Prevalence of regulatory T cell subtypes in preeclampsia

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Running head: Treg subtypes in preeclampsia

Abstract

Problem: The prevalence of regulatory T cells (Tregs) is lower in preeclampsia (PE) compared to healthy pregnancy (HP). However, the proportion of recently described Treg subtypes has not been investigated.

Method: Peripheral blood samples of 19 PE and 21 HP women in the third trimester were evaluated using flow cytometry for the prevalence of activated T cells and naive, effector, thymic, extrathymic, and exhausted Tregs.

Results: The prevalence of activated T cells and exhausted Tregs was higher in PE than in HP. The prevalence of the functionally most active effector Tregs is decreased, while naive Tregs appear to be unaffected in PE compared to HP. No difference was detected between Tregs according to their origin (thymic or extrathymic).

Conclusion: The combination of lower effector Treg and higher exhausted Treg prevalence may account for the decrease in the functionality of Tregs in PE.

Keywords: CD279, effector Treg, exhausted Treg, Helios, naive Treg, preeclampsia

Introduction

Preeclampsia (PE) is an immune-mediated syndrome usually developing in the third trimester of pregnancy characterized by an excessive maternal systemic inflammatory response with activation of both the innate and adaptive arms of the immune system.[1,2] The maternal immune tolerance towards the developing semi-allogeneic fetus present in healthy pregnancy (HP) is compromised in PE. Activated neutrophils, monocytes, and natural killer cells initiate inflammation, which induces endothelial dysfunction, and activated T cells may support inadequate maternal tolerance mechanisms. An important feature of systemic inflammation in PE is the absence of Th2 skewness characteristic for HP, and thus the predominance of a Th1-type immunity.[3]

Regulatory T cells (Tregs) are mediators of the maternal immune tolerance towards the developing fetus. The FoxP3 transcription factor has been widely used as an intracellular marker to identify this subset. Tregs modulate the functions of other T cells (both CD4+ and CD8+), primarily through secretion of cytokines, including IL-10 and TGF-β.[4,5] Evidence for the role of Treg cells in establishing fetal tolerance during pregnancy comes from animal studies, demonstrating a significantly increased rate of fetal resorption in allogeneic gestations of Treg-deficient mice.[6-8] Lower than normal Treg frequency may contribute to the exaggerated systemic inflammation also in humans in PE. Indeed, a number of groups including ours demonstrated that the prevalence of peripheral Tregs is lower in PE compared to healthy pregnancy.[9-13] While these studies mainly investigated peripheral blood, the distribution of Tregs in the decidua might differ. Earlier investigations provided evidence for the selective migration of fetus-specific Tregs from peripheral blood to the decidua, further emphasizing the importance of studying Tregs at the fetomaternal interface.[14-15] Another study, evaluating both peripheral blood and decidual Treg cells in mostly severe PE patients

confirmed that the proportion of Tregs was decreased in both locations compared to normal term controls.[16]

Based on different intra- and extracellular markers, recent investigations identified that Tregs are not a homogenous cell subset, but can divided into further subgroups. Tregs have been grouped according whether they originate from the thymus (naturally occurring or nTregs) or induced in the periphery (iTregs). The Helios transcription factor, belonging to the Ikaros family, has been identified as a marker of thymic-derived Treg cells.[17] Its function is not yet fully understood. Binding to the promoter region of FoxP3, it may have a role in regulating the expression of FoxP3.[18] It does not seem to have a direct effect on Treg prevalence or function.[19] Both nTregs and iTregs have a high expression of CD25 and CTLA-4, and low expression of CD127. These two subsets are different in the cytokines they produce and their potential to suppress other cells: iTregs produce IL-10 and IL-17 and have a higher regulatory potential.

