Seminal plasma protects human spermatozoa and pathogenic yeasts from capture by dendritic cells

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INTRODUCTION

The female genitourinary tract has particular immunological requirements. Tolerance to allogenic spermatozoa and, after successful fertilization, the conceptus has to be given. At the same time, however, a protection of the host from invading microbial pathogens also appears to be important (Iijima et al., 2008). Although the upper female reproductive tract, comprising the endocervix, uterus and fallopian tubes, is considered to be sterile, the lower part with the vagina and ectocervix is highly populated by a complex commensal microbial flora (Quayle, 2002; Horne et al., 2008; Iijima et al., 2008). In addition, candidate invading micro-organisms can also be transmitted by the male ejaculate, an issue that appears to be important for both sexually transmitted infections and reproductive failure. In patients suffering from infertility, diverse bacterial and fungal species have been identified in the endocervix or in ejaculates. In the latter case, the most frequently detected species were Staphylococcus epidermidis (40%), Enterococci (30%), group A Streptococci (18%), group B Streptococci (18%) and several others. Yeasts were observed in the vaginal fornix in 10% of the patients, with Candida...
albicans accounting for 8% (Eggett-Kruse et al., 1992). The opportunistic pathogenic yeast C. albicans is a harmless commensal organism on mucosal surfaces of the gastrointestinal and genitourinary tracts in most healthy individuals. However, in the immunocompromised host, C. albicans can cause superficial as well as life-threatening disseminated infections (Odds, 1988). Furthermore, vulvovaginal mycoses by Candida species affect ~70–75% of all women at least once in a lifetime and, in most cases, are induced by C. albicans (Sobel, 2007).

Once spermatozoa or microbes enter the female reproductive tract, they are confronted with host cells of the immune system. Here, dendritic cells (DCs) and macrophages are classical antigen presenting cells of mucosal surfaces, being involved in the coordination of primary immune responses. DC populations are located in genital mucosae and submucosae, and in addition, monocyte-derived DCs and plasmacytoid DCs are recruited from peripheral blood to the vaginal mucosae upon infection or inflammation (reviewed in Iijima et al., 2006). In particular, DCs are present in stratified squamous epithelial layers of ectocervix and vagina, which also represent deposition loci for seminal plasma and sites of entry for a variety of sexually transmitted infectious agents (Pudney et al., 2005). DCs communicate directly and indirectly with different cells of the immune system, including other types of leukocytes such as T-cells, B-cells and natural killer (NK) cells by a release of signaling molecules. The role of DCs in promoting immunity or tolerance appears to depend upon their maturation state. Although they are found throughout the body surfaces as immature precursor cells, upon exposure to inflammatory stimuli, DCs lose their phagocytic ability and migrate to the draining lymph nodes. Here, they present antigens via major histocompatibility complex molecules to T-cells, and antigen-directed responses are generated. Given their immunomodulatory potential, DCs presumably represent suitable candidates to fulfill regulatory functions in the uterine environment during the process of fertilization and implantation.

The major task of semen is thought to act as a carrier of spermatozoa. Before one single sperm can fertilize the oocyte, a huge population of spermatozoa has to pass through almost the entire female reproductive tract. During this passage, a strong selection is taking place, thereby maximizing the chance that fertilization is only achieved by the most potent gametes. Important selecting factors include sperm characteristics, e.g. motility and morphology, but also adverse environmental stimuli such as an acidic pH of the lower reproductive tract. These cervical barriers are primarily directed to defend the host against invading microbial pathogens; however, a putative attack on spermatozoa is also possible. Therefore, semen characteristics, which may support spermatozoa to withstand this hostile environment, have been identified. For example, the relative neutral pH of seminal plasma (pH 6.7–8.0) has the potential to neutralize the acidic pH of the vaginal fluid. Moreover, there is increasing evidence that seminal plasma is not only a sperm carrier and survival medium but also has immunomodulatory potential, influencing the inflammatory host responses in the female genital tract. Seminal plasma contains estrogen, testosterone, various prostaglandins and other signaling substances, including cytokines and growth factors. These molecules are known to recruit and activate macrophages, granulocytes and DCs in the vagina, the cervix and the uterus (Robertson, 2005). Here, these cell populations presumably fulfill functions not only in host defense against invading pathogens, but also in the elimination of post-capacitated spermatozoa. In the equine host, for example, polymorphonuclear neutrophils have been observed to phagocytose non-viable spermatozoa, in contrast to viable sperm, in the presence of seminal plasma (Alghamdi et al., 2004; Troedsson et al., 2005).

The complex interactions between host, gametes and microbes have received only little attention at the molecular level. In the present work, we set out to integrate the assumed functions of DCs in terms of pathogen attack, sperm tolerance and the putative immune modulating properties of seminal plasma. We show that seminal plasma protects both spermatozoa and pathogenic C. albicans cells from association with DCs. In deciphering the molecular nature of this inhibitory effect of seminal plasma, we identified distinct prostaglandins. These results should therefore contribute to a better understanding of the complex interactions during both human reproduction and host defense.

