numbers of pregnant asthmatics were associated with pre-eclampsia, a state of prostaglandin-E $_2$ deficiency [49]. The placental production of IFN- γ [50] and the apoptotic effect of this cytokine on human cytotrophoblasts [51] were reported to be increased in case of intrauterine growth retardation. IFN- γ has also been known to inhibit the synthesis of trophoblast DNA [52], actions that might be behind the present demonstration of an inverse relationship between the number of IFN- γ -synthesizing T cells and the birth weight of newborns of asthmatic pregnant women.

We have found no data on these T cell subsets in pregnant asthmatics, except a recent abstract [53] reporting similar

observations on a higher number of T cells synthesizing IFN- γ before than after delivery. In another new study, Murphy et al. [54] found evidence of increased maternal inflammation associated with a sex-specific (female) retardation of fetal growth in pregnant women suffering from asthma.

In summary, pregnancy in asthmatic women markedly increased an IFN- γ -, and, to a lesser extent IL-4-producing subset of T lymphocytes and there was a significant negative correlation between the volumes of these cellular pools and maternal PEF rates or birth weights of their babies. It is presumed that maternal hormones and other smooth muscle relaxant factors of pregnancy more or less counterbalanced the potentially deleterious effects of exaggerated T lymphocyte proliferation in pregnant asthmatics.

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References

- 1 Robinson DS, Hamid Q, Ying S et al. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326:298–304.
- 2 Maggi E, Parronchi P, Manetti R et al. Reciprocal regulatory effects of IFN- γ and IL-4 on the in vitro development of human Th1 and Th2 clones. *J Immunol* 1992; 148:2142–7.
- 3 Iwamoto I, Nakajima H, Endo H, Yoshida S. Interferon gamma regulates antigen induced eosinophil recruitment into the mouse airways by inhibiting the infiltration of CD4 T cells. *J Exp Med* 1993; 177:573–6.
- 4 Wegman TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol Today* 1993; 14:353–6.
- 5 Kühnert M, Strohmeier R, Stegmüller M, Halberstand E. Changes in lymphocyte subsets during normal pregnancy. *Eur J Obstet Gynecol* 1998; 76:147–51.
- 6 Makhseed M, Raghupathy R, Azizieh F, Omu A, Al-Shamali E, Ashkanani L. Th1 and Th2 cytokine profiles in recurrent aborters with successful pregnancy and with subsequent abortions. *Hum Reprod* 2001; 16:2219–26.
- 7 Lin H, Mosmann TR, Guilbert L, Tuntipipat S, Wegmann TG. Synthesis of T helper-2 type cytokines at the maternal-fetal interface. *J Immunol* 1993; 151:4562–73.
- 8 Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. *Human Reprod* 2000; 15:713–8.
- 9 Nagaeva O, Jonsson L, Mincheva-Nilsson L. Dominant IL-10 and TGF-beta mRNA expression in gamma-delta T cells of human early pregnancy decidua suggests immunoregulatory potential. *Am J Reprod Immunol* 2002; 48:9–17.
- 10 Reinhard G, Noll A, Schlebusch H. Shifts in TH1/TH2 Balance during human pregnancy correlate with apoptotic changes. *Biochem Biophys Res Commun* 1998; 245:933–8.
- 11 Piccinni MP, Beloni L, Livi C, Maggi E, Scarselli G, Romagnani S. Defective production of both leukaemia inhibitory factor and type