Miyara et al. categorized Tregs based on their expression of CD45RA and FoxP3. CD4+ FoxP3+ CD45RA+ cells were described as naive or resting Tregs, while CD4+ FoxP3hi CD45RA- cells were regarded as fully functional effector Tregs. CD4+ FoxP3+ CD45RA- cells are cytokine-secreting, non-suppressive T cells.[20] CD45RA was also used by other groups as a marker of distinction.[21,22] While naive Tregs have the potential to proliferate, effector Tregs are not capable of proliferation or further differentiation.[23]

An “exhausted” and dysfunctional phenotype of Tregs has been reported in conditions of chronic disease and infection.[24] Exhausted Tregs express CD279 or programmed death receptor 1 (PD-1), a negative regulatory molecule. Lines of evidence indicate that CD279 expression on T cells is associated with limited proliferative capacity and reduced immune suppression in vivo.[25-29] Interestingly, one of the ligands for CD279, CD274 (PD-L1), has been shown to negatively regulate Tregs by inhibiting STAT-5 phosphorylation at sites of

chronic inflammation. Thus PD-1 and its ligand appear to be a major inhibitory receptor pathway involved in T cell exhaustion.[26]

In this study, we aimed to compare the peripheral prevalence of activated T cells and Treg subtypes, such as naive and effector, thymic and extrathymic, as well as exhausted Tregs in PE and HP.

Methods

Sample collection

We enrolled 19 women with PE at on average the 34th gestational week of pregnancy. PE was diagnosed according to standard internationally accepted criteria. These include hypertension (defined as systolic blood pressure and/or diastolic blood pressure ≥ 140 mmHg and ≥ 90 mmHg, respectively) occurring after 20 weeks of gestation, and proteinuria (defined as presence of ≥ 0.3 g protein in a 24 hour urine specimen). As controls, 21 healthy, age-matched pregnant women at on average the 36th gestational week were enrolled. Clinical characteristics of participants are summarized in Table I. PE was regarded as severe if any of the following criteria was present: blood pressure ≥ 160 mmHg systolic or ≥ 110 mmHg diastolic, or proteinuria ≥ 5 g/24 h (or 3+ on dipstick). Early onset of PE was defined as onset of the disease before 34 weeks of gestation. Informed consent was obtained from all subjects, and our study was reviewed and approved by an independent ethical committee of the institution (Scientific and Research Ethics Committee, Semmelweis University, Budapest, Hungary). The study was adhered to the tenets of the most recent revision of the Declaration of Helsinki.

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were separated by a standard density gradient centrifugation (Ficoll Paque, Amersham Biosciences AB, Uppsala, Sweden, 25 minutes, 400 g, 22 °C) from freshly drawn blood collected in lithium heparin-treated tubes (BD Vacutainer, BD Biosciences, San Jose, CA, USA). Cells were kept at -80 °C in Fetal Bovine Serum containing 10% DMSO until analysis. After thawing, cells were washed twice in phosphate buffered saline.

PBMCs were stained for 30 min at room temperature in the dark with PE Cy7-conjugated CD4, APC-conjugated CD25, APC-Cy7-conjugated CD279 and FITC-conjugated CD45RA (BioLegend, San Diego, CA, USA). After washing, cells were fixed with Fixation/Permeabilization solution and treated with Permeabilization Buffer according to the manufacturer's instructions (eBioscience, San Diego, CA, USA). They were then stained with PE-conjugated FoxP3 PE, and PerCP-conjugated Helios for 30 min at 4 °C in the dark. Mouse IgG1 antibodies were used as isotype control.

After washing, cells were analyzed on a BD FACSAria flow cytometer (BD Biosciences). Data were processed using the FACSDiVa software. 200 000 cells per sample were recorded.

Statistics

Data are expressed as median and interquartile range (IQR). Comparisons between two sample populations were made with the Mann-Whitney *U* test, as a test of normality (according to Kolmogorov-Smirnoff) indicated non-normal distribution of data; p-values less than 0.05 were considered significant. Statistics were calculated using the GraphPad software (Prism version 5.00 for Windows, GraphPad Software, San Diego, CA, USA).