**Materials and Methods**

**Human spermatozoa, C. albicans and culture conditions**

Human semen samples obtained by masturbation from normozoospermic healthy donors were provided along with informed consent and local ethics committee approval (study file number 62/08 from 18 April 2008, University of Würzburg, Germany, Faculty of Medicine), and analyzed according to WHO guidelines (WHO, 1999). Sperm motility was assessed microscopically by scoring the percentage of progressive motile (A + B), non-progressive (C) and immotile spermatozoa (D). Progressive motile sperm were recovered by a two-step pure sperm gradient (Nidacon, Möln达尔, Sweden) and resuspended in Sperm Preparation Medium (Orgin, Berlin, Germany). In some of the experiments, the isolated spermatozoa were pooled, and in other cases, they were derived from individual donors. The observed effects were consistent despite the identity of the donor or whether they were derived from a single donor or from a pool. Seminal plasma was obtained as the supernatant from ejaculate by centrifugation at 1500 g for 15 min, and aliquots were stored at −20°C. The hypoosmotic swelling test was used to immobilize spermatozoa. Briefly, the swelling test was performed by diluting 20 μl of sperm suspension (2 × 10^6 sperm) with 200 μl of hypoosmotic solution (7.35 g sodium citrate and 13.51 g fructose in 1 l of distilled water). After incubation for 30 min at 37°C with 5% CO2, spermatozoa were centrifuged for 5 min at 900g. The pellet was resuspended in 100 μl of Sperm Preparation Medium (Orgin). To induce the acrosome reaction, motile spermatozoa were incubated in 10 μM calcimycin (A23187, Sigma, Taufkirchen, Germany) in Sperm Preparation Medium for 30 min as described previously (Rennemeier et al., 2009). For cryopreservation, motile spermatozoa in Sperm Preparation Medium were incubated 1:1 in Sperm Freezing Medium (Orgin). The mixture was left for incubation at room temperature for 10 min. The semen was loaded in cryo-straws and sealed. The straws were suspended above the surface of liquid nitrogen for 45 min and finally transferred into liquid nitrogen and stored at −196°C. For thawing, cryopreserved spermatozoa were resuspended 1:2 in Sperm Preparation Medium, centrifuged for 5 min at 900g and resuspended in fresh Sperm Preparation Medium.

Candida albicans strain SC5314 (Gillum et al., 1984) was routinely propagated on yeast peptone dextrose (YPD) agar plates (20 g peptone, 10 g yeast extract, 20 g glucose and 15 g agar per liter) at 30°C, and stored as frozen stock in liquid YPD medium with 15% glycerol at −80°C. A potential influence of seminal plasma on the survival of C. albicans under
conditions used for DC phagocytosis assays was tested as follows. An overnight C. albicans preculture was grown at 30 °C in SD medium [20 g glucose and 6.7 g yeast nitrogen base with ammonium sulfate (MP Biomedicals, Solon, OH, USA) per liter]. Of this culture, 1 × 10^6 fungal cells were mixed with 0.5 or 5.0% seminal plasma in a final volume of 1 ml RPMI 1640 supplemented with 1% fetal bovine serum (FBS) and incubated at 37 °C for 1 h. Thereafter, the samples were appropriately diluted and spread on YPD agar plates. The plates were incubated at 30 °C for 48 h before the number of colony forming units (CFUs) was recorded. In order to monitor a potential effect of seminal plasma on C. albicans germ tube formation, the cultures in RPMI 1640 medium (supplemented with 1% FBS) in the absence or presence of seminal plasma were analyzed by microscopic inspection after incubation at 37 °C for 2 h.

Preparation of human DCs
Human monocyte-derived DCs were obtained from peripheral blood mononuclear cells by a standard protocol (Romani et al., 1994). In brief, after dilution with 50 ml phosphate-buffered saline (PBS)/0.1 M citrate, blood was distributed over a density-gradient (leukocyte separation medium, PAA Laboratories, Co¨lbe, Germany) and centrifuged at 400 g for 30 min at room temperature. Monocytes were collected from the interface and washed with PBS, then resuspended in RPMI 1640 medium (PAA Laboratories), supplemented with 10% FBS, 2 mM glutamine and 50 μg/ml gentamicin and incubated for 1 h at 37 °C and 5.7% CO2 on cell culture plastic dishes (Greiner, Frickenhausen, Germany). The non-adherent cells were removed after 1 h by repeated washing. The adherent fractions (monocytes) were cultured for 2–3 days in RPMI 1640 medium supplemented with 10% FBS, 2 mM glutamine, 50 μg/ml gentamicin, 800 μl granulocyte-macrophage (GM)-colony-stimulating factor (CSF) (Leukine, Bayer Health Care, Seattle, WA, USA) and 250 U/ml interleukin (IL-4) (Cellgrow, CellGenix, Freiburg, Germany). Cytokines and medium were replaced every second day. Purity of immature DCs was indicated by fluorescence-activated cell sorter (FACS) analysis using a human leukocyte antigen-D related (HLA-DR) antibody. Primary Abs used for the characterization of DCs were CD25, CD83, CD14, DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) and HLA-DR (eBioscience, San Diego, CA, USA). Non-specific binding of Abs was calculated by incubating DCs with the respective isotype controls. The Abs were conjugated with phycoerythrin (PE) or fluorescein isothiocyanate (FITC). Surface marker expression was quantified by flow cytometry using a FACS Calibur (BD Biosciences).

