ORIGINAL ARTICLE

# Changes in the Subpopulation of CD25$^+$ CD4$^+$ and FOXP3$^+$ Regulatory T Cells in Decidua with Respect to the Progression of Labor at Term and the Lack of Analogical Changes in the Subpopulation of Suppressive B7-H4$^+$ Macrophages – A Preliminary Report

Krystyna Galazka[1], Lukasz Wicherek[1], Kazimierz Pitynski[1], Jacek Kijowski[2], Kszysztof Zajac[1], Wieslawa Bednarek[3], Magdalena Dutsch-Wicherek[4], Krzysztof Rytlewski[5], Jaroslaw Kalinka[6], Antoni Basta[1], Marcin Majka[2]

[1]Department of Gynecology and Oncology of the Jagiellonian University, Krakow, Poland;
[2]Department of Transplantation of the Jagiellonian University, Krakow, Poland;
[3]Department of Gynecology and Oncology, Medical University Lublin, Lublin, Poland;
[4]ENT Department Jagiellonian University, Krakow, Poland;
[5]Department of Perinatology of the Jagiellonian University, Krakow, Poland;
[6]Medical and Environmental Pregnancy Health Hazards Unit, Department of Perinatology, I Chair of Gynecology and Obstetrics, Medical University of Lodz, Lodz, Poland

**Keywords**
B7-H4$^+$ macrophages, CD25$^+$CD4$^+$FOXP3$^+$ regulatory T cells, cesarean section, labor at term, treg

**Correspondence**
Lukasz Wicherek, Gynecology, Obstetrics and Oncology Department of the Jagiellonian University, 23 Kopernika Str, 31-501 Krakow, Poland.
E-mail: mowicher@cyf-kr.edu.pl

Submitted September 24, 2008;
accepted November 20, 2008.

**Citation**
Galazka K, Wicherek L, Pitynski K, Kijowski J, Zajac K, Bednarek W, Dutsch-Wicherek M, Rytlewski K, Kalinka J, Basta A, Majka M. Changes in the subpopulation of CD25$^+$ CD4$^+$ and FOXP3$^+$ regulatory T cells in decidua with respect to the progression of labor at term and the lack of analogical changes in the subpopulation of suppressive B7-H4$^+$ macrophages – a preliminary report. Am J Reprod Immunol 2009; 61: 136–146

doi:10.1111/j.1600-0897.2008.00674.x

## Problem
The initiation of labor is accompanied by alterations in the level of maternal immune tolerance toward fetal antigens. It is a complex molecular response leading to a brief activation of the maternal immune system with an accompanying capacity to restrict this same activation. The aim of our study was to evaluate the subpopulation of regulatory T cells (Tregs) and B7-H4 macrophages in the decidua basalis during cesarean sections performed on patients in various stages of labor.

## Method of study
The decidual tissue samples evaluated in our study were obtained from 23 pregnant women who underwent cesarean sections at term. Moreover, the patients were divided into three subgroups according to the progression of labor at the time of the cesarean. The presence of Treg cells and B7-H4 positive macrophages were analysed by fluorescence-activated cell sorter.

## Results
The percentages of FOXP3$^+$ cells in the subpopulation of CD25$^+$ CD4$^+$ T lymphocytes found in the deciduas of patients decreased with the successive stages of labor, while the percentages of B7-H4 positive cells in the macrophage subpopulation remained almost constant.

## Conclusion
These changes in the Treg cell subpopulation in the decidua would seem to be related to a brief activation of the maternal immune system as labor begins and lack of analogical changes in the subpopulation of decidual suppressive B7-H4$^+$ macrophages that enable the restriction of this same activation as labor progresses.

## Introduction

Human regulatory T cells (Tregs) play an important role in immune tolerance and the negative regulation of the immune response.[1] Pregnancy is characterized by maternal immune tolerance toward fetal antigens, and the presence of Treg cells is pivotal for the maintenance of pregnancy.[2–7] Treg cell population can be identified by CD4 and CD25 antigen expression, and precise identification is now possible by means of the transcription factor forkhead box P3 (Foxp3).[8–10] Heikkinen et al. have demonstrated the presence of Treg cells in the decidua and peripheral blood in women throughout the trimesters of pregnancy.[11]. In a study by Tilburg et al., the percentage of FOXP3$^+$ cells within the CD4$^+$ CD25$^+$ lymphocyte subpopulation in the decidua basalis remained constant as pregnancy advanced from the second to third trimester.[12] It has further been demonstrated that fetus-specific Treg cells are recruited to the decidua from the periphery.[12] The way of Treg-cell activation is complex and related to the continuous antigen stimulation of CD4$^+$ CD25$^-$cells leading to the generation of adaptive CD4$^+$ CD25$^+$ FOXP3$^+$ regulatory T cells.[13] It has been observed that in mice estrogen (17-beta-estradiol-E2) enhances Foxp3 expression in CD25$^+$ T cells both *in vitro* and *in vivo*.[10] The number of Treg cells in human decidua would therefore seem to be related to the status of the microenvironment; it would also seem that the decidual microenvironment itself undergoes important changes during labor. The beginning of labor is determined not only by the molecular changes in maternal–fetal interface, but most likely also by the activity of the fetal adrenals and related cofactors. As the involvement of endocrine signals from the fetal adrenals during the initiation of labor is well known,[14] we have focused our study on the decidual microenvironment and regulatory immune cells that can be found there. Certainly, the initiation of labor is accompanied by alterations in the level of maternal immune tolerance toward fetal antigens.[15–29] It is a complex molecular response leading to a brief activation of the maternal immune system with an accompanying capacity to restrict this same activation.[1,18,20,22] An increase in lymphocyte activity during labor has been observed[18] as well as an alteration in the distribution of natural killer (NK) cells (CD56$^+$ CD16$^+$) and CD3 positive lymphocytes in the decidua basalis derived from both spontaneous vaginal delivery and cesarean sections.[16] At term, a higher percentage of CD4$^+$ CD25$^{dim}$ as also CD4$^+$ CD25$^{bright}$ T lymphocytes have been found in the decidua parietalis than in the decidua basalis.[30] Moreover, it has recently been suggested that the maternal immune system is not solely responsible for the proper initiation of labor, but that fetal macrophages also play an important role in this process.[19] Fetal macrophages produce and secrete interleukin (IL)-6 during the initiation of labor. IL-6 is responsible for the inhibition of Treg activity[31] and is necessary for the proper course of labor; its presence has been identified in the decidua and amnion.[32] For this reason, we have chosen the progression of labor as a research model for analyzing the number of Treg cells in decidua. Other inhibitory cells, such as CD8$^+$ CD28$^-$ lymphocytes, have also been analysed in the decidua basalis and the decidua parietalis at term pregnancy.[30] Until now, the presence of B7-H4 macrophages–cells responsible for the cytotoxic cell inhibition – in the decidua during labor had not been analysed. There are many molecular similarities between the phenomenon of maternal immune tolerance during pregnancy and that of tumor escape from the host immune surveillance.[2,33] The presence of Treg cells in the tumor microenvironment plays a key role in tumor development, and B7-H4 macrophages have been identified among the tumor-associated macrophages in the tumor microenvironment.[34,35] B7-H4 are co-stimulatory molecules, and the membrane protein B7 family is responsible for the negative regulation of T-cell-mediated immune response.[36,37] Kryczek et al. have shown that B7-H4 expression identifies a new suppressive macrophage population in humans.[35] He has also observed that IL-6 and IL-10 cytokines stimulate the expression of B7-H4 on macrophages. For this reason, we have analysed the Treg cells and the B7-H4 macrophages concurrently. The aim of our study has been to evaluate the subpopulation of Treg cells and B7-H4 macrophages in the decidua basalis during cesarean sections performed on patients in various stages of labor.

