Journal of Reproductive Immunology 136 (2019) 102613

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jri

Lower activation of CD4$^+$ memory T cells in preeclampsia compared to healthy pregnancies persists postpartum

Tom E.C. Kieffer[a], Sicco A. Scherjon[a], Marijke M. Faas[b], Jelmer R. Prins[a,*]

[a] Department of Obstetrics and Gynecology, University Medical Center Groningen, University of Groningen. PO Box 30001, 9700 RB Groningen, the Netherlands
[b] Division of Medical Biology, Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen. PO Box 30001, 9700 RB Groningen, the Netherlands

ARTICLE INFO

Keywords:
Preeclampsia
Postpartum
Pregnancy
Memory T cells
Effector memory cells
Central memory cells

ABSTRACT

Insufficient adaptations of the maternal immune system are associated with pregnancy complications such as preeclampsia. Memory T cells might be implicated in the pathophysiology of preeclampsia and its recurrence risk. Therefore, peripheral blood samples were taken from healthy pregnant women (n = 15), preeclamptic pregnant women (n = 15), formerly healthy pregnant women (n = 16), and formerly preeclamptic women (n = 15). CD4$^+$ and CD8$^+$ memory cells (CD45RO$^+$), central-memory cells (CM, CD45RO$^+$CCR7$^+$), effector-memory cells (EM, CD45RO$^+$CCR7$^-$), and their activated (CD69$^+$) proportions were analyzed using flow cytometry. A magnetic Luminex assay was performed on plasma samples from all groups to analyze 16 cytokines associated with memory T cells homeostasis and T cell differentiation. Proportions of CD4$^+$ and CD8$^+$ memory cell populations did not differ between preeclamptic and healthy pregnant women or between formerly preeclamptic and formerly healthy pregnant women. However, activated proportions of the general CD4$^+$ memory, CD4$^+$ EM, and CD4$^+$ CM population in the peripheral blood were lower during preeclampsia compared to healthy pregnancies and were also lower postpartum in formerly preeclamptic compared to formerly healthy pregnant women. This was accompanied by lower IL2 concentrations in plasma from formerly preeclamptic compared to formerly healthy pregnant women. The lower activated proportions of memory T cells after a preeclamptic pregnancy were not associated with altered memory T cell associated cytokine plasma concentrations. These findings showed lower activation of memory CD4$^+$ T cell subsets in and after preeclampsia as compared with healthy pregnancies, which makes their implication in the preeclampsia recurrence risk likely.

1. Introduction

During pregnancy, adaptations of the maternal immune system are necessary to prevent immune rejection of the semi-allogeneic fetus. Insufficient adaptations of the maternal immune system are associated with pregnancy complications such as pregnancy loss, fetal growth restriction, and preeclampsia (Cudihy and Lee, 2009; Sibai et al., 2005; Zenclussen, 2013). Preeclampsia occurs in 5–8% of pregnancies, and has short- and long term consequences for fetal and maternal health (Abalos et al., 2013; Saftlas et al., 1990). Long term consequences on maternal health include increased risk of hypertension, ischemic heart disease, and stroke (Bellamy et al., 2007; Veerbeek et al., 2015). Besides these consequences, pre-eclampsia has an average recurrence risk of 10–20% (Sibai et al., 1991; van Oostwaard et al., 2015). Many factors are implicated in the pathophysiology of preeclampsia, but increasing evidence shows that especially insufficient adaptations of the maternal immune system are involved (Kieffer et al., 2019; Nguyen et al., 2017; Wagner et al., 2015).

Several immune mechanisms are implicated in the pathophysiology of preeclampsia (Redman and Sargent, 2010), including alterations in levels and function of regulatory T cells (Toldi et al., 2015, 2012). Another implicated immune mechanism could be formation of immunologic memory; i.e. the ability of the immune system to memorize antigens, which is mainly regulated by memory T cells (Kieffer et al., 2019). Memory T cells are formed during an immune response and remain latent to induce rapid cell expansion and secretion of cytokines on a second encounter with the cognate antigen to ensure quick clearance (Lanzavecchia Federica, 2005; Sallusto et al., 1999). In peripheral blood, the main memory T cell subsets are effector-memory (EM) cells and central-memory (CM) cells with either a CD4$^+$ or CD8$^+$

Abbreviations: T cell, T-lymphocyte; CM cell, central memory T cell; EM cell, effector memory T cell; BMI, body mass index; UMCG, University Medical Center Groningen; WBC, white blood cell

* Corresponding author at: Department of Obstetrics and Gynecology, University Medical Center Groningen, PO Box 30001, 9700 RB Groningen, the Netherlands.
E-mail addresses: t.e.c.kieffer@umcg.nl (T.E.C. Kieffer), s.a.scherjon@umcg.nl (S.A. Scherjon), m.m.faas@umcg.nl (M.M. Faas), j.r.prins@umcg.nl (J.R. Prins).

https://doi.org/10.1016/j.jri.2019.102613
Received 6 May 2019; Received in revised form 19 August 2019; Accepted 17 September 2019

background (Kieffer et al., 2019; Sallusto et al., 2004, 1999). Upon activation with the cognate antigen, CM cells induce quick expansion of the T cell population and differentiate into EM cells (Gray et al., 2018; Rivino et al., 2004; Sallusto et al., 2004; Zaph et al., 2004). The EM cell population also rapidly expands upon reactivation and secretes large amounts of pro-inflammatory cytokines such as interferon-gamma (IFN-gamma), interleukin-4 (IL4), and IL5 (MacLeod et al., 2010; Pepper and Jenkins, 2011; Sallusto et al., 2004).

Previous studies indicated that memory T cells may have a different function in reproduction, and might contribute to fetal-maternal tolerance, reducing risks of pregnancy complications (Kieffer et al., 2019; Tilburgs et al., 2010; van der Zwan et al., 2018). However, studies on memory T cells in preeclampsia are limited and contradictory. Two studies found higher levels of the general $CD4^+$ memory T cell population in peripheral blood of women with preeclampsia compared to healthy pregnant women (Chaiworapongsa et al., 2002; Darmochwal-Kolarz et al., 2007). Whether the differences in these studies were observed in the CM or the EM cell subset was not addressed. Moreover, another study found comparable levels of general $CD4^+$ cells (Nguyen et al., 2017). Locally, at the fetal-maternal interface, impaired function of memory T cells has been shown in preeclamptic patients (Nguyen et al., 2017). Possibly, during a preeclamptic pregnancy memory T cell populations are formed differently, which could have effects on memory T cell populations postpartum.

We have previously shown that levels of $CD4^+$ memory cells are higher and activated proportions are more frequent in women after a healthy pregnancy compared to nulligravid women, suggesting a persistent influence of pregnancy on the memory T cell population (Kieffer et al., 2017). We hypothesized that memory T cell populations differ in preeclampsia compared to healthy pregnancies and that these differences are also present postpartum. Therefore, in the present study we evaluated CM and EM cell populations in and after preeclamptic pregnancies versus healthy pregnancies.

2. Materials and methods

2.1. Patient samples

Peripheral blood samples were taken from healthy pregnant women (n = 15), preeclamptic pregnant women (n = 15), formerly healthy pregnant women (n = 16), and formerly preeclamptic women (n = 15). Raw data from peripheral blood memory T cell subsets from 12 women with uncomplicated pregnancies, and 13 formerly healthy pregnant women were used from a previously published study (Kieffer et al., 2017). Pregnant women were between 25 and 35 weeks of gestation. Preeclampsia was defined as gestational hypertension with a diastolic blood pressure ≥90 mmHg and systolic blood pressure ≥140 mmHg, in two different measurements, accompanied with proteinuria ≥300 mg / 24 h, maternal organ dysfunction, or uteroplacental dysfunction, according to the definition of the International Society for the Study of Hypertension (Brown et al., 2019). Formerly pregnant women were at least 6 months after birth, did not breastfeed, and had no intra uterine contraceptive devices. Samples were taken on one of the first 8 days of the menstrual cycle or on one of the first 7 days of the week without the contraceptive pill. None of the women smoked, used drugs or alcohol. All women had a body mass index (BMI) ≤30, were between 20 and 40 years of age, reported pregnancies with only one partner, and had no known immune associated disorders other than preeclampsia. Apart from oral contraceptive pill use in formerly pregnant women, only the pregnant women with preeclampsia used medication (antihypertensive drugs and/or corticosteroids for fetal lung maturation). Blood samples were taken in a 10 ml lithium heparin tube (BD Biosciences, USA).

This study was approved by the Medical Ethical Committee of the University Medical Center Groningen (protocol number: NL46127.042.13 and NL55954.042.15). All women gave written informed consent.