Quantification of spermatozoal and C. albicans association with human DCs by light microscopy
For our binding assays, DCs were seeded into 24-well culture plates (Greiner Bio-One) at a density of 1 × 10^5 cells/well. To perform light microscopy analysis, DCs were seeded on glass-coverslips (diameter: 12 mm). The attachment of DCs to the surface of the dishes occurred within 1 h at 37 °C under 5% CO2. The prepared DCs were incubated with 10 spermatozoa, 5 C. albicans cells or 50 fluorescent glass beads (diameter: 2 μm) per DC in RPMI 1640 supplemented with 1% FBS at 37 °C under 5% CO2. Coincubation experiments were started with a centrifugation step at 100g for 4 min. For inhibition experiments with seminal plasma, DCs were preincubated with 0.05–10% seminal plasma for 30 min and then washed three times with fresh RPMI 1640 medium supplemented with 1% FBS prior to the addition of spermatozoa, C. albicans cells or glass beads. In further inhibition assays, coincubation was carried out in the presence of fructose, glucose, mannosse (50 mM each), mannan (1 mg/ml), anti-DC-SIGN antibody (1:100) (R&D Systems, MAB1621) or different prostaglandins (1–12 μg/ml). The number of DC-associated sperm or C. albicans cells in binding assays was quantified by light microscopy. Briefly, non-adherent spermatozoa or C. albicans cells were removed by rinsing the DCs several times in PBS. For microscopy, DCs were washed and then fixed with 3.7% paraformaldehyde (AppliChem, Darmstadt, Germany). Light microscopy was performed with an Olympus IX 51 microscope equipped with an Octax Eye USB2 camera. The cells were observed with a × 40 achromat objective. The imaging software was Octax EyeWare Mx.

Visualization of DC cytoskeleton by immunofluorescent staining and microscopy
For the visualization of the cytoskeleton, the fixed samples were washed with PBS/10% FBS and permeabilized with 0.1% Triton-X-100 for 5 min. After several washings with PBS, DCs were incubated with a monoclonal phallolidin antibody coupled to Alexa 488 (green). Fluorescence microscopy was performed with a Zeiss LSM 510 inverted confocal laser scanning microscope equipped with a Zeiss Axiovert 100 microscope. Imaging scans were acquired with an argon laser with a wavelength of 488 nm and corresponding filter settings for FITC and parallel transmission images and the CLSM software. The cells were observed with a × 63 immersion oil objective. Each bar in the images represents 10 μm.

Flow cytometric analysis
For labeling with FITC, 1 × 10^5 sperm cells were incubated with FITC (10 μg/ml, Sigma) in 1 ml Sperm Preparation Medium for 30 min at 37 °C and 5% CO2. After incubation, spermatozoa were washed twice with the same medium and resuspended in appropriate medium for further experiments. To investigate the association of spermatozoa with DCs, 1 × 10^5 FITC-labeled spermatozoa were incubated for the indicated times with 1 × 10^5 DCs in a total volume of 500 μl of RPMI medium at 37 °C under 5% CO2. Cells were washed in PBS/0.5% FBS and FITC-positive DCs were measured by flow cytometry. DCs were detected using log forward- and log side-scatter dot plots and density plots. FACS analysis was performed by use of the FACSscan 2.0 cytometry system equipped with an argon laser emitting at 488 nm (Becton Dickinson). The fluorescence was measured on the FL1 fluorescence channel equipped with a 488-nm band-pass filter. Per sample, 10,000 cells were analyzed and counted at a low flow rate. Fluorescence data were collected by using logarithmic amplifiers, and forward scatter data were collected using linear amplifiers. FACS analysis detected apoptotic and necrotic DCs by the use of the annexin V-FITC kit (Bender Medsystems, Vienna, Austria). In brief, aliquots of 1 × 10^5 DCs were incubated with 0.05–5% seminal plasma or 1 × 10^5 spermatozoa for 3 and 24 h, respectively. After washing, the pellet was incubated with 195 μl of prediluted binding buffer and 5 μl of annexin V-FITC. The cells were incubated in the dark at room temperature for 10 min. The cell suspension was centrifuged at 900g for 5 min and resuspended in 190 μl of prediluted binding buffer with 10 μl propidium iodide. The cells were detected using log forward- and log side-scatter dot plots and density plots. Evaluation of the results was performed using WinMDI (Version 2.8). At a minimum, three independent experiments were performed.

Statistical analysis
All data were expressed as the mean ± SD. Differences were analyzed by the two-tailed unpaired Student’s t-test. In all analyses, a P-value of <0.05 was considered statistically significant.
Results

DCs interact with human spermatozoa

Given that spermatozoa are phagocytosed by macrophages in the female genital tract (Oren-Benaroya et al., 2007), we tested whether spermatozoa also interact with immature DCs. For these experiments, DCs were obtained from human monocytes cultured for 3–5 days in the presence of GM-CSF and IL-4. DCs (1 × 10⁶) were incubated for different time periods (0–24 h) with increasing numbers of FITC-labeled sperm cells (0.1 × 10⁶–5 × 10⁶). The results of the flow cytometric analysis demonstrated a strong time-dependent association of human spermatozoa with DCs (Fig. 1A). In addition, this interaction was dose-dependent and increased with higher numbers of spermatozoa. Although a large variability was observed between experiments carried out with DCs and/or sperm samples from different donors, the relative results were similar.

