Regulatory T-cell Subpopulations in Severe or Early-onset Preeclampsia

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ABSTRACT

Problem

A deficiency in regulatory T (Treg) cells causing reduced immune regulatory capacity has been proposed in preeclampsia.

Objective

Utilizing recent advances in flow cytometry phenotyping, we aimed to assess whether a deficiency of Treg subpopulations occurs in preeclampsia.

Method of Study

Six-color flow cytometry was used for Treg phenotyping in 18 preeclamptic women (1 early-onset, 1 severe and 16 both), 20 women with normal pregnancy and 20 non-pregnant controls.

Results

No differences were found in major Treg populations including $CD127^{low}CD25^+/CD127^{ow}FOXP3^+$, resting ($FOXP3^{dim}CD45RA^+$) and activated ($FOXP3^{bright}CD45RA^-$) Treg cells, whereas preeclamptic women showed increased CTLA-4^+ and CCR4$^+$ proportions within resting/activated Treg populations. Corticosteroid-treatment prior to blood sampling (n=10) affected the distribution of Treg populations.

Conclusions

Although we found no major alterations in circulating Treg frequencies, differences in CTLA-4^+ and CCR4$^+$ frequencies suggest a migratory defect of Treg cells in preeclampsia. Corticosteroid treatment should be taken into account when evaluating Treg cells.

INTRODUCTION

Preeclampsia and eclampsia are among the most frequent causes of maternal and fetal mortality and morbidity affecting 2-10 % of the pregnant population worldwide (1). There is still no generally accepted etiology of preeclampsia, but an increasing amount of data indicate involvement of the immune system, with a defective tolerance to the conceptus being an integral part of the pathogenesis, in particular in early-onset and severe preeclampsia (2). The end result is a state of exaggerated systemic inflammation (3-7) with activated circulating T cells (8-10) and increased production of interferon-gamma (IFN-γ) (8, 11, 12). It is therefore reasonable to suspect that a defect in the regulation of T cells could be involved in the development of preeclampsia.

Regulatory T (Treg) cells represent one of the major immune regulators(13), suppressing many cell types including T cells (14) via mechanisms that are incompletely understood but involving cell-cell contact through for example CTLA-4 as well as secretion of soluble mediators like the cytokines interleukin 10 (IL-10), transforming growth factor β (TGF-β) and IL-35 (15, 16). In humans, Treg cells are identified as CD4^{+} T helper cells expressing forkhead box P3 (FOXP3) (14) and high levels of the IL-2 receptor α-chain, CD25 (14, 15). Early reports indicated that the circulating Treg pool was expanded in normal human pregnancy (17-20). However, with increasing insights in the complexity of Treg markers in humans, subsequent studies did not report increased frequency in the circulation (21-25). Instead it seems that highly suppressive Treg cells are enriched in the decidua (23, 26-28). One mechanism for this enrichment is through abundant decidual CCL17 expression (29) and recruitment of CCR4 expressing Treg cells (28).

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In preeclampsia, reduced numbers of circulating Treg cells have been reported (for review see 30) although these findings were not confirmed in other studies (31-33). One reason for the inconsistency could be the selection of cases in a heterogeneous condition like preeclampsia. Since severe and early-onset preeclampsia with growth-retarded fetuses is associated with increased disease severity and placental pathology, this entity might represent a more homogeneous placental disease. Thus, research on this sub-group has been encouraged (34). Also, treatment with corticosteroids could influence Treg cell frequencies and phenotype (35), which is important in preeclampsia where corticosteroids are often administered at an early time point to enhance fetal lung maturation. The most prominent factor explaining inconsistent results is likely how Treg cells were defined. One major problem is that both CD25 and FOXP3, the key phenotypic markers, are expressed upon activation of conventional CD4^{+} T cells, which implies difficulties in enumeration of "true" Treg cells in diseases with systemic T cell activation such as preeclampsia. Different flow cytometric strategies and markers have evolved to circumvent the inclusion of activated non-suppressive ("false positive") Treg cells; (1) lower CD4 expression on suppressive Treg cells than conventional CD4 cells ("CD4^{dim}") (36); (2) low or no expression of the IL-7 alpha receptor subunit CD127 (37); (3) CD45RA to distinguish suppressive ("true") resting Treg cells (FOXP3^{dim}CD45RA^{+}) from non-suppressive, activated FOXP3 expressing T helper cells (FOXP3^{dim}CD45RA^{-}) (38). This strategy, which has not been used in studies of preeclampsia, not only excludes false positive (non-suppressive FOXP3^{+}) cells, but also defines the balance between resting (FOXP3^{dim}CD45RA^{+}) and activated (FOXP3^{bright}CD45RA^{-}) Treg cells.

The aim of our study was to assess whether the frequency and phenotype of circulating Treg cells differs in women with preeclampsia compared with pregnant and non-pregnant controls when using a set of updated Treg and phenotype markers. Since early onset and

severe preeclampsia is associated with significant morbidity and mortality and may constitute a homogeneous group, we focused our work around these patients, and we also paid special attention to effects of corticosteroid treatment.