2.2. Flow cytometry

Blood samples were processed within 1 h after blood withdrawal. Total white blood cell (WBC) count was measured on a Sysmex, PocH 100-i (Sysmex, Japan). Thereafter, the sample was centrifuged (250 x g, 10 min, 20 °C) to separate plasma from blood cells. Plasma was used for Magnetic Luminex assay. The volume of plasma was replaced with PBS (Phosphate Buffered Saline), after which ammonium chloride was added, followed by 10 min incubation on ice, to lyse red blood cells. The sample was centrifuged (600 x g, 5 min, 4 °C), the supernatant was removed, and the red blood cell lysis steps were repeated. Cells were washed with FACS buffer (PBS and 2% Fetal Calf Serum). Remaining cells were counted using a coulter counter (Beckman, USA), and 1.000.000 cells were added per well in a 96 well plate for flow cytometric staining. Cells were blocked using 20% mouse serum (Sanquin, The Netherlands) in FACS buffer to reduce non-specific binding (Andersen et al., 2016). After centrifugation (600 x g, 3 min, 4 °C) and removal of supernatant, cells were incubated (30 min, 4 °C) with monoclonal antibodies CD3 (APC-Efluor 780, SK7) (eBioscience, USA), CD4 (FITC, OKT4) (BioLegend, USA), CD8 (Percp-cy5.5, RPA-T8) (BioLegend, USA), CD45RO (PeCy7, UCHL1) (BioLegend, USA), CCR7 (BV605, G043H7) (BioLegend, USA), and CD69 (PE, FN50) (BioLegend, USA). Thereafter, cells were washed with FACS buffer and fixed using FACS fix solution (BD biosciences, USA). Approximately 500.000 cells per sample were acquired for analysis using a FACSVerse flow cytometer (BD Biosciences) using BD FACS Suite™ software (BD Biosciences). UltraComp eBeads (eBiosciences, USA) stained with a single monoclonal antibody were used for compensation settings. Isotype controls were used to control for non-specific binding and fluorescence minus one controls were used to set gates.

Data were analyzed using Flowjo V10 software (LLC, USA) (Fig. 1). First, single cells were gated and lymphocytes were selected using forward and sideward scatterplots (Fig. 1A, B). Within the lymphocyte population, $CD3^+$ cells were selected (Fig. 1C) followed by identification of $CD4^+$ and $CD8^+$ cells (Fig. 1D). $CD4^+$ and $CD8^+$ cells were further subdivided into CM and EM cell populations by using CD45RO and CCR7 expression (Fig. 1E, G). Within the different memory cell populations, CD69 expression was used to identify activated memory T cell proportions (Fig. 1F, H).

2.3. Magnetic luminex assay

Plasma was centrifuged again (1000 x g, 4 °C) and was stored in cryotubes at −80 °C until analysis. Magnetic Luminex assay (R&D systems, USA) was used to analyze concentrations of cytokines associated with memory T cell formation (IL4, IL7, IL15, and IL23 (Berard et al., 2003; Duvallet et al., 2011; Lanzavecchia Federica, 2005; Li et al., 2016; Weng et al., 2002)) or cytokines associated with Th1, Th2, or Th17 differentiation (IFN-gamma, IL1-beta, IL2, IL5, IL6, IL10, IL12, IL17-alpha, IL18, IL21, and IL27 (Berger, 2000; Hartigan-O'Connor et al., 2011; Lockwood et al., 2008; Pinheiro et al., 2013; Zhu and Paul, 2008)). A single measurement was performed per sample according to manufacturer's instructions. In brief, plasma was diluted in a 1:2 dilution in Calibrator Diluent RD6-52 (R&D systems, USA). Microparticle Cocktail (R&D systems, USA) was added to each individual sample and incubated on a shaker overnight (800 rpm, 4 °C). Samples were washed and Biotin-Antibody Cocktail (R&D systems, USA) was added to each sample and incubated on a shaker for 1 h (800 rpm, 20 °C). Samples were washed and incubated with Streptavidin-PE (R&D systems, USA) for 30 min (800 rpm, 20 °C). Samples were washed and resuspended in 100 µl Wash Buffer (R&D systems, USA), and analyzed with a Luminex 100/200 system (R&D systems, USA) with xPONENT software (R&D systems, USA). A standard curve was created on each plate with each concentration in duplo.

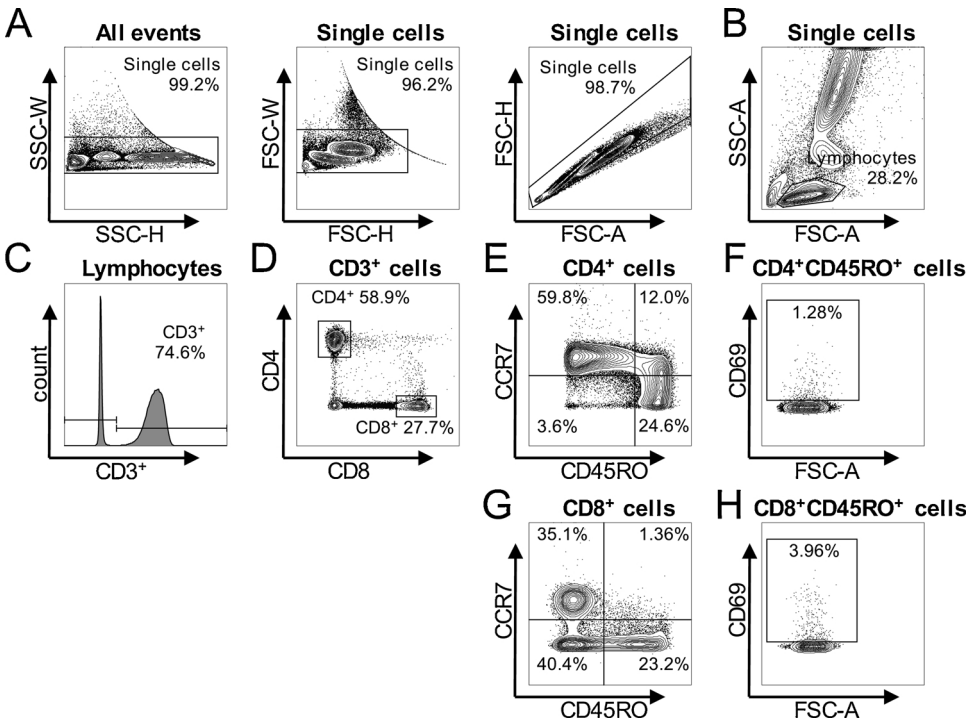

Fig. 1. Flow cytometric analysis of memory T cell subsets in peripheral blood. First, single cells were gated from doublets **(A)**. Lymphocytes were selected in a forward/sideward (FSC/SSC) scatterplot of single cells **(B)**. Within the lymphocyte population, $CD3^+$ cells were gated **(C)**. Then, $CD4^+$ and $CD8^+$ cells were distinguished **(D)**. Within the $CD4^+$ cell population, $CD4^+$ central memory (CM) cells ($CCR7^+CD45RO^+CD4^+$) **(E)**, $CD4^+$ effector memory (EM) cells ($CCR7^-CD45RO^+CD4^+$) **(E)**, and general $CD4^+$ memory cells (CM + EM) ($CD45RO^+CD4^+$) **(E)**, were identified. Within each $CD4^+$ memory cell subset activated ($CD69^+$) proportions were identified **(F)**. Within the $CD8^+$ cell population, $CD8^+$ CM cells ($CCR7^+CD45RO^+CD8^+$) **(G)**, $CD8^+$ EM cells ($CCR7^-CD45RO^+CD8^+$) **(G)**, general $CD8^+$ memory cells (CM + EM) ($CD45RO^+CD8^+$) **(G)**, were identified. Within each $CD8^+$ memory cell subset activated ($CD69^+$) proportions were identified **(H)**.

2.4. Statistics

Data were tested for normality using Kolmogorov-Smirnov test. As some of the data sets were nonparametric, all data are shown as median with interquartile range. Outliers were excluded from the data using the ROUT method (Motulsky et al., 2006). A Kruskal-Wallis test with Dunn's test for multiple comparisons was performed to compare the healthy pregnant group with the preeclamptic group, the healthy pregnant group with the formerly healthy pregnant group, the preeclamptic group with the formerly preeclamptic group, and the formerly healthy pregnant group with the formerly preeclamptic group. Categorical patient characteristics were analyzed using Fisher's exact test. To investigate the effect of gestational age at onset of preeclampsia on activated memory cell proportions, a linear regression analysis was performed. Data were analyzed using Graphpad Prism 6.0 h for mac OS X, (GraphPad Software, USA), and IBM SPSS for Windows Version 20 (IBM, USA). Differences were considered significant if $p < 0.05$.

3. Results

3.1. Baseline characteristics

In Table 1 an overview of the characteristics of the women enrolled in this study is shown. The maternal age, ethnicity, gestational age at blood withdrawal, day of menstrual cycle, gravidity, and fetal sex, were similar between the groups. Parity of formerly healthy pregnant women and formerly preeclamptic women was higher compared to healthy pregnant women ($p < 0.0001$) and preeclamptic women ($p < 0.01$) respectively. The median BMI in all groups was below 25 kg/m^2, but the BMI of the formerly preeclamptic women was higher compared to formerly healthy pregnant women ($p < 0.05$). White blood cell counts were higher in the pregnant groups compared to the formerly pregnant groups, but comparisons between the preeclamptic and healthy pregnant groups, and between the formerly preeclamptic and formerly healthy pregnant groups did not show a difference. Formerly preeclamptic women delivered more through caesarean section compared to formerly healthy pregnant women ($p < 0.01$). All other patient characteristics did not differ between the groups.

