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Seminal plasma components stimulate interleukin-8 and interleukin-10 release

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Human seminal plasma has potent anti-inflammatory properties which are thought to confer a survival advantage to the spermatozoa within the hostile female genital tract. In contrast, a profound pro-inflammatory leukocytosis has been observed post-coitus in animals and humans. Whether components of seminal plasma are involved in initiating this leukocytic reaction is not known. This study investigated the effect of human seminal plasma, a seminal plasma fraction and its principal constituent prostaglandins, prostaglandin E_2 (PGE_2) and 19-hydroxy PGE, on the release of the pro-inflammatory neutrophil chemotactic factor interleukin-8 (IL-8) and the anti-inflammatory cytokines interleukin-10 (IL-10) and secretory leukocyte protease inhibitor (SLPI). The tissues studied were non-pregnant cervical explants, peripheral blood and the monocyte cell line U937. Seminal plasma fraction (SPF) significantly ($P < 0.05$) stimulated release of IL-8 and inhibited release of SLPI from non-pregnant cervical explants. SPF, PGE_2 and 19-hydroxy PGE significantly ($P < 0.005$) stimulated IL-8 release from peripheral blood and U937 cells. 19-hydroxy PGE was significantly ($P < 0.005$) more effective than PGE_2 in stimulating IL-8 release. Seminal plasma, SPF and PGE_2 significantly ($P < 0.05$) stimulated IL-10 release from U937 cells. 19-hydroxy PGE stimulated IL-10 release from U937 cells but this failed to reach significance. Release of IL-10 by cervical explants and SLPI by peripheral blood and U937 cells were below the detection limit of the assays employed. We suggest that the anti- and pro-inflammatory immune responses which seminal plasma induces might act in combination initially to promote sperm survival and then to facilitate their removal from the female genital tract.

Key words: human seminal plasma/19-hydroxprostaglandin/interleukins/prostaglandin E_2/secretory leukocyte protease inhibitor

Introduction

Human seminal plasma has powerful immunosuppressive properties containing high concentrations of the soluble p55 tumour necrosis factor-α (TNF-α) receptor (Liabakk *et al*., 1993), receptors for the Fc portion of γ-globulin, transforming growth factorβ (TGFβ) (Nocera and Chu, 1993), spermine (Evans *et al*., 1995) and complement inhibitors (Kelly, 1995). However, it is the prostaglandins prostaglandin E_2 (PGE_2) and 19-hydroxyprostaglandin E (19-hydroxy PGE), present in mM concentrations within semen (Taylor and Kelly, 1974; Templeton *et al*., 1978) which are thought to be the principal effectors of the immunosuppression. They are potent stimulators of cAMP, thus inhibiting lymphocyte proliferation and natural killer cell activity, and are likely to modify cytokine release from antigen presenting cells (Kelly, 1995). In addition, human seminal plasma and its component prostaglandins stimulate release of the immunosuppressive T-helper-2 (Th-2) cytokine interleukin-10 (IL-10) and inhibit the release of the Th-1 cytokine interleukin-12 (IL-12) from lipopolysaccharide-stimulated whole blood (Kelly *et al*., 1997). The effect of PGE_2 and the subsequent cytokine shift would be to induce an anergic response in T-cells (Mannie *et al*., 1995; Groux *et al*., 1996) and may therefore have implications for non-recognition of both sperm antigen and viral pathogen.

In contrast, coitus and ejaculation of semen deposits millions of potentially pro-inflammatory immunogenic spermatozoa into the vagina in vaginal inseminators, such as humans and into the uterus in intra-uterine inseminators, such as the horse. Since in most animals only a handful of spermatozoa are required for fertilization, then the majority of spermatozoa are rendered redundant and need to be removed from the reproductive tract. How this is achieved is not well understood but there is good evidence in horses (Kotilainen *et al*., 1994), rabbits (Tyler, 1977), goats (Mattner, 1968), cattle (Howe and Black, 1963) and mouse (De *et al*., 1991; Parr and Parr, 1991) that a profound pro-inflammatory leukocytosis develops within the uterus and cervix post-coitus. The invading cells, which are predominately neutrophils, are then thought to phagocytose those spermatozoa not destined to be involved in fertilization. A similar leukocytic reaction is thought to occur post-coitally in humans (Thompson *et al*., 1992) but whether this performs the same physiological function remains to be established. In addition, the factor or factors which initiate the leukocytosis are unknown and pure spermatozoa, seminal plasma stripped of spermatozoa and a combination of both have all been implicated (Howe and Black, 1963; Mattner, 1968). Finally, heat stable enhancers of neutrophil chemotaxis identified in human semen have not been fully characterized and their role,

if any, in initiating leukocytosis remains uncertain (Clarke and Klebanoff, 1976).