Since the susceptibility to macrophage phagocytosis was reported to be enhanced for post-capacitated spermatozoa compared with freshly isolated sperm (Oren-Benaroya et al., 2007), we analyzed whether capacitation or other sperm parameters influence the interaction of DCs with spermatozoa. We incubated spermatozoa for different time periods in Sperm Preparation Medium (for details, see Materials and Methods) that allows capacitation, thereby producing sperm populations of different ages. To monitor a potential influence of sperm motility on DC–sperm -interaction, another sperm population was immobilized by incubating in a hypoosmotic medium for 15 min. Sperm samples treated with calcimycin, a known inducer of the acrosome reaction, were used to uncover a potential effect of premature acrosomal exocytosis. Sperm samples that had been cryopreserved and thawed were also tested. Immature DCs were incubated for 120 min with the different aged or otherwise treated sperm populations and analyzed by flow cytometry for sperm-positive DCs. A high proportion of sperm-positive DCs was found in all of the tested sperm samples, and not in the case of post-capacitated (2 day old) spermatozoa only. The interaction of DCs with spermatozoa was therefore not significantly related to the tested sperm parameters, age, motility, acrosomal status or previous cryopreservation and thawing (Fig. 1B).

Seminal plasma inhibits the capture of spermatozoa by DCs

In order to detect a potential effect of seminal plasma on the interaction of spermatozoa with DCs, binding assays were carried out in the presence of different concentrations of human seminal plasma. Flow cytometric analysis showed a dose-dependent inhibition of the sperm–DC -association in the presence of seminal plasma, indicating the specificity of this interaction. A 50% reduction in spermatozoal adherence to DCs was observed in the presence of 1% seminal plasma (Fig. 2A). This result was confirmed by microscopy, where completely phagocytosed spermatozoa were detected already after 30 min of coincubation. Manual counting of DC-associated spermatozoa by microscopic inspection revealed that after 120 min the number of adherent/intracellular sperm cells per DC was 0.6 on average. In contrast, this number was reduced to 0.4–0.1 in the presence of different concentrations of seminal plasma (0.05–5%; Fig. 3B and C). To addressing the question of whether the inhibitory influence of seminal plasma on sperm–DC -association is related to sperm quality, we used seminal plasma of individual donors, characterized by different sperm qualities (for details, see Materials and Methods), for the binding experiments. FACS analysis attested a strong inhibitory effect on the binding of spermatozoa to DCs for all of the inspected seminal plasma samples. Only a slight difference in the degree of inhibition was observed, which however did not correlate with the quality of the different sperm samples (Fig. 2B). Therefore, the ability of seminal plasma to inhibit the association of spermatozoa with DCs appeared to be conserved.

Seminal plasma protects C. albicans cells from capture by DCs

Functioning in both innate and adaptive immunity, DCs are important in the recognition of pathogens and play a particular role in the
dose-dependent inhibition of this interaction in the presence of C. albicans analysis. Enumeration of DC-associated C. albicans cells indicated a dose-dependent inhibition of this interaction in the presence of seminal plasma (Fig. 3A and C). By contrast, treatment of DCs with seminal plasma had no significant effect on their interaction with fluorescent glass beads (Fig. 3B and C). Therefore, it is possible that the receptors on DCs which contribute to the interaction with glass beads are different to those interacting with spermatozoa and/or C. albicans. The presence of seminal plasma had no effect on the survival of C. albicans or the ability of the fungus to form germ tubes under the tested conditions (Fig. 3D and E). In summary, these findings suggest that seminal plasma components display major immunomodulatory functions on DCs, which are likely exploitable by microbial pathogens.

**Seminal plasma influences the morphology of DCs**

The observed inhibitory effect of seminal plasma on the attachment and phagocytosis of spermatozoa by DCs coincided with characteristic morphological alterations of the DC population. Phalloidin staining of the actin cytoskeleton and light microscopy revealed an altered DC morphology already in the presence of low concentrations of seminal plasma (1%). Untreated immature DCs formed numerous short pseudopodia, whereas seminal plasma treated cells appeared globular (Fig. 4A). Previous studies have shown that seminal plasma exerts cytotoxic effects on human lymphocytes and human NK cells in long-term cultures (Rees et al., 1986; Vallely et al., 1988). Therefore, viability assays were carried out to test the possibility that the observed morphological changes are due to seminal plasma induced cytotoxic effects. After DCs were incubated for 3 and 24 h in the presence of seminal plasma, respectively, an assay was applied for the detection of apoptotic and/or necrotic cells, i.e. apoptotic DCs were visualized via binding of annexin V-FITC to externalized phosphatidylserine, whereas necrotic cells were detected with propidium iodide. As demonstrated by flow cytometry, adverse effects of seminal plasma at the tested concentrations were not detected after 3 h. A slight decrease of viable DCs was only observed after 24 h at the concentration of 5% seminal plasma (Fig. 4B). From these results, we concluded that no significant cytotoxic effects of seminal plasma on DCs were exerted in our DC sperm or DC C. albicans inhibition assays, which were conducted within time periods of 120 min.

**Seminal plasma induces maturation of DCs**

Although a cytotoxic effect of seminal plasma on DCs under the tested conditions was excluded, the observed morphological alteration of immature DCs in the presence of seminal plasma potentially coincided with other cellular modulations. To address this possibility, we monitored the expression of characteristic surface markers on the DCs in response to seminal plasma. Immature DCs, which are negative for CD83 and positive for CD14, were incubated with 1 and 5% seminal plasma, respectively, or in the presence of 1 x 10^6 sperm cells for 24 h. Expression levels of the maturation-associated surface markers CD83 and CD25 were monitored by flow cytometry. As a result, CD83 and CD25 expression was proved to be significantly up-regulated in the presence of 1 or 5% seminal plasma, but not as strongly when compared with the positive control, which contained a maturation cocktail (1000 U/ml IL-1β, 1000 U/ml tumor necrosis factor-α (TNF-α), 1000 U/ml IL-6, 10^-8 mol/l PGE_2). Incubation of