3.2. Preeclampsia does not affect general $CD4^+$ and $CD8^+$ cell proportions

To investigate alterations of the general T cell subsets in and after preeclampsia, general lymphocyte, $CD3^+$, $CD4^+$ and $CD8^+$ cell proportions were analyzed using flowcytometry in peripheral blood of healthy pregnant women, preeclamptic pregnant women, formerly healthy pregnant women, and formerly preeclamptic women. The lymphocyte proportion of all white blood cells was higher in formerly healthy pregnant women compared to healthy pregnant women ($p < 0.001$), and was also higher in formerly preeclamptic women compared to preeclamptic women ($p < 0.01$) (Fig. 2A). The lymphocyte proportion did not differ between healthy pregnant and preeclamptic women, or between formerly healthy pregnant and formerly preeclamptic women (Fig. 2A). No differences were found in $CD3^+$, $CD4^+$, and $CD8^+$ cell frequencies or their activated proportions between the groups (Fig. 2).

3.3. Lower activated proportions of $CD4^+$ memory cells in preeclampsia compared to healthy pregnancies persists postpartum

Memory T cell subsets were analyzed in pregnant women with and without preeclampsia, and women at least half a year postpartum after a pregnancy with or without preeclampsia. Between healthy pregnant women and preeclamptic pregnant women, during and after pregnancy, no differences were found for proportions of $CD4^+$ memory, $CD4^+$ CM, and $CD4^+$ EM cells (Fig. 3A, C, E). Also, no differences were observed when comparing these proportions between formerly healthy pregnant women and healthy pregnant women, or formerly preeclamptic women and preeclamptic women (Fig. 3A, C, E).

Despite a lack of differences in the proportions of the $CD4^+$ memory T cell subsets, we found clear differences in the proportions of activated memory T cell subsets. The pregnant group with preeclampsia had lower proportions of activated memory T cells ($p < 0.001$), $CD4^+$ CM cells ($p < 0.0001$), and $CD4^+$ EM cells ($p < 0.05$), compared to the healthy pregnant group (Fig. 3B, D, F). In the postpartum groups, formerly preeclamptic women had lower activated proportions of $CD4^+$ memory ($p < 0.01$), $CD4^+$ CM ($p < 0.001$) and $CD4^+$ EM cells ($p < 0.05$) compared to formerly healthy pregnant women (Fig. 3B, D,

Table 1
Baseline Characteristics.

	Pregnant Healthy (n = 15)	Pregnant PE (n = 15)	Formerly Healthy Pregnant (n = 16)	Formerly PE (n = 15)
Maternal age (years)	29.8 (28.9-33.2)	28.7 (25.0-32.1)	31.0 (28.0-35.1)	30.5 (26.7-33.1)
Ethnicity (%)				
White	83	80	84	83
Other	17	20	16	17
Gestational age at blood withdrawal (weeks)	30.6 (27.3-33.3)	30.6 (27.3-31.9)	NA	NA
Day of menstrual cycle (days)	NA	NA	4 (2-6)	3 (2-4)
Oral contraceptive pill use (% of women)		26,7		26,7
Months after last delivery (months)	NA	NA	16.0 (11.5-23.5)	17.7 (13.2-32.2)
Gravidity	1(1-1.25)	1(1-2)	1(1-2)	1(1-2)
Parity	0 (0-0.25)	0 (0-1)	1 (1-2)****	1(1-1)§§
Body Mass Index (kg/m^2)	23.1 (21.6-24.3)	25.5 (23.6-28.0)	21.2 (20.1-22.8)	24.0 (22.0-26.2)†
White blood cell count (10^9/L)	10.7 (8.7-11.8)	10.4 (8.2-15.5)	6.2 (5.3-7.2)***	5.4 (4.8-6.4)****
Gestational age at onset of preeclampsia (weeks)	NA	30.0 (26.6-31.0)	NA	34.7 (30.3-36.3)§§
Preeclampsia subtype (%)				
Early-onset	NA	80	NA	40§§
Late-onset	NA	20	NA	60§§
Gestational age at delivery (weeks)	40.2 (39.1-41.0)	31.0 (28.5-33.85)****	40.1 (39.5-40.4)	35.7 (30.0-37.1)†††
Corticosteroid therapy for fetal lung maturation (% of women)	0	20	0	20
Mode of delivery last pregnancy (%)				
Vaginal	80	33.3*	87.5	40††
CS	20	66.7*	12.5	60††
Fetal sex last pregnancy (%)				
Female	60	50	56	40
Male	40	50	44	60

Data as median (interquartile range); PE, preeclampsia; CS, caesarean section; Kruskal-wallis test with Dunn's test for multiple comparisons to compare the pregnant groups, the formerly pregnant groups, the healthy pregnant with the formerly healthy pregnant group, and the preeclamptic with the formerly preeclamptic group; Mann-Whitney *U* test to compare data only available in the pregnant or the postpartum group; Fisher's exact test to compare categorical data; *Compared to healthy pregnant group; †Compared to formerly healthy pregnant group; §Compared to preeclamptic pregnant group.

F). Activated memory T cell proportions did not differ between formerly healthy pregnant women and healthy pregnant women, or between formerly preeclamptic women and preeclamptic women (Fig. 3B, D, F).

3.4. CD8$^+$ memory cell activation is not persistently affected by preeclampsia

Between healthy pregnant women and preeclamptic pregnant women, during and after pregnancy, no differences in CD8$^+$ memory, CD8$^+$ CM, and CD8$^+$ EM cell proportions were observed (Fig. 4A, C, E). Comparison of these proportions between healthy pregnant and formerly healthy pregnant women, or between preeclamptic and formerly preeclamptic women also did not show differences (Fig. 4A, C, E).

Activated proportions of CD8$^+$ memory T cells, CD8$^+$ CM cells, and CD8$^+$ EM cells did not differ between the healthy and preeclamptic women, or between the formerly healthy pregnant and formerly preeclamptic women (Fig. 4B, D, F). Formerly preeclamptic women had significantly lower activated proportions of CD8$^+$ EM cells compared to preeclamptic women ($p < 0.05$) (Fig. 4F). Activated proportions of CD8$^+$ EM cells did not differ between the formerly healthy pregnant and the healthy pregnant group (Fig. 4F). No differences were observed in activated proportions of CD8$^+$ memory cells and CD8$^+$ CM cells between formerly preeclamptic and preeclamptic women, or between formerly healthy pregnant and healthy pregnant women (Fig. 4B, D).

3.5. Lower IL2 plasma concentrations in formerly preeclamptic women compared to formerly healthy pregnant women

Using magnetic Luminex assay analysis, concentrations of cytokines associated with memory T cell homeostasis and Th1, Th2, or Th17 cell differentiation were analyzed in the plasma of all participating women. Lower IL2 concentrations were found in plasma of formerly preeclamptic women compared to formerly healthy pregnant women ($p < 0.05$) (Fig. 5C). The IL2 concentrations did not differ between preeclamptic and healthy pregnant women, between formerly healthy pregnant and pregnant women, or between formerly preeclamptic and preeclamptic women. The IL6 concentration was lower in plasma of formerly preeclamptic women compared to preeclamptic women ($p < 0.05$) (Fig. 5D). IL27 plasma concentrations were significantly higher in pregnant women compared to formerly pregnant women ($p < 0.001$) and in preeclamptic compared to formerly preeclamptic women ($p < 0.0001$), but concentrations did not differ between healthy and preeclamptic groups (Fig. 5M). All other cytokines measured did not show any significant differences between the pregnant groups, between the postpartum groups, between the preeclamptic and formerly preeclamptic group, or between the healthy pregnant and formerly healthy pregnant group (Fig. 5). Concentrations of IL4, IL5, and IL23 were below the detection level of the Luminex analysis and were therefore excluded from further analysis.

4. Discussion

This study showed that activated proportions of the general CD4$^+$ memory cell, CD4$^+$ EM cell, and CD4$^+$ CM cell population in the peripheral blood were lower during and after preeclampsia compared to women during and after healthy pregnancies. The lower activated memory T cell proportions after preeclampsia were accompanied by lower IL2 concentrations in plasma of formerly preeclamptic women compared to formerly healthy pregnant women.