Results

Our results are summarized in Figure 1 and Table II. Within CD4+ T cells, the prevalence of CD4+ CD25hi FoxP3+ regulatory T cells was lower in women with PE than in HP women (4.63 (4.22-5.56) % vs. 3.69 (3.32-4.09) %, p = 0.0003).

In PE, the prevalence of CD45RA- effector regulatory T cells among CD4+ cells was also decreased (2.44 (1.02-6.78) % vs. 0.87 (0.45-1.22) %, p = 0.0098), while that of CD45RA+ naive regulatory T cells did not differ between the two groups. However, when investigating the prevalence of naive Tregs within the Treg subset, an increase in PE was detected (57.3 (30.9-77.8) % vs. 75.2 (46.0-87.9) %, p = 0.0014).

The percentage of activated CD4+ T cells was higher in women with PE than in the control group (2.56 (0.55-7.02) % vs. 4.30 (1.78-7.85) %, p = 0.0456).

Within the regulatory T cells, the prevalence of CD279+ exhausted Tregs was higher in women with PE than in HP women (8.08 (4.16-13.5) % vs. 18.2 (9.27-36.3) %, p = 0.0223).

The prevalence of other regulatory T cell subtypes (Helios+ effector Tregs, Helios+ naive Tregs, thymic Tregs, extrathymic Tregs) did not differ between the two groups.

Discussion

In this study, we analyzed the prevalence of recently described regulatory T cell subsets in PE compared to HP. As many studies have noted before,[9-13] the percentage of Tregs, identified as CD4+ CD25hi FoxP3+ cells, was lower in PE than in HP, contributing to the compromised maternal immune tolerance towards the semi-allogeneic fetus characteristic for PE, and to the loss of control over activated T cells. Indeed, the proportion of CD4+ CD25+ FoxP3+ CD45RA- activated T cells was higher among CD4+ T cells in PE compared to HP. This might in turn contribute to the increased Th1 type response and imbalance of Th1/Th2 ratio.[3] Nevertheless, our results indicate that the decrease in the prevalence of Treg cells is specific for certain Treg subtypes, while other subsets of these regulatory cells remain unaffected in PE. Interestingly, of all investigated subsets, the prevalence of the functionally most active effector Tregs (CD4+ FoxP3++ CD45RA-) is decreased to the highest extent, while naive Treg cells appear to be unaffected. A further factor that may contribute to the decreased functionality of Tregs in PE is that the prevalence of exhausted, functionally less active Tregs (CD4+ CD25hi FoxP3+ CD279+) is higher compared to HP. In line with these findings in pregnancy, an exhausted and dysfunctional phenotype of Tregs has been reported before in chronic disease and infection.[24] The combination of lower effector Treg and higher exhausted Treg prevalence may account not only for the lower Treg proportion, but also for the observed decrease in the functionality of Tregs in PE.[30]

The Helios transcription factor belongs to the Ikaros family and is expressed by thymic-derived Treg cells.[17] Samstein et al. reported that extrathymic generation of regulatory T cells enforces maternal-fetal tolerance in placental mammals.[7] In addition, Hsu et al. demonstrated that the expansion of CD4+ Helios- Foxp3+ iTreg cells, rather than CD4+ Helios+ Foxp3+ nTreg cells, accounts for Treg expansion in HP. This expansion was found to be even more

pronounced in the decidua, where an overrepresentation of iTreg cells was found. In PE, however, impaired systemic iTreg cell expansion was described, associated with a lack of iTreg cell overrepresentation in the decidua.[31] On the other hand, Inada et al. recently observed that Helios+ effector nTreg cells are decreased in the decidua of miscarriage cases with normal fetal chromosomal content, and their data suggest that these cells might play an important role in the maintenance of pregnancy in humans.[32] According to our results, the origin of Tregs in the periphery does not seem to play an important role in their activity in PE, since no alteration was detected in the proportion of thymic and extrathymic Tregs (CD4+ CD25hi FoxP3+ Helios+ and CD4+ CD25hi FoxP3+ Helios-, respectively) between the two study groups. Furthermore, Helios+ effector and naive Tregs are also of comparable prevalence in HP and PE.

