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J Immunol (2012) 188 (5): 2445–2454.

<https://doi.org/10.4049/jimmunol.1102736>

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Seminal Fluid Induces Leukocyte Recruitment and Cytokine and Chemokine mRNA Expression in the Human Cervix after Coitus

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In mice, seminal fluid elicits an inflammation-like response in the female genital tract that activates immune adaptations to advance the likelihood of conception and pregnancy. In this study, we examined whether similar changes in leukocyte and cytokine parameters occur in the human cervix in response to the male partner's seminal fluid. After a period of abstinence in proven-fertile women, duplicate sets of biopsies were taken from the ectocervix in the periovulatory period and again 48 h later, 12 h after unprotected vaginal coitus, vaginal coitus with use of a condom, or no coitus. A substantial influx of CD45⁺ cells mainly comprising CD14⁺ macrophages and CD1a⁺ dendritic cells expressing CD11a and MHC class II was evident in both the stratified epithelium and deeper stromal tissue after coitus. CD3⁺CD8⁺CD45RO⁺ T cells were also abundant and increased after coitus. Leukocyte recruitment did not occur without coitus or with condom-protected coitus. An accompanying increase in *CSF2*, *IL6*, *IL8*, and *IL1A* expression was detected by quantitative RT-PCR, and microarray analysis showed genes linked with inflammation, immune response, and related pathways are induced by seminal fluid in cervical tissues. We conclude that seminal fluid introduced at intercourse elicits expression of proinflammatory cytokines and chemokines, and a robust recruitment of macrophages, dendritic cells, and memory T cells. The leukocyte and cytokine environment induced in the cervix by seminal fluid appears competent to initiate adaptations in the female immune response that promote fertility. This response is also relevant to transmission of sexually transmitted pathogens and potentially, susceptibility to cervical metaplasia. *The Journal of Immunology*, 2012, 188: 2445–2454.

Seminal fluid is conventionally thought to have a single purpose: to deliver spermatozoa to the oocyte to achieve conception and commence pregnancy. However, studies in several species show that seminal fluid also contains signaling molecules that interact with epithelial cells lining the female reproductive tract to trigger gene expression, leukocyte recruitment, and activation of innate and adaptive immune events in a sequence that resembles an inflammatory response (1, 2). This postcoital leukocytic response has been described in mice (3, 4), pigs, rabbits, sheep, and other mammals (5–8), and is thought to have a fundamental role in reproduction because seminal fluid signaling

proteins linked with immunity and defense are present in such diverse organisms as flies, mosquitos, crickets, honeybees, rodents, and primates (9).

Immune changes induced by seminal fluid facilitate preparation of the female reproductive tissues for pregnancy through clearance of debris and pathogens, sperm selection, and induction of immune tolerance toward the semiallogeneic embryo (1, 2). The full effect of seminal fluid on female immune parameters is best characterized in the mouse, where within hours of mating, macrophages, dendritic cells (DCs), and granulocytes are recruited into the endometrial stroma and lumen (3, 10, 11). Through a process of cross-presentation by female DCs in lymph nodes draining the genital tract, seminal fluid Ags activate and expand inducible regulatory T cell populations (12, 13) that subsequently migrate into the endometrium to mediate immune tolerance of the conceptus at implantation (14). The regulatory T cell response to seminal fluid depends on seminal plasma factors originating in the seminal vesicle gland (4), notably TGF- β , which is synthesized in the latent form and activated in the female tract after ejaculation (15).

In humans, the female immune response to seminal fluid and its relevance to fertility have received little attention. In studies conducted decades ago, samples recovered from the cervix using a cervical brush or spatula revealed that after artificial insemination with donor spermatozoa, there is an influx of neutrophils into the cervical canal, with low numbers of macrophages and lymphocytes also detected (16, 17). Leukocytosis was evident by 4 h and maximal at 12 h before diminishing by 24 h (16). However, these previous studies explored only the superficial surface of the cervical mucosa and provide no indication of the cellular changes within the stratified epithelium and stroma, where on the basis of animal experiments the major cellular changes are expected to

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Received for publication September 21, 2011. Accepted for publication December 14, 2011.

This work was supported by a program grant and fellowship from the National Health and Medical Research Council, Australia (to S.A.R.).

The microarray data presented in this article have been submitted to the National Center for Biotechnology Information's Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33745>) under Series accession number GSE33745.

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The online version of this article contains supplemental material.

Abbreviations used in this article: COX-2, cyclooxygenase 2; DAB, diaminobenzidine tetrahydrochloride; DC, dendritic cell; LH, luteinizing hormone; MHCII, MHC class II; MMP, matrix metalloproteinase; NHS, normal human serum; qRT-PCR, quantitative RT-PCR; VIA, video image analysis.

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occur. In addition, both studies used artificial insemination with frozen or fresh spermatozoa, raising the question of whether similar responses occur in the physiological situation, after vaginal intercourse.

The imperative to explore the influence of seminal fluid on the immune response in the human female reproductive tract is now increased as its potential importance to fertility (18), transmission of sexually transmitted pathogens such as HIV (19, 20), and development and progression of cervical metaplasia (21, 22) has become evident. In this study, we recovered small-needle biopsies from the ectocervix of ovulating women before and after vaginal intercourse. The specific effects of seminal fluid were discriminated from other physical effects of coitus by including two control groups who either used condoms to prevent seminal fluid contact or abstained completely from intercourse. Immunohistochemistry was used to examine the effects on leukocyte populations throughout the cervical mucosa, and quantitative RT-PCR (qRT-PCR) and microarray were used to quantify effects on cytokine and chemokine gene expression. We report in this article that seminal fluid exposure at coitus results in accumulation of macrophages and T cells in both the epithelial and stromal compartments of the ectocervix, and this is accompanied by elevated expression of inflammatory cytokine genes including *CSF2*, *IL1A*, *IL6*, and *IL8*. Together, these findings suggest that in women, as in other mammals, the role of seminal fluid extends beyond simple provision of male gametes and potentially involves activating changes in the innate and adaptive immune response that could influence female tract receptivity for pregnancy and defense from infection.

Materials and Methods

Subject population

Participants for this study were recruited and cervical biopsies obtained at the Karolinska University Hospital, Stockholm, Sweden. Ten healthy women aged 18–40 y, with regular menstrual cycles (between 25 and 35 d) and current involvement in a sexual relationship with a proven fertile regular partner, volunteered for the study. All women had previously undergone tubal ligation, and none of the women used steroidal contraceptives or an intrauterine device for a minimum of 3 mo preceding the study. A complete gynecological examination of each subject was performed upon admission. Couples were also screened for any sign of bacterial or viral infection including *Chlamydia* (by PCR analysis of cervical and urethral swabs), hepatitis B, hepatitis C, and HIV (by serology) and human papilloma virus (by cytology). A negative test result was a prerequisite for inclusion in the study. Subjects with male partners who were either vasectomized or known azoospermic were excluded from this study. The study was approved by the local ethics committee of Karolinska University Hospital. All women gave their written informed consent before entering the study.