We found similar proportions of CM and EM cell subsets within the CD4$^+$ cell compartments between preeclamptic and healthy pregnant women. Similar to our study, Nguyen et al. also did not find any differences for the CD4$^+$ EM, CD8$^+$ CM and CD8$^+$ EM cell population between healthy and preeclamptic women, but they did report higher CD4$^+$ CM cells in preeclamptic women compared to healthy pregnant women (Nguyen et al., 2017). The discrepancy in the findings of the CD4$^+$ CM cell subset in preeclampsia could be due to selection of the subtype of preeclampsia. In our study, we selected mainly early-onset preeclamptic women, while Nguyen et al. mainly included women with

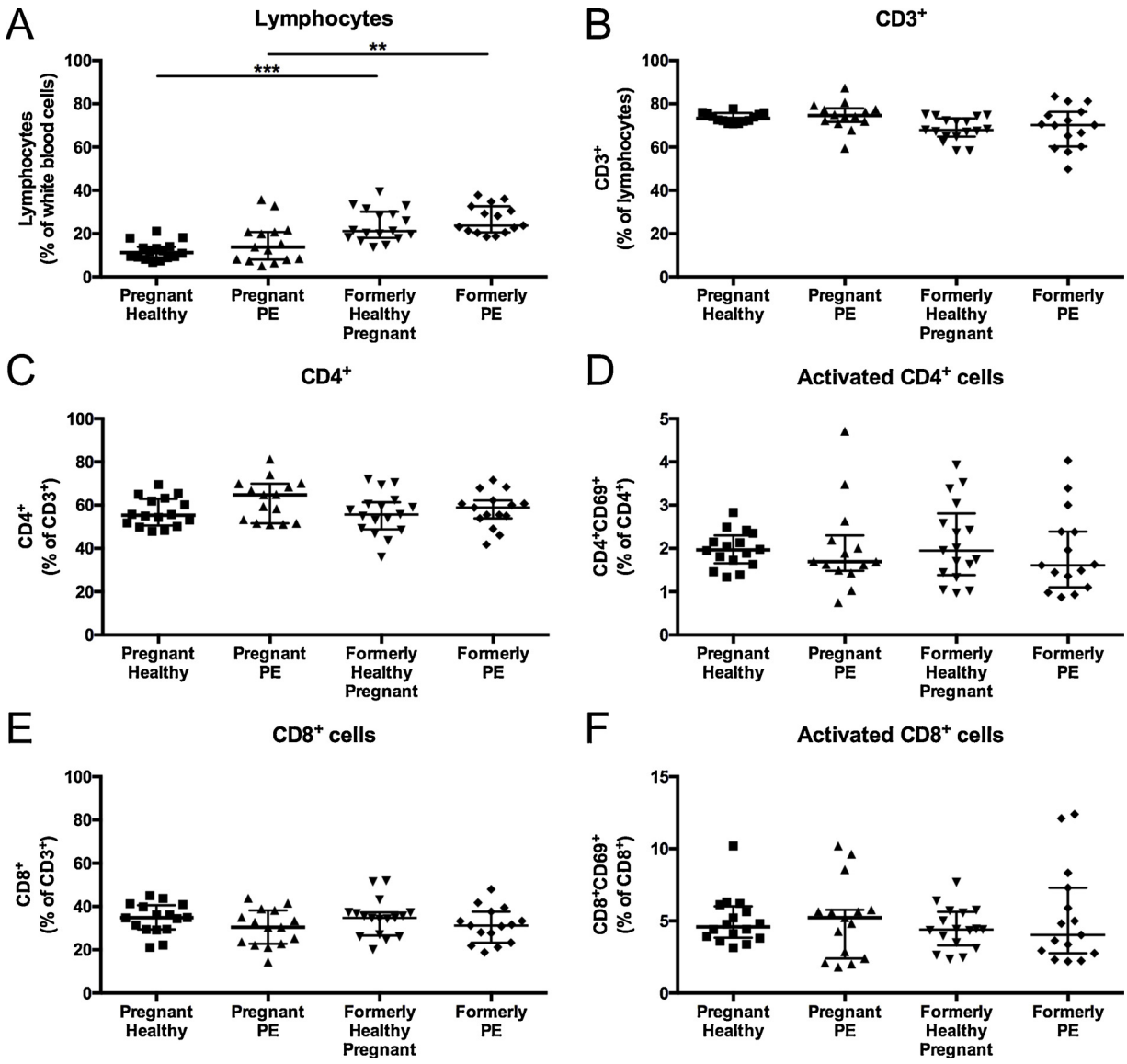

Fig. 2. General lymphocyte populations in and after preeclamptic and healthy pregnancies. Proportions of lymphocytes (% of white blood cells) **(A)**, proportions of T-lymphocytes ($CD3^+$) (% of lymphocytes) **(B)**, proportions of $CD4^+$ cells **(C)**, activated ($CD69^+$) proportions of $CD4^+$ cells **(D)**, proportions of $CD8^+$ cells **(E)**, activated ($CD69^+$) proportions of $CD8^+$ cells **(F)** in peripheral blood of healthy pregnant women, pregnant women with preeclampsia, formerly healthy pregnant women, and formerly preeclamptic women. $CD4^+$ and $CD8^+$ cell proportions are shown as proportion of the $CD3^+$ cell population. Symbols represent individual values per patient with data shown as median with interquartile range. Analysis by Kruskal-Wallis and Dunn's test for multiple comparisons; $^{**}p < 0.01$, $^{***}p < 0.001$.

late-onset preeclampsia (Nguyen et al., 2017). It has become more and more accepted that early-onset and late-onset preeclampsia are different subtypes of preeclampsia (Tranquilli et al., 2013). In our study, the median gestational age at diagnosis of preeclampsia in the formerly preeclamptic group was 34.7 weeks and the preeclamptic women had a median of 30.0 weeks and therewith our study represents more early-onset preeclampsia.

To the best of our knowledge, there are no studies on memory T cell subsets comparing postpartum women after a healthy pregnancy and postpartum women after a preeclamptic pregnancy. Some studies have reported altered maternal immune responses in formerly preeclamptic women (Bonney et al., 2017; van Rijn et al., 2016), including altered plasma cytokine levels and a different immune response after stimulation with influenza vaccination in women after preeclamptic pregnancies compared to women after a healthy pregnancy (Freeman et al., 2004; Hubel et al., 2008; van Rijn et al., 2016). Our finding of lower IL2 plasma concentrations in formerly preeclamptic women compared to formerly healthy pregnant women is in line with these studies. However, we did not find differences in the proportions of $CD4^+$ or $CD8^+$ EM or CM cells in formerly preeclamptic pregnant women compared to formerly healthy pregnant women.

The main differences in our study are differences in the proportions of activated (i.e. CD69 expressing) $CD4^+$ memory cells in both the CM and EM cell subset. We found lower proportions of these activated memory cells in preeclampsia as compared to healthy pregnancies, both during pregnancy and postpartum. The lower activated proportions we found seemed specific for the $CD4^+$ memory T cell population, since we did not find higher proportions of $CD69^+$ cells in the general T cell and $CD8^+$ memory population. The exact function of CD69 is unknown, but CD69 has been found to have regulatory effects on the immune system (Esplugues et al., 2003; Sancho et al., 2005). It may inhibit inflammatory responses, since it has been shown that $CD69^+$ suppresses the differentiation of T cells towards the Th1/Th17 cell lineage *in vitro* through production of tumor growth factor beta (Esplugues et al., 2003;