## Material and methods

### Patients

The decidual tissue samples evaluated in our study were obtained from 23 pregnant women who underwent cesarean sections at term. The women in our study were selected from those undergoing such

cesarean sections between January and December of 2007 in the Department of Gynecology, Obstetrics and Oncology at the Jagiellonian University, Krakow, Poland. The following conditions were indications for performing a cesarean: fetal malpresentation (mainly breech presentation), repeat cesarean section, cephalopelvic disproportion, myoma, and myopia. Furthermore, the patients were divided into three subgroups according to the progression of labor at the time of the cesarean (we have also used this division in our recent studies concerning the regulation of immune system activity during labor[18,20,38]) Patients with multiple pregnancies or existing complications of pregnancy (such as pre-term deliveries, hypertension, and diabetes mellitus) were excluded from this study, as were cases of fetal demise. The tissue samples were obtained from patients during curettage of the decidua basalis just after effecting the placenta detachment during cesarean section. From the obtained samples, mononuclear cells were isolated and immediately frozen in a freezing medium containing 10% DMSO, 5% albumin and 85% of a cell culture medium.

The patient's consent was obtained in each case. Prior to the present study we also obtained the approval of the Jagiellonian University Ethical Committee for our research program (KBET/135/B/2007).

### Decidual Mononuclear Cell Isolation

The decidua was cut into small fragments and disintegrated by smashing it through a 40 $\mu$m cell-strainer. The cells so obtained were then centrifuged and the resulting pellet was subjected to ammonium chloride lysis for elimination of contaminating red cells. Following lysis, the cells were washed in phosphate-buffered saline (PBS). Thereafter, the cells were re-suspended in PBS, counted, and used either immediately for staining or frozen for future analysis.

### Flow Cytometry

The cell phenotype was analysed with the panel of monoclonal antibodies (mAb) – CD4 fluorescein isothiocyanate (FITC), CD25 allophycocyanin (APC), and FOXP3 phycoerythrin PE (BD Biosciences Pharmingen, San Jose, CA, USA). Briefly stated, to the $1 \times 10^6$ cells suspended in 60 $\mu$L of staining buffer (PBS, 2% FBS), 20 $\mu$L of each mAb (CD4, CD25) was added. Thereafter, the cells were incubated in the dark for 30 min at 4°C. After incubation, the cells were washed twice in PBS and were permeabilized with FoxP3 permeabilization buffer (BD Biosciences Pharmingen) for 30 min at room temperature in the dark and stained with anti-FOXP3 antibodies for 30 min at room temperature in the dark. The stained cells were then washed and collected using the FACSCanto-cytometer (BD Biosciences Immunocytometry Systems, San Diego, CA, USA), and finally were analysed with FACS Diva software (Becton Dickinson). Each time, $3 \times 10^4$ events were saved for analysis. Logical gates were used to analyse particular populations of cells.

The CD14/B7-H4 cells were stained using monoclonal antibodies – CD14 FITC and B7-H4 P (Pharmingen) – as were the Treg cells described above except that the permeabilization step was omitted. The analysis was also performed in the same way and the percentage of CD14/B7-H4 positive cells was estimated in the whole population of isolated cells.

### Statistical Analysis

The statistical significance between the groups was determined by the ANOVA test (the number of lymphocytes) and by the Kruskal–Wallis analysis of variance (clinical characteristics). The data was presented in terms of median value and intra-quartile range (clinical characteristics) as well as in terms of mean and standard deviation (the number of lymphocytes).

## Result

### Clinical Comparison of Analysed Groups of Patients with Cesarean Section at Term with Respect to the Progression of Labor

As there are different indications for performing a cesarean section, it would seem to be important to compare the parameters characterizing the course of pregnancy and labor in the different groups of patients considered (Table I).