Experimental design and biopsy collection

Subjects were randomly allocated, according to the protocol shown in Fig. 1, into one of three groups: 1) no coitus ($n = 7$), 2) coitus with a condom ($n = 5$), or 3) unprotected coitus ($n = 6$). To synchronize the timing of the biopsies, all subjects determined their luteinizing hormone (LH) peak in urine samples taken twice daily from approximately day 10 after the onset of menstruation to the time at which an LH increase was detected (LH0-LH+1), using a rapid self-test (Clearplan; Searle Unipath, Bedford, U.K.). All subjects presented for a first biopsy (B1) during the peri-ovulatory stage of the menstrual cycle (at 9:00 AM to 12:00 PM on day LH0 to LH+1), and again 48 h later for a second biopsy (B2) (at 9:00 AM to 12:00 PM on day LH+2 to LH+3). Some couples involved in the study volunteered to participate on more than one occasion and were allocated to two or three of the different treatment groups. Couples who had already been included in the study were required to wait for a minimum of 3 mo from their previous biopsy before reinclusion in the study.

All couples totally abstained from penetrative intercourse for 2 d before the first biopsy, and condoms were used for 5 d before this to ensure that no sperm would be present in the cervical region at the first biopsy. Before biopsy, the cervix was washed with 10 ml saline solution to clear mucus and debris. All cervical washings were collected and examined microscopically

for the presence of sperm to confirm that abstinence was adhered to. Two small-needle biopsies (~50–100 mg tissue each) were taken from adjacent sites in the ectocervix, ~1 cm from the transformation zone. Couples then abstained from intercourse for 36 h to enable hemostasis and healing of the biopsy site. Couples allocated to groups 2 and 3 had intercourse, with or without condom use, respectively, on one occasion ~36 h after the first biopsy and 12 h before the second biopsy. Abstinent couples did not have intercourse between the two biopsies. At second biopsy, the cervix was again washed with 10 ml saline solution, which was collected and examined to confirm compliance. Again, two small-needle biopsies were taken from adjacent sites in the ectocervix positioned 180° from the site of the first biopsy. At both first and second biopsy, one sample destined for immunohistochemical analysis was immediately embedded in O.C.T. Tissue Tek (Miles Scientific, Elkhart, IN), frozen by immersion in isopropanol (Ajax Chemicals, Auburn, NSW, Australia) cooled by liquid N₂, and then stored at –80°C. The duplicate sample, for analysis by qRT-PCR, was immediately snap frozen in liquid N₂ and stored at –80°C. At the completion of sample collection, all biopsies were shipped on dry ice to Adelaide and then stored at –80°C until processing and analysis by immunohistochemistry and qRT-PCR.

mAbs and immunohistochemistry

mAbs specific for a range of leukocyte cell lineages (Supplemental Table I) were used to determine the distribution and phenotype of leukocytes in cervical tissue. Mouse anti-human mAbs including CD1a (HI149), CD11a (HB202), CD14 (FMC17), CD15 (FMC13), CD45 (FMC51), CD57 (TIB200), CD80 (L307.4), CD86 (IT2.2), FOXP3 (236A/E7), and MHC class II (MHCII; FMC52) were purchased from BD Pharmingen (San Diego, CA), eBioscience (San Diego, CA), or provided as hybridoma supernatants (P. McCardle, Flinders Medical Centre, Adelaide, SA, Australia). The specificity of all mAbs was validated by FACS analysis and immunohistochemical staining of smears of mononuclear cells from human peripheral blood (not shown). Sections of cervical tissue (6 µm) were cut from cervical biopsies, air-dried, and stored at –80°C with silica gel (Ajax Finechem, Auburn, NSW, Australia) until staining was performed. All sections to be stained for a given mAb were prepared and processed together. Sections of cervical tissue were fixed in 96% ethanol (4°C, 10 min), then washed three times in PBS and incubated for 2 min with 1% (w/v) BSA in PBS. Duplicate sections were incubated with primary mAbs (diluted to 10 µg/ml in PBS with 1% BSA and 10% normal human serum [NHS]) at 4°C for 2 h or overnight for FOXP3. Negative control sections were incubated with an irrelevant, isotype-matched mouse mAb. All sections were then incubated with biotinylated goat anti-mouse secondary Ab (1:300 in PBS with 1% BSA and 10% NHS; Dako Corporation, Carpinteria, CA) at 4°C for 45 min, washed in PBS, and incubated with HRP-conjugated avidin (1:400 in PBS with 1% BSA and 10% NHS; Dako Corporation). To visualize HRP, we incubated slides in diaminobenzidine tetrahydrochloride (DAB; 5 mg/ml in 0.05 M Tris-HCl pH 7.2 plus 0.02% hydrogen peroxide; Sigma-Aldrich, St. Louis, MO) for 8 min at room temperature. Tissue sections were counterstained in hematoxylin (Sigma-Aldrich), dehydrated in two changes of absolute ethanol, cleared in two changes of Salsolvent (Ajax Finechem), and mounted in Depex (BDH Laboratory Supplies, Toronto, ON, Canada).

Video image analysis (VIA) software (Video Pro 32; Leading Edge, Blackwood, SA, Australia) was used to quantify the density of DAB staining in the cervical tissue sections. Staining was analyzed separately in the cervical epithelium and stroma. Cervical stroma was defined as being the region of tissue located directly beneath the basement membrane and was up to 2 mm deep. The mean area of positive staining “% positivity” value [(mean area of DAB stain/mean area of hematoxylin + DAB stain) × 100] was quantified in 5–10 low-power fields (×20 objective), depending on tissue size. Before each session of data collection, the VIA system was calibrated using a standard field of tissue. The interassay coefficient of variation was determined to be ~6%, calculated by analyzing an individual field (10 times) on 5 separate occasions. The intra-assay coefficient of variation was ~2%. Images of sections stained for FOXP3 were captured using a NanoZoomer digital system (Hamamatsu Photonics, Hamamatsu City, Japan), and numbers of FOXP3⁺ cells per square millimeter of epithelial and stromal ectocervical tissue were counted manually.

Quantitative real-time RT-PCR

RNA was recovered from cervical tissue biopsies by disruption in 500 µl TRIzol solution (Invitrogen, Carlsbad, CA) by homogenization using a pestle mixer (Scientific Specialties, Randallstown, MD) and extraction in 50 µl chloroform (Sigma-Aldrich). RNA was then precipitated from the aqueous phase in isopropanol (Sigma, Castle Hill, NSW, Australia), and the pellet was washed in ice-cold 75% ethanol, dried, and resuspended in

100 μ l Milli-Q water. After treatment with RNase-free DNase I (500 IU/ml; 60 min/37°C; Roche, Basel, Switzerland), first-strand cDNA was reverse transcribed from 5 μ g RNA using a Superscript-I Reverse Transcriptase kit (10 min/30°C, 45 min/42°C; Invitrogen). Primer pairs specific for published cytokine and chemokine cDNA sequences were designed and PCR conditions were optimized for each primer pair as previously described (23). Primer sequences, product sizes, gene target sites, and GenBank accession numbers for target sequences are shown in Supplemental Table II.