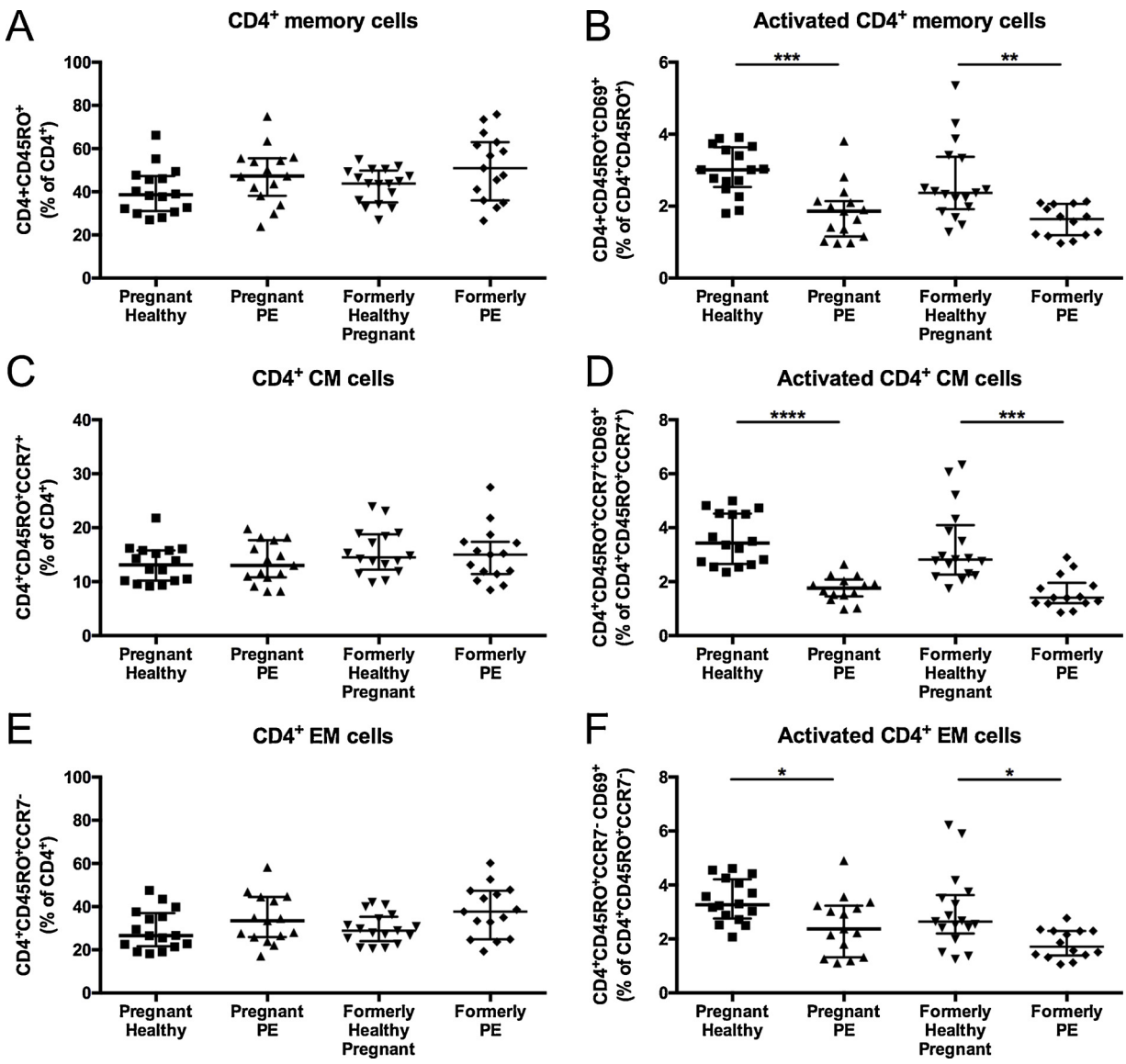

Fig. 3. CD4$^+$ memory T cell populations in and after preeclamptic and healthy pregnancies. Proportions of general CD4$^+$ memory cells **(A)**, activated (CD69$^+$) proportions of general CD4$^+$ memory cells **(B)**, proportions of CD4$^+$ central memory (CM) cells **(C)**, activated proportions of CD4$^+$ CM cells **(D)**, proportions of CD4$^+$ effector memory (EM) cells **(E)**, activated proportions of CD4$^+$ EM cells **(F)** in peripheral blood of healthy pregnant women, pregnant women with preeclampsia, formerly healthy pregnant women, and formerly preeclamptic women. Memory cell proportions are shown as proportion of the CD4$^+$ cell population. Symbols represent individual values per patient with data shown as median with interquartile range. Analysis by Kruskal-Wallis and Dunn's test for multiple comparisons; *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Sancho et al., 2003). The findings of decreased CD69 expression on various memory T cell subsets during preeclampsia as compared to healthy pregnancies may be related to the increased Th1 and Th17 responses in this condition (Saito, 2010; Saito and Sakai, 2003). The persistently upregulated CD69 on memory T cells in healthy pregnancies (Kieffer et al., 2017), may be an immune mechanism to control inflammatory responses, such as the Th1 and Th17 response, during healthy pregnancy.

An explanation for the lower CD69$^+$ proportions in preeclampsia could be non-adequate or altered antigen presentation in preeclampsia causing altered induction of T cells and with that lower CD69 expression. In preeclampsia, altered levels and subsets of antigen presenting cells, such as dendritic cells and macrophages have been found (Huang et al., 2008; Scholz et al., 2008). Hence, antigen presentation causing differentiation and activation of T cells might be altered in preeclampsia and CD69$^+$ expression on T cells in accordance. The lower activated proportions of memory T cells during preeclampsia also seemed to result in lower proportions of activated memory cells after preeclampsia.

An alternative explanation for the lower activated proportions of memory T cells could be an altered plasma cytokine milieu which is necessary for memory T cell formation (Kondrack et al., 2003; Li et al., 2016; Richer et al., 2015). Therefore, we measured plasma concentrations of cytokines associated with T cell differentiation, such as interferon-gamma, IL7 and IL15. We did not find differences in the levels of these cytokines between healthy pregnant and preeclamptic women during pregnancy, suggesting that the reduced activated proportions of memory T cells found in preeclampsia versus healthy pregnant women are not affected by altered levels of these plasma cytokines. We did find lower IL2 plasma concentrations in formerly preeclamptic women compared to formerly healthy pregnant women which do suggest an altered cytokine milieu postpartum after preeclampsia. It is known that IL2 is both an inducer of effector and memory responses (Kalia and Sarkar, 2018; McKinstry et al., 2014). Interestingly, it has also been

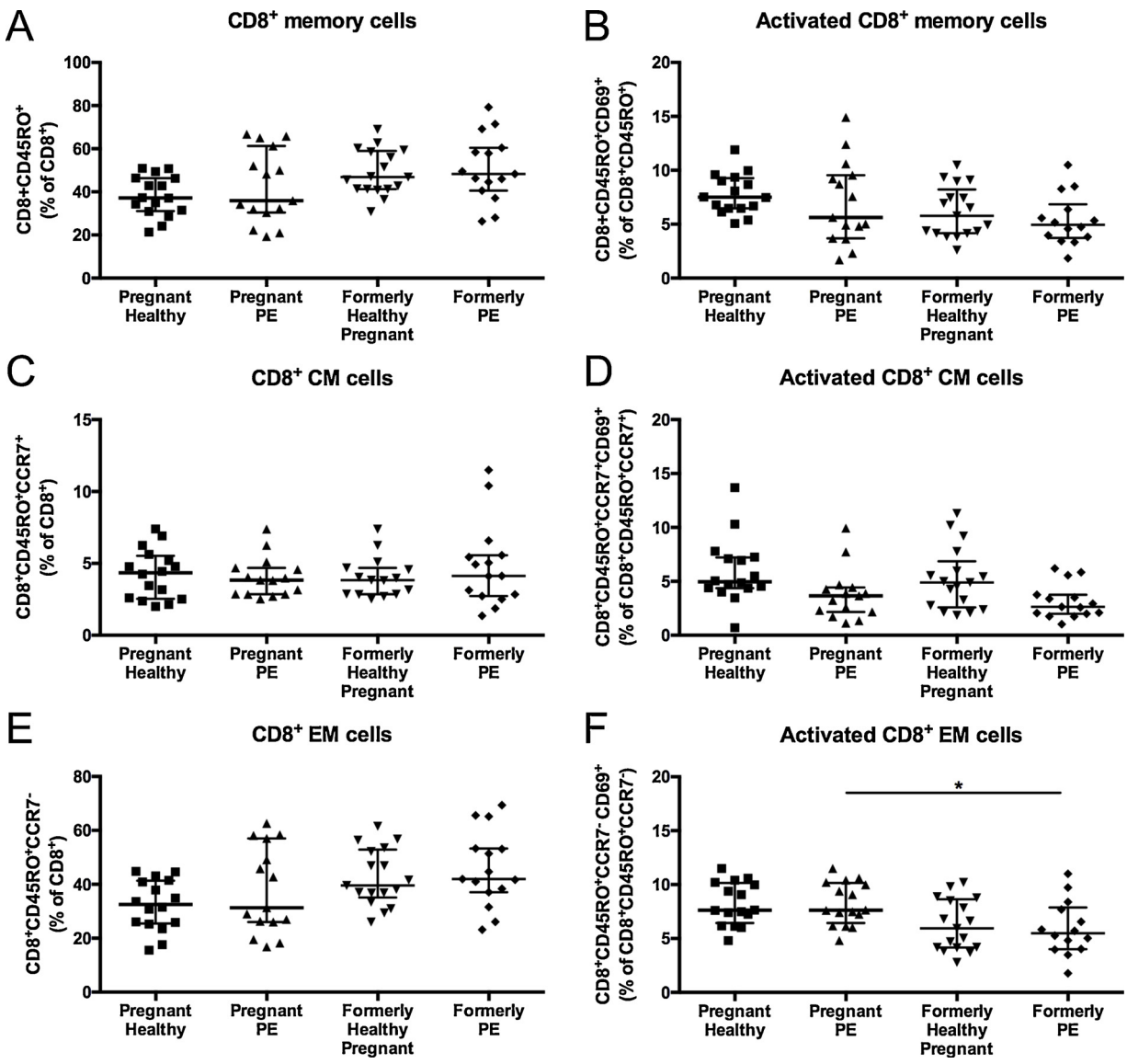

Fig. 4. $CD8^+$ lymphocyte populations in and after preeclamptic and healthy pregnancies. Proportions of general $CD8^+$ memory cells **(A)**, activated ($CD69^+$) proportions of general $CD8^+$ memory cells **(B)**, proportions of $CD8^+$ central memory (CM) cells **(C)**, activated proportions of $CD8^+$ CM cells **(D)**, proportions of $CD8^+$ effector memory (EM) cells **(E)**, activated proportions of $CD8^+$ EM cells **(F)** in peripheral blood of healthy pregnant women, pregnant women with preeclampsia, formerly healthy pregnant women, and formerly preeclamptic women. Memory cell proportions are shown as proportion of the $CD8^+$ cell population. Symbols represent individual values per patient with data shown as median with interquartile range. Analysis by Kruskal-Wallis and Dunn's test for multiple comparisons; $^*p < 0.05$.

shown that IL2 concentrations particularly affect the memory T-cell subtype distribution of CD4+ cells, with higher IL2 levels leading to a decrease in CM cells (Kaartinen et al., 2017). Whether these higher IL2 levels are indeed linked to the reduced $CD4^+$ memory T cell activation in these women remains to be investigated. Even though we did correct for multiple comparisons per cytokine, the results should be interpreted carefully considering the number of cytokines that was measured.