The host responses elicited by seminal plasma are therefore highly complex and potentially conflicting. This study initially investigated the effect of seminal plasma extracts on cytokine release by human cervical explants. The pro-inflammatory cytokine studied was the neutrophil chemotactic factor interleukin-8 (IL-8) and the anti-inflammatory cytokines were secretory leukocyte protease inhibitor (SLPI), an inhibitor of neutrophil function and IL-10. These responses were further characterized by examining the effect of whole seminal plasma, a seminal plasma extract, PGE_2 and 19-hydroxy PGE on release of these mediators by peripheral blood and the monocyte cell line U937.

Materials and methods

Tissue collection

Pooled human seminal plasma was obtained from healthy young men involved in the ongoing semen donor programme (more than 20 men). Cervical biopsies, ~20–35 mg in weight, 15–20 mm in length and 2–3 mm in diameter, were obtained from healthy, non-pregnant women (n = 15, ages 29–45) with regular menstrual cycles undergoing a hysterectomy for a non-malignant condition. These were taken from the anterior lip of the cervix immediately post-hysterectomy. Peripheral blood was taken using a Li-heparin tube (Monovette, Sarstedt, Amsterdam, Holland) from healthy non-pregnant women (n = 6, aged 20–35 years) with normal menstrual cycles not using any hormonal form of contraception. The women were at various stages of the menstrual cycle. Ethical approval was obtained for these studies from the Local Ethics Committee.

Preparation of seminal plasma fraction (SPF)

All reagents were from Sigma (Poole, UK), unless otherwise stated. Human seminal plasma (700 ml) was treated with ethanol (1000 ml) and centrifuged at 4°C for 20 min at 1500 *g*. The supernatant was evaporated at 23°C to a final volume of 450 ml then passed through ENV + extraction columns (International Sorbent Technology Ltd., YstradMynach, Hengoed, UK) which had previously been washed with 1:1 ethanol/hexfluoropropanol (HFP; Aldrich, Poole, UK). Lipid was eluted with 1:1 ethanol/HFP and the liquid evaporated at 23°C to 10 ml. Chloroform (15 ml; Merck, Poole, UK) was added to leach the residue and the chloroform layer evaporated and redissolved in ethanol (4 ml) to give the seminal plasma fraction. PGE_2 and 19-hydroxy PGE were assayed as described below. The seminal plasma fraction contained 165 μg/ml PGE_2 and 705 μg/ml 19-hydroxy PGE. The lipid extraction of the seminal plasma removes the majority of the polyamines such as spermine and spermidine which may have cytotoxic effects (Allen and Roberts, 1986).

Tissue culture

Cervical biopsies

Cervical biopsies were placed immediately in Roswell Park Memorial Institute (RPMI) 1640 medium at 4°C for transport. Explants were washed in phosphate-buffered saline (PBS), dissected into small pieces 1–2 mm^3 then placed in 1 ml complete medium (RPMI 1640 supplemented with 10% fetal calf serum (FCS); 2 mM L-glutamine; 50 μg/ml streptomycin, Gibco, Paisley, UK; 20 μg/ml gentamicin and 50 IU/ml penicillin) in a 24-well plate (Costar, High Wycombe, UK). Explants were treated in quadruplicate with seminal plasma fraction diluted 1:2000 then cultured for 24 h at 37°C in 95% air and 5% carbon dioxide under humid conditions. The harvested media were frozen at –20°C until analysed.

Peripheral blood

Peripheral blood was diluted immediately 1:10 in RPMI 1640 prior to plating out at 1 ml/well and culturing for 24 h at 37°C in 5% CO_2. Media were stored at –20°C until analysis.

U937 cells

U937 cells, a human pro-monocytic cell line, were plated out at 2×10^5 cells/ml in complete media, treated with whole seminal plasma (1–0.0001%), seminal plasma fraction (1–0.0001%), PGE_2 (10^{-6} to 10^{-10} M) and 19-hydroxy PGE (Cayman Chemicals, Ann. Arbor, Michigan, USA; 10^{-6} to 10^{-10} M) at the time of addition. In addition, cells were treated with 10^{-7} M phorbol ester (PMA) for the IL-10 experiments because no IL-10 was released from cells without PMA treatment. U937 cells were cultured for 24 h at 37°C in 5% CO_2 under humid conditions and the media were stored at –20°C until analysis.