All cervical tissue samples were reverse transcribed in a single batch and were analyzed for a given primer set in the same PCR run. The PCR amplification used reagents supplied in a SYBR Green PCR Master Mix (Applied Biosystems), and each reaction volume (20 μ l total) contained 3 μ l cDNA, and 5' and 3' primers at concentrations of 0.1–1.0 μ M as previously described (23). All cytokine and chemokine primer sets were used with reaction cycle conditions of 95°C for 15 s and 60°C for 1 min, except for IL-6 primers, which were used at 95°C for 20 s, 60°C for 20 s, and 72°C for 1 min. The negative control included in each reaction consisted of H₂O substituted for cDNA. PCR amplification was performed in either an ABI Prism 5700 or 7000 Sequence Detection System (Applied Biosystems) to allow amplicon quantification using the arithmetic equation $2^{-Ct} \times 100 K^{-1}$ according to the manufacturer's instructions (Applied Biosystems User Bulletin #2), where Ct is the cycle number at which 50% maximal amplicon synthesis is achieved. Reaction products were analyzed by dissociation curve profile and by electrophoresis in 2% agarose gel (Promega, Madison, WI) containing 0.5 μ g/ml ethidium bromide followed by visualization over a UV light box and image capture using a Kodak digital camera. Representative PCR products were purified and sequenced at the Institute of Medical and Veterinary Science (Adelaide, SA, Australia) using Big Dye version 2 or 3 (Applied Biosystems) to confirm primer specificity. The efficiency of each PCR was confirmed to be >80% as determined by regression analysis of amplicon abundance versus cycle number.

Affymetrix GeneChip microarray

DNase-treated RNA from one pair of first and second biopsies from each treatment group was further purified using RNeasy Mini Spin columns (Qiagen, Valencia, CA) and eluted into RNase-free Milli-Q water according to the manufacturer's instructions. Five hundred nanograms of each RNA preparation was then sent to the Adelaide Microarray Facility for single-cycle labeling and hybridization to Affymetrix Human Gene 1.0 ST arrays (Affymetrix, Santa Clara, CA). This microarray chip is composed of 764,885 probes covering 28,869 genes (for the complete list, see <http://www.affymetrix.com>). RNA integrity analysis, hybridization, and washing were performed according to the manufacturer's instructions. The array data were analyzed using Partek Genomics Suite (Partek, St. Louis, MO). In brief, .cel files were imported using Robust Multichip Average background correction, guanosine/cytidine content correction, and mean probe summarization. The difference between signal for first biopsy (B1) and second biopsy (B2) was calculated (M value), and probe sets were classified as differentially expressed when M values were >1.0. Fold changes between probe sets were calculated as fold change = 2^M . Ingenuity Pathway Analysis software was used to identify gene pathways regulated by seminal fluid exposure.

The microarray data discussed in this publication have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (24) and are accessible through Gene Expression Omnibus Series accession no. GSE33745 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33745>).

Statistical analysis

SPSS version 17 (SPSS, Chicago, IL) was used to analyze complete data sets. Data were analyzed by paired *t* tests to determine differences between cell number or mRNA abundance values in first biopsy (B1) and second biopsy (B2) tissues within individual subjects. Statistical significance in differences between groups was concluded when $p < 0.05$. Gene symbols and protein names are according to nomenclature specified by HUGO Gene Nomenclature Committee.

Results

Seminal fluid elicits leukocyte influx into the cervical epithelium and stroma

To investigate whether exposure to semen at coitus alters leukocyte populations in the cervix, we collected ectocervical tissue biopsies from groups of women assigned to one of three groups: 1) no coitus, 2) vaginal coitus with use of a condom, or 3) unprotected vaginal coitus. Two sets of biopsies were recovered from all women: the

first biopsy close to the time of ovulation (LH+1) and the second biopsy 48 h later, 12 h after coitus (Fig. 1).

Immunohistochemical analysis of all leukocytes using CD45 (leukocyte common Ag) and several leukocyte lineage-restricted markers showed substantial effects of seminal fluid contact in both the stratified epithelial cell layers and the deeper stromal compartment. CD45⁺ leukocytes were detected within the cervical epithelium and stroma of all cervix tissues, with similar abundance in all women regardless of treatment group before coitus. Exposure to semen at coitus elicited an increase in total leukocytes evident throughout the tissue and particularly in the vicinity of the epithelial-stromal junction (Fig. 2A). Increased CD45⁺ leukocyte numbers were detected in women after unprotected coitus, with mean increases of 42 and 79% in the epithelium and stroma, respectively (both $p < 0.001$, paired *t* test; Fig. 3A). In contrast, there was little change in CD45⁺ leukocytes between the first and second biopsies in cervical tissues in the abstain group or condom-protected coitus group (Fig. 3A).

Macrophage and DC recruitment after coitus

Macrophages and DCs were the most abundant leukocytes in cervical tissue. CD11a (integrin α -chain, ITGAL), expressed by both macrophages and DCs, as well as neutrophils and some lymphocytes, was widely expressed in both the epithelial and stromal compartments of the cervix and showed mean increases after coitus of 130% ($p = 0.002$) and 133% ($p < 0.001$), respectively (Fig. 2B, 3B). The majority of these cells were macrophages, because CD14 expression increased 104% in the epithelium and 116% in the stroma (both $p < 0.001$), whereas little change was evident in the condom use and abstain groups (Fig. 3C, Table I). DCs and Langerhans cells identified using CD1a comprised a substantial population localized predominantly within the cervical epithelium with fewer cells in the stroma (Fig. 2C). CD1a⁺ cells present in the epithelium increased 41% after coitus ($p = 0.052$), but no consistent change was seen in the stroma (Fig. 3D). After coitus, macrophages and DCs appeared to accumulate predominantly in the stromal tissue in close proximity to the lamina propria at the epithelial interface (Fig. 2B, 2C).

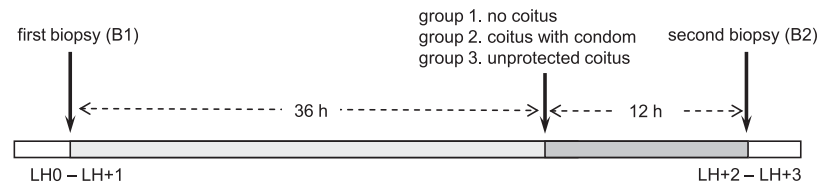
MHCII expression by activated macrophages and DCs was prevalent and was consistently increased in both the epithelium and stroma after unprotected coitus (Fig. 2D), with mean increases of 54 and 117%, respectively (both $p < 0.001$; Fig. 3E), but no change occurred in either control group. The costimulatory molecules CD80 (B7-1) and CD86 (B7-2) expressed by some activated macrophages and DCs were also examined. Both markers were sparsely and variably expressed in the cervical epithelium and stroma. CD86 was increased by 54% ($p = 0.012$) in DCs within the epithelial layer in tissues from the unprotected coitus group, but not in control group tissues (Table I).

CD15⁺ neutrophils comprised only a minor population of the leukocytes present in the cervix, and their number did not change significantly after coitus (Table I).

Lymphocyte recruitment after coitus

CD3⁺ T lymphocytes comprised a substantial population of leukocytes present in the cervix. Exposure to semen after coitus elicited a 33 and 47% increase (both $p = 0.040$) in the mean number of CD3⁺ T cells in the epithelium and stroma, respectively (Figs. 4A and 3F). The T cells present in the cervix at ovulation and recruited after unprotected coitus were mainly CD8⁺ T cells, with mean increases of 51 and 85% (both $p < 0.001$) in the epithelium and stroma, respectively (Fig. 4B, Table I). CD4⁺ T cells were less prevalent in both compartments and were decreased by 40% in the cervical stroma after coitus ($p = 0.033$; Table I).