We did not observe any statistically significant differences in the clinical parameters among the groups examined. The lack of differences in the clinical parameters characterizing the course of pregnancy enabled us to compare the respective percentages of $FOXP3^+$ cells in the subpopulations of $CD25^+$ $CD4^+$ T lymphocytes as well as the respective percentage of $B7\text{-}H4^+$ cells in the subpopulation of CD14 positive cells in the decidua basalis.

**Table I** Clinical Characteristics of Patients Who Underwent Cesarean Section at Different Stages in the Progression of Labor

| Variables | Cesarean section without labor ($n$ = 5) | Cesarean section with symptoms of spontaneous beginning of labor ($n$ = 9) | Cesarean section with advanced labor ($n$ = 9) | $P$-value |
|---|---|---|---|---|
| Maternal age (mean ± S.D.) | 26.14 ± 3.23 | 28.8 ± 4.54 | 28.4 ± 3.13 | 0.82 |
| Parity (median ± S.E.M.) | 1 ± 0.13 | 1 ± 0.19 | 2 ± 0.19 | 0.42 |
| Gestational age (mean ± S.D.) | 37.8 ± 2.03 | 37.6 ± 1.83 | 38.6 ± 2.07 | 0.5 |
| Newborn mass (average ± S.D.) | 3224.2 ± 368.9 | 3028 ± 417.6 | 3510 ± 439.3 | 0.65 |
| Apgar score, 1 min (median ± S.E.M.) | 9 ± 0.13 | 9 ± 0.18 | 9.5 ± 0.12 | 0.8 |

S.D., standard deviation.

### The Analysis of the CD25$^+$ CD4$^+$ FOXP3$^+$ T-regulatory Cells and Suppressive B7-H4$^+$ Macrophages in Decidua with Respect to the Progression of Labor

The changes in the percentage of the subpopulation of immune cells in the decidua basalis were identified according to the successive stages of labor. The study group of patients who underwent cesarean sections at term was divided into three subgroups according to the degree of uterine cervical ripening and the presence of uterine contractions during the surgical procedure. Group A consisted of patients without any uterine contractions and with closed uterine cervical os (i.e. 'cesarean without labor'); group B consisted of patients with irregular uterine contractions or who were beginning regular uterine contractions with a cervical dilation between 1 and 3 cm (i.e. cesarean section with the spontaneous beginning of labor); group C consisted of patients with regular uterine contractions and a cervical dilation above 3 cm (i.e. 'cesarean with advanced labor') (Figs 1 and 2).

We found a statistically significant lower percentage of FOXP3$^+$ cells in the subpopulation of CD25$^+$ CD4$^+$ T lymphocytes in the decidua basalis when the cesarean section was performed during advanced labor (C) in comparison with that when the cesarean section was performed without labor (A) (respectively $P = 0.02$). The percentage of CD25$^+$ CD4$^+$ FOXP3$^+$ T lymphocytes decreased with the successive stages of labor, but the difference in the percentage of these cells found in the decidua between patients on whom the cesarean sections were performed after the spontaneous beginning of labor (B) and on whom the cesarean sections were performed during advanced labor was not statistically significant ($P = 0.055$).

We have noted a comparable percentage of CD4$^+$ lymphocytes T in the following groups of patients in accordance with the progression of labor [respectively as follows: group A – 23.28% (±5.98); group B – 29.04% (±6); and group C – 22.94% (±8.04)]. Additionally, we have also observed a comparable percentage of CD4$^+$ CD25$^+$ cells within the CD4$^+$ T lymphocyte subpopulation in these groups [respectively group A – 2.6% (±3.33); group B – 3.21% (±2.05); and group C – 97% (±4.23)] (Fig. 3).

No differences in the percentages of the decidual B7-H4 positive cells in the subpopulation of CD14 positive cells were found among the tissue samples derived from patients who had undergone caesareans at different stages of labor (Fig. 4).

## Discussion

The percentages of FOXP3$^+$ cells in the subpopulation of CD25$^+$ CD4$^+$ T lymphocytes found in the decidua basalis of patients decreased with the successive stages of labor, while the percentages of B7-H4 positive cells in the macrophage subpopulation remained almost constant.

To our knowledge, this is the first investigation to focus on the alterations in Treg cells in the decidua relative to the progression of labor. Tilburg et al. have investigated the distribution of Treg CD25$^+$ CD4$^+$ lymphocytes in the decidua basalis, decidua parietalis, and peripheral blood in women in early pregnancy (between 17 and 24 weeks) and at term (up to week 37).[30] Although the FOXP3 antigen has not been used for the identification of these cells, two types of lymphocytes were distinguished, CD4$^+$ CD25$^{bright}$ and CD4$^+$ CD25$^{dim}$. In this study,[30] the percentage of CD4+ CD25$^{bright}$ T lymphocytes in both the decidua basalis and decidua parietalis remained constant throughout the course of