Earlier studies demonstrated that the prevalence of Tregs is decreased not only on the systemic level in peripheral blood of PE patients, but also locally, within the decidual tissue.[14,16] A limitation of our study is that our findings represent circumstances in peripheral blood only. Therefore, further studies on the expression of the investigated Treg cell subtypes in the decidua are required to identify their role played in mediating tolerance at the fetomaternal interface. Moreover, the functional activity of Treg cell subsets should also be examined in healthy pregnancy and preeclampsia in future studies.

Conclusion

In summary, the decrease in the prevalence of Treg cells in PE is specific for certain Treg subtypes. No difference was detected between Tregs according to their origin (thymic or extrathymic). The prevalence of the functionally most active effector Tregs is decreased to the highest extent, while naive Treg cells appear to be unaffected. The combination of lower effector Treg and higher exhausted Treg prevalence may account for the decrease in the functionality of Tregs in PE.

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References

1. Redman CW, Sacks GP, Sargent IL: Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol 1999;180:499-506.
2. Saito S, Shiozaki A, Nakashima A, Sakai M, Sasaki Y: The role of the immune system in preeclampsia. Mol Aspects Med 2007;28:192-209.
3. Saito S, Sakai M: Th1/Th2 balance in preeclampsia. J Reprod Immunol 2003;59:161-173.
4. Saito S, Nakashima A, Shima T, Ito M: Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol 2010;63:601-610.
5. Sakaguchi S, Yamaguchi T, Nomura T, Ono M: Regulatory T cells and immune tolerance. Cell 2008;133:775-787.
6. Quinn KH, Parast MM: Decidual regulatory T cells in placental pathology and pregnancy complications. Am J Reprod Immunol 2013;69:533-538.
7. Samstein R, Josefowicz S, Arvey A, Treuting P, Rudensky A: Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. Cell 2012;150:29-38.
8. Aluvihare V, Kallikourdis M, Betz A: Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol 2004;5:266-271.
9. Sasaki Y, Darmochwal-Kolarz D, Suzuki D, Sakai M, Ito M, Shima T, Shiozaki A, Rolinski J, Saito S: Proportion of peripheral blood and decidual CD4(+) CD25(bright) regulatory T cells in preeclampsia. Clin Exp Immunol 2007;149:139-145.
10. Darmochwal-Kolarz D, Saito S, Rolinski J, Tabarkiewicz J, Kolarz B, Leszczynska-Gorzelak B, Oleszczuk J: Activated T lymphocytes in pre-eclampsia. Am J Reprod Immunol 2007;58:39-45.

11. Prins JR, Boelens HM, Heimweg J, Van der Heide S, Dubois AE, Van Oosterhout AJ, Erwich JJ: Preeclampsia is associated with lower percentages of regulatory T cells in maternal blood. Hypertens Pregnancy 2009;28:300-311.
12. Steinborn A, Haensch GM, Mahnke K, Schmitt E, Toermer A, Meuer S, Sohn C: Distinct subsets of regulatory T cells during pregnancy: is the imbalance of these subsets involved in the pathogenesis of preeclampsia? Clin Immunol 2008;129:401-412.
13. Toldi G, Svec P, Vásárhelyi B, Mészáros G, Rigó J, Tulassay T, Treszl A: Decreased number of FoxP3+ regulatory T cells in preeclampsia. Acta Obstet Gynecol Scand 2008;87:1229-1233.
14. Tilburgs T, Roelen DL, Van der Mast BJ, Van Scip JJ, Kleijburg C, de Groot-Swings GM, Kanhai HH, Claas FH, Scherjon SA: Differential distrubution of CD4+CD25bright and CD8+CD28- T-cells in decidua and maternal blood during human pregnancy. Placenta 2006;27 Suppl. A: S47-S53.
15. Tilburgs T, Roelen D, van der Mast B, de Groot-Swings GM, Kleijburg C, Scherjon S, Claas F: 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.
16. Sasaki Y, Darmochwal-Kolarz D, Suzuki D, Sakai M, Ito M, Shima T, Shiozaki A, Rolinski J, Saito S: Proportion of peripheral blood and decidual CD4+ CD25bright regulatory T cells in pre-eclampsia. Clin Exp Immunol 2007;149:139-145.
17. Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, Shevach EM: Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. J Immunol 2010;184:3433-3441.