- 2 T-helper cytokines by decidual T cells in unexplained recurrent abortion. *Nat Med* 1998; 4:1020–4.
- 12 Raghupathy R, Tangri S. Immunodystrophism, T cells, cytokines, and pregnancy failure. *Am J Reprod Immunol* 1996; 35:291–6.
- 13 Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. *Hum Reprod* 2000; 15:713–8.
- 14 Hill JA, Polgar K, Anderson DJ. T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion. *JAMA* 1995; 273:1933–6.
- 15 Tangri S, Wegmann TG, Lin H, Raghupathy R. Maternal antiplacental reactivity in natural immunologically mediated fetal resorptions. *J Immunol* 1994; 152:4903–11.
- 16 Davidson A, Keiser HD. Diagnosing and treating the predominantly female problems of systemic autoimmune diseases. *Medscape Womens Health* 1997; 2:6.
- 17 Berner IC, Theumann N, Dudler J. Rheumatoid arthritis and pregnancy. *Rev Med Suisse* 2005; 1:694–8.
- 18 Palmer GW, Claman HN. Pregnancy and immunology: selected aspects. *Ann Allergy Asthma Immunol* 2002; 89:350–9.
- 19 Schatz M. Interrelationships between asthma and pregnancy: a literature review. *J Allergy Clin Immunol* 1999; 103:S330–6.
- 20 Schatz M, Harden K, Forsythe A et al. The course of asthma during pregnancy, post partum, and with successive pregnancies: a prospective analysis. *J Allergy Clin Immunol* 1988; 81:509–17.
- 21 Liu S, Wen SW, Demissie K, Marcoux S, Kramer MS. Maternal asthma and pregnancy outcomes. A retrospective cohort study. *Am J Obstet Gynecol* 2001; 184:90–6.
- 22 Triche EW, Safflas AF, Belanger K, Leaderer BP, Bracken MB. Association of asthma diagnosis, severity, symptoms, and treatment with risk of preeclampsia. *Obstet Gynecol* 2004; 104:585–93.
- 23 Namazy JA, Schatz M. Update in the treatment of asthma during pregnancy. *Clin Rev Allergy Immunol* 2004; 26:139–48.
- 24 Pocket Guide for Asthma Management and Prevention, Global Initiative for Asthma, updated November 2003. (Accessed 14 September 2004 at <http://www.ginasthma.com>)
- 25 Nagy G, Pallinger E, Antal-Szalmas P et al. Measurement of intracellular interferon-gamma and interleukin-4 in whole blood T lymphocytes from patients with systemic lupus erythematosus. *Immunol Lett* 2000; 74:207–10.
- 26 de Swiet M, ed. Diseases of the respiratory system in medical disorders in obstetric practice, 3rd Edn. Oxford: Blackwell, 1995; 1–32.
- 27 Schatz M, Zeiger RS, Hoffman CP et al. Intrauterine growth is related to gestational pulmonary function in pregnant asthmatic women. *Chest* 1990; 98:389–92.
- 28 Kramer MS, Coates AL, Michoud MC et al. Maternal asthma and idiopathic preterm labor. *Am J Epidemiol* 1995; 142:1078–88.
- 29 Berkowitz RS, Hill JA, Kurtz CB, Anderson DJ. Effects of products of activated leukocytes (lymphokines and monokines) on the growth of malignant trophoblast cells in vitro. *Am J Obstet Gynecol* 1988; 158:199–203.
- 30 Ashkar AA, Di Santo JP, Croy BA. Interferon- γ contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J Exp Med* 2000; 192:259–69.
- 31 Macaubas C, de Klerk NH, Holt BJ et al. Association between antenatal cytokine production and the development of atopy and asthma at age 6 years. *Lancet* 2003; 362:1192–7.
- 32 Brown M, Gustafson M, Saldana S, Baradaran A, Miller H, Halonen M. Correlation of human decidual and cord blood mononuclear cell cytokine production. *Hum Immunol* 2004; 65:1336–43.
- 33 Krug N, Madden J, Redington AE et al. T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. *Am J Respir Cell Mol Biol* 1996; 14:319–26.
- 34 Pene J, Lagier B, Rivier A, Chanez P, Vendrell JP, Bousquet J. Phenotype of T-cell clones obtained from bronchial biopsies and peripheral blood from three asthmatics. *Cell Biol Int* 1993; 17:353–7.