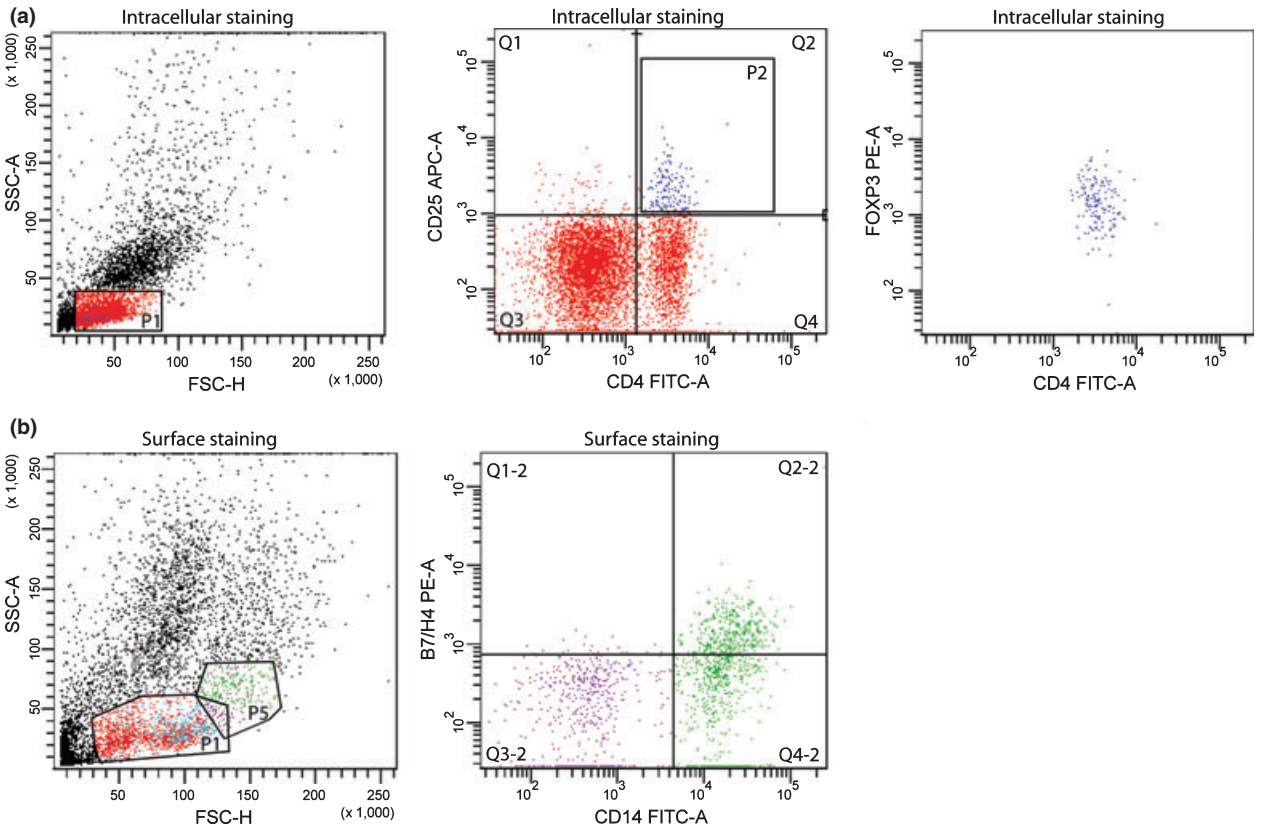


**Fig. 1** Staining of Treg cells and suppressive macrophages in decidua. (a) Intra-cellular staining CD4/CD25/FOXP3. P1 – lymphocyte gate; P2 – $CD4^+$ $CD25^+$ population. (b) Surface staining CD14/B7-H4. P1 – lymphocyte gate; P5 – monocyte gate.

pregnancy. In early pregnancy, there was no difference between the percentage of these cells in the decidua basalis and those in the decidua parietalis. At term, however, the percentage of CD4+ $CD25^{bright}$ T lymphocytes was statistically significantly higher in the decidua parietalis than in the decidua basalis.[30] Next, Tilburg et al. presented an analysis of the percentage of $FOXP3^+$ cells within the subpopulation of both $CD4^+$ $CD25^{bright}$ and $CD4^+$ $CD25^{dim}$ T lymphocytes, but did not identify any differences in the distribution of these cells in the decidua basalis when compared with the decidua parietalis at term. Moreover, no differences in the percentage of these cells were observed between the second and third trimesters of pregnancy.[12] In our study, only the lymphocytes infiltrating the decidua basalis were analysed. Like Tilburg et al., we identified a comparable percentage of $CD4^+$ $CD25^+$ T lymphocytes in decidua basalis[12,30] and additionally found that the percentage of these cells did not differ as labor progressed. In contrast to Tilburg, who in his study analysed healthy women after uncomplicated pregnancies without noting the stage of the labor in which the decidual samples were collected, we analysed decidua obtained during cesarean sections performed during the various stages of labor. Decidua obtained during the various stages of labor can provide the necessary information to establish a research model for the analysis of the molecular changes that occur in the decidua basalis as labor progresses (Such a research model was also applied in our previous studies concerning the molecular changes in the decidua during labor[18,20,38]) As we evaluated the presence of Treg cells during three different phases of the first stage of the labor, we were able to demonstrate a decrease in the number of FOXP3 positive cells within the $CD4^+$ $CD25^+$ lymphocyte subpopulation, as the labor advanced.

The initiation of labor is related to the activity of the maternal immune system as well as to the fetal immune response. TGF-beta is a pivotal cytokine in the conversion of $CD4^+$ $CD25^-$ lymphocytes into