MATERIALS AND METHODS

Subjects

Eighteen women with *de novo* proteinuria and hypertension appearing after gestational week 20 were included (Table 1). Inclusion criteria were either severe or early onset preeclampsia. Severe preeclampsia was defined as highest measured blood pressure $\geq$ 160/110 mmHg and/or with signs of renal, hepatic or neurological impairment or thrombocytopenia (39), while early-onset preeclampsia was defined by onset at gestational age of less than 34 weeks (40, 41). Included patients had either severe (n=1) or early-onset (n=1) preeclampsia or both (n=16). Women with diabetes, BMI >35, chronic hypertension, thrombophilia and twin pregnancies were excluded. The majority (72.2 %) of preeclampsia patients delivered small for gestational age babies as a sign of placental involvement in pathogenesis. Treatment with antenatal betamethasone to facilitate fetal lung maturity, consisting of two intramuscular injections of 12 mg with an interval of 24 hours, was started prior to blood sampling in 10 out of 18 preeclamptic women. Blood sampling was done in close proximity to treatment, in most cases between the first and the second injection. Median time from first injection of betamethasone to blood sampling was 23.5 hours (range 12-100 hours) and in 70 % of the cases the sampling was done within 2 days from first injection. The patients were recruited from the obstetrical departments of Linköping University Hospital and County Hospital Ryhov in Jönköping. As controls, 20 women with normal pregnancy and 20 healthy non-pregnant women were included (Table 2). The normal pregnant women were included (gestational week 24-27) at their regular second trimester control at the antenatal clinic. Ten of the normal pregnant and ten of the

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non-pregnant women were also included in a previous paper (36). There was no significant difference across the three groups with regard to age, parity and body mass index, whereas the gestational age was significantly lower (p<0.001) in the normal pregnant compared with the preeclamptic women. Informed consent was obtained from all participants. The local ethics committee at Linköping University approved the study.

Six-colour flow cytometry

One million peripheral blood mononuclear cells (PBMC), prepared from EDTA blood as described previously (36), were incubated with fluorochrome-conjugated antibodies against human CD3 (APC-Cy7, clone SK7), CD4 (PerCP, clone SK3), CD25 (APC, clone 2A3), CD45RA (FITC, clone L48), CD45R0 (PE-Cy7, clone UCHL1), CD127 (PE, clone hIL-7R-M21) and CCR4 (PE, clone 1G1), all from BD Biosciences, San Jose/San Diego, CA USA. Intracellular staining was done for anti-human FOXP3 (PE or FITC, clone PCH101, eBioscience, San Diego, CA, USA) and CTLA-4 (PE, clone BNI3, BD Biosciences) after fixation/permeabilization as described before (31). Background fluorescence was assessed by antibodies against irrelevant protein (isotype controls). Absolute leukocyte (CD45), T lymphocyte (CD3) and T helper (CD4) cell counts in whole EDTA blood were determined by using bead-based TruCount tubes (BD Biosciences) as described by the manufacturer.

Flow cytometric gating

PBMC were collected using the FACSCanto II system (BD Biosciences) and analyzed with the Kaluza software (version 1.2, Beckman Coulter, Miami, USA). All gating analyses were performed in a blinded manner, *i.e.* the evaluator was unaware of the diagnosis.

The lymphocyte gate was set according to characteristic forward and side scatter properties and cells were subsequently gated for co-expression of CD3 and CD4 (Fig.1a). Treg cells were defined as resting (FOXP3^{dim}CD45RA^{+}) or activated (FOXP3^{bright}CD45RA^{-}) (Fig.1b) according to the gating strategy described by Miyara *et al* (38). A gate was also set for the non-suppressive FOXP3^{+} T helper cells (FOXP3^{dim}CD45RA^{-}). The FOXP3^{bright} gate was set to include cells expressing the very highest levels of FOXP3, while FOXP3^{dim} gated cells were slightly lower in FOXP3 expression but still positive. Owing to the reciprocal nature of CD45RA^{+} and CD45RO^{-}, we tested whether they could be used inter-changeably as markers of resting (or naïve) status of T cells. We found that Treg cells defined by either CD45RA^{-} or CD45RO^{+} were highly correlated in all comparisons (rho = 0.99, p <0.001). The definitions of CD45RA and CD45RO were determined by identifying the contours of positive populations (Fig. 1 b-c). Our observation that resting and activated Treg cells can be defined also as FOXP3^{dim}CD45RO^{-} and FOXP3^{bright}CD45RO^{+}, respectively, made it feasible to evaluate CTLA-4 and CCR4 expression within these Treg subpopulations (Fig. 1d-e). To define the cut-off for positivity of CCR4 and CTLA-4, negative populations were used in combination with isotype controls.

For comparison with previous findings (36), gating of CD4^{dim}CD25^{bright} cells was also performed (Fig. 1). The CD4^{dim}CD25^{bright} gate was adjusted to contain cells expressing slightly lower levels of CD4 compared with conventional CD4^{+} cells, in combination with the highest levels of CD25. We previously showed this population to contain highly suppressive Treg cells avoiding activated non-suppressive cells (36). Reduced or absent expression of CD127, in combination with positivity for FOXP3 or CD25, defined the CD127^{low} gate (Fig.1g) (37).