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**Figure 2** Seminal plasma inhibits the association of spermatozoa with DCs. (A) 1 x 10^5 DCs were incubated with various final concentrations (0; 0.05; 0.5; 1; 5 or 10%) of human seminal plasma and 1 x 10^6 FITC-labeled spermatozoa for 120 min. DCs were washed in PBS and binding of FITC-labeled spermatozoa to DCs was analyzed by flow cytometry. The percentage of FITC-positive DCs incubated with spermatozoa without seminal plasma was set to 100%. The results are the means ± SD from three independent experiments; the symbol ‘*’ indicates that the detected differences were significant (P < 0.05). (B) 1 x 10^5 DCs were incubated with 1 x 10^6 FITC-labeled spermatozoa for 120 min in the presence of 1% seminal plasma from individual donors with different sperm qualities (N, normozoospermia; T, teratozoospermia; A, astheno-zoospermia; O, oligozoospermia; Azoo, azoospermia; Cry, cryptozoospermia; OAT, oligoasthenoteratozoospermia in severity levels I–III). After washing, the interaction of FITC-labeled spermatozoa with DCs was analyzed by flow cytometry. The percentage of FITC-positive DCs incubated with spermatozoa with the absence of seminal plasma was set to 100%. The results are the means ± SD from three independent experiments.

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defense against fungi (Buentke and Scheynius, 2003; De Bernardis et al., 2006). Since the association of spermatozoa with DCs was inhibited by seminal plasma, we investigated that whether a similar effect could also protect C. albicans cells from DC action. Treatment of DCs with seminal plasma (0.5 and 5%) strongly inhibited the adherence of C. albicans cells to DCs as determined by light microscopy analysis. Enumeration of DC-associated C. albicans cells indicated a
Figure 3 (A) $1 \times 10^5$ DCs were seeded on glass-coverslips and, after attachment, incubated with $1 \times 10^5$ C. albicans cells for 0.5 or 1 h at 37°C (for details, see Materials and Methods). These coincubations were conducted in the absence or presence of different concentrations of seminal plasma (0.5 and 5%). Glass-coverslips with DCs were washed and fixed before the association of C. albicans cells to DCs was determined by light microscopy. The results are the means ± SD from three independent experiments. (B) Association of $1 \times 10^6$ spermatozoa or $5 \times 10^6$ glass beads (diameter: 2 μm) to DCs in the presence of seminal plasma after 120 min of coincubation was determined by light microscopy. The results are the means ± SD from three independent experiments; the symbol * indicates that the detected differences were significant ($P < 0.05$). (C) Light microscopy of associated C. albicans cells, spermatozoa and glass beads to DCs in the presence of seminal plasma after 30 or 120 min of incubation. A representative experiment is shown (scale bar: 10 μm). (D) and (E) Seminal plasma (0.5 or 5%) displays no detectable effect on the survival or germ tube formation of C. albicans. $1 \times 10^5$ C. albicans cells were mixed with 0.5 or 5% seminal plasma and incubated at 37°C for 1 h. The CFUs were determined by plating appropriate dilutions on YPD agar plates, incubated for 48 h before counting. The results are the means ± SD from three independent experiments. Germ tube formation of C. albicans was assayed under the same conditions except that the samples were incubated for 2 h (scale bar: 10 μm).
DCs with spermatozoa alone did not result in increasing levels of the maturation markers (Fig. 5). These data suggested that the seminal plasma induced changes in the morphology of DCs and the concomitant loss of their phagocytic capacity might be, to some extent, associated with an altered DC maturation.

The DC-specific adhesion receptor DC-SIGN contributes to DC–sperm -interaction

The DC-SIGN is known to function as a universal pathogen receptor (Geijtenbeek and van Kooyk, 2003), which interacts with glycostructures on the surface of Mycobacterium spp., Helicobacter pylori and C. albicans (Cambi et al., 2008; Geurtsen et al., 2009). In addition, a recent study demonstrated that DC-SIGN plays an important role in the attachment of HIV-1 to immature DCs, an interaction that, interestingly, was inhibited by the addition of seminal plasma (Sabatte et al., 2007). To determine whether the attachment of spermatozoa to human DCs is mediated via the DC-SIGN receptor, 1 mg/ml mannan or anti-DC-SIGN (1:100) antibodies were used in our inhibition assays (Geijtenbeek et al., 2000). The presence of mannan or anti-DC-SIGN antibodies resulted in a slight but significant reduction of DC-positive spermatozoa (Fig. 6A). The effect was not as strong as the inhibitory effect of 1% seminal plasma, suggesting that additional, yet unknown receptors may be responsible for the attachment of spermatozoa to DCs.

Characterization of the antiphagocytic activity of human seminal plasma

The nature of the inhibitory activity of seminal plasma on the sperm–DC -binding remained enigmatic. Therefore, different parameters were addressed in the following experiments to narrow down the chemical composition of the putative effector molecule(s). As a first step, we tested whether the inhibitory seminal plasma component is heat-stable. Heat pretreatment (95°C, 10 min) of seminal plasma did not detectably abolish its capacity to inhibit the spermatozoal adherence to DCs when compared with untreated seminal plasma (Fig. 6B). According to previous studies, human seminal plasma contains, among others, albumin and a complex mixture of glycopeptides with different proportions of carbohydrates (Lopes et al., 1998). To determine whether such carbohydrates can block the interaction between DCs and spermatozoa, adherence assays in the presence of 0.1 and 5% human serum albumin (HSA), 50 mM fructose, glucose or mannose were performed. These experiments revealed that neither albumin nor the tested sugars retained a significant inhibitory effect on the analyzed phenotype (Fig. 6B and C).

