Journal of Reproductive Immunology 119 (2017) 1–8

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

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

Pregnancy persistently affects memory T cell populations

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ARTICLE INFO

Article history:
Received 10 July 2016
Received in revised form 28 October 2016
Accepted 10 November 2016

Keywords:
Central memory t cells
Effector memory t cells
Immunologic memory
Maternal immune tolerance
T-lymphocytes

ABSTRACT

Pregnancy is an immune challenge to the maternal immune system. The effects of pregnancy on maternal immunity and particularly on memory T cells during and after pregnancy are not fully known. This observational study aims to show the short term and the long term effects of pregnancy on the constitution, size and activation status of peripheral human memory T-lymphocyte populations. Effector memory (EM) and central memory (CM) T-lymphocytes were analyzed using flow cytometry of peripheral blood from 14 nulligravid, 12 primigravid and 15 parous women that were on average 18 months postpartum. The short term effects were shown by the significantly higher CD4+ EM cell and activated CD4+ memory cell proportions in primigravid women compared to nulligravid women. The persistent effects found in this study were the significantly higher proportions of CD4+ EM, CD4+ CM and activated memory T cells in parous women compared to nulligravid women. In contrast to CD4+ cells, activation status of CD8+ memory cells did not differ between the groups. This study shows that pregnancy persistently affects the pre-pregnancy CD4+ memory cell pool in human peripheral blood. During pregnancy, CD4+ T-lymphocytes might differentiate into EM cells followed by persistent higher proportions of CD4+ CM and EM cells postpartum. The persistent effects of pregnancy on memory T cells found in this study support the hypothesis that memory T cells are generated during pregnancy and that these cells could be involved in the lower complication risks in multiparous pregnancies in humans.

1. Introduction

During pregnancy, the maternal immune system delicately regulates inflammation at the fetal-maternal interface and prevents rejection of the semi-allogeneic cells of the fetus at the fetal-maternal interface. The mechanisms responsible for these immune responses are only partly known. Immune cells, such as T regulatory lymphocytes (Treg cells), natural killer cells, macrophages and monocytes, have been shown to play an important role in the immune response during pregnancy (Guerin et al., 2011; Ishida et al., 2015; Luppi et al., 2002; Prins et al., 2009; Saito et al., 2007a,b; Veenstra van Nieuwenhoven et al., 2002; Yuan et al., 2009). Non-optimal regulation of the maternal immune responses during pregnancy is implicated in the pathophysiology of pregnancy disorders such as miscarriage, infertility and preeclampsia (Cudihy and Lee, 2009; Larsen et al., 2013; Prins et al., 2009; Saito et al., 2007a; Sibai et al., 2005; Zenclussen, 2013). The risk of immune mediated pregnancy complications seems to be lower in subsequent pregnancies from the same father (Dekker, 2002; Saftlas et al., 2003). As the maternal immune system is challenged during pregnancy, most likely memory cells will be generated, such as memory T cells, which might play an important role in immunological tolerance in (subsequent) pregnancies (Lo et al., 2000; Nelson, 2008).

Memory T cells are long living antigen experienced lymphocytes that have different migration patterns, enhanced functional activity and, depending on the T cell receptor expressed, some can be activated without need for accessory cell co-stimulation which allows them to respond quicker to antigens that they encountered before (Mueller et al., 2013). Antigen experienced T-lymphocytes express CD45RO which is therefore used as a marker to identify memory T cells (Sallusto et al., 2004). Within the CD45RO+ cell population, central memory (CM) and effector memory (EM) cells can be identified. CM cells express CCR7, a chemokine receptor that

Abbreviations: Treg cell, T regulatory cell; 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; mAb, monoclonal antibody; WBC, white blood cell.

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http://dx.doi.org/10.1016/j.jri.2016.11.004

enables them to enter secondary lymph nodes (Förster et al., 1999; Sallusto et al., 2004). These cells display reactive behavior, they are highly sensitive for antigen stimulation and effectively stimulate dendritic cells and B cells (Sallusto et al., 2004). After activation, CD69 is expressed on the cell surface, CM cells irreversibly lose their CCR7 expression and proliferate to EM cells (Sallusto et al., 2004; Ziegler et al., 1994). Next to this, CM cells are able to renew themselves and maintain a permanent population whereas EM cell populations decrease over time (Lanzavecchia and Sallusto, 2005). EM cells do not express CCR7, therefore they are able to migrate to infectious areas (Lanzavecchia and Sallusto, 2000). Immediately after activation through antigen, EM cells express CD69 and display effector cell like behavior by producing cytokines such as interferon gamma and interleukin 4 (Lanzavecchia and Sallusto, 2000; Ziegler et al., 1994).