FIGURE 1. Diagrammatic illustration of the study design, with timing of collection of first biopsy (B1) and second biopsy (B2) from subjects in three groups: 1) no coitus, 2) coitus with a condom, or 3) unprotected coitus. Details are given in *Materials and Methods*.



Expression of CD45RO, a marker of activated and memory T lymphocytes, was prevalent at ovulation and further elevated in the cervical epithelium and stroma after coitus, with mean increases of 131 and 118%, respectively (both $p < 0.001$; Fig. 4C, Table I). Naive or virgin T lymphocytes expressing CD45RA⁺ comprised a smaller proportion of the T cells present and were unchanged after coitus. No substantial changes were seen in any of these T cell markers in control group tissues (Table I).

Expression of FOXP3, a marker of regulatory T cells, was detected on a minor subpopulation of cells within the stromal compartment of all of the cervical tissue samples examined; however, there was substantial variation between individuals, and no significant effect of coitus was evident (Fig. 4D, Table I).

CD57⁺ NK cells were present in low numbers at ovulation. After unprotected coitus, their mean numbers increased by 93% in the epithelium and 101% in the stroma ($p = 0.025$ and $p = 0.008$). A significant increase of 40% was also seen in the stromal tissue

of women after condom-protected coitus ($p = 0.023$), but no change was seen in either tissue compartment in women who abstained (Table I).

Cytokine and chemokine mRNA expression after coitus: qRT-PCR analysis

To evaluate whether exposure to semen at coitus alters expression of cytokines in the cervix, we undertook qRT-PCR analysis on cDNA prepared from the duplicate of each cervical tissue biopsy collected at the time of ovulation or 48 h later. Expression of several cytokine genes shown previously to be induced by seminal fluid in mice or in human cells in vitro (25, 26) was evaluated.

Expression of several cytokine mRNAs was induced by semen exposure after unprotected coitus. The mean increase in *CSF2* mRNA expression was 2.5-fold ($p = 0.015$, paired *t* test; Fig. 5A). *IL1A* mRNA expression was increased 3.5-fold ($p = 0.028$; Fig. 5B). *IL6* mRNA expression was increased 2.6-fold ($p = 0.025$; Fig. 5C), and *IL8* mRNA expression was increased 17.3-fold ($p = 0.037$; Fig. 5D). No significant change in expression in *CSF2*, *IL1A*, *IL6*, or *IL8* was seen in tissues recovered from women after condom-protected coitus or in the abstinent control group. The increase in expression of these cytokines was evident regardless of whether expression in first (B1) and second (B2) biopsies from the same woman were compared by paired *t* test analysis, or alternatively whether data sets were combined according to group and compared by unpaired nonparametric analysis.

Several additional cytokine mRNAs were readily detected in cervical tissue at ovulation including *TNFA*, *IFNG*, and *LIF*, as well as chemokines *CCL2* (MCP-1), *CCL4* (MIP-1 β), *CCL5* (RANTES), and *CCL20* (MIP-3 α). Although there was evidence of increased expression after coitus in some women, these changes were not consistent and did not reach statistical significance (data not shown).

Cytokine and chemokine mRNA expression after coitus: microarray analysis

Finally, we used the remainder of the mRNA from cervical biopsies in a microarray experiment to further explore the effect of seminal fluid on gene expression in cervical tissue. One pair of first biopsy and second biopsy RNA samples from each treatment group were reverse transcribed into cDNA and hybridized to Affymetrix Human Gene 1.0 ST arrays. A total of 713 probe sets were identified as differentially expressed (fold change > 2) between first and second biopsies after unprotected coitus, with 436 genes upregulated and 277 genes downregulated. Ingenuity Pathway Analysis revealed that gene pathways including inflammatory response, immune response, immune cell trafficking, cellular movement, and Ag presentation were significantly affected by seminal fluid exposure. Among these were genes encoding several chemokines that target granulocytes, monocyte/macrophages, DCs and lymphocytes, proinflammatory cytokines and regulators of cytokine synthesis, PG pathway genes including *PTGS2* (cyclooxygenase 2 [COX-2]), and several matrix metalloproteinases (MMPs). Of these genes, no change or a substantially smaller change was seen between first and second biopsies obtained after coitus with condom use or abstinence (Table II).

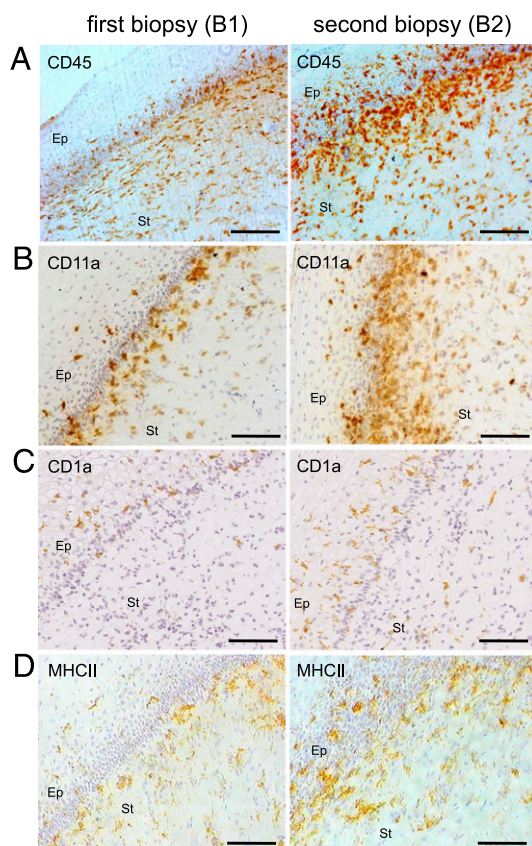


FIGURE 2. Effect of semen exposure after intercourse on myeloid cell populations in the cervix. Representative photomicrographs of first biopsy (B1) and second biopsy (B2) from subjects in the unprotected coitus group, depicting changes in leukocyte populations within the cervix after exposure to semen. Tissue sections were incubated with mAbs specific for CD45 (A), CD11a (B), CD1a (C), and MHCII (D). Original magnification $\times 20$. Scale bar, 100 μm . Ep, stratified epithelium; St, stromal tissue.