Seminal plasma prostaglandins inhibit the association of C. albicans and spermatozoa with DCs

Seminal plasma is rich in prostaglandins, and the levels of these messenger molecules are several fold higher in semen than elsewhere in the human body. The comparatively high concentrations of prostaglandins in normal semen were previously quantified: 19-OH-PGE (267 μg/ml), PGE1 and PGE2 (73 μg/ml) and small amounts of PGF (2 μg/ml) were found to be the major prostaglandins in seminal plasma (Templeton et al., 1978). To determine a potential contribution of prostaglandins to the inhibitory effect of seminal plasma on DC sperm or DC – C. albicans interaction, blocking experiments with 8 μg/ml of 19-OH-PGE1, PGE1, PGE2 or PGF1α were carried out. The results showed a significant inhibition of C. albicans and sperm cell adherence to DCs in the presence of 19-OH-PGE1, PGE1 and PGE2, which are present in particularly high concentrations in human seminal plasma. In contrast, PGF1α, which is found in lower concentrations in seminal plasma, displayed only a slight inhibitory effect (Fig. 7A and B). The effect of PGE1 was dose-dependent (Fig. 7C and D), indicating the specificity of prostaglandin interaction with DCs. Further, we discovered that 6 μg/ml 19-OH-PGE1 or PGE1 induce similar drastic morphological changes in DCs as observed before with seminal plasma (Fig. 7E). However, DC incubation in the presence of PGE1 and subsequent analysis of the above-mentioned

**Figure 4** Seminal plasma induces phenotypic changes of human DCs. (A) Fluorescence microscopy of DCs in the absence of (0%) or after treatment with 1% seminal plasma (sp) for 1 h. After fixation with 3.7% paraformaldehyde (PFA), the DC actin cytoskeleton was stained for 1 h with phalloidin-FITC (1:30 in 1% bovine serum albumin/PBS). Representative images are shown (scale bar: 10 μm). (B) 1 × 10⁵ DCs were treated for 3 and 24 h, respectively, with increasing concentrations of seminal plasma (0; 0.05; 0.5 or 5%). Viable, apoptotic and necrotic DCs were quantified by flow cytometry using the annexin V-FITC/ PI assay, and the results of a representative experiment are shown.
surface markers showed that the seminal plasma induced DC maturation is not attributable to this prostaglandin (data not shown). In conclusion, these data demonstrated that prostaglandins 19-OH-PGE1, PGE1 and PGE2 from human seminal plasma are likely to be the major factors responsible for the inhibition of capture of spermatozoa and C. albicans by human DCs.

**Discussion**

DCs are likely among the first classical antigen-presenting cells that encounter spermatozoa as well as pathogens upon entry into the female reproductive tract. Therefore, a fine-tuned balance between antigen tolerance and elimination is required, a tightrope walk that may be influenced by multiple stimuli. Here we show that spermatozoa are captured by human DCs, a process that, however, was found to be inhibited by the presence of small concentrations of human seminal plasma. This inhibitory effect was attributed to specific seminal plasma prostaglandins, is accompanied by enhanced DC maturation, and is exploitable by pathogenic yeasts. Therefore, the presence of seminal plasma during fertilization may impact not only reproduction but also host–microbe interactions.

Human macrophages have previously been reported to phagocytose preferentially post-capacitated spermatozoa (Oren-Benaroya et al., 2007). In addition, phagocytosis of spermatozoa in the female genital tract was found to be carried out also by vaginal epithelial cells, neutrophils and isthmic epithelial cells (Eisenbach, 2003). The molecular mechanisms underlying these interactions have not been intensely investigated so far. Yet, a role of specific markers on the sperm surface, which are associated with the capacitation process, was suggested to be involved in sperm phagocytosis. In the present work, we report that another population of uterine immune cells, human DCs, showed a strong association with capacitated spermatozoa. Moreover, the DC–sperm interaction was also evident in experiments with freshly ejaculated spermatozoa, and was not significantly related to sperm motility, acrosomal status or preceding cryopreservation. These findings support the idea that even fertile sperm are readily

**Figure 5** Surface marker expression by DCs incubated with seminal plasma. (A) and (B) $1 \times 10^5$ DCs were incubated with maturation cocktail (final concentration: 1000 U/ml IL-1β, 1000 U/ml TNF-α, 1000 U/ml IL-6, 10⁻⁹ mol/l PGE2), 5 or 1% of seminal plasma, or $1 \times 10^6$ sperm cells for 24 h before CD25 and CD83 surface marker expression was measured by flow cytometry with antibodies CD25-PE (2 µg/20 µl) and CD83-FITC (2 µg/200 µl), respectively. The fluorescence values were monitored after gating the main population of events and subtracting the corresponding isotype controls. For the flow cytometric analysis, the means ± SD of three independent experiments are shown. (C) Representative histograms are presented (isotype controls are shown as bold black curves).
Eliminated and that effective mechanisms have to shelter spermatozoa in the female reproductive tract from phagocytosis by uterine DCs. A potential protective role of seminal plasma in this scenario is supported by the present results.