Statistics

Since the majority of the data sets followed a Gaussian distribution, data were analyzed using ANOVA followed by Student's unpaired t-test if the ANOVA indicated $p \leq 0.05$. For correlation analyses Pearson's correlation test was used. A significance level of $p \leq 0.05$ was used.

RESULTS

The total numbers of lymphocytes, $CD3^+$ and $CD4^+$ cells (expressed as cells/uL of whole blood) were similar in non-pregnant, normal pregnant and preeclamptic women ($p = 0.59$-0.70, data not shown). Thus, Treg populations could safely be reported as the proportion of $CD4^+$ cells (expressed as percentage of $CD3^+CD4^+$ cells). Corticosteroid treatment neither affected the total number of lymphocytes nor the number of $CD3^+$ or $CD4^+$ cells ($p=0.67$ - 0.84) when comparing corticosteroid treated (n=10) and untreated (n=8) women.

Similar frequencies of resting and activated circulating Treg subpopulations in women with severe or early-onset preeclampsia and normal pregnancy

The proportions of the resting and activated Treg subpopulations were similar in pregnant women with and without preeclampsia and in non-pregnant women, as was the frequency of the non-suppressive $FOXP3^{dim}CD45RA^-$ population (Fig.2 a-c). Also when adding the resting and activated Treg populations, as well as when defining Treg cells by $CD127^{low}$ combined with CD25 or FOXP3, there were no differences in between groups. When Treg cells were defined as $CD4^{dim}CD25^{bright}$ (Fig.1f) (36) pregnant women, both with preeclampsia and healthy pregnancy, showed lower proportions as compared with non-pregnant women (Fig.2d).

Corticosteroid treatment affects the frequency of circulating Treg cells

As can be seen in Figure 2 (unfilled versus filled circles in the preeclampsia group), the Treg populations seemed to be differently distributed in corticosteroid treated *versus* non-treated women. We therefore stratified the preeclampsia group according to status of corticosteroid treatment given prior to blood sampling (Fig.3). Thereby, among women with preeclampsia, corticosteroid treatment was associated with a reduced proportion of resting Treg cells (Fig.3a), whereas there were no significant differences with regard to activated Treg cells (Fig.3b) or the activated non-suppressive $FOXP3^{dim}CD45RA^-$ population (Fig.3c).

However, in an attempt to relate the Treg cells to the effector CD4 cells, we found that the ratio (Fig.3d) between the sum of resting and activated Treg ($FOXP3^{dim}CD45RA^+$ and $FOXP3^{high}CD45RA^-$) and the activated non-suppressive $FOXP3^{dim}CD45RA^-$ population, was significantly higher in non-corticosteroid treated preeclamptic women compared with normal pregnant women. This difference was also found in the entire group of preeclamptic women ($p=0.01$), while there was no significant increase in the corticosteroidtreated preeclamptic women compared with normal pregnant women. Non-pregnant women had a higher ratio than normal pregnant women (Fig. 3d).

Finally, the $CD4^{dim}CD25^{bright}$ Treg population was lower in the corticosteroid treated group, but not in the untreated group, as compared with non-pregnant women (Fig.3e).

Severe or early-onset preeclampsia is associated with divergent CTLA-4 and CCR4 expression in circulating Treg subpopulations

When looking at expression within Treg subpopulations, we found that the proportions of $CTLA-4^+$ and $CCR4^+$ were higher both in resting and in activated Treg populations (Fig.4a-d) in untreated preeclamptic compared with normal pregnant women. In contrast, normal pregnancy was associated with lower proportions of $CTLA-4^+$ (Fig.4b) and $CCR4^+$

cells (Fig.4d) within the activated Treg population as compared with non-pregnant women. Thus, differences in CTLA-4 and CCR4 expression within Treg populations were found in preeclamptic women. However, these differences were confined to the non-corticosteroid-treated group, again indicating an influence of corticosteroid treatment on the Treg phenotype.

DISCUSSION

In this study we used different up-to-date phenotyping strategies to assess Treg frequencies in early onset and severe preeclampsia. We found no major alterations in circulating Treg frequencies compared with healthy pregnant and non-pregnant women. However, by analyzing expression of functional and migratory markers (CTLA-4 and CCR4) within subpopulations we found alterations of Treg cells in preeclampsia. We also observed that corticosteroid treatment affected the Treg phenotype, thus important to consider when evaluating Treg cells in preeclampsia. Most previous studies on Treg cells in preeclampsia have reported decreased levels compared with normal pregnancy (30). Factors that may affect results and explain divergent findings include strategy and markers of Treg phenotyping, patient selection and influence of corticosteroid treatment.