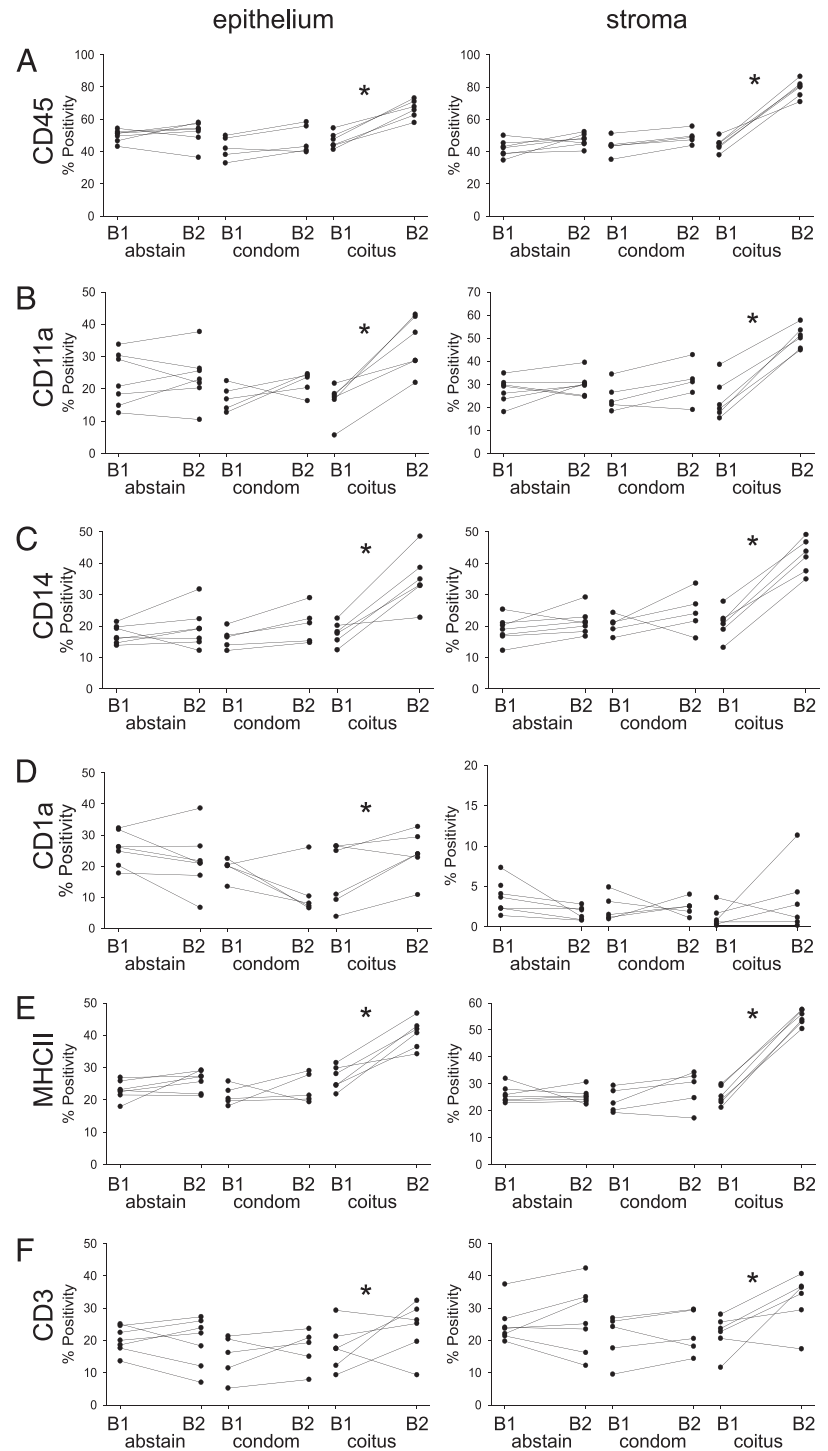


FIGURE 3. Effect of exposure to semen after intercourse on leukocyte numbers in the cervical epithelium and stroma. Mean percentage positivity was determined by VIA for CD45 (A), CD11a (B), CD14 (C), CD1a (D), MHCII (E), and CD3 (F) staining within cervical epithelium or stroma in first biopsy (B1) and second biopsy (B2) in subjects from three groups: 1) no coitus (abstain; $n = 7$), 2) coitus with a condom (condom; $n = 5$), or 3) unprotected coitus (coitus; $n = 6$). Data are values in first biopsy (B1) and second biopsy (B2) for each individual. Data were evaluated by paired t test. *Significant difference between B1 and B2; $p < 0.05$.

Discussion

By virtue of its rich population of DCs, macrophages, and lymphocytes, the cervix is recognized to be the major inductive and effector site for immune responses in the female genital tract (19). This study clearly demonstrates that exposure to seminal fluid at intercourse elicits substantial changes in the leukocyte populations in the cervix, initiating an inflammation-like response with effects that penetrate through the stratified epithelial layer and up to several millimeters deep into the stroma of the ectocervix. The actions of seminal fluid in the cervix are linked with induction of several cytokines and chemokines including GM-CSF, IL-6, IL-8, and IL-1A.

Infiltration of macrophages, DCs, and T lymphocytes into the cervix was observed in all seven women after unprotected coitus

and was evident across the full thickness of the cervical epithelium and subjacent stromal tissue. In contrast, condom-protected intercourse did not elicit any substantial change and was not distinguishable from tissues of abstinent women. The only significant effect attributable to coitus with a condom was an increase in cervical NK cells, but this was considerably smaller than in women with unprotected coitus. Together, the results indicate that a direct interaction between seminal constituents and cervical epithelial cells is an essential requirement for the postcoital inflammatory response, and that mechanical stimulation or injury sustained by the cervix after intercourse is not responsible.

The ectocervix was chosen as the preferred site to investigate after *in vitro* studies identified that ectocervical epithelial cells were

Table I. Leukocyte populations present within the cervical epithelium and stroma before and after abstinence, condom-protected intercourse, or unprotected intercourse