Cytokine assays

IL-8 assay

IL-8 was measured by enzyme-linked immunosorbent assay (ELISA) using matched pairs of capture and biotinylated-labelled detection antibodies for IL-8 (R&D Detection Systems, Oxford, UK) as described in detail previously (Denison *et al*., 1997). The highest concentration of standard was 500 pg/ml, the intra- and inter-assay precision was 9.1% relative standard deviations (r.s.d) and 11% r.s.d. respectively; the detection limit of the assay was 15 pg/ml.

Secretory leukocyte protease inhibitor (SLPI) assay

SLPI was measured by a competitive ELISA. Plates (96-well; Nunc Maxi Sorp; Gibco) were coated with recombinant SLPI (R&D Detection Systems; 0.025 μg/ml in PBS/1% carbonate buffer; 400 mM $NaHCO_3$, 40 mM Na_2CO_3 in 500 ml distilled H_2O, pH 9.6) at 300 μl/well for 60 min at 23°C, blocked with milk powder (0.1 mg/ml in distilled water) at 400 μl/well for 30 min at 23°C then washed (150 mM NaCl, 100 mM Tris, 0.05% Tween-20 to final pH of 7–7.5). ELISA assay buffer (150 mM NaCl; 100 mM Tris; 50 mM Phenol Red solution; 2 mM EDTA; 1 mM 2-methylisothiazolone, Boehringer Mannheim, Lewes, UK; 1 mM bromonitrodioxane, Boehringer Mannheim; 2 mg/ml bovine serum albumin; 0.05% Tween-20 to final pH of 7.2) was added for the non-specific binding (NSB) in singleton at 250 μl/well. Recombinant standards (R&D Detection Systems; highest concentration 50 ng/ml) and samples were added at 200 μl/well and anti-SLPI (polyclonal anti-SLPI, R&D Detection Systems; 2 μg/ml in ELISA buffer) added at 50 μl/well to all wells except the NSB. Plates were incubated for 120 min at 23°C on an orbital shaker then washed and anti-sheep/goat immunoglobulin (Ig)G-peroxidase *Fab* fragments (Boehringer Mannheim; diluted 1:500 in ELISA buffer) added at 100 μl/well. Finally, plates were incubated for 120 min at 23°C on an orbital shaker, washed and substrate (0.3 g/l urea-hydrogen peroxide; 0.1 g/l tetramethyl benzidine in 100 mM sodium acetate, pH 6.0) added at 200 μl/well for 10 min prior to quenching with 2 N H_2SO_4 at 50 μl/well. Absorption was read at 450 nm within 30 min of quenching. The correlation between the results from this assay and those obtained by a commercial assay (n = 10; R&D Detection Systems) was 0.85. The intra- and inter-assay precision was 9.2% r.s.d and 10.1% r.s.d respectively, and the detection limit of the assay was 9.8 pg/ml.

IL-10 assay

All dilutions were in 10% FCS in PBS unless otherwise stated. Plates were coated with capture antibody (Pharmingen, San Diego, USA; 200 ng/ml in PBS) added at 100 μl/well for 60 min at 23°C then blocked (10% FCS in PBS) at 300 μl/well for a further 60 min at

23°C. The plates were then washed and both samples and recombinant standards (Pharmingen) added at 100 µl/well with the top standard being 500 pg/ml. Plates were then incubated on an orbital shaker for 60 min, washed, detection antibody (Pharmingen; 125 ng/ml) added at 100 µl/well and incubated for 60 min at 23°C. After a further wash, polyperoxidase (CLB laboratories, Amsterdam, Holland; 1 ng/ml in ELISA buffer without Tween) was added at 100 µl/well for 30 min. Finally, plates were washed and read as for SLPI. The intra- and inter-assay precision was 6.4% r.s.d and 10.1% r.s.d respectively, and the detection limit of the assay was 15 pg/ml.

PGE_2 ELISA

Samples were treated 1:1 with methyloximating solution (0.1 M methoxyamine hydrochloride in 10% ethanol diluted in 1 M sodium acetate, pH 5.6) overnight at 4°C. Plates (Costar Amine-binding plates, Paisley, UK) were coated with donkey anti-rabbit (DAR serum; Scottish Antibody Production Unit, Carluke, UK) using the direct γ-globulin binding procedure. Briefly, they were coated with rabbit IgG (1 mg/ml diluted in PBS/1% carbonate buffer, pH 9.6) at 200 µl/well for 16 h at 4°C, the solution flicked out and blocking solution (50 mM glycine; bovine serum albumin 10 mg/ml Sigma-A7888 in distilled H_2O) added at 250 µl/well for 120 min at 23°C. They were then washed, DAR serum added at 150 µl/well, incubated for 16 h at 4°C then washed, air-dried and stored with a desiccant at 4°C.