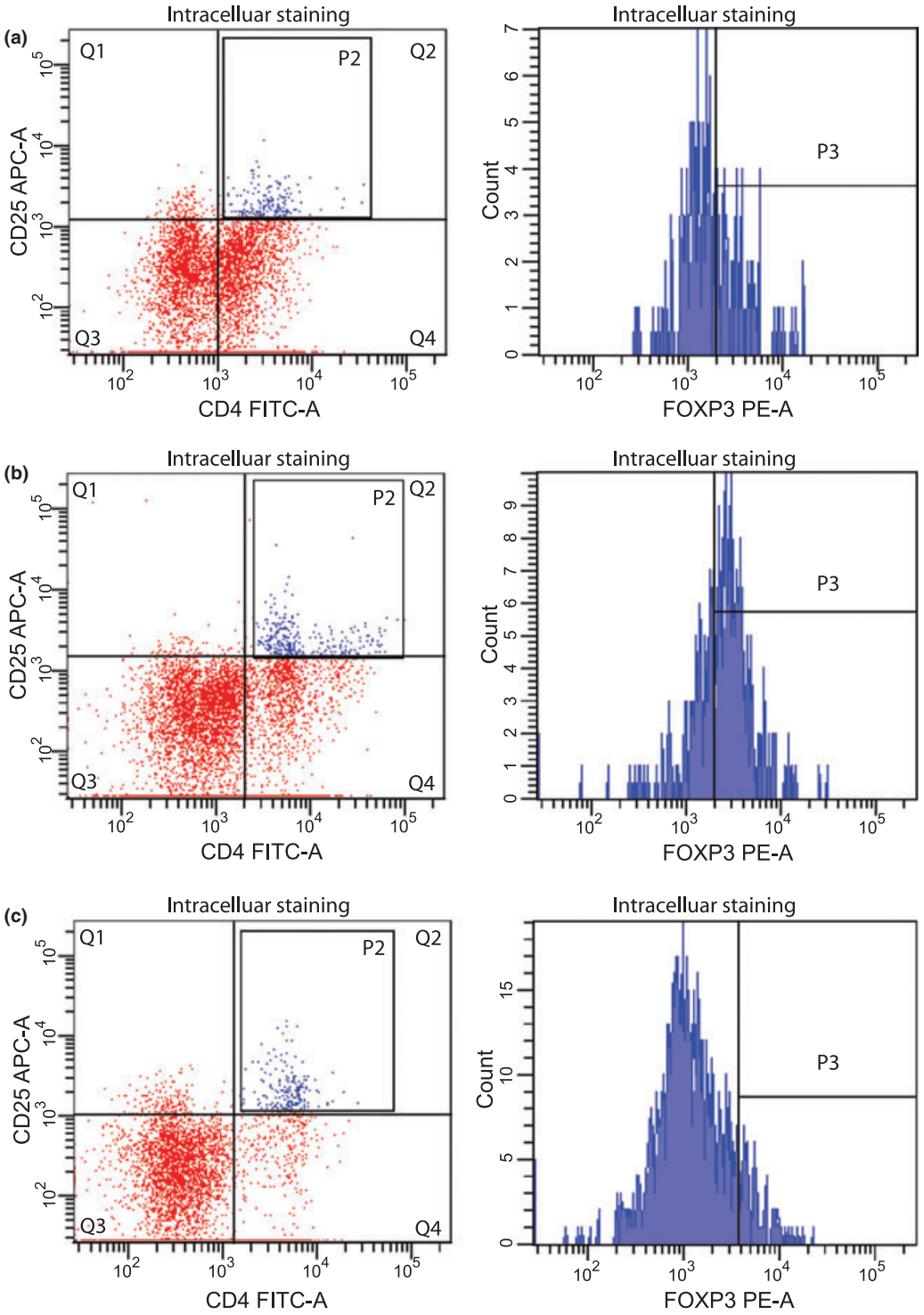


**Fig. 2** Intra-cellular staining CD4/CD25/FOXP3 relative to the progression of labor (a) cesarean without labor; (b) cesarean section with spontaneous beginning of labor; (c) cesarean with advanced labor. Cells were gated from lymph gate. P2 – $CD4^+$ $CD25^+$ population; P3 – $CD4^+$ $CD25^+$ $FOXP3^+$ population.

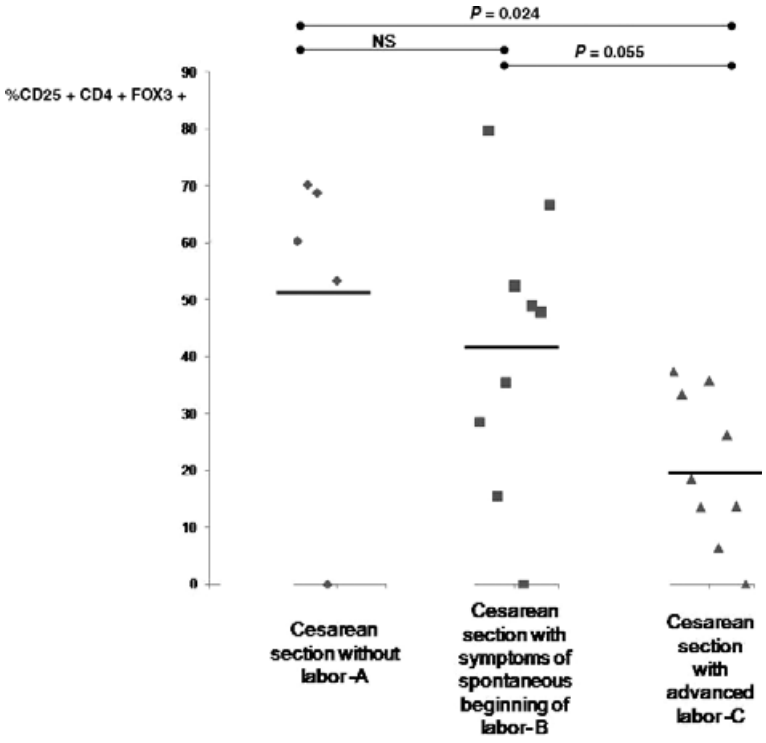


**Fig. 3** The results of the analysis of the percentages of FOXP3$^+$ cells in the subpopulation of CD25$^+$ CD4$^+$ T lymphocytes in decidua basalis relative to the progression of labor in patients on whom cesarean sections were performed (Group A – cesarean without labor; group B – cesarean section with spontaneous beginning of labor; group C – cesarean with advanced labor).