Although there is still no perfect marker of Treg cells, there has been a gradual improvement in phenotyping strategies and markers in order both to define Treg cell subpopulations and to exclude non-suppressive $CD4^+$ cells that express FOXP3 and CD25 after activation. A major breakthrough was the report by Miyara *et al*., demonstrating that by combining FOXP3 with CD45RA, Treg cells were classified as either resting ($FOXP3^{dim} CD45RA^+$) or activated ($FOXP3^{high}CD45RA^-$), while at the same time $FOXP3^{dim}CD45RA^-$ cells were classified as activated non-suppressive T cells ("false positive" FOXP3 expression) and therefore correctly excluded from the Treg cell

enumeration (30, 38, 42). When using this strategy we found that neither the total Treg frequency nor the balance between resting and activated Treg cells was altered in preeclampsia *versus* normal pregnancy. Furthermore, also when using other strategies that avoid activated non-suppressive FOXP3^{+} T cells (CD127^{low} (37) and CD4^{dim}CD25^{bright} (36)) we found no differences between preeclampsia and normal pregnancy. Our findings do not support the majority of previous studies that in general showed lower frequencies of Treg cells in blood of preeclamptic as opposed to healthy pregnant women (19, 24, 43-49), although some previous studies did not show a difference between these groups (32, 33, 44). In a more recent study, by using an updated phenotyping strategy, Steinborn *et al*. did not find any differences with regard to the whole Treg cell population, while alterations in the preeclampsia group were found in Treg subpopulations (31), in line with our findings (see below). Taken together, the phenotyping strategy has a major impact on Treg cell frequencies and must be taken into account when evaluating previous studies that may have included activated non-suppressive FOXP3^{+} or CD25^{+} cells.

In addition to the phenotyping strategy, the selection of material can affect findings. Although previous studies have enrolled patients with mainly moderate to severe preeclampsia, none has focused on early-onset disease and in several studies findings were not related to gestational age. Hence, some of the Treg disturbances reported previously may have been confined to the group of women presenting with late-onset preeclampsia, possibly having had a subclinical disease for a long time and findings could therefore reflect the effect of, rather than the cause of the disease. We focused our attention on women with severe or early-onset preeclampsia, hypothesizing that this would provide us with a homogenous group of diseased women presenting with a more distinct placental disease (34, 50). This is also the patient group suffering the most severe consequences of

their disease. However, our findings do not support a numerical defect in Treg cells in early-onset preeclampsia.

Due to the early onset of the disease and the high risk of premature birth, ten of the preeclamptic women in our study received the potent glucocorticoid betamethasone, which was given in almost all cases in close proximity to blood sampling. This treatment is normally started directly at admission of a patient with early onset preeclampsia. The women in the preeclampsia group were recruited at the County Hospital Ryhov in Jönköping and the University Hospital in Linköping, but several women were transferred from smaller hospitals and corticosteroid treatment had already been started at admission. Importantly, we found that the corticosteroid-treated group showed significantly decreased frequencies of resting Treg cells, and corticosteroid treatment also affected the composition of Treg subpopulations. In humans, dexamethasone has been shown to promote Treg cells (35, 51, 52), while BALB/c mice treated with dexamethasone showed enhanced $CD4^+CD25^+$ but suppressed $CD4^+FOXP3^+$ Treg cell numbers (53). Further, Treg cell numbers were unaffected by hydrocortisone in a murine model of autoimmune disease (54). Obviously, the glucocorticoid effects on Treg cells are complex and related to the dose and duration of the treatment. Hence, using samples from non-glucocorticoid-treated women is desirable. In previous studies the use of corticosteroid treatment was in general not reported, and its effects on Treg cells has not been previously evaluated in preeclamptic women.

The aberrant findings in preeclamptic compared with normal pregnant women in this study were related to function (CTLA-4) and chemotaxis (CCR4) of Treg cells. CTLA-4 is a key element in the suppressive effect of Treg cells (55), while CCR4 mediates migration of $CCR4^+$ T cells, such as T_H2-like cells and subsets of activated Treg cells, to the fetal-maternal interface, which is rich for the CCR4 ligand CCL17 (29, 56). In normal

pregnancy compared with the non-pregnant state, we found a reduced frequency in blood of both CTLA-4$^+$ and CCR4$^+$ cells within the activated Treg population (FOXP3^{high}CD45RO$^+$). This reduction in blood is in line with an accumulation of suppressive Treg cells in the decidua, as reported previously (23). Consequently, the presently noted increase in preeclampsia of the CTLA$^+$ and CCR4$^+$ fractions of Treg cells could reflect a failure to accumulate Treg cells in the decidua, hence leading to a lack of anti-inflammatory regulation at the fetal-maternal interface. Indeed, a lowered frequency of Treg cells in the decidua has been found in preeclampsia (46, 57). In an attempt to relate the Treg cell frequency to effector CD4 cells, we used the resting plus the activated Treg cells as reliable measures of true Treg cells, and the activated non-suppressive CD4 population as an established activated population (38). When making a ratio between resting and activated Treg cells divided by the effector T cells, we found a higher ratio in non-corticosteroid treated women compared with both normal pregnant and corticosteroid treated women. This finding is in line with a possible migratory defect of Treg cells in preeclampsia, *i.e.* that Treg cells do not properly reach their destination in the uterus, while the effector cells are recruited to the uterus, hence occurring at lower level in the circulation relative to the Treg cells. Of note, the differences in the ratio must be interpreted with great caution since there were no significant differences when evaluating the fractions one by one.