Seminal plasma has widely been supposed to support fertilization and implantation by various potential mechanisms (Robertson et al., 2006). Individual molecules of seminal fluid are thought to approach the endometrium after sexual intercourse and likely exhibit fertilization-related biological properties in these tissues. In this context, experimental insights suggested that seminal plasma may play a role in implantation events as its components were shown to be responsible for a post-mating inflammatory response in mice and the infiltration of stromal tissues by macrophages, DCs and granulocytes (Tremellen et al., 1998). Further studies demonstrated improved live birth rates in couples undergoing IVF/ICSI treatment when patients engage in intercourse around the time of embryo transfer (Tremellen et al., 2000). Published reports on the immunological effects of seminal plasma in vivo are scarce, but clinical evidence was provided by von Wolff et al. (2009) who deposited seminal plasma into the cervix after follicle aspiration for IVF/ICSI treatment. Herewith, the authors found an increased clinical pregnancy rate of 37.3% in comparison with 25.7% of the placebo group. This finding and other studies support the concept that seminal plasma provides the female with paternally derived antigens, thereby modulating the female immune system to better tolerate the implantation of the embryo. However, possible detrimental effects of seminal fluid on reproduction were also described, showing that direct application of seminal plasma to endometrial cells stimulated the proliferation of endometrial cells.

**Figure 6** Blocking of spermatozoal binding to DCs. (A) Binding of anti-DC-SIGN antibody to human DCs affects the association of spermatozoa to DCs. Association of spermatozoa to DCs was investigated in the presence of anti-DC-SIGN antibody (1:100), the isotype control (1:100) or 1 mg/ml mannan. The numbers of associated spermatozoa was determined by light microscopy and the number of DC-associated spermatozoa without further pre-incubation was set to 100%. Results represent the means ± SD of three independent experiments (ctrl, control; Ab, antibody). (B) Association of sperm cells (1 × 10⁶) to DCs (1 × 10⁵) was investigated in the presence of 0.1 or 5% seminal plasma (sp), heat-inactivated seminal plasma or HSA. After incubation, the cells were washed in PBS, fixed in 3.7% paraformaldehyde and the number of associated spermatozoa was monitored by light microscopy. The percentage of the number of spermatozoa binding to DCs without seminal plasma was set to 100%. The results are the means ± SD from three independent experiments. (C) Adherence assays of sperm cells (1 × 10⁶) with DCs (1 × 10⁵) were conducted in the presence of 50 mM fructose, glucose or mannose, or 0.5% HSA. After incubation, the cells were washed in PBS, fixed in 3.7% paraformaldehyde and the number of associated spermatozoa was monitored by light microscopy. The percentage of the number of spermatozoa binding to DCs in the absence of seminal plasma was set to 100%. The results are the means ± SD from three independent experiments; the symbol ‘*’ (A–C) indicates that the detected differences were significant (P < 0.05).
and endometriotic cells in women with endometriosis (Khan et al., 2010).

The presence of seminal plasma in our binding assays displayed a strong influence on the interaction of spermatozoa with DCs as well as on the DC phenotype per se. Our studies revealed that seminal plasma efficiently inhibits the binding of spermatozoa to DCs even at very low concentrations. These results raised the question whether such dramatic effects are related to sperm donor and
quality. Therefore, seminal plasma samples of donors with normal and reduced sperm parameters were compared for their influence on the interaction of spermatozoa with DCs. However, no differences were detected, indicating that the effect likely represents a basic common property of seminal plasma. Other immunomodulatory effects of seminal plasma have also been found to be unrelated to sperm quality, such as the stimulatory effect of seminal plasma on the recruitment of immune cells into the cervix (von Wolff et al., 2007). Likewise, the function of seminal plasma in the physiology of reproduction does not seem to be dependent on the number of spermatozoa within a given sample. It has to be noted that in vivo secretions of seminal plasma can be differentiated in fractions originating from various glands associated with the male reproductive tract, including epididymis, seminal vesicles, prostate and bulbourethral glands. Seminal plasma used during in vitro experiments, however, is commonly derived from the preparation of entire ejaculate according to standard laboratory procedures, which do not allow a differentiation between various secretions. Therefore, unraveling the in vivo role of seminal plasma may also require further in-depth analyses of seminal plasma fractions and the identification of individual factors therein.

A detailed identification of immunosuppressive and/or other effector molecules in seminal plasma has been of particular interest; yet its analysis has been hampered by its complex composition. Proteomic analysis detected >900 unique proteins and the average overall protein concentration in seminal fluid was determined to be between 35 and 55 g l\(^{-1}\) (Pilch and Mann, 2006). The immunosuppressive effects of seminal plasma have been attributed to diverse components, e.g. zinc, polyamines, lactoferrin, \(\beta\)-2-microglobulin and prostaglandins, all of which are present in seminal plasma at high concentrations (James and Hargreave, 1984; Quayle et al., 1989). A direct sperm-protecting effect of porcine seminal plasma was recently attributed to seminal plasma factor H, which was suggested to protect spermatozoa from complement attack (Sakaue et al., 2010). Heating human seminal plasma to 100°C prior to its addition to our binding assays did not reduce its inhibitory activity, already suggesting that the protein fraction of seminal plasma was unlikely to be responsible for the inhibition of the described interaction. Hence, we have focused our analysis on other putative effector molecules. Seminal plasma components also include a variety of carbohydrates, including fructose, mannose and glucose, however, in our experiments none of the tested carbohydrates inhibited the sperm–DC -interaction. In contrast, our results suggest that the inhibitory effect of seminal plasma on DC sperm or DC C. albicans interaction is mediated by specific seminal plasma prostaglandins. Immunosuppressive properties of these lipid mediators have widely been reported before in a broad range of research areas (Boyce, 2008; Legler et al., 2010). In the field of human reproduction, a role of seminal plasma prostaglandins was already linked to the putative prevention of sensitization of the female to allogeneic spermatozoa after coitus (James and Hargreave, 1984). In addition, seminal plasma prostaglandins PGE\(_1\) and PGE\(_2\) and 19-OH-PGE\(_1\) were proposed to contribute to seminal plasma induced suppression of T-cell responses and NK-cell-mediated cytolysis (Tarter et al., 1986; Valley et al., 1988). An interest in a potential role of seminal plasma in host–microbe interaction has also been raised in the past, yet little is known on the underlying processes on the molecular level. In this context, an inhibition of antibody/complement-mediated killing of Neisseria gonorrhoeae by seminal plasma was observed, as well as a reduced association of these bacteria with polymorphonuclear cells (Brooks et al., 1981). Whether the latter finding is also attributable to specific seminal plasma prostaglandins will be of particular interest in future studies. A possible correlation might be feasible, supported by another early report, which suggested that the suppression of macrophage phagocytosis of Listeria monocytogenes by spleen culture supernatant was potentially mediated by PGE\(_2\) (Hutchison and Myers, 1987).