During childhood and adulthood most, if not all, women had multiple immune responses towards pathogens and vaccines and therefore they have a memory T cell population (Jaigirdar and MacLeod, 2015). We hypothesize that pregnancy in itself as an immune challenge leads to activation, generation and expansion of the pre-pregnancy EM and CM cell pools and that pregnancy causes persistent higher levels of EM and CM cell subsets postpartum. These persistent alterations could be of use in a subsequent pregnancy by establishing more adequate immunity during pregnancy. Although several studies have reported on memory T cells during pregnancy, persistent alterations of memory T cell populations due to pregnancy has not been shown in humans yet (Gomez-Lopez et al., 2013; Kinder et al., 2014; Piper et al., 2007; Rosenblum et al., 2015, 2011; Rowe et al., 2012; Tilburgs et al., 2010). Therefore, this study aims to evaluate the short term and the persistent effects of pregnancy on effector memory T cell and central memory T cell populations, the size of these populations and their activation status.

2. Materials and methods

2.1. Patient details

Memory T cell subsets were analyzed in peripheral blood of 14 healthy non-pregnant women that had never been pregnant before (nulligravid), 12 healthy pregnant women (primigravid) (blood withdrawal between 27 and 34 weeks of pregnancy) and 15 non-pregnant women that had been pregnant (parous) and were at least 6 months postpartum. All women were between 20 and 40 years of age, had a body mass index (BMI) between 19 and 30, did not have symptoms of illness or fever, and did not smoke. Women with fertility disorders, intra uterine contraceptive devices or immune associated diseases were excluded. All primigravid and parous women gave birth at term and had uncomplicated pregnancies.

The parous women and the nulligravid women were recruited from staff members of the University Medical Center Groningen (UMCG), and blood was collected in the first eight days of their menstrual cycle. Pregnant women were recruited from the midwife practice of Groningen and the department of Obstetrics and Gynecology of the UMCG.

This study was approved by the Medical Ethical Committee of the UMCG (protocol number: NL46127.042.13). All women gave written informed consent

2.2. Flow cytometry

A monoclonal antibody (mAb) cocktail was used to identify memory T cell subsets (Table 1). Isotype controls were used at the same concentration as the primary mAb (Table 1).

Samples were processed within one hour after blood withdrawal. Firstly, a total white blood cell count was performed using Sysmex, PocH 100-i. Thereafter RBCs were lysed with ammonium chloride. Cells were incubated on ice for 10 min followed by centrifuging at 4 °C, and RBC lysis was repeated. After the cells were washed with FACS buffer (PBS and 2% FCS), they were counted using a coulter counter (Beckman Coulter). Cells were incubated in a 96-well plate with FACS buffer and 20% mouse serum (Sanquin, Amsterdam, The Netherlands) per 1,000,000 cells to reduce non-specific binding (Andersen et al., 2016). Cells were then centrifuged, supernatant was discarded and the mAb or isotype control cocktail was added. An unstained control was added to every row in the 96 well plate to control for possible contamination. No background signals were found in the unstained control wells. Cells were incubated on ice and were washed with FACS buffer afterwards. To preserve the staining, the cells were fixed using FACS fix solution (BD biosciences). Then, cells were resuspended in FACS buffer and analyzed with a FACSVerse™ flow cytometer (BD Biosciences) using BD FACS Suite™ software (BD Biosciences). Approximately 500,000 cells per sample were acquired for analysis.

Cells or UltraComp eBeads (eBiosciences) stained with a single mAb were used for setting compensation settings.

Data analysis was done with FlowJo v10 software (Fig. 1). T-lymphocytes were selected based on forward and side scatter plots and staining for CD3. Within the CD3+ cell population, CD4+ and CD8+ cells were distinguished. To identify memory T cells, gates were set within these populations based on CD45RO. Within the CD45RO positive population, CCR7 was used to distinguish CM and EM cells and CD69 to identify the activated cell proportion. Isotype controls and FMO controls were used to control for non-specific characteristics of the antibodies and to set gates. Absolute cell counts of the different populations were calculated with the dual platform approach using the total white blood cell counts performed directly after blood withdrawal and the proportions calculated with FlowJo (Barnett and Reilly, 2007).

2.3. Statistics

Data were analyzed with GraphPad Prism 6.0 h for mac OS X, (GraphPad Software, CA, USA), and IBM SPSS for Windows Version 20. Statistical outliers were excluded using the ROUT method (Motulsky et al., 2006). One-way ANOVA followed by Tukey's test for multiple comparisons was performed. To investigate the effect of parity and to control for the possible confounding effect of age, a linear regression analysis was performed. Differences were considered statistically significant if $p < 0.05$.

3. Results

3.1. Characteristics of donors

An overview of the characteristics of the women that participated in this study is displayed in Table 2. Parous women were on average 18 months postpartum. White blood cell counts and age differed significantly between the groups. No significant differences were found for any of the other characteristics.

Pregnancy affects the total memory T cell count and the proportion of memory T cells in the total T cell population, therefore absolute counts and proportions are displayed. The total white blood cell count is higher in primigravid women compared to both nulligravid ($p < 0.0001$) and parous women ($p < 0.0001$) (Table 2). Fig. 2A shows that the proportion of T-lymphocytes within the total white blood cell population differs between the groups. The T-lymphocyte proportion of all white blood cells is lower in primigravid women as compared with nulligravid ($p < 0.0001$)