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The immunomodulating effect of seminal plasma on T cells

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ABSTRACT

Seminal plasma (SP) contains immunomodulatory factors that may contribute to the formation of a tolerogenic environment at the embryo implantation site. The main focus of this study was to investigate the influence of SP on female T cells in the presence and absence of antigen-presenting cells (APCs) in an in vitro model. Female PBMCs and T cells were incubated with SP from seminal fluid samples of known and variable sperm quality. The immediate effect of SP on the mRNA expression of CD25, IL-10, IFN-γ, and Foxp3 was measured. Furthermore, proliferative responses, cytokine production, and CD25 expression were determined. Exposure to SP leads to increased mRNA expression of CD25, IL-10, and Foxp3 in T cells. Induction of mRNA for IL-10 and CD25 was dependent on the presence of APCs. Both PBMCs and T cells exposed to SP showed a proliferative response and produced several cytokines. The proliferative effects of SP on T cells observed were independent of sperm quality parameters, cytokines or soluble HLA molecules in SP. Furthermore, the presence of SP induced a higher expression of CD25 on the membrane of CD4+ T cells. SP has a direct immunomodulatory effect on T cells, as reflected in a proliferative response and upregulation of Foxp3. The presence of APCs is needed to induce IL-10 and CD25 upregulation in T cells exposed to SP. In conclusion, SP has both a direct and an indirect effect mediated through APCs on T cells.

1. Introduction

During pregnancy the maternal immune system has to tolerate the presence of the semi-allogeneic fetus. Allorecognition takes place at the site of embryo implantation, where trophoblast cells invade and are confronted with maternal PBMCs.

In mice, regulatory T cells (Tregs), usually implicated in the maintenance of tolerance to self-antigens (Sakaguchi, 2000), are present in the decidua and are responsible for maternal tolerance to fetal alloantigens (Aluvihare et al., 2004; Zhao et al., 2007). In humans, too, Tregs are increased in the decidua during pregnancy (Sasaki et al., 2004). In women with complicated pregnancies, decreased numbers of Tregs were found in the decidua (Yang et al., 2008) and placenta (Sasaki et al., 2007) suggesting that these Tregs might play a pivotal role in uncomplicated pregnancies.

Other studies in mice have shown that during copulation, long before implantation, maternal tolerance toward

Abbreviations: SP, seminal plasma; fPBMCs, female peripheral blood mononuclear cells; APCs, antigen-presenting cells; sHLA, soluble human leukocyte antigen; Tregs, regulatory T cells; IDO, indoleamine 2,3-dioxygenase; cpm, counts per minute; pg/ml, picogram per milliliter; ACTB, β-actin; VCM, volume × concentration × motility; DCs, dendritic cells.

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fetal alloantigens is induced (Moldenhauer et al., 2009; Zenclussen et al., 2005). In fact, alloantigens are present in human SP (Koelman et al., 2000; Politch et al., 2007) and may be responsible for the Treg expansion, as was previously shown in mice (Robertson et al., 2009). In addition, within two days of insemination, Tregs with an upregulation of Foxp3 expression can be found in the draining lymph nodes in mice (Aluvihare et al., 2004).

Dendritic cells (DCs), with a great presence in the decidua, are partly responsible for this antigen-specific Treg expansion (Moldenhauer et al., 2010). SP also contains a large variety of cytokines, which may modulate the maternal immune response (Baratelli et al., 2005; Sharkey et al., 2012a). TGFβ is highly present in human SP and is thought to inhibit a type 1 immune response against the semi-allogeneic fetus by initiating a type 2 or Treg-dominated immune response associated with partner-specific tolerance (Robertson et al., 2002). In addition, TGFβ elicits the expression of pro-inflammatory cytokines such as IL-6 and GM-CSF in human cervical epithelial cells (Sharkey et al., 2012a), which may also contribute to improved antigen presentation by DCs (Moldenhauer et al., 2010).

In humans, most SP is deposited at the cervix (Sobrero and Macleod, 1962), where it may affect the function of multiple cell types, including immune cells and the endometrium (Gutsche et al., 2003). Balandya et al. showed that exposure of human PBMCs to SP resulted in an increased intracellular expression of markers of Tregs and TGFβ (Balandya et al., 2012).