In the present study, we used strict selection criteria for preeclamptic women resulting in homogenous groups, minimizing variation, but this unfortunately also resulted in a small sample size, which could explain that we could not confirm earlier studies regarding altered cytokine levels in and after preeclampsia. 80% of the preeclamptic women in this study had early-onset preeclampsia, whereas 40% of the formerly preeclamptic women had early-onset preeclampsia. Interestingly, the gestational age at delivery, which reflects severity of disease, did not differ significantly between the preeclamptic and the formerly preeclamptic group. To assess whether gestational age at onset of preeclampsia influenced the activated proportions of memory T cells, we performed linear regression analyses. No effect of gestational age at onset of preeclampsia on the activated proportions of memory T cells was found, either during pregnancy or postpartum, indicating that the gestational age of onset of preeclampsia did not affect the proportion of activated memory T cells. Due to the strict selection criteria, all formerly preeclamptic women and formerly healthy pregnant women were healthy postpartum, did not use any medication, had similar parity, had similar gravidity, and had blood withdrawn in the first 8 days of the menstrual cycle to minimize effects of sex hormone fluctuations (Arruvito et al., 2007; Hughes et al., 2013). As the proportion of former preeclamptic women and formerly healthy pregnant women that used oral contraceptives did not differ, we believe that the use of oral contraceptives did not influence our results significantly. Although there was a statistical significant difference in BMI of formerly healthy pregnant women compared to formerly preeclamptic women (median 21.2, and 24.0 respectively), both medians were

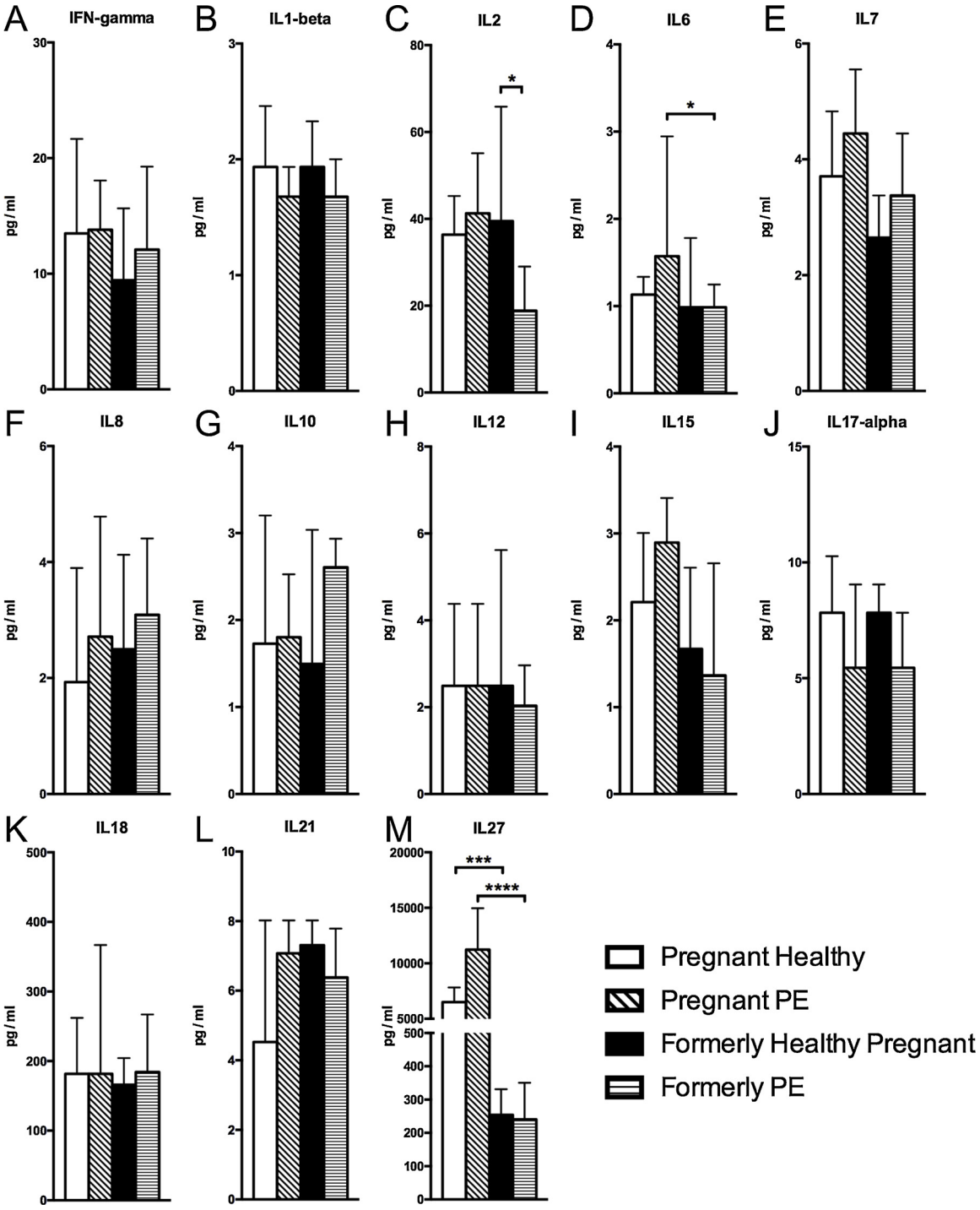

Fig. 5. Cytokine plasma concentrations in and after preeclamptic and healthy pregnancies. Interferon-gamma (IFN-gamma) **(A)**, interleukin-beta (IL1-beta) **(B)**, IL2 **(C)**, IL6 **(D)**, IL7 **(E)**, IL8 **(F)**, IL10 **(G)**, IL12 **(H)**, IL15 **(I)**, IL17-alpha (J), IL18 **(K)**, IL21 **(L)**, and IL27 **(M)** concentrations in plasma of healthy pregnant women, pregnant women with preeclampsia, formerly healthy pregnant women, and formerly preeclamptic women. Data shown as median with interquartile range. Analysis by Kruskal-Wallis and Dunn's test for multiple comparisons; *$p < 0.05$, *** $p < 0.001$, ****$p < 0.0001$.

within the normal BMI range. Using linear regression analyses, we could not find an effect of BMI on the (activated) proportions of memory T cells in our study.

In conclusion, we are the first to show persistent effects of preeclampsia on the memory T cell populations. Preeclamptic women during and after preeclampsia had lower activated proportions of $CD4^+$ memory T cells as measured by CD69 expression. These findings make the involvement of memory T cells in preeclampsia and its recurrence risk more likely. Our results encourage larger longitudinal studies, as well as functional studies in preeclampsia on memory T cells to investigate an association of memory T cells with the pathophysiology and recurrence risks of preeclampsia

Funding

This work was supported by the University Medical Center Groningen, the University of Groningen, a Junior Scientific Masterclass MD/PhD grant (awarded to T.E.C. Kieffer) and a Mandema Stipendium

(awarded to J.R. Prins). The funding sources had no involvement in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Declaration of Competing Interest

None.

Acknowledgements

We are grateful to all women who participated in this study and for the HELLP-foundation for their assistance with patient recruitment.

References

Abalos, E., Cuesta, C., Grosso, A.L., Chou, D., Say, L., 2013. Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur. J. Obstet. Gynecol. Reprod. Biol. 170, 1–7. https://doi.org/10.1016/j.ejogrb.2013.05.005.

Andersen, M.N., Al-Karradi, S.N.H., Kragstrup, T.W., Hokland, M., 2016. Elimination of erroneous results in flow cytometry caused by antibody binding to Fc receptors on human monocytes and macrophages. Cytometry A. https://doi.org/10.1002/cyto.a.22995.

Arruvito, L., Sanz, M., Banham, A.H., Fainboim, L., 2007. Expansion of CD4 + CD25 + and FOXP3 + regulatory T cells during the follicular phase of the menstrual cycle: implications for human reproduction. J. Immunol. 178, 2572–2578. https://doi.org/10.4049/JIMMUNOL.178.4.2572.