18. Zabransky DJ, Nirschl CJ, Durham NM, Park BV, Ceccato CM, Bruno TC, Tam AJ, Getnet D, Drake CG: Phenotypic and functional properties of Helios+ regulatory T cells. PLoS One 2012;7:e34547.
19. Cai Q, Dierich A, Oulad-Abdelghani M, Chan S, Kastner P: Helios deficiency has minimal impact on T cell development and function. J Immunol 2009;183:2303-2311.
20. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, Parizot C, Taflin C, Heike T, Valeyre D, Mathian A, Nakahata T, Yamaguchi T, Nomura T, Ono M, Amoura Z, Gorochov G, Sakaguchi S: Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity 2009;30:899-911.
21. Pan X, Yuan X, Zheng Y, Wang W, Shan J, Lin F, Jiang G, Yang YH, Wang D, Xu D, Shen L: Increased CD45RA+ FoxP3(low) regulatory T cells with impaired suppressive function in patients with systemic lupus erythematosus. PLoS One 2012;7:e34662.
22. Steinborn A, Schmitt E, Kisielewicz A, Rechenberg S, Seissler N, Mahnke K, Schaier M, Zeier M, Sohn C: Pregnancy-associated diseases are characterized by the composition of the systemic regulatory T cell (Treg) pool with distinct subsets of Tregs. Clin Exp Immunol 2012;167:84-98.
23. Saito S, Shima T, Inada K, Nakashima A: Which types of regulatory T cells play important roles in implantation and pregnancy maintenance? Am J Reprod Immunol 2013;69:340-345.
24. Shen T, Zheng J, Liang H, Xu C, Chen X, Zhang T, Xu Q, Lu F: Characteristics and PD-1 expression of peripheral CD4+CD127loCD25hiFoxp3+ Treg cells in chronic HCV infected-patients. Virol J 2011;88:279.
25. Franceschini D, Paroli M, Francavilla V, Videtta M, Morrone S, Labbadia G, Cerino A, Mondelli MU, Barnaba V : PD-L1 negatively regulates CD4+CD25+Foxp3+ Tregs by

limiting STAT-5 phosphorylation in patients chronically infected with HCV. J Clin Invest 2009;119:551-564.

26. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R: Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006;439:682-687.
27. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, Mackey EW, Miller JD, Leslie AJ, DePierres C, Mncube Z, Duraiswamy J, Zhu B, Eichbaum Q, Altfeld M, Wherry EJ, Coovadia HM, Goulder PJ, Klenerman P, Ahmed R, Freeman GJ, Walker BD: PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 2006;443:350-354.
28. Brown JA, Dorfman DM, Ma FR, Sullivan EL, Munoz O, Wood CR, Greenfield EA, Freeman GJ: Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. J Immunol 2003;170:1257-1266.
29. Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ: The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nat Immunol 2007;8:239-245.
30. Darmochwal-Kolarz D, Kludka-Sternik M, Tabarkiewicz J, Kolarz B, Rolinski J, Leszczynska-Gorzelak B, Oleszczuk J: The predominance of Th17 lymphocytes and decreased number and function of Treg cells in preeclampsia. J Reprod Immunol 2012;93:75-81.
31. Hsu P, Santner-Nanan B, Dahlstrom JE, Fadia M, Chandra A, Peek M, Nanan R: Altered decidual DC-SIGN+ antigen-presenting cells and impaired regulatory T-cell induction in preeclampsia. Am J Pathol 2012;181:2149-2160.
32. Inada K, Shima T, Ito M, Ushijima A, Saito S: Helios-positive functional regulatory T cells are decreased in decidua of miscarriage cases with normal fetal chromosomal content. J Reprod Immunol 2015;107:10-19.