The assay used a PGE–biotin link as a pro-label. To prepare the link, 0.06 M synthetic PGE_2 (kind gift of Applied Therapeutics, Paisley, UK) was added to 320 µl dry dimethylformamide (DMF; Aldrich, Poole, UK), the solution cooled to 4°C and 6 µl tributylamine (Aldrich) and 3 µl butylchloroformate (Aldrich) were added with stirring for 30 min at 4°C. Next 0.05 M biocytin, in 300 µl 1:1 DMF/distilled H_2O was added and the vial left at 4°C for 30 min then at 23°C for 30 min. Finally, the solution was ether extracted and purified by reverse phase chromatography. Rabbit anti-sera were raised against PGE_2 complexed to keyhole limpet haemocyanin and have been previously characterized (Kelly *et al.*, 1989). Samples and synthetic standards (Applied Therapeutics, Paisley, UK; highest concentration 5120 pg/ml) were added in duplicate at 100 µl/well, link (1 in 1.5×10^6) at 50 µl/well and anti-sera (1 in 50 000) at 50 µl/well to all wells except the NSB. The final concentration of methyloximating solution in standards and samples was 12.5%. Plates were incubated at 4°C for 16 h, washed and 100 µl/well of 0.2 IU/ml streptavidin peroxidase (Boerhringer Mannheim) added. The plates were then incubated for 20 min at 23°C on an orbital shaker, washed, substrate added and plates read as for SLPI. The intra- and inter-assay coefficients of variation were 7.8% and 15.0% respectively and the ED_{50} was 195 pg/ml.

19-hydroxy PGE assay

The plates and anti-sera were prepared as described above. 19-hydroxy PGE standards containing equal amounts of 19-hydroxy PGE_1 and 19-hydroxy PGE_2 were prepared as follows. Human seminal plasma (65 ml) was centrifuged at 4°C for 15 min at 1000 *g*, mixed with acetonitrile (Merck; 65 ml), centrifuged at 4°C for 15 min at 2000 *g* prior to freeze-drying the supernatant overnight. The residue was leached with methanol, evaporated, added to a silica column and the prostaglandins (PGE_2 and 19-hydroxy PGE) were eluted with increasing concentrations of methanol (Merck) in methyl formate (Merck). The fractions were analysed by thin layer chromatography and that corresponding to the 19-hydroxy PGE fraction was quantified, using crystalline synthetic PGE_2 as a standard, by treatment with 0.05 M NaOH and measurement of UV absorption at 280 nm in ethanol. To prepare the link, ~1 mg 19-hydroxy PGE was added to 200 µl dry DMF, the solution cooled to 4°C and 3 µl tributylamine and 1.5 µl butylchloroformate were added with stirring for 30 min at 4°C. Finally, 12 mM Pro-Gly-Tyr-Biotin (synthesized in house) in 300 µl 1:1 DMF/distilled H_2O was added and the solution extracted as described above. Standards (5120–78 pg/ml), link (1:20 000) and anti-sera (1:20 000) were diluted in ELISA buffer without Tween and the assay set up as for PGE_2. The intra-assay coefficient of variation was 8.46% and the ED_{50} was 196 pg/ml.

Statistical analysis

Statistical analysis of the data was performed using Statview 4.1 (Abacus Inc, Berkeley, CA, USA). The data were normally distributed and are expressed as pg/ml (mean ± SEM) with a statistically significant difference defined as $P < 0.05$.

Results

Cervical explants

Seminal plasma fraction significantly stimulated ($P < 0.005$) IL-8 and inhibited ($P < 0.005$) SLPI release (Figure 1; $n = 15$ different women, treatments in quadruplicate). IL-10 release was below the detection limit of the assay employed. Pure seminal plasma was not used to treat cervical explants due to possible immunological interactions between tissues and cytotoxic effects. Cervical biopsies are scarce and difficult to obtain. Hence the experiments performed were limited and insufficient explants were obtained to treat with PGE_2 and 19-hydroxy PGE.

