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Peripheral Gamma Delta T cells secrete inflammatory cytokines in women with idiopathic recurrent pregnancy loss

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ABSTRACT

Background: Gamma delta (γδ) T cells are known to link innate and adaptive immunity. Decidual γδ T cells are known to provide immunotolerance by producing IL-10 and TGF-β. In recurrent pregnancy loss (RPL) females, the role of peripheral γδ T cells remain unstudied.
Objective: To investigate the different phenotypes of γδ T cells in the peripheral blood of women with idiopathic RPL and their possible involvement in RPL condition.
Methods: A total of 120 women were recruited for the study. Peripheral blood lymphocytes were isolated and they were stained with appropriate antibodies to determine the phenotype of γδ T cells and major cytokines produced by them in the blood using flow cytometry.
Results: We observed a significant decrease in the proportion of $CD3^+CD4^-CD8^-$γδ T cells ($p < 0.001$) and increase in the percentage of IFN-γ ($p < 0.05$) and IL-17 ($p < 0.001$) producing γδ T cells in RPL pregnant as compared to normal pregnant females.
Conclusion: Increase in IFN-γ and IL-17-producing $CD3^+$ $CD4^-CD8^-$ γδ T cells is associated with creating inflammatory cytokine milieu, thereby, may contribute towards pregnancy loss in RPL females.

1. Introduction

Recurrent pregnancy loss (RPL), a condition of two or more consecutive pregnancy losses before the 20th week of pregnancy, affects 1–3% of couples trying to conceive. About 50% among these cases are idiopathic; ie, the etiology is not known [1]. Although many causes have been proposed such as genetic, anatomic, endocrine, infectious and thrombophilic, but immune pathology can be determined as one of the potential etiology of RPL.

During pregnancy, a complex interplay of maternal and fetal factors are required for the occurrence of immunological interactions so that a successful pregnancy can be maintained throughout the period of gestation [2]. Upon cell infection or stress, one of the important component of the innate immune system that recognizes self and non-self antigens are the γδ T cells [3]. Γδ T cells are a group of unconventional T cells that recognizes antigens by expressing γδ T cell receptor. In humans and adult rodents, γδ T cells represents approximately 5–10% of total peripheral mononuclear cells [4] but are more abundant in the epithelial regions of skin, reproductive organs and intestine [5]. Most γδ T cells lack CD4 and CD8 surface markers [6]. A major difference between αβ T cells and γδ T cells is the lack of requirement of antigen processing and presentation by major histocompatibility (MHC) molecules for γδ T cells, thereby allowing more rapid response against their respective antigens [7].

γδ T cells act as early sensors of cellular stress and infection. Although γδ T cells constitute a small proportion of circulating and tissue associated T lymphocytes in the human immune system, they function in a very broad manner like pathogen clearance, inflammation, tissue homeostasis in response to tissue stress [8]. Szekerez-Bartho et al. have studied the role of γδ T cells in the peripheral blood of women with normal pregnancy and with recurrent abortions. They had also observed a significant increase in the ratio of activated $\gamma\delta TCR^+$ cells in normal pregnancies as compared to that of recurrent abortions [9,10].

Recently, it has been reported that on the basis of the microenvironment, γδ T cells can assume the features similar to that of Th1, Th2, Th17, regulatory T cells (T regs) and antigen presenting cells [11]. Like αβ T cells, γδ T cells have been reported to show Th1 (IFN-γ), Th2 (IL-4, IL-10) and Th17 (IL-17) and Treg type phenotype by producing cytokines, thus, play a major role in modulating the immune responses in case of host defense, inflammation and tumor immunity [12–15].

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Table 1
Characteristics of the patients recruited for the study. Data were expressed as mean ± SD.

	Healthy pregnant (n = 32) **(H/P)**	Healthy non-pregnant (n = 16) **(H/NP)**	RPL pregnant (n = 35) **(RPL/P)**	RPL non-pregnant (n = 37) **(RPL/NP)**
Age (yrs)	27.25 ± 4.02	28.95 ± 4.17	27.4 ± 4.7	26.65 ± 3.52
Gravidity	1.85 ± 0.87	–	4.45 ± 1.53	–
Parity	0.65 ± 0.74	1.8 ± 0.83	0.3 ± 0.57	0.45 ± 0.6
Sp. abortions	0	0	3.05 ± 1.19	3.75 ± 1.45
VDRL	NR	NR	NR	NR
HIV	−ve	−ve	−ve	−ve
HbSAg	−ve	−ve	−ve	−ve
aCL	−ve	−ve	−ve	−ve
Anti-β2GPI	−ve	−ve	−ve	−ve
LAC	−ve	−ve	−ve	−ve

Where VDRL: Venereal Disease Research Laboratory, HIV: human immunodeficiency virus, HbSAg: hepatitis B surface antigen, aCL: anti-cardiolipin antibody, β2GP1: Beta-2 Glycoprotein 1 Antibodies, LAC: Lupus Anticoagulant.

Also, it has been suggested that murine and human γδ T cells provide help to B cells, correlating positively with their production of IL-4, a Th2 cytokine [16,17]. Jensen et al have demonstrated that naive γδ T cells preferentially secrete IL-17, whereas antigen encountered T cells secrete IFN-γ [18].

Various studies have been done on the proportion and function of decidual γδ T cells and the types of cytokines they secrete in the decidua which decide for the survival of the fetus. Hence, the main aim of the study is to determine the proportion of different phenotypes of circulating γδ T cells in the idiopathic RPL females and their possible role in maintaining the cytokine environment favourable for fetus survival.