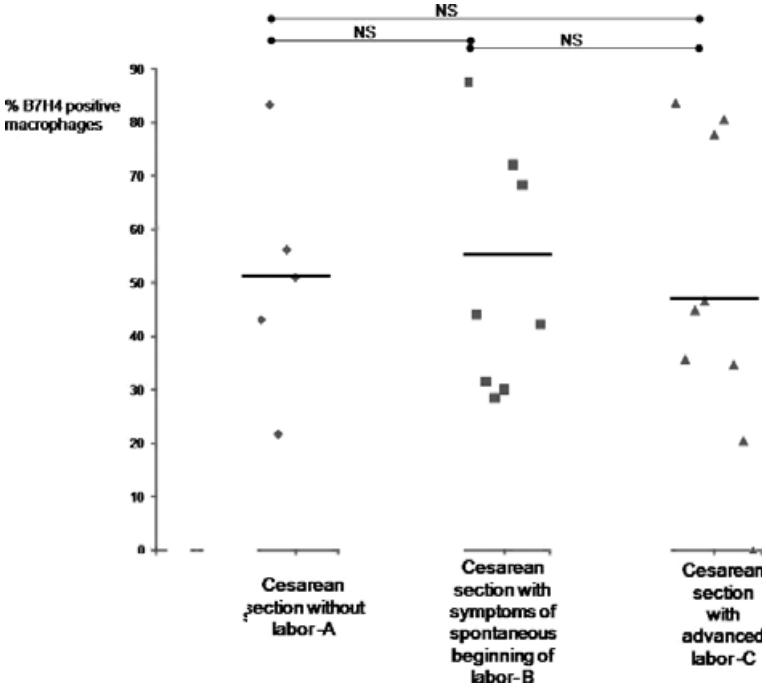


**Fig. 4** The results of the analysis of the percentages of B7-H4 positive cells in the decidual CD14 positive cells subpopulation in relation to the progression of labor in patients on whom caesarean sections were performed (Group A – cesarean without labor; group B – cesarean section with the spontaneous beginning of labor; group C – cesarean with advanced labor).

CD4$^+$ CD25$^+$ FOXP3$^+$ T cells. An observed increase in the TGF-beta1 concentration level in the maternal blood in late pregnancy was significant; however, this level did not alter as labor progressed. This has been confirmed through the analysis of TGF-beta1 secretion by decidual leukocytes that spontaneously


American Journal of Reproductive Immunology **61** (2009) 136–146

secrete TGF-beta1 at a constant level, both with and without labor.[39] The level of TGF-beta1 concentration in the umbilical blood did change, however, as labor progressed.[40] Consequently, we know that the TGF-beta1 concentration level at the maternal–fetal interface may change as labor advances. TGF-beta-induced Treg cells can be inhibited under IL-6 influence, and a dichotomy Treg/Th17 is moved into pro-inflammatory T-cell response related to Th17 cell.[41] Steinborn et al. have demonstrated that fetal macrophages secrete IL-6 during labor and that this cytokine is also produced by fetal membranes.[19] Such an immune system reaction is necessary for the initiation of labor, and this has been confirmed by many studies.[15–18,29]

It has also been demonstrated that CD4$^+$ CD25$^{bright}$ T cells are able to inhibit both the specific and non-specific immune responses in a dose-dependent manner.[12] Changes in the number of cytotoxic immune cells during the menstrual cycle with the infiltration of NK cells in decidua have also been found.[42] Arruvito et al. have observed an increase in the percentage of FOXP3$^+$ cells in the subpopulation of CD4$^+$ CD25$^+$ T cells in the peripheral blood in women just ahead of ovulation[4,24] and a decrease in the percentage of these cells in women with recurrent spontaneous abortion. Treg cells have also been shown to inhibit NK cells and to induce lower expression of NKG2D receptor on NK cells;[43,44] the changes in the NK cell population during labor have been already demonstrated.[16] Alterations in the number of Treg cells in the decidua are most likely related to the increase in immune cytotoxic cell activity necessary for the proper course of labor.

The changes in the decidua microenvironment during the initiation of labor are probably responsible for the observed decrease in the subpopulation of Treg cells. However, the factor responsible for the restriction of the maternal immune system activity that increases with the initiation of labor is not clear. In our previous study, one of the mechanisms observed to restrict the maternal immune system activity was the increase in the inhibitory activity of the decidua that is related to membrane proteins expressed by decidual cells and secreted to the feto-placental microenvironment.[20,22,45] In the current study, we have observed the presence of B7-H4 macrophages at the maternal–fetal interface. To our knowledge, this is the first investigation to focus on the presence of B7-H4 suppressive macrophages in the decidua relative to the progression of labor. Up until now, the presence of these cells had been identified in the tumor microenvironment.[34,35] The expression of the B7-H4 molecule on macrophages is induced by IL-6.[34] It has been confirmed in *in vitro* study that the presence of Treg cells sensitizes macrophages to the action of IL-6 and subsequent B7-H4 expression.[34] The B7-H4 molecule shares 23% sequence identity with CD80 and CD86, but does not possess a CTL-4/CD28 binding site, nor does it bind CTLA-4 or CD28.[46] The B- and T-lymphocyte attenuator that is the member of CD28 family is probably a ligand for this molecule.[47] B7-H4 inhibits T cells by cell cycle arrest and the inhibition of cytokine production. B7-H4 action is mediated in a fashion similar to that of CTLA-4. In sum, the B7-H4 molecule constitutes a checkpoint for the negative control of T cells.[46]

The analysis of the presence of macrophages (CD14 positive cells) in the decidua did not reveal any differences in the CD14$^+$ cell subpopulation when samples from patients experiencing vaginal labor were compared with those from the women undergoing cesarean sections.[48] The alterations in macrophage activity, as measured by such antigens as CD80, CD86, and HLA-DR, have been documented in this study as being associated with the development of pregnancy, and their activity has been shown to be significantly higher during the second than during the third trimester of pregnancy.[48] The lack of alterations in the percentage of B7-H4 positive cells in the subpopulation of CD14 positive cells observed in our current study may account for the restriction of the immune cell action that is initiated as the labor–homeostatic mechanism in the reproductive tract is set in motion. The fetus probably actively participates in this process by producing IL-6. The development of immune tolerance during pregnancy thus seems to require a combination of regulatory mechanisms. Moreover, the restriction of immune-cell response connected with the progression of labor is a complex phenomenon related to the function of both the maternal decidua and the fetal immune system.