Peripheral blood data

Whole seminal plasma significantly ($P < 0.05$) inhibited IL-8 release at concentrations of >0.1%. Maximum inhibition of IL-8 release down to 8% of control values ($P < 0.005$) was achieved when seminal plasma was added at 1%. In contrast,

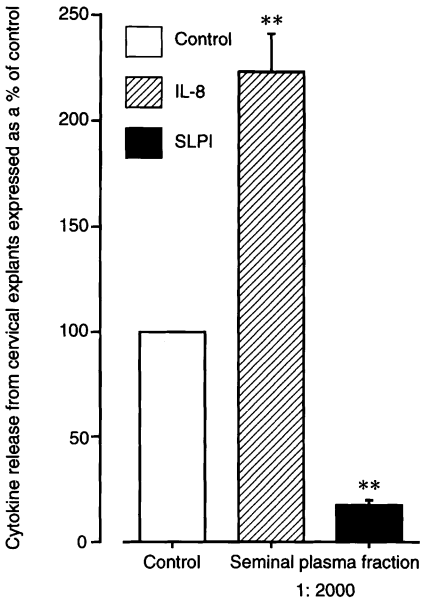

Figure 1. Effect of seminal plasma fraction on interleukin (IL)-8 and secretory leukocyte protease inhibitor (SLPI) release from non-pregnant cervical explants ($n = 15$ different women, treatments in quadruplicate). IL-8 release was significantly stimulated and SLPI release significantly inhibited by seminal plasma fraction at 1:2000. Values are expressed as cytokine release as percentage of control ± SEM. **Results are significantly different ($P < 0.005$).

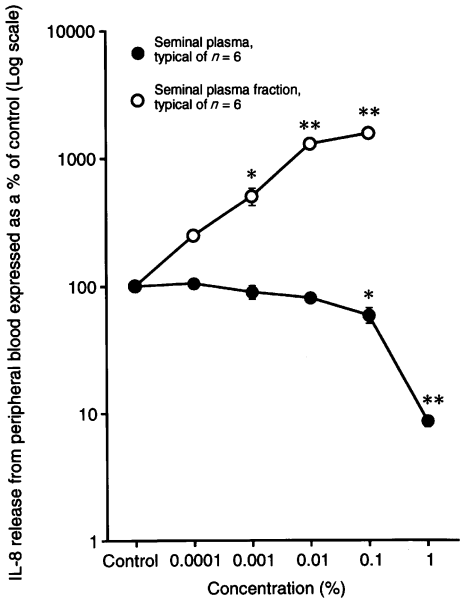

Figure 2. The effect of seminal plasma and seminal plasma fraction on interleukin (IL)-8 release from peripheral blood (typical of six separate experiments, treatments in quadruplicate). IL-8 release was significantly stimulated by the seminal plasma fraction whereas seminal plasma significantly inhibited IL-8 release. Values are expressed as the log of IL-8 release as percentage of control ± SEM. Significantly different from control; $*P < 0.05$; $**P < 0.005$.

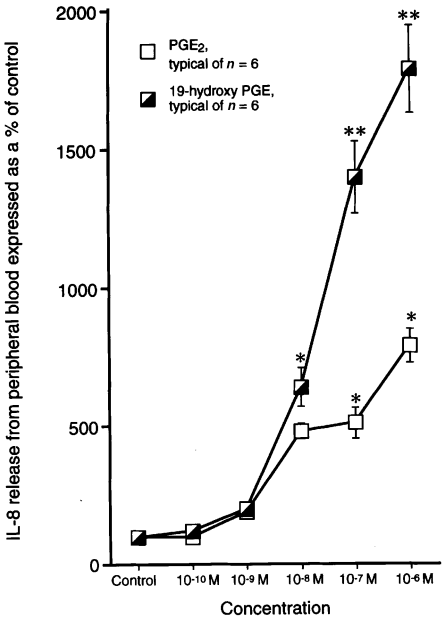

Figure 3. The effect of prostaglandin E_2 (PGE_2) and 19-hydroxy PGE on interleukin (IL)-8 release from peripheral blood (typical of six separate experiments, treatments in quadruplicate). IL-8 release was significantly stimulated by both PGE_2 and 19-hydroxy PGE. At concentrations of $>10^{-8}$ M, 19-hydroxy PGE was significantly ($P < 0.005$) more effective in stimulating IL-8 release than PGE_2. Values are expressed as IL-8 release as percentage of control ± SEM. Significance of difference from control; $*P < 0.05$; $**P < 0.005$.

seminal plasma fraction significantly ($P < 0.05$) stimulated IL-8 release when used at 0.001% with a 10-fold stimulation achieved when peripheral blood was cultured with 0.1% seminal plasma fraction A ($P < 0.005$; Figure 2; $n = 6$ separate experiments, treatments in quadruplicate). Both PGE_2 and 19-hydroxy PGE significantly stimulated ($P < 0.05$; $P < 0.005$ respectively) IL-8 release with maximal stimulation achieved at 10^{-6} M. 19-hydroxy PGE was significantly ($P < 0.05$) more effective in stimulating IL-8 release than PGE_2 at concentrations of $>10^{-8}$ M (Figure 3; $n = 6$ separate experiments, treatments in quadruplicate). SLPI release was below the detection limit of the assay used.