2. Materials and methods

2.1. Study population

A total of 120 subjects were recruited from the Department of Obstetrics & Gynecology, All India Institute of Medical Sciences, New Delhi, India. Seventy two women [35 pregnant (RPL/P) and 37 non-pregnant (RPL/NP) during recruitment] with a history of ≥2 recurrent miscarriage and with no genetic, endocrine, uterine or autoimmune abnormalities and without any other infections were included for the study. They have been categorised as RPL in this study. Forty eight age-matched healthy women with no previous history of miscarriage and without any complications or infections were considered as controls, both during pregnancy (H/P) and non-pregnancy (H/NP), for this study. The detailed characteristics of the patients are given in Table 1.

The study was approved by the institute ethics committee **(Ref. No. IEC/NP-410/2013 RP-01/2013)** and informed consent was obtained from each subject.

2.2. Sample collection & processing

Ten millilitre blood was collected from the patients and a part was used to isolate the serum for cytokine analysis and from the remaining blood, PBMCs were isolated by density gradient centrifugation using histopaque (density 1.077 g/ml) (Sigma–Aldrich, USA) as previously described [19]. Then these cells were stained for immunophenotyping using flow cytometry. Briefly, the cells were washed with FACS buffer (Phosphate saline buffer containing 2% Bovine Albumin Serum) and incubated with fluorochrome tagged-monoclonal antibodies specific for

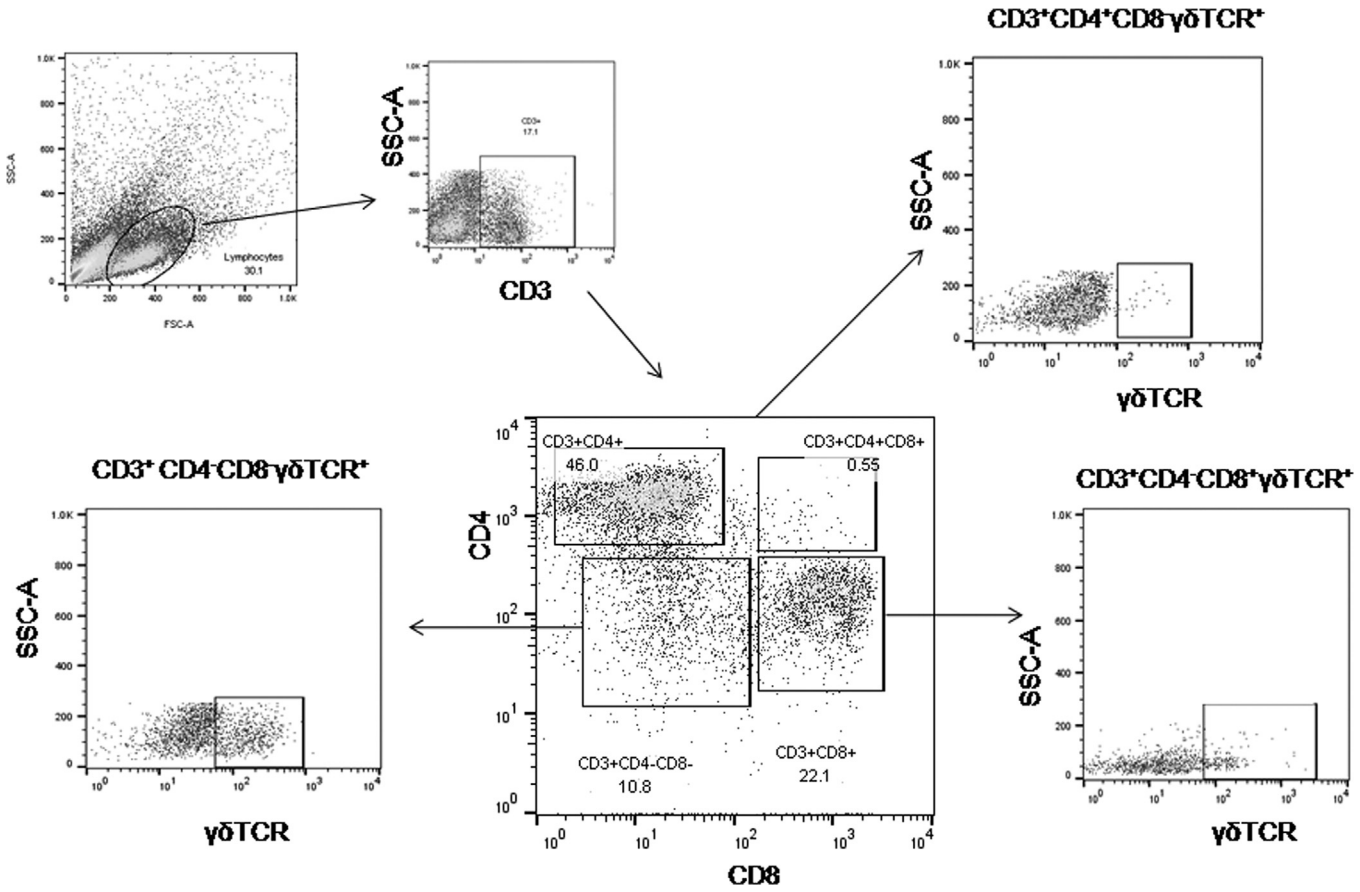

Fig. 1. Representative figure shows the gating strategy using flow cytometry for $CD3^+CD4^+$ γδ T cells, $CD3^+CD8^+$ γδ T cells and $CD3^+CD4^-CD8^-$ γδ T cells in the peripheral lymphocytes of healthy pregnant women.