A limitation of our study is that we have only studied Treg cell frequency in peripheral blood and not locally in decidua. A strength of the study is that only women with early-onset or severe preeclampsia with clear signs of placental pathology were included, and so is our stratifying for corticosteroid treatment as well as the use of an up-dated phenotyping panel assessing Treg subpopulations and excluding non-suppressive activated FOXP3-expressing cells. The sample size is not huge but similar to most previous studies that have

noted differences in Treg cell frequencies. When stratifying for corticosteroid treatment, the subgroups are rather small and our findings need to be confirmed. However, significant differences were still noted and they are sufficient to draw the attention to the issue of corticosteroid treatment in future studies.

There was also a difference of approximately four weeks in mean gestational age between the group of preeclamptic women and the normal pregnant women. Steinborn *et al* found that the suppressive action of Treg cells was quite stable after 20 weeks of pregnancy until time close to labor (31). None of the women in the study were close to spontaneous labor in the study and we therefore believe that this difference has not affected the results in any significant way.

In conclusion, although we found no major alterations in circulating Treg numbers, increased fractions of CTLA-4 and CCR4$^+$ cells within Treg subpopulations are in line with a migratory defect of Treg cells in preeclampsia, potentially associated with a reduced number of suppressive Treg cells at the fetal maternal interface. Since corticosteroid treatment seems to affect the distribution of Treg subpopulations, this variable should be accounted for in studies of Treg cells in preeclampsia.

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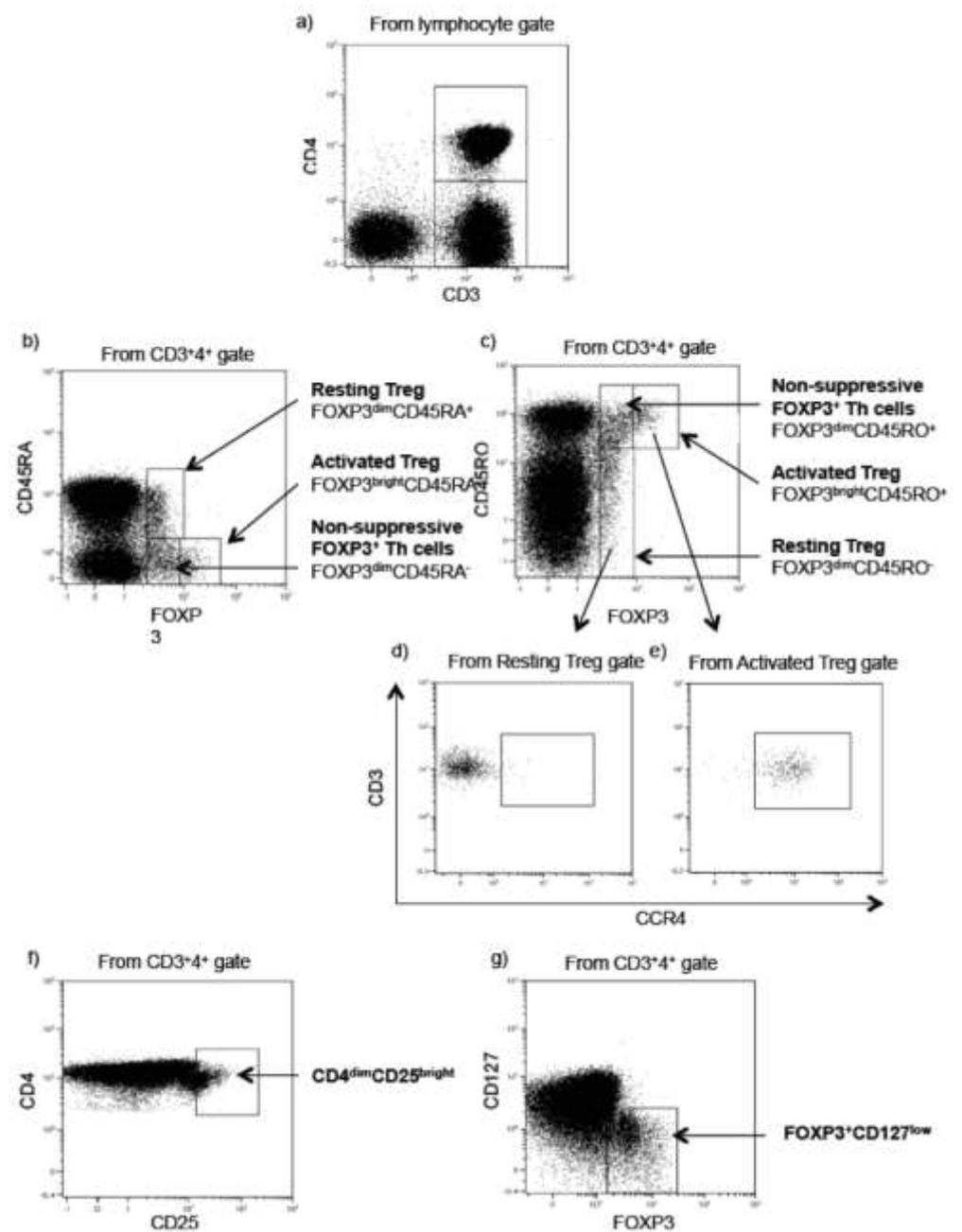
Acknowledgements

FIGURES

Figure 1.