U937 monocyte cell line

Seminal plasma and seminal plasma fraction significantly (both $P < 0.005$) stimulated IL-8 release from U937 cells cultured without PMA by comparable amounts with maximum stimulation at 0.1% (Figure 4; $n = 6$ separate experiments, treatments in quadruplicate). PGE_2 significantly ($P < 0.05$) stimulated IL-8 release from U937 cells with maximum stimulation at 10^{-6} M. 19-hydroxy PGE also stimulated ($P < 0.005$) IL-8 release and as demonstrated in peripheral blood it was significantly ($P < 0.05$) more effective in inducing IL-8 release than PGE_2 (Figure 5; $n = 6$ separate experiments, treatments in quadruplicate). SLPI release was not detectable from U937 cells.

Seminal plasma and seminal plasma fraction significantly stimulated ($P < 0.05$) IL-10 release from U937 cells with maximum stimulation at 0.1 and 0.01% respectively, for seminal plasma and fraction (Figure 6; $n = 6$ separate experiments, treatments in quadruplicate). Seminal plasma

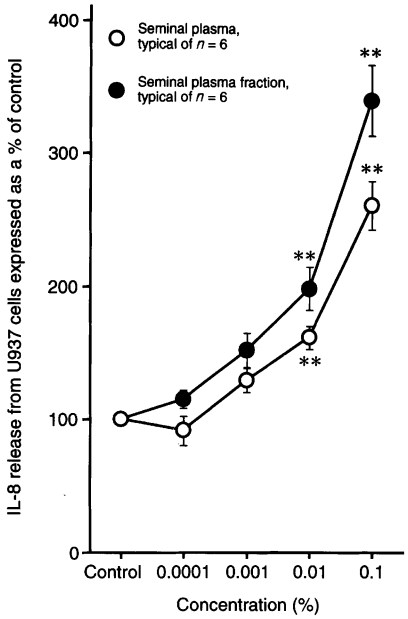

Figure 4. The effect of seminal plasma and seminal plasma fraction on interleukin (IL)-8 release from U937 cells (typical of six separate experiments, treatments in quadruplicate). IL-8 release was significantly stimulated by both seminal plasma and seminal plasma fraction. Values are expressed as IL-8 release as a percentage of control ± SEM. Significance of difference from control; $**P < 0.005$.

fraction inhibited secretion of IL-10 back to control values when added at 0.1%. PGE_2 significantly ($P < 0.05$) stimulated IL-10 release from U937 cells with maximum stimulation at

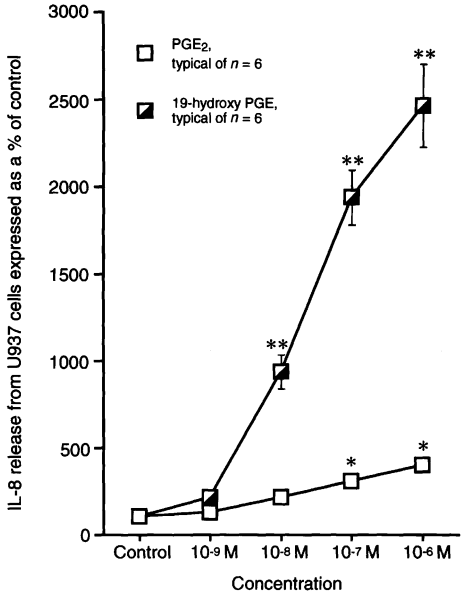

Figure 5. The effect of prostaglandin E_2 (PGE_2) and 19-hydroxy PGE on interleukin (IL)-8 release from U937 cells (typical of six separate experiments, treatments in quadruplicate). IL-8 release was significantly stimulated by both PGE_2 and 19-hydroxy PGE. 19-hydroxy PGE was significantly ($P < 0.005$) more effective in stimulating IL-8 release than PGE_2. Values are expressed as IL-8 release as a percentage of control ± SEM. Significance of difference from control; $*P < 0.05$; $**P < 0.005$.