## Conclusion

These changes in the Treg cell subpopulation in decidua would seem to be related to a brief activation of the maternal immune system as labor begins and a lack of analogical changes in the subpopula-

tion of decidual suppressive B7-H4$^+$ macrophages that enable the restriction of this same activation as labor progresses. This could indicate that the immune processes that occur during labor require a combination of regulatory mechanisms.

### Acknowledgments

We wish to thank Professors J. Kotarski and L. Fainboim for their advice, helpful discussions, and friendly words of support. I would also like to thank Christine Maisto and Dr Tomasz Banas for their assistance. This work was funded by the Polish Ministry of Science, Grant Number: 0888/B/P01/2008/35 in 2008/2009.

### References

1 Hori S, Sakaguchi S: Foxp3: a critical regulator of the development and function of regulatory T cells. *Microbes Infect* 2004; 6:745–751.
2 Wilczynski JR, Kalinka J, Radwan M: The role of the T-regulatory cells in pregnancy and cancer. *Front Biosci* 2008; 13:2275–2289.
3 Wilczynski JR, Radwan M, Kalinka J: The characterization and role of regulatory T cells in immune reactions. *Front Biosci* 2008; 13:2266–2274.
4 Aluvihare VR, Betz AG: The role of regulatory T cells in alloantigen tolerance. *Immunol Rev* 2006; 212:330–343.
5 Aluvihare VR, Kallikourdis M, Betz AG: Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004; 5:266–271.
6 Saito S, Sasaki Y, Sakai M: CD4(+)CD25high regulatory T cells in human pregnancy. *J Reprod Immunol* 2005; 65:111–120.
7 Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S: Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. *Mol Hum Reprod* 2004; 10:347–353.
8 Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY: Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity* 2005; 22:329–341.
9 Fontenot JD, Gavin MA, Rudensky AY: Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003; 4:330–336.
10 Polanczyk MJ, Carson BD, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, Offner H: Cutting edge: estrogen drives expansion of the CD4+ CD25+ regulatory T cell compartment. *J Immunol* 2004; 173:2227–2230.
11 Heikkinen J, Möttönen M, Alanen A, Lassila O: Phenotypic characterization of regulatory T cells in the human decidua. *Clin Exp Immunol* 2004; 136:373–378.
12 Tilburgs T, Roelen DL, van der Mast BJ, de Groot-Swings GM, Kleijburg C, Scherjon SA, Claas FH: Evidence for a selective migration of fetus-specific CD4+CD25bright regulatory T cells from the peripheral blood to the decidua in human pregnancy. *J Immunol* 2008; 180:5737–5745.
13 Mahic M, Henjum K, Yaqub S, Bjørnbeth BA, Torgersen KM, Taskén K, Aandahl EM: Generation of highly suppressive adaptive CD8(+)CD25(+)FOXP3(+) regulatory T cells by continuous antigen stimulation. *Eur J Immunol* 2008; 38:640–646.
14 Smith R, Nicholson RC: Corticotrophin releasing hormone and the timing of birth. *Front Biosci* 2007; 12:912–918.
15 Abadia-Molina AC, Ruiz C, Montes MJ, King A, Loke YW, Olivares EG: Immune phenotype and cytotoxic activity of lymphocytes from human term decidua against trophoblast. *J Reprod Immunol* 1996; 31:109–123.
16 Sindram-Trujillo AP, Scherjon SA, van Hulst-van Miert PP, Kanhai HH, Roelen DL, Claas FH: Comparison of decidual leukocytes following spontaneous vaginal delivery and elective cesarean section in uncomplicated human term pregnancy. *J Reprod Immunol* 2004; 62:125–137.
17 Osman I, Young A, Jordan F, Greer IA, Norman JE: Leukocyte density and proinflammatory mediator expression in regional human fetal membranes and decidua before and during labor at term. *J Soc Gynecol Investig* 2006; 13:97–103.
18 Wicherek L, Galazka K: The possible correlation between the patient's immune tolerance level during cesarean section and the incidence of subsequent emergency peripartum hysterectomy. *Clin Dev Immunol* 2007; 2007:63596 (doi 10.1155/2007/63596).
19 Steinborn A, Sohn C, Sayehli C, Baudendistel A, Huwelmeier D, Solbach C, Schmitt E, Kaufmann M: Spontaneous labor at term is associated with fetal monocyte activation. *Clin Exp Immunol* 1999; 117:147–152.
20 Wicherek L, Basta P, Galazka K, Mak P, Dancewicz L, Kalinka J: RCAS1 decidual immunoreactivity and RCAS1 serum level during cesarean section with respect to the progression of labor. *Am J Reprod Immunol* 2008; 59:152–158.
21 Tskitishvili E, Komoto Y, Kinugasa Y, Kanagawa T, Song M, Mimura K, Tomimatsu T, Kimura T,