mAb	Group	Epithelium		Stroma	
		First Biopsy (B1) ^a	Second Biopsy (B2)	First Biopsy (B1)	Second Biopsy (B2)
CD1a	Abstain	25.8 ± 2.0%	21.9 ± 3.6%	3.7 ± 0.8%	1.7 ± 0.3%
	Condom	19.3 ± 1.5%	11.7 ± 3.7%	2.3 ± 0.8%	2.4 ± 0.5%
	Coitus	17.0 ± 4.1%	24.0 ± 3.1%*	1.2 ± 0.5%	3.4 ± 1.7%
CD3	Abstain	20.3 ± 1.6%	19.6 ± 2.9%	25.0 ± 2.3%	26.5 ± 3.9%
	Condom	14.9 ± 3.0%	17.4 ± 2.8%	20.9 ± 3.3%	22.5 ± 3.1%
	Coitus	17.9 ± 2.9%	23.8 ± 3.4%*	22.1 ± 2.3%	32.5 ± 3.4%*
CD4	Abstain	7.7 ± 0.5%	7.9 ± 0.4%	9.0 ± 0.9%	9.4 ± 0.9%
	Condom	5.9 ± 0.7%	6.4 ± 0.9%	8.5 ± 1.2%	9.1 ± 1.4%
	Coitus	6.9 ± 0.5%	4.2 ± 1.4%	7.8 ± 1.3%	3.8 ± 0.5%*
CD8	Abstain	17.6 ± 1.4%	18.1 ± 1.4%	14.6 ± 1.1%	16.8 ± 0.9%
	Condom	16.7 ± 0.9%	17.7 ± 1.1%	18.3 ± 1.0%	21.1 ± 1.2%
	Coitus	15.3 ± 1.5%	23.1 ± 2.3%*	16.2 ± 1.3%	29.6 ± 1.7%*
CD11a	Abstain	22.9 ± 3.1%	23.6 ± 3.1%	27.5 ± 2.1%	30.0 ± 1.8%
	Condom	17.1 ± 1.8%	21.9 ± 1.6%	24.6 ± 2.8%	30.3 ± 3.9%
	Coitus	16.4 ± 2.3%	33.8 ± 3.5%*	23.5 ± 3.5%	50.5 ± 2.0%*
CD14	Abstain	17.3 ± 1.1%	19.4 ± 2.4%	18.9 ± 1.5%	21.4 ± 1.5%
	Condom	16.1 ± 1.4%	20.5 ± 2.6%	20.4 ± 1.3%	24.5 ± 2.9%
	Coitus	17.8 ± 1.4%	35.2 ± 3.5%*	20.8 ± 2.0%	42.4 ± 2.2%*
CD15	Abstain	5.2 ± 1.2%	5.6 ± 0.9%	6.3 ± 1.4%	7.3 ± 1.4%
	Condom	5.1 ± 2.1%	5.5 ± 1.3%	5.7 ± 1.9%	6.5 ± 1.3%
	Coitus	4.8 ± 1.6%	6.7 ± 3.0%	8.1 ± 2.0%	10.6 ± 3.0%
CD45	Abstain	49.8 ± 1.4%	42.3 ± 3.2%	41.9 ± 1.9%	47.3 ± 1.6%
	Condom	42.3 ± 3.2%	47.6 ± 3.9%	43.6 ± 2.6%	49.1 ± 1.9%
	Coitus	46.9 ± 2.0%	66.4 ± 2.3%*	44.3 ± 1.7%	79.3 ± 2.2%*
CD45RA	Abstain	2.6 ± 0.2%	2.7 ± 0.1%	4.4 ± 0.2%	4.7 ± 0.3%
	Condom	2.4 ± 0.3%	2.4 ± 0.2%	4.6 ± 0.5%	5.2 ± 0.4%
	Coitus	2.5 ± 0.2%	3.2 ± 0.3%	4.6 ± 0.3%	6.0 ± 0.8%
CD45RO	Abstain	9.2 ± 1.6%	8.9 ± 0.7%	11.4 ± 1.2%	12.0 ± 1.1%
	Condom	6.8 ± 0.9%	7.9 ± 0.8%	10.4 ± 2.7%	12.7 ± 1.6%
	Coitus	8.6 ± 1.3%	19.3 ± 2.4%*	12.0 ± 1.3%	25.6 ± 2.3%*
CD57	Abstain	3.2 ± 0.5%	2.6 ± 0.4%	3.7 ± 0.3%	4.0 ± 0.4%
	Condom	2.3 ± 0.4%	2.5 ± 0.5%	3.1 ± 0.5%	4.2 ± 0.6%*
	Coitus	2.3 ± 0.1%	4.6 ± 0.8%*	3.3 ± 0.4%	6.3 ± 1.2%*
CD80	Abstain	0.20 ± 0.03%	0.22 ± 0.04%	0.52 ± 0.11%	0.74 ± 0.22%
	Condom	0.30 ± 0.07%	0.37 ± 0.07%	0.58 ± 0.15%	0.99 ± 0.40%
	Coitus	0.22 ± 0.04%	0.26 ± 0.04%*	0.49 ± 0.13%	1.72 ± 0.88%
CD86	Abstain	0.15 ± 0.01%	0.16 ± 0.01%	0.17 ± 0.02%	0.18 ± 0.02%
	Condom	0.17 ± 0.02%	0.22 ± 0.02%	0.30 ± 0.09%	0.47 ± 0.21%
	Coitus	0.14 ± 0.01%	0.21 ± 0.02%*	0.17 ± 0.02%	1.68 ± 0.71%*
FOXP3 ^b	Abstain	3.7 ± 0.6/mm ²	8.1 ± 2.0/mm ²	22.1 ± 11.7/mm ²	12.5 ± 1.8/mm ²
	Condom	7.2 ± 1.8/mm ²	6.0 ± 2.1/mm ²	28.3 ± 10.3/mm ²	24.6 ± 12.4/mm ²
	Coitus	8.8 ± 2.0/mm ²	6.7 ± 2.3/mm ²	19.4 ± 12.0/mm ²	17.7 ± 7.4/mm ²
MHCII	Abstain	23.0 ± 1.1%	26.0 ± 1.2%	26.0 ± 1.2%	25.3 ± 1.0%
	Condom	21.4 ± 1.3%	23.6 ± 2.0%	23.8 ± 2.0%	28.0 ± 3.1%
	Coitus	26.7 ± 1.5%	40.5 ± 1.9%*	25.5 ± 1.4%	54.7 ± 1.1%

^aData are mean ± SEM percentage positivity in the epithelial and stromal cell compartments of ectocervix biopsy tissue collected at first (B1) and second biopsy (B2) according to the protocol shown in Fig. 1 in subjects from three groups: 1) no coitus (abstain; *n* = 7), 2) coitus with a condom (condom; *n* = 5), or 3) unprotected coitus (coitus; *n* = 6). Data were evaluated by paired *t* test.

^bFOXP3 data are mean ± SEM number of positively labeled cells per square millimeter of cervical tissue in the epithelial and stromal cell compartments.

*Significant difference between B1 and B2; *p* < 0.05.

more responsive to seminal factors than epithelial cells from the endocervix or vagina (26). Differences between women in baseline leukocyte numbers can be attributed to factors including genetic differences, composition and load of bacterial microflora and viruses, ovarian steroid hormone levels, or sexual and reproductive history. Small changes in leukocyte numbers observed between the first and second biopsy in some women not exposed to seminal fluid were most likely caused by natural fluctuations of menstrual cycle stage. However, we cannot rule out the alternative explanation that the sampling procedure impacted local leukocyte parameters, with the first biopsy causing some slight disseminated inflammation.

Several previous reports identify macrophages and DCs as prevalent within the ectocervical epithelium and stroma (19, 27,

28). Their relative densities and distribution do not fluctuate substantially over the course of the menstrual cycle (19, 27), although leukocytes increase with infection (19) and can decrease with cervical neoplasia (29). Seminal fluid caused ~2-fold increases in both CD14⁺ macrophages and CD11a⁺ DCs. MHCII and CD11a were also substantially increased, consistent with a more activated phenotype in these cells after coitus. The costimulatory molecules CD80 and CD86 were expressed by only a minor subpopulation of DCs, indicating a diversity of maturation phenotypes. CD86 was increased ~10-fold after coitus, which together with elevated MHCII suggests competent Ag-presenting function in at least a subset of cervical DCs (30). Substantially smaller increases in macrophages were noted in superficial scrapings of the cervical epithelium after insemination (16, 17,

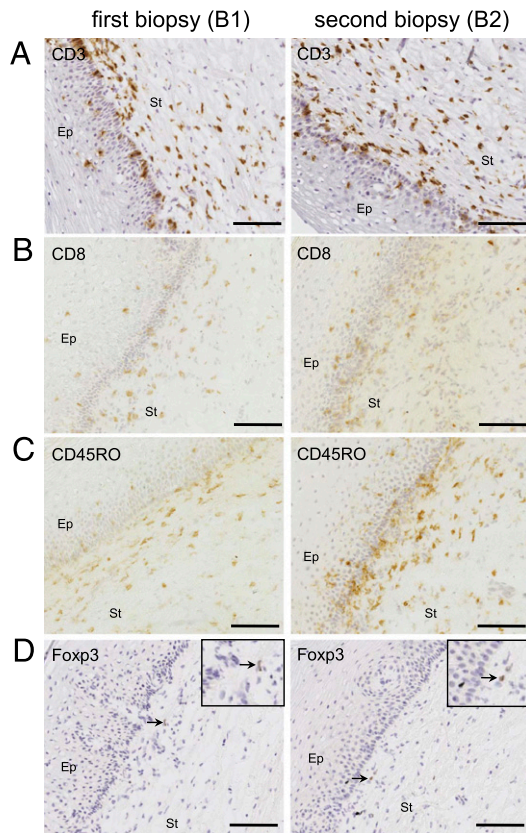


FIGURE 4. Effect of semen exposure after intercourse on lymphocyte populations in the cervix. Representative photomicrographs of first biopsy (B1) and second biopsy (B2) from subjects in the unprotected coitus group, depicting changes in lymphocytes within the cervix after exposure to semen. Tissue sections were incubated with mAbs specific for CD3 (**A**), CD8 (**B**), CD45RO (**C**), and FOXP3 (**D**). Insets in (**D**), original magnification $\times 100$; all other images, original magnification $\times 20$. Scale bar, 100 μm . Ep, stratified epithelium; St, stromal tissue.