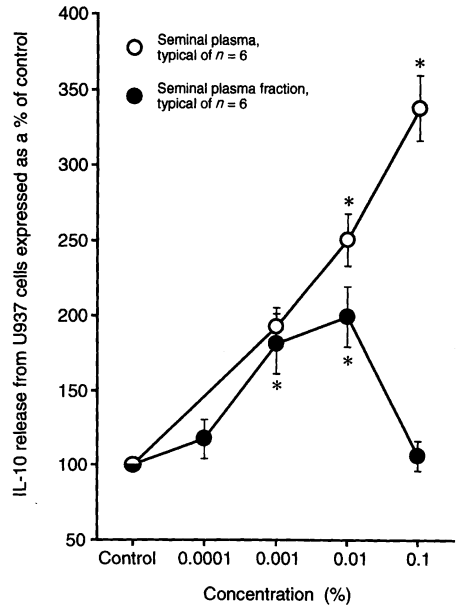

Figure 6. The effect of seminal plasma and seminal plasma fraction on interleukin (IL)-10 release from U937 cells (typical of $n = 6$ separate experiments, treatments in quadruplicate). Seminal plasma significantly ($P < 0.05$) stimulated IL-10 release from U937 cells. Seminal plasma fraction significantly ($P < 0.05$) stimulated IL-10 release at 0.001 and 0.01%, but inhibited its release back to control values at 0.1%. Values are expressed as IL-10 release as a percentage of control ± SEM. Significance of difference from control; $*P < 0.05$.

10^{-6} M. Although 19-hydroxy PGE also stimulated IL-10 release this failed to reach significance (Figure 7; $n = 6$ separate experiments, treatments in quadruplicate).

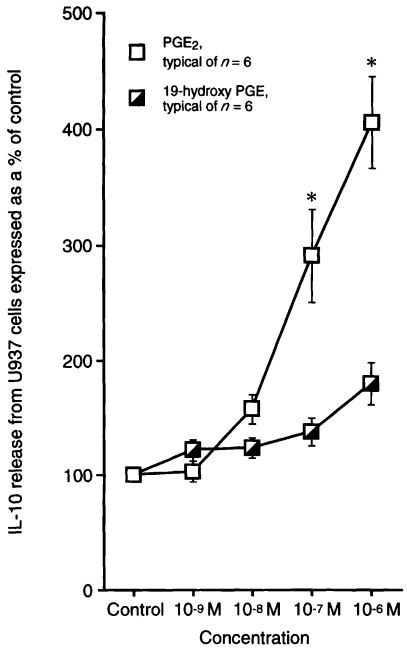

Figure 7. The effect of prostaglandin E_2 (PGE_2) and 19-hydroxy PGE on interleukin (IL)-10 release from U937 cells (typical of six separate experiments, treatments in quadruplicate). PGE_2 significantly stimulated IL-10 release from U937 cells. 19-hydroxy PGE stimulated IL-10 release but this failed to reach significance. Values are expressed as IL-10 release as a percentage of control ± SEM. Significance of difference from control; $*P < 0.05$.

Discussion

In this study, it is reported for the first time that seminal plasma and its principal constituent prostaglandins, PGE_2 and 19-hydroxy PGE stimulated IL-8 release from peripheral blood and the monocyte cell line U937. Moreover, 19-hydroxy PGE was significantly more potent than PGE_2 in inducing IL-8 release. In addition, seminal plasma extracts were effective in stimulating IL-8 release from cultured cervical explants. It was also demonstrated that IL-10 release was stimulated in U937 cells by seminal plasma, seminal plasma fractions, PGE and 19-hydroxy PGE, although the latter was not significant. This confirms previous data (Kelly *et al.*, 1997) which showed stimulation of IL-10 release from lipopolysaccharide-stimulated whole blood under similar conditions.

Human seminal plasma contains very high concentrations of PGE_2 (Samuelsson, 1963) and 19-hydroxy PGE (Taylor and Kelly, 1974) which are mainly synthesized *de novo* within the seminal vesicles (Pourian *et al.*, 1995). Seminal plasma PGE_2 acts primarily to suppress the female genital tract immune system thus conferring a survival advantage to spermatozoa within the hostile female reproductive tract (Kelly and Critchley, 1997). In addition to altering the ratio of IL-10 to IL-12 release, it also reduces expression of the T-cell co-stimulator ligands B7-1 and B7-2 required for normal T-cells activation and antigen recognition (Iglesias *et al.*, 1997). In contrast, in other situations, PGE_2 can act as a potent pro-inflammatory agent by virtue of its vasodilatory properties. Within human skin it synergizes with the neutrophil chemotactic and activating agent IL-8 to promote neutrophil chemotaxis and activation (Colditz, 1990) and in IL-1α stimulated synovial

fibroblasts PGE_2 stimulates IL-8 release (Agro *et al*., 1996). Whether seminal plasma PGE_2 is capable of inducing a pro-inflammatory response either within semen or the female genital tract is not known. Elevated concentrations of IL-8 within seminal plasma are, however, associated with leukocytospermia, a condition characterized by abnormally high concentrations of pro-inflammatory leukocytes in seminal plasma (Shimoya *et al*., 1993).