Our results suggest a prominent effect of seminal plasma on DCs, rather than on sperm or C. albicans cells. Nevertheless, this effect on DCs appears to be related to interactions with the specific binding partners, indicated by the observation that the attachment/phagocytosis of glass beads was only slightly blocked in the presence of seminal plasma under the tested conditions. This finding also supports the idea that specific cellular elements mediating binding between sperm or fungal cells to DCs are of interest in terms of gamete and pathogen recognition, respectively. The microbial cell wall, which is mostly composed of different glycoproteins embedded within a polysaccharide matrix, likely plays an important role in this cellular interaction. Previous findings demonstrate that DCs bind and internalize C. albicans through the interaction of fungal cell wall poly saccharide mannann and the DC-specific adhesion molecule DC-SIGN (Cambi et al., 2008). Further studies unraveled that the capture of human immunodeficiency virus type 1 (HIV-1) by DCs was also mediated by this C-type lectin and high-mannose glycoproteins on the envelope of the virus, an interaction that, interestingly, was shown to be markedly inhibited in the presence of small amounts of seminal plasma (Sabatte et al., 2007). In contrast to these findings, however, our results reveal that spermatozoal binding to DCs was significantly, but only slightly dependent on DC-SIGN, suggesting additional mechanisms or interacting molecules, respectively. Potential candidates for future research include, for example, yeast and/or sperm-surface-associated carbohydrates. Sperm surface components have also been investigated in terms of transmission of HIV-1, revealing that glycocalyx glycoproteins on human spermatozoa are critical for the virus–sperm interaction (Ceballos et al., 2009). Whether transmission of C. albicans or commensal and/or pathogenic growth of the fungus during vaginal Candida infections is directly influenced by seminal plasma will be of particular clinical interest. At least DC action appears to be of major importance for host defense against fungal pathogens at mucosal surfaces of the vagina, a preferred site of C. albicans infections (De Bernardis et al., 2006; LeBlanc et al., 2006; Cambi et al., 2008). In addition, Candida may be transmitted by the male ejaculate, and in comparison with many bacteria, the fungal cells exhibit increased resistance to the antimicrobial activity of seminal plasma (Mardh and Colleen, 1975; Horowitz et al., 1987). Supporting the latter observation, in our experiments, C. albicans survival or growth was not affected by the presence of the tested seminal plasma concentrations.

Incubation of DCs with small amounts of seminal plasma resulted in an altered phenotype of the immune cells, which was demonstrated by microscopic inspection of the morphology and by FACS analysis of the expressed surface receptors. Seminal plasma functioned as a stimulus for the maturation of DCs, which may be related to immunomodulatory components therein. Seminal plasma contains cytokines, growth factors, prostaglandines and hormones, and was also shown
to induce the production of GM-CSF in female uterine epithelium (Robertson, 2005). Specific prostaglandins, e.g. PGE1, displayed a similar antiphagocytic effect on DCs as seminal plasma; yet, this molecule was not proved—in our experiments—to induce comparable DC maturation.

In conclusion, our results bring together aspects of host–microbe interaction and human reproduction, by a common shared mechanism, i.e. the blockage of sperm or C. albicans phagocytosis by DCs in the presence of seminal plasma or specific components thereof. The seminal plasma mediated prevention of phagocytosis by DCs may be of particular importance for the survival of spermatozoa in the female reproductive tract and hence for successful reproduction. At the same time, such antiphagocytic effects may help us to avoid that the female becomes sensitized by repeated exposure to given sperm antigens during coitus, which would imply an adverse effect on the fertility of couples. On a pathogenic view, however, such inhibitory effects on phagocyte function may, at the same time, evolve negative consequences on host defense mechanisms against infectious agents. Fulfilling both these major tasks simultaneously is likely one of the most fundamental challenges in human reproduction, and a better understanding of the underlying regulatory immune functions will be of major future interest.

**Authors’ roles**

C.R. and P.S. conceived the project, designed experiments, performed experiments, analyzed data and drafted the manuscript; M.S. and T.F. analyzed the data and obtained reagents and materials; U.K., J.M. and J.D. analyzed the data and participated in critical discussion; U.L. and C.A. performed experiments; all authors critically revised and approved the manuscript.

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