Table I. Clinical characteristics of study participants

	Healthy pregnant women (n = 21)	Preeclamptic women (n = 19)
Age (years)	32 (28-33)	33 (28-34)
Systolic blood pressure (mmHg)	115 (100-125)	160* (140-180)
Diastolic blood pressure (mmHg)	70 (55-80)	95* (85-120)
Gestational age at blood sample collection (week)	36 (35-37)	34 (31-37)
Gestational age at birth (week)	39 (37-41)	35* (31-38)
Fetal birth weight (g)	3340 (2870-3650)	2770* (2100-3450)
Early onset preeclampsia	-	5 (26%)
Severe preeclampsia	-	7 (37%)

Data are presented as median (interquartile range) for continuous variables and as number (percentage) for categorical variables. * $p < 0.05$ versus healthy pregnant women

Table II. Prevalence of the investigated cell subsets

Subset	Marker	HP (n = 21)	PE (n = 19)	p
Treg	CD4+ CD25hi FoxP3+/CD4+	4.63 (4.22-5.56) %	3.69 (3.32-4.09) %	**0.0003**
Effector Treg	CD4+ FoxP3++ CD45RA-/CD4+ FoxP3+	30.5 (17.7-40.2) %	17.1 (9.88-25.6) %	**0.0022**
Naive Treg	CD4+ FoxP3+ CD45RA+/CD4+ FoxP3+	57.3 (30.9-77.8) %	75.2 (46.0-87.9) %	**0.0014**
Effector Treg	CD4+ FoxP3++ CD45RA-/CD4+	2.44 (1.02-6.78) %	0.87 (0.45-1.22) %	**0.0098**
Naive Treg	CD4+ FoxP3+ CD45RA+/CD4+	4.58 (2.35-7.89) %	3.76 (2.11-5.67) %	0.1098
Activated T	CD4+ CD25+ FoxP3+ CD45RA-/CD4+	2.56 (0.55-7.02) %	4.30 (1.78-7.85) %	**0.0456**
Helios+ Effector	CD4+ FoxP3++ CD45RA- Helios+/CD4+ FoxP3++ CD45RA-	72.0 (49.1-96.8) %	73.1 (50.2-92.3) %	0.9159
Helios+ Naive	CD4+ FoxP3+ CD45RA+ Helios+/CD4+ FoxP3+ CD45RA+	56.9 (40.5-67.7) %	63.0 (37.2-75.3) %	0.8973
Thymic Treg	CD4+ CD25hi FoxP3+ Helios+/CD4+ CD25hi FoxP3+	60.5 (39.9-87.2) %	60.2 (28.7-90.0) %	0.7053
Extrathymic Treg	CD4+ CD25hi FoxP3+ Helios-/CD4+ CD25hi FoxP3+	39.5 (12.8-60.1) %	39.8 (10.1-71.8) %	0.6202
Exhausted Treg	CD4+ CD25hi FoxP3+ CD279+/CD4+ CD25hi FoxP3+	8.08 (4.16-13.5) %	18.2 (9.27-36.3) %	**0.0223**

Data are presented as median (interquartile range). HP – healthy pregnancy, PE – preeclampsia

Figure legend

Figure 1. Box-plots representing the frequency of the investigated cell subsets in healthy pregnancy (HP) and preeclampsia (PE). Horizontal line: median; Box: interquartile range (25-75 percentile); Whisker: range. * $p < 0.05$ versus HP