Less is known about the function of 19-hydroxy PGE_2 within seminal plasma although, like PGE_2 it is thought to act principally as an immunosuppressive agent inhibiting natural killer cell activity (Tarter *et al*., 1986) and elevating concentrations of cAMP (Kelly *et al*., 1994). Despite the fact that 19-hydroxy PGE is a less potent immunosuppressive agent than PGE_2 *in vitro*, its three-fold higher concentrations within seminal plasma may confer on it greater immunosuppressive activity *in vivo*. In addition, 19-hydroxy PGE is a relatively selective cAMP prostaglandine (EP)-2 receptor agonist (Woodward *et al*., 1993) unlike PGE_2 which binds to all of the EP receptors. Given that the primate cervix contains mainly EP-2 receptors (Smith *et al*., 1998) then the higher affinity of 19-hydroxy PGE for the EP-2 receptor may make it even more active *in vivo*. A pro-inflammatory effect for 19-hydroxy PGE has not been demonstrated previously.

These results demonstrate that seminal plasma and its component prostaglandins stimulate IL-8 release. The only exception to this was in peripheral blood where whole semen inhibited IL-8. However, seminal plasma is a potent stimulator of IL-10 release in peripheral blood and given that there is mutual repression between release of IL-10 and IL-8 (Lu *et al*., 1995; Kang *et al*., 1998) then the high concentrations of IL-10 generated may have inhibited IL-8 release. In addition, other factors within seminal plasma may also affect IL-8 release in peripheral blood preparations. We therefore suggest that PGE_2 and 19-hydroxy PGE, by stimulating IL-8 release from the female genital tract mucosa, may act as potent neutrophil leukoattractants within seminal plasma. Neutrophils invading into the upper vaginal tract, cervical mucus and stroma would then be ideally placed to phagocytose any spermatozoon not involved in fertilization. In addition, 19-hydroxy PGE may be useful in inducing cervical ripening at term, given that IL-8 induces tissue remodelling (Chwalisz *et al*., 1994).

It has been suggested that pure seminal plasma stripped of spermatozoa is not capable of leukocytosis and therefore the hypothesis proposed would be invalid. The published data are however conflicting and this may be due to different methods of collecting and purifying the seminal plasma and the age of the semen used for the studies. In addition, pure spermatozoa washed of seminal plasma have also been reported to induce leukocytosis (Thompson *et al*., 1992). However, washing spermatazoa may induce alterations in membrane structure including lipid peroxidation generating oxygen free radicles and leukotrienes both of which are chemotactic for neutrophils (Krauss *et al*., 1994; Wizemann and Laskin, 1994). This could account for the observed leukocytosis post insemination of pure spermatozoa. It is likely therefore that *in vivo* a combination of seminal plasma constituents such as PGE_2, 19-hydroxy PGE and spermatozoa would act together to induce leukocytosis.

In this study, we have also demonstrated that seminal plasma and its constituent prostaglandins stimulate release of IL-10 from U937 cells, thus supporting earlier data (Kelly *et al*., 1997) with PGE_2 being more effective than 19-hydroxy PGE in stimulating IL-10 release. This immunosuppressive action of seminal plasma may seem to conflict with the pro-inflammatory effect of stimulating IL-8 release. However, IL-8 is also a potent chemotactic factor for T-lymphocytes (Taub *et al*., 1996). It could therefore induce their entry into the upper vaginal tract where they could be presented with antigen in the presence of high concentrations of immunomodulatory agents such as IL-10, PGE_2 and TGF-β which would all favour development of anergy (Groux *et al*., 1996; Tremellen *et al*., 1998). In addition, seminal plasma prostaglandins could be transported to draining lymph nodes together with foreign cells (Ibata *et al*., 1997). These nodes enlarge post-coitus (Alexander and Anderson, 1987) and could be a further site of IL-10 mediated immunosuppression.

In conclusion, it has been demonstrated that seminal plasma prostaglandins can induce release of both pro-and anti-inflammatory cytokines. Moreover, PGE_2 and 19-hydroxy PGE have differing capacities to induce anti- and pro-inflammatory cytokine release respectively. We suggest that these observations are not mutually exclusive and that the different cytokines released may act in combination initially to promote sperm survival, and then to facilitate their removal from the female reproductive tract.

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