31), implying that macrophages and DCs largely remain within the stromal tissue rather than traverse the epithelial surface.

The number of neutrophils detected both before and after intercourse were much lower than that observed in cervical scrapings after donor insemination (16, 17), which was surprising considering that neutrophils comprise $\sim 70\%$ of all leukocytes in the cervical mucus of nonpregnant women (32). Neutrophils are also prevalent after mating in the cervical and uterine tissues of mice, rats, rabbits, and pigs (3, 5–7). In earlier studies, neutrophils were recovered from the luminal surface 4 h after insemination (16, 17); therefore, it seems plausible that neutrophil migration into the lumen was complete before the second postcoital biopsy was taken. As previously reported (33), NK cells were infrequently found in the cervix even after coitus.

CD3⁺ T lymphocytes comprised a major population in the cervix, consistent with previous studies (27). Exposure to seminal fluid at coitus elicited a substantial increase in CD3⁺ T cells within both the epithelium and stroma. CD8⁺ cells were more common than CD4⁺ cells, in contrast with an earlier study where CD4⁺ cells outnumbered CD8⁺ cells (27). Few CD8⁺ T cells were present in the superficial epithelium and cervical mucus after donor insemination (17), implying that recruited T cells are generally retained within the tissue and do not emigrate into the lumen. Elevated CD4⁺ T cells in the cervical mucus are also linked with recent intercourse (31).

The majority of T cells recruited into the ectocervix expressed CD45RO but not CD45RA, indicating a memory phenotype. This

is consistent with previous encounter and priming to seminal fluid Ags, which would be expected in sexually active women not using barrier contraception. This contrasts with experiments in virgin mice, where reproductive tract T cells are less abundant and seminal fluid-induced population expansion requires several days to become detectable (14, 34). Notwithstanding the more rapid and predominantly memory T cell response we observed in women, the time frame of this study is not ideal for detecting T cell responses, which generally take >12 h to fully evolve.

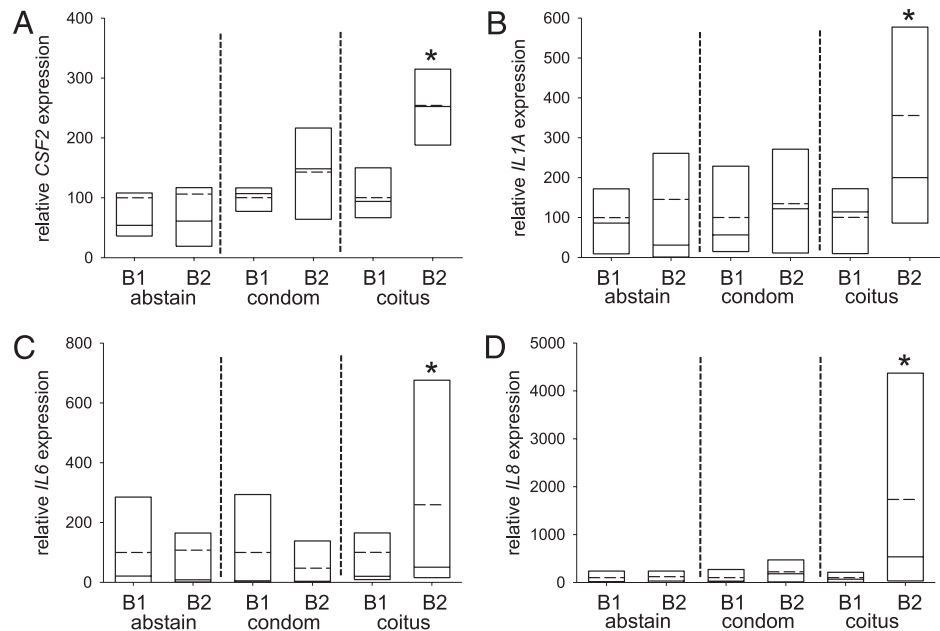
Consistent increases in cervical expression of genes encoding GM-CSF, IL-1A, IL-6, and IL-8 were demonstrated in cervical tissue after unprotected coitus, whereas no change occurred after coitus with a condom or abstinence. Elevated expression of a wide range of genes associated with inflammation and immune response pathways, notably in several chemokines and MMPs, as well as PTGS2 (COX-2), was indicated by microarray data. Many of these genes were previously demonstrated to be induced by seminal plasma in ectocervical epithelial cells (26) and cervical tissue explants (35) in vitro. Similar patterns of cytokines have been detected in cervical fluid recovered in vivo, with variations in abundance attributed to resident microflora and infection, and incidence of cigarette smoking (36, 37). In situ localization studies identify epithelial cells as the principal cellular source of GM-CSF (38, 39), IL-6 (39, 40), and IL-8 (41, 42) in the cervix.

Null mutation and cytokine add-back experiments in mice show that seminal fluid-induced cytokine synthesis in reproductive tract epithelial cells is the first step in the female response to seminal fluid and occurs upstream of inflammatory leukocyte recruitment (43–45). The data reported in this study are consistent with seminal plasma-induced cytokines acting to control leukocyte recruitment and activation. GM-CSF is identified as a key regulator of DC recruitment by human papillomavirus-transformed cervical epithelium (38), and IL-8 acts in synergy with GM-CSF to control neutrophil chemotaxis (46). In reproductive tract epithelium of rats, IL-6 production is reported to increase Ag presentation and subsequent Ab production (47), and induction of *IL6* expression in the cervix after intercourse may similarly facilitate Ag presentation. Nonimmune actions of cervical cytokines are also possible: IL-6 may enhance the fertilizing capacity of sperm, because this cytokine has been shown to trigger sperm capacitation in vitro (48, 49).

The identity of the signaling factors present in human seminal fluid that interact with female cervical cells is not fully defined, but TGF- β cytokines are implicated (50). TGF- β has been shown to mimic some aspects of the seminal response (35, 51) and is present in high concentrations in human seminal fluid (52). In addition, leukocyte recruitment into the cervical epithelium and stromal tissue is likely to be facilitated by chemokines present in the seminal plasma, which include IL-8, CCL2, CCL3, CCL4, and CCL5 (52, 53), and could be further influenced by the presence of microorganisms in the seminal fluid (54).