Gating strategy for regulatory (Treg) cells. (a) Representative dot plot of CD3$^+$CD4$^+$cells in lymphocytes. (b) Subpopulations of CD3$^+$CD4$^+$ cells defined by expression of CD45RA and FOXP3: resting Treg cells (FOXP3^{dim}CD45RA$^+$), activated Treg cells (FOXP3^{bright}CD45RA$^-$) and non-suppressive Th cells (FOXP3^{dim}CD45RA$^-$). (c) Corresponding resting and activated subpopulations, defined by CD45RO and FOXP3. (d) Expression of CCR4 in resting and (e) activated Tregs. (f) The CD4^{dim}CD25^{bright} gate was set to contain cells expressing high levels of CD25 and slightly lower levels of CD4 than the total CD4$^+$ population. (g) Low or absent expression of CD127 in combination with FOXP3 in CD4$^+$ cells (FOXP3$^+$CD127^{low}).
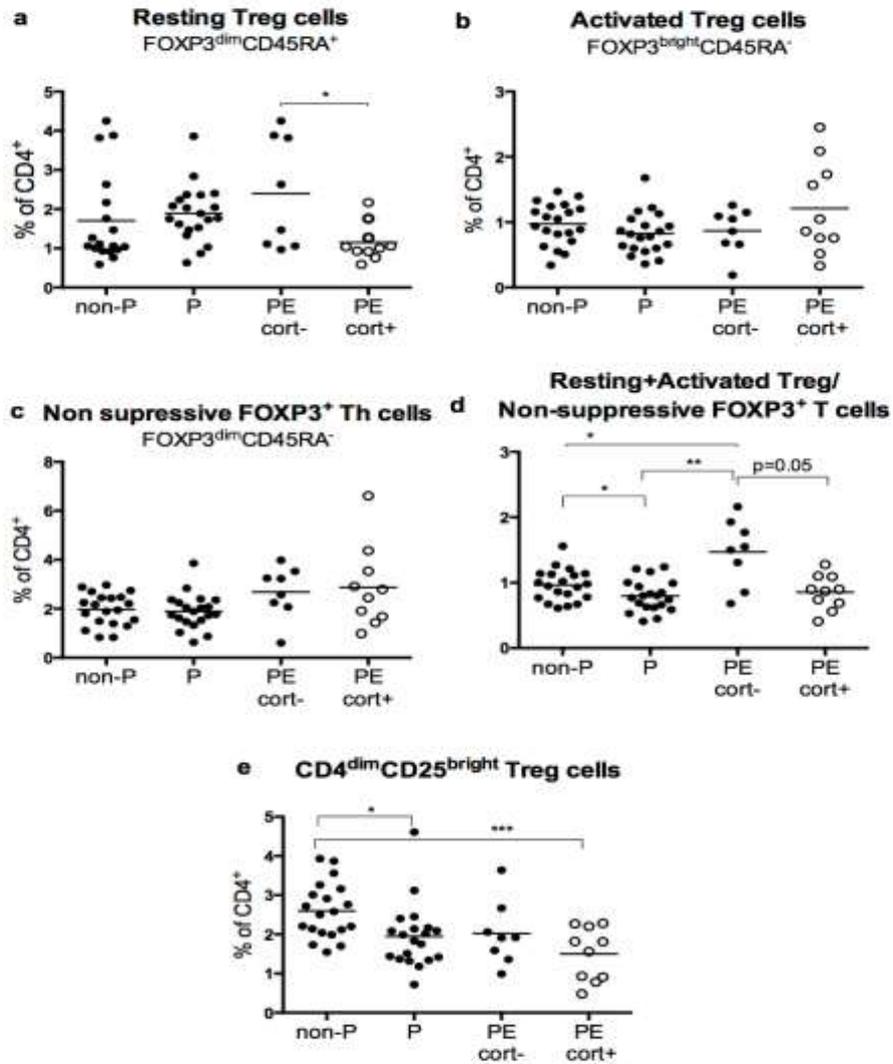
Figure 2
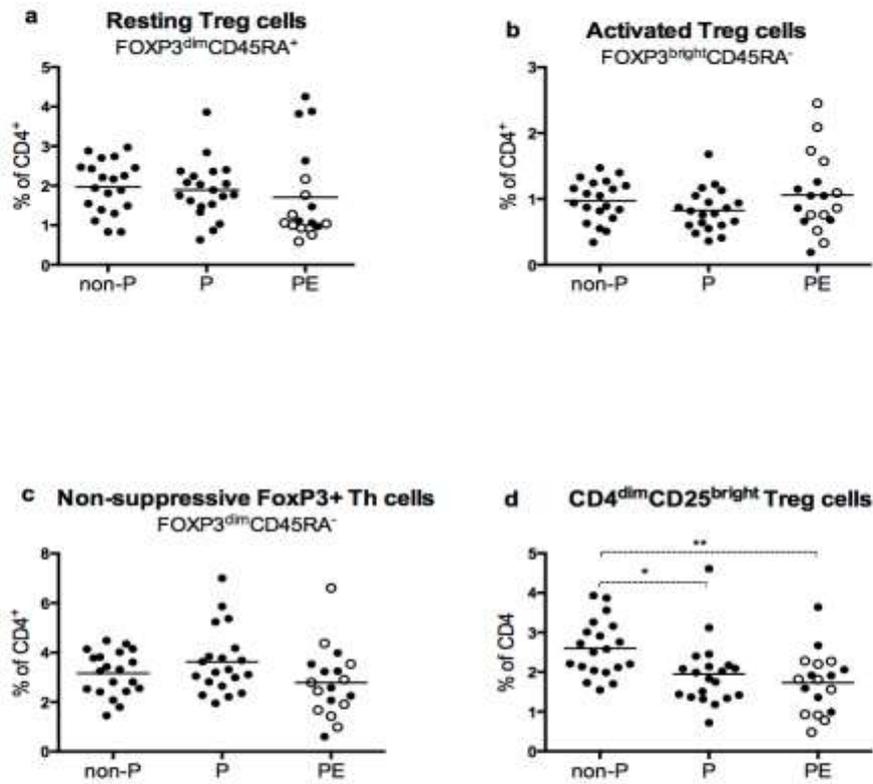
Figure 2.
Proportions (%) of Treg subpopulations in non-pregnant (non-P; n=20), normal pregnant (P; n=20) and preeclamptic (PE; n=18) women: (a) resting Treg cells (FOXP3^{dim}CD45RA); (b) activated Treg cells (FOXP3^{high}CD45RA$^-$); (c) non-suppressive FOXP3$^+$ Th cells (FOXP3^{dim}CD45RA$^-$); (d) Treg cells defined as CD4^{dim}CD25^{bright} cells. Lines indicate mean values. Empty circles indicate corticosteroid treated preeclamptic women.
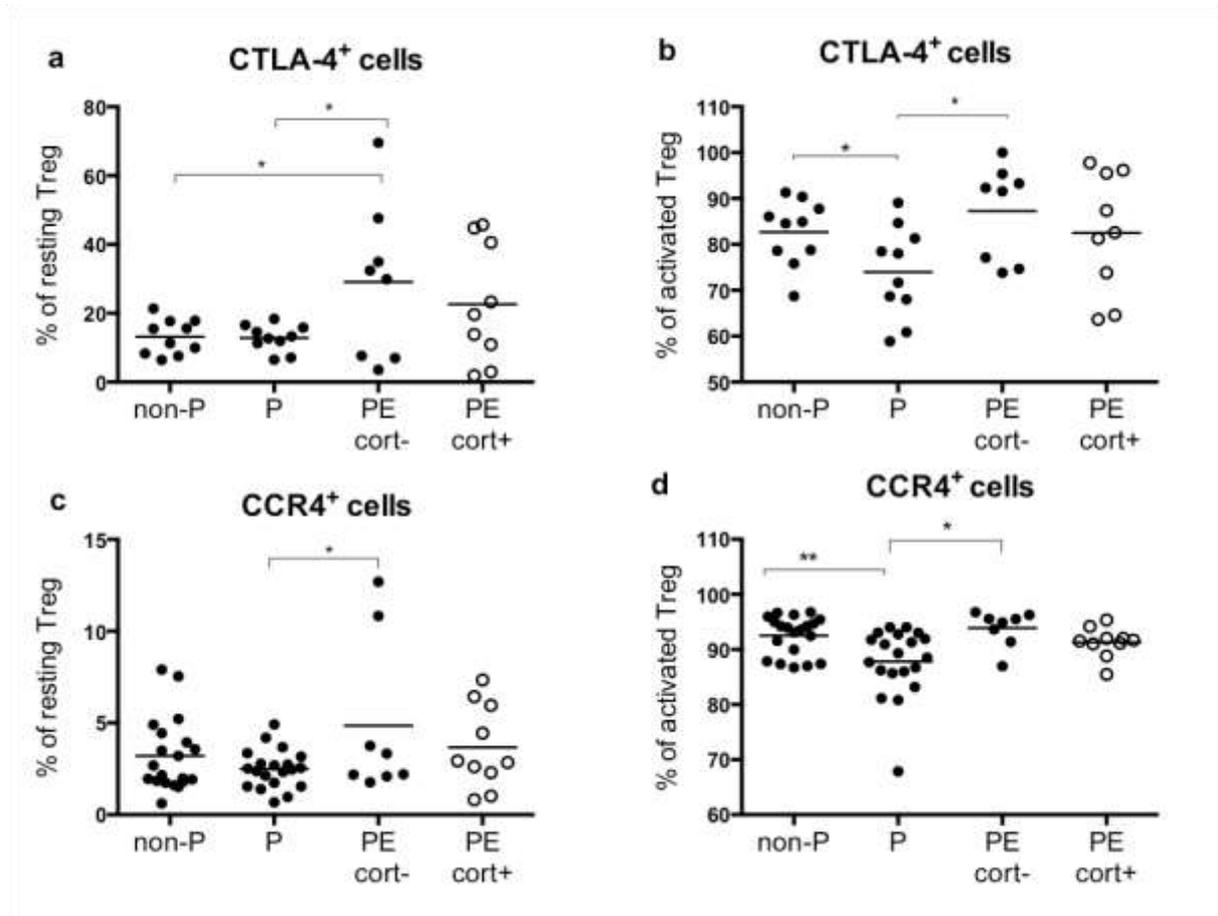* p< 0.05; **p<0.01 by Student's unpaired t-test if the ANOVA indicated p ≤ 0.05