- 35 Ten Hacken NHT, Oosterhoff Y, Kauffman HF et al. Elevated serum interferon- γ in atopic asthma correlates with increased airways responsiveness and circadian peak expiratory flow variation. *Eur Respir J* 1998; 11:312–6.
- 36 Krouwels FH, Hol BEA, Bruinier B, Lutter R, Jansen HM, Out TA. Properties of pulmonary T-cell clones from patients with allergic asthma. *Am Rev Respir Dis* 1993; 147:A782.
- 37 Maestrelli P, Del Prete GF, De Carli M et al. Activated CD8 lymphocytes producing IFN- γ and IL-5 in bronchial mucosa of subjects sensitized to toluene diisocyanate. *J Allergy Clin Immunol* 1993; 91:220.
- 38 Cho SH, Stanciu LA, Begishvili T, Bates PJ, Holgate ST, Johnston SL. Peripheral blood CD4+ and CD8+ T cell type 1 and type 2 cytokine production in atopic asthmatic and normal subjects. *Clin Exp Allergy* 2002; 32:427–33.
- 39 Magnan AO, Mély LG, Camilla CA et al. Assessment of the Th1/Th2 paradigm in whole blood in atopy and asthma. *Am J Respir Crit Care Med* 2000; 161:1790–6.
- 40 O'Sullivan S, Cormican L, Faul JL et al. Activated, cytotoxic CD8+ T lymphocytes contribute to the pathology of asthma death. *Am J Respir Crit Care Med* 2001; 164:560–4.
- 41 Brown V, Warke TJ, Shields MD, Ennis M. T cell cytokine profiles in childhood asthma. *Thorax* 2003; 58:311–6.
- 42 Luft BJ, Remington JS. Effect of pregnancy on augmentation of natural killer cell activity by *Corynebacterium parvum* and *Toxocara gondii*. *J Immunol* 1984; 132:2375–80.
- 43 Lieberman D, Lieberman D, Printz S et al. Atypical pathogen infection in adults with acute exacerbation of bronchial asthma. *Am J Respir Crit Care Med* 2003; 167:406–10.
- 44 Walker C, Bode E, Boer L, Hansel TT, Blaser K, Virchow JC. Allergic and non-allergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am Rev Respir Dis* 1992; 146:109–15.
- 45 Sacks G, Sargent I, Redman C. An innate view of human pregnancy. *Immunol Today* 1999; 20:114–8.
- 46 Stallmach T, Hebisch G, Orban P, Lü X. Aberrant position of trophoblast and lymphocytes in the feto*-maternal interface with preeclampsia. *Virchows Arch* 1999; 434:207–11.
- 47 Wilczynski JR, Tchorzewski H, Banasik M et al. Lymphocyte subset distribution and cytokine secretion in third trimester deciduas in normal pregnancy and preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 2002; 109:8–15.
- 48 Szekeres-Bartho J, Barakonyi A, Polgar B et al. The role of γ/δ T cells in progesterone-mediated immunomodulation during pregnancy: a review. *Am J Reprod Immunol* 1999; 42:44–8.
- 49 Pedersen EB, Christensen NJ, Christensen P et al. Preeclampsia a state of prostaglandin deficiency? *Hypertension* 1983; 5:105–11.
- 50 Banerjee S, Smallwood A, Moorhead J et al. Placental expression of interferon-gamma (IFN- γ) and its receptor OFN- γ R2 fail to switch from early hypoxic to late normotensive development in preeclampsia. *J Clin Endocrinol Metab* 2005; 90:944–52.
- 51 Crocker IP, Cooper S, Ong SC, Baker PN. Differences in apoptotic susceptibility of cytotrophoblasts and syncytiotrophoblasts in normal pregnancy to those complicated with preeclampsia and intrauterine growth retardation. *Am J Pathol* 2003; 162:637–43.
- 52 Starkey PM. The deciduas and factors controlling placentation. In: Redman CWC, Sargent LL, Starkey PM, eds. *The human placenta*. Oxford, UK: Blackwell Scientific Publication, 1998; 362–413.
- 53 Rastogi D, Lendor C, Horton MK, Blythe D, Miller RL. Systemic Th2 polarization among pregnant asthmatics. *Am J Respir Crit Care Med* 2004; 169:A501.
- 54 Murphy VE, Gibson PG, Giles WB et al. Maternal asthma is associated with reduced fetal growth. *Am J Respir Crit Care Med* 2003; 168:1317–23.