Bellamy, L., Casas, J.-P., Hingorani, A.D., Williams, D.J., 2007. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ 335, 974. https://doi.org/10.1136/bmj.39335.385301.BE.

Berard, M., Brandt, K., Bulfone-Paus, S., Tough, D.F., 2003. IL-15 promotes the survival of naive and memory phenotype CD8 + T cells. J. Immunol. 170, 5018–5026. https://doi.org/10.4049/JIMMUNOL.170.10.5018.

Berger, A., 2000. Th1 and Th2 responses: what are they? BMJ 321, 424. https://doi.org/10.1136/bmj.321.7258.424.

Bonney, E.A., Howard, A., Krebs, K., Begin, K., Veilleux, K., Gokina, N.I., 2017. Impact of immune deficiency on remodeling of maternal resistance vasculature 4 weeks postpartum in mice. Reprod. Sci. 24, 514–525. https://doi.org/10.1177/1933719116678691.

Brown, M.A., Magee, L.A., Kenny, L.C., Karumanchi, S.A., McCarthy, F.P., Saito, S., Hall, D.R., Warren, C.E., Adoyi, G., Ishaku, S., International Society for the Study of Hypertension in Pregnancy (ISSHP), 2018. Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. Hypertension 72, 24–43. https://doi.org/10.1161/HYPERTENSIONAHA.117.10803.

Chaiworapongsa, T., Gervasi, M.T., Refuerzo, J., Espinoza, J., Yoshimatsu, J., Berman, S., Romero, R., 2002. Maternal lymphocyte subpopulations (CD45RA + and CD45RO +) in preeclampsia. Am. J. Obstet. Gynecol. 187, 889–893. https://doi.org/10.1067/mob.2002.127309.

Cudihy, D., Lee, R., 2009. The pathophysiology of pre-eclampsia: current clinical concepts. J. Obstet. Gynaecol. 29, 576–582. https://doi.org/10.1080/01443610903061751.

Darmochwal-Kolarz, D., Saito, S., Rolinski, J., Tabarkiewicz, J., Kolarz, B., Leszczynska-Gorzelak, B., Oleszczuk, J., 2007. Activated T lymphocytes in pre-eclampsia. Am. J. Reprod. Immunol. 58, 39–45. https://doi.org/10.1111/j.1600-0897.2007.00489.x.

Duvallet, E., Semerano, L., Assier, E., Falgarone, G., Boissier, M.-C., 2011. Interleukin-23: a key cytokine in inflammatory diseases. Ann. Med. 43, 503–511. https://doi.org/10.3109/07853890.2011.577093.

Esplugues, E., Sancho, D., Vega-Ramos, J., Martínez, C., Syrbe, U., Hamann, A., Engel, P., Sánchez-Madrid, F., Lauzurica, P., 2003. Enhanced antitumor immunity in mice deficient in CD69. J. Exp. Med. 197, 1093–1106. https://doi.org/10.1084/jem.20021337.

Freeman, D.J., McManus, F., Brown, E.A., Cherry, L., Norrie, J., Ramsay, J.E., Clark, P., Walker, I.D., Sattar, N., Greer, I.A., 2004. Short- and long-term changes in plasma inflammatory markers associated with preeclampsia. Hypertension 44, 708–714. https://doi.org/10.1161/01.HYP.0000143849.67254.ca.

Gray, J.I., Westerhof, L.M., MacLeod, M.K.L., 2018. The roles of resident, central and effector memory CD4 T-cells in protective immunity following infection or vaccination. Immunology 154, 574–581. https://doi.org/10.1111/imm.12929.

Hartigan-O'Connor, D.J., Hirao, L.A., McCune, J.M., Dandekar, S., 2011. Th17 cells and regulatory T cells in elite control over HIV and SIV. Curr. Opin. HIV AIDS 6, 221–227. https://doi.org/10.1097/COH.0b013e32834577b3.

Huang, S., Chen, C.-P., Schatz, F., Rahman, M., Abrahams, V., Lockwood, C., 2008. Pre-eclampsia is associated with dendritic cell recruitment into the uterine decidua. J. Pathol. 214, 328–336. https://doi.org/10.1002/path.2257.

Hubel, C.A., Powers, R.W., Snaedal, S., Gammill, H.S., Ness, R.B., Roberts, J.M., Arngrímsson, R., 2008. C-reactive protein is elevated 30 years after eclamptic pregnancy. Hypertension 51, 1499–1505. https://doi.org/10.1161/HYPERTENSIONAHA.108.109934.

Hughes, G.C., Clark, E.A., Wong, A.H., 2013. The intracellular progesterone receptor regulates CD4 + T cells and T cell-dependent antibody responses. J. Leukoc. Biol. 93, 369–375. https://doi.org/10.1189/jlb.1012491.

Kaartinen, T., Luostarinen, A., Maliniemi, P., Keto, J., Arvas, M., Belt, H., Koponen, J., Loskog, A., Mustjoki, S., Porkka, K., Ylä-Herttuala, S., Korhonen, M., Korhonen, M., 2017. Low interleukin-2 concentration favors generation of early memory T cells over effector phenotypes during chimeric antigen receptor T-cell expansion. Cytotherapy 19, 689–702. https://doi.org/10.1016/j.jcyt.2017.03.067.

Kalia, V., Sarkar, S., 2018. Regulation of effector and memory CD8 t cell differentiation by IL-2-A balancing act. Front. Immunol. 9, 2987. https://doi.org/10.3389/fimmu.2018.02987.

Kieffer, T.E.C., Faas, M.M., Scherjon, S.A., Prins, J.R., 2017. Pregnancy persistently affects memory T cell populations. J. Reprod. Immunol. 119, 1–8. https://doi.org/10.1016/j.jri.2016.11.004.

Kieffer, T.E.C., Laskewitz, A., Scherjon, S.A., Faas, M.M., Prins, J.R., 2019. Memory t cells in pregnancy. Front. Immunol. 10, 625. https://doi.org/10.3389/fimmu.2019.00625.

Kondrack, R.M., Harbertson, J., Tan, J.T., McBreen, M.E., Surh, C.D., Bradley, L.M., 2003. Interleukin 7 regulates the survival and generation of memory CD4 cells. J. Exp. Med. 198, 1797–1806. https://doi.org/10.1084/jem.20030735.

Lanzavecchia Federica, A.S., 2005. Understanding the generation and function of memory T cell subsets. Curr. Opin. Immunol. 17, 326–332. https://doi.org/10.1016/j.coi.2005.04.010.

Li, Y., Wang, H., Lu, H., Hua, S., 2016. Regulation of memory t cells by Interleukin-23. Int. Arch. Allergy Immunol. 169, 157–162. https://doi.org/10.1159/000445834.

Lockwood, C.J., Yen, C.-F., Basar, M., Kayisli, U.A., Martel, M., Buhimschi, I., Buhimschi, C., Huang, S.J., Krikun, G., Schatz, F., 2008. Preeclampsia-related inflammatory cytokines regulate interleukin-6 expression in human decidual cells. Am. J. Pathol. 172, 1571–1579. https://doi.org/10.2353/ajpath.2008.070629.

MacLeod, M.K.L., Kappler, J.W., Marrack, P., 2010. Memory CD4 T cells: generation, reactivation and re-assignment. Immunology 130, 10–15. https://doi.org/10.1111/j.1365-2567.2010.03260.x.

McKinstry, K.K., Strutt, T.M., Bautista, B., Zhang, W., Kuang, Y., Cooper, A.M., Swain, S.L., 2014. Effector CD4 T-cell transition to memory requires late cognate interactions that induce autocrine IL-2. Nat. Commun. 5, 5377. https://doi.org/10.1038/ncomms6377.

Motulsky, H.J., Brown, R.E., Barnett, V., Lewis, T., Hampel, F., Ronchetti, E., Rousseeuw, P., Stahel, W., Hoaglin, D., Mosteller, F., Tukey, J., Press, W., Teukolsky, S., Vettering, W., Flannery, B., Benjamini, Y., Hochberg, Y., Benjamini, Y., Yekutieli, D., Hochberg, Y., Benjamini, Y., Storey, J., Storey, J., Rousseeuw, P., Croux, C., Croux, C., Rousseeuw, P., Rousseeuw, P., Verboven, S., Draper, N., Smith, H., Seber, G., Wild, C., Levenberg, K., Marquardt, D., 2006. Detecting outliers when fitting data with nonlinear regression – a new method based on robust nonlinear regression and the false discovery rate. BMC Bioinformatics 7, 123. https://doi.org/10.1186/1471-2105-7-123.

Nguyen, T.A., Kahn, D.A., Loewendorf, A.I., 2017. Maternal—fetal rejection reactions are unconstrained in preeclamptic women. PLoS One 12, e0188250. https://doi.org/10.1371/journal.pone.0188250.