Figure 3.

Proportions (%) of Treg subpopulations in non-pregnant (non-P; n=20), normal pregnant (P; n=20), non-corticosteroid treated preeclamptic (PEcort-; n=8) and corticosteroid treated (PEcort+; n=10) women: (a) resting Treg cells (FOXP3^{dim}CD45RA); (b) activated Treg cells (FOXP3^{high}CD45RA^{-}); (c) non-suppressive FOXP3^{+} T cells (FOXP3^{dim}CD45RA^{-}); (d) ratio between the sum of resting and activated Treg defined as in a-b, divided by the proportion of non-suppressive FOXP3^{+} T cells (e) Treg cells defined as CD4^{dim}CD25^{bright} cells. Lines indicate mean values.

* p< 0.05; ** p<0.01 by Student's unpaired t-test if the ANOVA indicated p ≤ 0.05

Figure 4.

Proportions (%) of CTLA-4$^+$ and CCR4$^+$ cells within (a, c) resting (FOXP3^{dim}CD45RO$^-$) and (b, d) activated (FOXP3^{bright}CD45RO$^+$) Treg subpopulations in non-pregnant (non-P; n=20), normal pregnant (P; n=20), non-corticosteroid treated preeclamptic (PEcort-; n=8) and corticosteroid treated (PEcort+; n=10) women. Lines indicate mean values.

*p< 0.05; **p<0.01 by Student's unpaired t-test if the ANOVA indicated p ≤ 0.05

TABLES

Table I. Clinical data of the preeclamptic women included in the study

	Age	BP*	Urine	Onset (GW)	FW deviation %	Cortico-steroid treatment	Complications
1	27	160/105	1+	29+0	-28.1	yes	
2	26	170/125	3+	28+0	-33.3	yes	
3	28	180/105	2+	30+6	-38.0	yes	abrubtio placentae
4	36	170/120	3+	31+2	-19.4	yes	
5	34	200/120	3+	33+4	-26.4	no	
6	31	185/115	3+	26+3	-28.3	no	
7	17	190/120	3+	23+0	-13.8	no	
8	21	180/110	3+	27+0	-27.4	yes	eclampsia
9	46	195/115	3+	31+3	-31.7	no	oliguria
10	35	170/105	3+	30+0	-6.0	yes	HELLP
11	27	180/110	3+	27+0	-6.7	yes	
12	24	200/120	3+	30+0	-32.9	yes	
13	38	220/120	3+	29+4	-33.0	yes	
14	31	160/90	3+	30+0	-34.8	no	
15	39	180/100	3+	35+0	-20.0	no	
16	31	170/100	3+	30+1	-24.8	no	
17	38	140/90	3+	34+0	-15.3	no	
18	27	140/95	1+	29+4	-32.7	yes	thrombocytopenia

*BP, highest blood pressure during admission GW=Gestational Weeks; FW=Fetal Weight

HELLP=Hemolysis, Elevated Liver enzymes and Low Platelet Count

Table II. Demographic and clinical data, given as median (and range within brackets) or as categorical data.

Subject characteristics	Preeclamptic women (n=18)	Healthy pregnant women (n=20)	Non-pregnant women (n=20)
Age at inclusion	29 (17-46)	28 (19-38)	27 (20-36)
Blood pressure	177/108 (140/90 - 220/120)	115/70 (110/60-130/80)	no information
Proteinuria (dipstick grading)	3+ (1+ - 3+)	no proteinuria	no information
Gestational week at inclusion (weeks)	29 (23-35)**	25 (24-27)	NA
Partus (weeks)	30 (26-37)	41 (35-42)	NA
Previous pregnancies (n)	2 (1-5)*	0 (0-2)	0 (0-2)
Previous births (n)	0 (0-2)	0 (0-1)	0 (0-2)
BMI	27(21-35)	23(17-31)	no information

No significant differences for age at inclusion, parity or BMI.

*p=0.029 **p<0.001   NA; not applicable