The aim of the present study was to study the immunomodulating effect of SP on human T cells. To investigate the direct effect of SP on T cells, T cells were enriched and isolated from female PBMCs (fPBMCs) and the effect of SP on the mRNA expression of CD25, IL-10, IFN-γ, and Foxp3 was measured. Furthermore, the proliferative response and cytokine production were measured. As a T cell response is often the result of the interaction between T cells and APCs, the possible role of APCs was studied as well.

2. Subjects and methods

2.1. SP samples

In all couples with infertility for at least 1 year visiting the reproductive medicine clinic at the Leiden University Medical Centre (LUMC), an exploratory study of fertility is performed, which includes determination of sperm quality (volume, concentration, motility, morphology, and viscosity). In this study sperm quality was defined as VCM: (volume × concentration × motility) × 10^6 (World Health Organization, 1999). Low quality was defined as a VCM below 10×10^6 and high quality as a VCM above 100×10^6. SP samples were collected via masturbation, and sperm quality was assessed the same day. All males were HIV-negative and asymptomatic for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. In addition, SP samples containing leukocytes, as a marker for infection, were excluded from this study.

Within 2 h of determination of the quality, samples were centrifuged at 2000 rpm for 10 min, the sperm was discarded and only SP was stored at −20° C. For the cultures, samples were thawed at room temperature and centrifuged at 14,000 rpm for 4 min.

2.2. Messenger RNA transcript analysis

Cultures were performed to demonstrate the effect of SP on fPBMCs or T cells (CD3+ fraction) either enriched by the depletion of non-T cells from fPBMCs according to magnetic cell sorting (Pan T Cell isolation Kit II, no. 130-091-156, MACS) or isolated. Briefly, in the procedure to isolate T cells, APCs were depleted from PBMCs. PBMCs were stained for CD14-FITC, CD19-FITC, CD40-FITC, CD56-FITC, CD36-FITC (Beckton Dickinson, Franklin Lakes, NJ, USA). FACS (FACS-Aria II with FACS-Diva software; Beckton Dickinson) sorted into a viable CD45+ population depleted of all FITC-stained cells and washed with culture medium containing RPMI 1640 with 10% human serum and L-glutamine. To confirm that this procedure does indeed lead to the depletion of APC fractions, they were stained with CD14-FITC, CD19-PE, CD3-PercCP, and CD45-APC (Beckton Dickinson). We added the autologous APC fractions to the isolated T cells.

The fPBMCs were selected from a panel of healthy HLA-typed volunteers, who, after informed consent, donated blood for transplantation and pregnancy-related research. SP used in these cultures was from unrelated men.

The fPBMCs enriched T cells, isolated T cells or isolated T cells with the addition of autologous APCs (500 μl of 2×10^6 per ml) were separately cultured with 500 μl of SP (1:100) or with culture medium (negative control) in round-bottom 24-well plates (Greiner Bio-one) for 1 day, and stored in 50 μl of RNA*later* (RNA stabilization buffer; Qiagen, Venlo, the Netherlands) at −20° C. RNA extraction was performed using NucleoSpin columns (Macherey-Nagel, Düren, Germany). To synthesize cDNA, RNA was combined with oligo dT (Invitrogen; 0.25 mg) and random nucleotide hexamers (Invitrogen; 0.25 mg), and incubated at 65 °C for 5 min (Eikmans et al., 2013). SuperScript III RT (200 U; Invitrogen), 0.5 mM dNTP, 40 U of RNAse OUT rRNAse inhibitor, and 5 mM DTT were added on ice. Reactions were allowed to proceed at 25 °C for 5 min and at 50 °C for 1 h. Reactions were terminated at 70 °C for 5 min. PCR assays were carried out using iQ™ SYBR® Green Supermix and a MyiQ Real-Time PCR detection system (Bio-Rad). The PCR program consisted of 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Primer pairs were selected in the coding sequence of the mRNA transcripts using Primer 3 (v. 0.4.0; SourceForge). Primers spanned at least one intron with a size of 800 bp or more. To ensure high specificity, primer sets were tested on control cDNA (from Human Reference Total RNA; Clontech, Mountain View, CA, USA) and genomic DNA. The expected size of the amplicons was checked on agarose gels. The efficiency of each PCR assay was between the 90 and 110%. A final melting curve analysis during the PCR runs was performed to check assay specificity.

The levels of mRNA transcripts for CD25, Foxp3, IL-10, IFN-γ were normalized to the geometric mean signal of reference genes GAPDH and β-actin. In five