Pepper, M., Jenkins, M.K., 2011. Origins of CD4(+) effector and central memory T cells. Nat. Immunol. 12, 467–471.

Pinheiro, M.B., Martins-Filho, O.A., Mota, A.P.L., Alpoim, P.N., Godoi, L.C., Silveira, A.C.O., Teixeira-Carvalho, A., Gomes, K.B., Dusse, L.M., 2013. Severe preeclampsia goes along with a cytokine network disturbance towards a systemic inflammatory state. Cytokine 62, 165–173. https://doi.org/10.1016/j.cyto.2013.02.027.

Redman, C.W.G., Sargent, I.L., 2010. Immunology of pre-eclampsia. Am. J. Reprod. Immunol. 63, 534–543. https://doi.org/10.1111/j.1600-0897.2010.00831.x.

Richer, M.J., Pewe, L.L., Hancox, L.S., Hartwig, S.M., Varga, S.M., Harty, J.T., 2015. Inflammatory IL-15 is required for optimal memory T cell responses. J. Clin. Invest. 125, 3477–3490. https://doi.org/10.1172/JCI81261.

Rivino, L., Messi, M., Jarrossay, D., Lanzavecchia, A., Sallusto, F., Geginat, J., 2004. Chemokine receptor expression identifies pre–T helper (Th)1, pre–Th2, and non-polarized cells among human CD4 $^{+}$ central memory t cells. J. Exp. Med. 200, 725–735. https://doi.org/10.1084/jem.20040774.

Saftlas, A.F., Olson, D.R., Franks, A.L., Atrash, H.K., Pokras, R., 1990. Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986. Am. J. Obstet. Gynecol. 163, 460–465. https://doi.org/10.1016/0002-9378(90)91176-D.

Saito, S., 2010. Th17 cells and regulatory T cells: new light on pathophysiology of pre-eclampsia. Immunol. Cell Biol. 88, 615–617. https://doi.org/10.1038/icb.2010.68.

Saito, S., Sakai, M., 2003. Th1/Th2 balance in preeclampsia. J. Reprod. Immunol. 59, 161–173. https://doi.org/10.1016/S0165-0378(03)00045-7.

Sallusto, F., Geginat, J., Lanzavecchia, A., 2004. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu. Rev. Immunol. 22, 745–763. https://doi.org/10.1146/annurev.immunol.22.012703.104702.

Sallusto, F., Lenig, D., Förster, R., Lipp, M., Lanzavecchia, A.A., 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 401, 708–712. https://doi.org/10.1126/science.1058867.

Sancho, D., Gómez, M., Sánchez-Madrid, F., 2005. CD69 is an immunoregulatory molecule induced following activation. Trends Immunol. 26, 136–140. https://doi.org/10.1016/j.it.2004.12.006.

Sancho, D., Gómez, M., Viedma, F., Esplugues, E., Gordón-Alonso, M., Angeles García-López, M., de la Fuente, H., Martínez-A, C., Lauzurica, P., Sánchez-Madrid, F., 2003. CD69 downregulates autoimmune reactivity through active transforming growth factor-β production in collagen-induced arthritis. J. Clin. Invest. 112, 872–882. https://doi.org/10.1172/JCI19112.

Scholz, C., Toth, B., Santoso, L., Kuhn, C., Franz, M., Mayr, D., Jeschke, U., Friese, K., Schiessl, B., 2008. Distribution and maturity of dendritic cells in diseases of insufficient placentation. Am. J. Reprod. Immunol. 60, 238–245. https://doi.org/10.1111/j.1600-0897.2008.00619.x.

Sibai, B., Dekker, G., Kupferminc, M., 2005. Pre-eclampsia. Lancet 365, 785–799.
Sibai, B.M., Mercer, B., Sarinoglu, C., 1991. Severe preeclampsia in the second trimester: recurrence risk and long-term prognosis. Am. J. Obstet. Gynecol. 165, 1408–1412.
Tilburgs, T., Schonkeren, D., Eikmans, M., Nagtzaam, N.M., Datema, G., Swings, G.M., Prins, F., van Lith, J.M., van der Mast, B.J., Roelen, D.L., Scherjon, Sa, Claas, F.H., 2010. Human decidual tissue contains differentiated CD8+ effector-memory T cells with unique properties. J. Immunol. 185, 4470–4477. https://doi.org/10.4049/jimmunol.0903597.
Toldi, G., Saito, S., Shima, T., Halmos, A., Veresh, Z., Vásárhelyi, B., Rigó, J., Molvarec, A., 2012. The frequency of peripheral blood CD4+ CD25high FoxP3+ and CD4+ CD25- FoxP3+ regulatory T cells in normal pregnancy and pre-eclampsia. Am. J. Reprod. Immunol. 68, 175–180. https://doi.org/10.1111/j.1600-0897.2012.01145.x.
Toldi, G., Vásárhelyi, Z.E., Rigó, J., Orbán, C., Tamássy, Z., Bajnok, A., Shima, T., Saito, S., Molvarec, A., 2015. Prevalence of regulatory T-Cell subtypes in preeclampsia. Am. J. Reprod. Immunol. 74, 110–115. https://doi.org/10.1111/aji.12380.
Tranquilli, A.L., Brown, M.A., Zeeman, G.G., Dekker, G., Sibai, B.M., 2013. The definition of severe and early-onset preeclampsia. Statements from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Pregnancy Hypertens. An Int. J. Women's Cardiovasc. Heal. 3, 44–47. https://doi.org/10.1016/j.preghy.2012.11.001.
van der Zwan, A., Bi, K., Norwitz, E.R., Crespo, Â.C., Claas, F.H.J., Strominger, J.L., Tilburgs, T., 2018. Mixed signature of activation and dysfunction allows human decidual CD8+ T cells to provide both tolerance and immunity. Proc. Natl. Acad. Sci. U. S. A. 115, 385–390. https://doi.org/10.1073/pnas.1713957115.
van Oostwaard, M.F., Langenveld, J., Schuit, E., Papatsonis, D.N.M., Brown, M.A., Byaruhanga, R.N., Bhattacharya, S., Campbell, D.M., Chappell, L.C., Chiaffarino, F., Crippa, I., Facchinetti, F., Ferrazzani, S., Ferrazzi, E., Figueiró-Filho, E.A., Gaugler-Senden, I.P.M., Haavaldsen, C., Lykke, J.A., Mbah, A.K., Oliveira, V.M., Poston, L., Redman, C.W.G., Salim, R., Thilaganathan, B., Vergani, P., Zhang, J., Steegers, E.A.P., Mol, B.W.J., Ganzevoort, W., 2015. Recurrence of hypertensive disorders of pregnancy: an individual patient data metaanalysis. Am. J. Obstet. Gynecol. 212 (624), e1–e17. https://doi.org/10.1016/j.ajog.2015.01.009.
van Rijn, B.B., Bruinse, H.W., Veerbeek, J.H., Post Uiterweer, E.D., Koenen, S.V., van der Bom, J.G., Rijkers, G.T., Roest, M., Franx, A., 2016. Postpartum circulating markers of inflammation and the systemic acute-phase response after early-onset pre-eclampsia. Hypertension 67, 404–414. https://doi.org/10.1161/HYPERTENSIONAHA.115.06455.
Veerbeek, J.H.W., Hermes, W., Breimer, A.Y., van Rijn, B.B., Koenen, S.V., Mol, B.W., Franx, A., de Groot, C.J.M., Koster, M.P.H., 2015. Cardiovascular disease risk factors after early-onset preeclampsia, late-onset preeclampsia, and pregnancy-induced hypertension. Hypertension 65, 600–606. https://doi.org/10.1161/HYPERTENSIONAHA.114.04850.
Wagner, M.I., Mai, C., Schmitt, E., Mahnke, K., Meuer, S., Eckstein, V., Ho, A.D., Schaier, M., Zeier, M., Spratte, J., Fluhr, H., Steinborn, A., 2015. The role of recent thymic emigrant-regulatory T-cell (RTE-Treg) differentiation during pregnancy. Immunol. Cell Biol. 93, 858–867. https://doi.org/10.1038/icb.2015.51.
Weng, N.-P., Liu, K., Catalfamo, M., Li, Y., Henkart, P.A., 2002. IL-15 is a growth factor and an activator of CD8 memory T cells. Ann. N. Y. Acad. Sci. 975, 46–56.
Zaph, C., Uzonna, J., Beverley, S.M., Scott, P., 2004. Central memory T cells mediate long-term immunity to Leishmania major in the absence of persistent parasites. Nat. Med. 10, 1104–1110. https://doi.org/10.1038/nm1108.
Zenclussen, A.C., 2013. Adaptive immune responses during pregnancy. Am. J. Reprod. Immunol. 69, 291–303. https://doi.org/10.1111/aji.12097;10.1111/aji.12097.
Zhu, J., Paul, W.E., 2008. CD4 T cells: fates, functions, and faults. Blood 112, 1557–1569. https://doi.org/10.1182/blood-2008-05-078154.