Shimoya K: Relationship between human tumor-associated antigen RCAS1 and gestational diabetes mellitus. *Am J Reprod Immunol* 2007; 58:440–446.
22 Wicherek L, Gałązka K, Lazar A: RCAS1 decidual immunoreactivity during placental abruption: immune cell presence and activity. *Am J Reprod Immunol* 2007; 58:46–55.
23 Binder T, Hajek Z, Zoban P, Plavka R: Conducting labor in women with previous caesarean section in a low gestational week. A prospective case-controlled study. *Gynecol Obstet Invest* 2008; 66:197–202.
24 Magann EF, Doherty DA, Briery CM, Niederhauser A, Chauhan SP, Morrison JC: Obstetric characteristics for a prolonged third stage of labor and risk for postpartum hemorrhage. *Gynecol Obstet Invest* 2008; 65:201–205.
25 Szekeres-Bartho J, Wilczynski JR, Basta P, Kalinka J: Role of progesterone and progestin therapy in threatened abortion and preterm labour. *Front Biosci* 2008; 13:1981–1990.
26 Raghupathy R, Kalinka J: Cytokine imbalance in pregnancy complications and its modulation. *Front Biosci* 2008; 13:985–994.
27 Weiss A, Goldman S, Shalev E: The matrix metalloproteinases (MMPS) in the decidua and fetal membranes. *Front Biosci* 2007; 12:649–659.
28 Olson DM, Ammann C: Role of the prostaglandins in labour and prostaglandin receptor inhibitors in the prevention of preterm labour. *Front Biosci* 2007; 12:1329–1343.
29 Szekeres-Bartho J, Varga P, Pacsa AS: Immunologic factors contributing to the initiation of labor–lymphocyte reactivity in term labor and threatened preterm delivery. *Am J Obstet Gynecol* 1986; 155:108–112.
30 Tilburgs T, Roelen DL, van der Mast BJ, van Schip JJ, Kleijburg C, de Groot-Swings GM, Kanhai HH, Claas FH, Scherjon SA: Differential distribution of CD4(+)CD25(bright) and CD8(+)CD28(−) T-cells in decidua and maternal blood during human pregnancy. *Placenta* 2006; 27(Suppl. A):S47–S53.
31 Lehner T: Special regulatory T cell review: the resurgence of the concept of contrasuppression in immunoregulation. *Immunology* 2008; 123:40–44.
32 Malmström E, Sennström M, Holmberg A, Frielingsdorf H, Eklund E, Malmström L, Tufvesson E, Gomez MF, Westergren-Thorsson G, Ekman-Ordeberg G, Malmström A: The importance of fibroblasts in remodelling of the human uterine cervix during pregnancy and parturition. *Mol Hum Reprod* 2007; 13:333–341.
33 Wicherek L: The role of the endometrium in the regulation of immune cell activity. *Front Biosci* 2008; 13:1018–1035.
34 Kryczek I, Wei S, Zhu G, Myers L, Mottram P, Cheng P, Chen L, Coukos G, Zou W: Relationship between B7-H4, regulatory T cells, and patient outcome in human ovarian carcinoma. *Cancer Res* 2007; 67:8900–8905.
35 Kryczek I, Zou L, Rodriguez P, Zhu G, Wei S, Mottram P, Brumlik M, Cheng P, Curiel T, Myers L, Lackner A, Alvarez X, Ochoa A, Chen L, Zou W: B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. *J Exp Med* 2006; 203:871–881.
36 Choi IH, Zhu G, Sica GL, Strome SE, Cheville JC, Lau JS, Zhu Y, Flies DB, Tamada K, Chen L: Genomic organization and expression analysis of B7-H4, an immune inhibitory molecule of the B7 family. *J Immunol* 2003; 171:4650–4654.
37 Miyatake T, Tringler B, Liu W, Liu SH, Papkoff J, Enomoto T, Torkko KC, Dehn DL, Swisher A, Shroyer KR: B7-H4 (DD-O110) is overexpressed in high risk uterine endometrioid adenocarcinomas and inversely correlated with tumor T-cell infiltration. *Gynecol Oncol* 2007; 106:119–127.
38 Galazka K, Pitynski K, Skret-Magierlo J, Mach P, Knafel A, Sikora J, Niemiec T, Dobrogowski J, Basta A, Wicherek L. The increase in metallothionein eutopic and ectopic decidual immunoreactivity with respect to the progression of labor at term and the lack of analogical changes in placental abruption. *Am J Reprod Immunol* 2008; 60:513–522. DOI 10.1111/j.1600-0897.2008.00615x.
39 Gustafsson C, Hummerdal P, Matthiesen L, Berg G, Ekerfelt C, Ernerudh J: Cytokine secretion in decidual mononuclear cells from term human pregnancy with or without labour: ELISPOT detection of IFN-gamma, IL-4, IL-10, TGF-beta and TNF-alpha. *J Reprod Immunol* 2006; 71:41–56.
40 Power LL, Popplewell EJ, Holloway JA, Diaper ND, Warner JO, Jones CA: Immunoregulatory molecules during pregnancy and at birth. *J Reprod Immunol* 2002; 56:19–28.
41 Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK: Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; 441:235–238.
42 Arruvito L, Sanz M, Banham AH, Fainboim L: Expansion of CD4+CD25+ and FOXP3+ regulatory T cells during the follicular phase of the menstrual cycle: implications for human reproduction. *J Immunol* 2007; 178:2572–2578.
43 Ghiringhelli F, Ménard C, Terme M, Flament C, Taieb J, Chaput N, Puig PE, Novault S, Escudier B, Vivier E, Lecesne A, Robert C, Blay JY, Bernard J,

Caillat-Zucman S, Freitas A, Tursz T, Wagner-Ballon O, Capron C, Vainchencker W, Martin F, Zitvogel L: CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner. *J Exp Med* 2005; 202:1075–1085.

44 Salih HR, Holdenrieder S, Steinle A: Soluble NKG2D ligands: prevalence, release, and functional impact. *Front Biosci* 2008; 13:3448–3456.

45 Skret-Magierlo J, Wicherek L, Basta P, Galazka K, Sikora J, Wilk M, Fudali L, Skret A: RCAS1 decidual immunoreactivity during cesarean section in scar deciduosis: immune cell presence and activity. *Gynecol Obstet Invest* 2008; 65:187–194.

46 Sica GL, Choi IH, Zhu G, Tamada K, Wang SD, Tamura H, Chapoval AI, Flies DB, Bajorath J, Chen L: B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. *Immunity* 2003; 18:849–861.

47 Martin-Orozco N, Dong C: Inhibitory costimulation and anti-tumor immunity. *Semin Cancer Biol* 2007; 17:288–298.

48 Repnik U, Tilburgs T, Roelen DL, van der Mast BJ, Kanhai HH, Scherjon S, Claas FH: Comparison of macrophage phenotype between decidua basalis and decidua parietalis by flow cytometry. *Placenta* 2008; 29:405–412.