The precise physiological function of the postcoital inflammatory response in humans is not clear. Neutrophil efflux into the cervical canal is implicated in promoting the survival of viable, fertilizing sperm by selectively phagocytosing dead, abnormal, or nonfertilizing sperm (55), and could also assist in the removal of pathogens introduced at insemination (56). A third and potentially critical function for the macrophages and DCs recruited into the cervical stroma and epithelium after coitus is to activate immune responses to sperm and microbial Ags contained within the ejaculate. The increase in APCs induced by seminal fluid in the human cervix is reminiscent of findings in mice and pigs, where macrophages and DCs expressing MHCII, scavenger receptor, and other activation markers invade the uterine endometrium and en-

FIGURE 5. Effect of exposure to semen after intercourse on cytokine and chemokine mRNA expression in the cervix. Relative mRNA expression for *CSF2* (A), *IL1A* (B), *IL6* (C), and *IL8* (D) was determined by qRT-PCR in first biopsy (B1) and second biopsy (B2) cervical tissue in subjects from three groups: 1) no coitus (abstain; $n = 7$), 2) coitus with a condom (condom; $n = 5$), or 3) unprotected coitus (coitus; $n = 6$). Relative mRNA expression was calculated by normalizing data to *ACTB* mRNA expression, and data are plotted as relative expression in arbitrary units adjusted such that the mean value for B1 in each group is assigned a value of 100. Data are box plots showing the median and 95% confidence intervals, and the mean value is shown as a broken line. Data were evaluated by paired t test. *Significant difference between B1 and B2; $p < 0.05$.



gulf ejaculate material after mating (4, 7, 45, 57). Studies in mice show that these cells have a key function in cross-presenting seminal fluid Ags to activate cognate T cells (13). When seminal fluid recruitment of macrophages and DCs is compromised because of genetic *CSF2* deficiency, both the capacity to induce T cell responses to seminal fluid Ags and to regain tract sterility after mating is impaired (45, 58).

Based on our findings in mice (12, 14), we speculate that cervical T cells play a crucial role in the development of tolerogenic immune responses to male transplantation Ags contained within the ejaculate. The phenotype of any T cell response is relevant to the fate of sperm and, thus, future fertility. Moreover, in the event of conception, the immune response to seminal fluid influences

whether the female tract will allow conceptus implantation into the uterus, when the same paternal transplantation Ags are re-encountered on invading placental trophoblast cells (2). Studies in mice indicate that seminal fluid activates and expands populations of inducible regulatory T cells in lymph nodes draining the genital tract, which are subsequently recruited into the uterus to mediate immune tolerance at implantation (12, 14). In women, extent of previous exposure to seminal fluid Ags is linked with alloimmunization to partner's HLA Ags (59), and a benefit for fertility is shown in protection from pregnancy pathologies including preeclampsia and fetal growth disturbances (60). In this study, only a small proportion of the T cells recruited into the cervix after coitus expressed the signature regulatory T cell

Table II. Microarray analysis of seminal fluid regulation of cervical tissue gene expression

Accession No.	Gene ^a	Gene Description/Synonym	Abstain FC ^b	Condom FC	Coitus FC
NM_004591	<i>CCL20</i>	CCL20, MIP-3 α	↑ 1.87	↑ 1.13	↑ 4.04
NM_002989	<i>CCL21</i>	CCL21, 6Ckine	↓ 2.36	↑ 1.21	↑ 6.07
NM_001511	<i>CXCL1</i>	CXCL1; MGSA α	↓ 1.77	↑ 1.00	↑ 19.44
NM_002089	<i>CXCL2</i>	CXCL2, MIP-2 α	↑ 1.33	↓ 1.20	↑ 2.53
NM_002090	<i>CXCL3</i>	CXCL3, MIP-2 β	↑ 1.60	↓ 1.03	↑ 2.02
NM_002994	<i>CXCL5</i>	CXCL5, ENA-78	↓ 3.08	↓ 1.35	↑ 9.22
NM_002993	<i>CXCL6</i>	CXCL6, GCP-2	↓ 1.54	↓ 2.37	↑ 2.91
NM_018725	<i>IL17RB</i>	IL17R β	↑ 1.04	↑ 1.81	↑ 2.90
NM_000576	<i>IL1B</i>	IL-1 β	↑ 1.19	↑ 1.32	↑ 2.95
NM_000877	<i>IL1R1</i>	IL1R1	↓ 4.34	↓ 2.62	↑ 2.24
NM_181078	<i>IL4R</i>	IL4R	↑ 1.00	↑ 1.23	↑ 2.10
NM_000584	<i>IL8</i>	IL-8, CXCL8	↓ 1.18	↑ 1.82	↑ 6.93
NM_002310	<i>LIFR</i>	LIFR	↓ 3.68	↓ 1.52	↑ 2.07
NM_002421	<i>MMP1</i>	MMP1	↑ 1.71	↑ 1.30	↑ 6.43
NM_004530	<i>MMP2</i>	MMP2	↓ 2.54	↓ 1.78	↑ 2.16
NM_002422	<i>MMP3</i>	MMP3	↑ 1.37	↑ 1.01	↑ 2.49
NM_002423	<i>MMP7</i>	MMP7	↓ 1.51	↓ 1.22	↑ 4.66
NM_002425	<i>MMP10</i>	MMP10	↓ 1.24	↑ 1.35	↑ 7.42
NM_000963	<i>PTGS2</i>	PTGS2, COX-2	↓ 4.05	↓ 3.81	↑ 6.28
NM_003955	<i>SOCS3</i>	SOCS3	↑ 1.90	↓ 1.48	↑ 2.16
NM_176823	<i>S100A7</i>	S100A7	↑ 1.04	↑ 1.70	↑ 3.63
NM_024873	<i>TNFAIP3</i>	TNFAIP3	↓ 1.42	↑ 1.19	↑ 2.40

^aListed genes are identified as differentially expressed (>2-fold change) and are representative of several genes associated with inflammatory response, immune response, immune cell trafficking, cellular movement, and Ag presentation pathways according to Ingenuity Pathway Analysis.

^bGene expression data are given as fold change (FC; either upregulated [↑] or downregulated [↓]) from first to second biopsy, for one subject in each of the abstain, coitus with condom, and unprotected coitus groups.

transcription factor FOXP3 and could be expected to have a regulatory T cell phenotype, and this did not change substantively after coitus. Consistent with this, quantitative RT-PCR analysis of *FOXP3* mRNA did not reveal any consistent increase in expression after coitus, and similarly, no change in expression of mRNAs encoding the Th1 and Th2 transcription factors GATA3 and TBET was detectable (data not shown). However, firm conclusions on T lymphocyte phenotypes cannot be drawn from this experiment given the relatively small proportion of T cell mRNA in biopsy samples, and accurate phenotyping will require a flow cytometry or laser capture-based approach, preferably using samples recovered over a longer time course after coitus.

The large number of T lymphocytes within the cervix and evidence of their capacity to exert cytolytic activity suggests that cell-mediated immunity is an important function in the prevention of sexually transmitted infection (19, 56). The macrophages, DCs, and T cells recruited into the cervix presumably express CCR5 and CXCR4 receptors for HIV, and it can be envisaged that the increased number of target cells present in the cervix would increase the opportunity for HIV transmission. However, this may be counterbalanced by an inverse relationship between alloimmunization associated with seminal fluid exposure and susceptibility to HIV infection (59).

Together, the experiments described in this article indicate a hitherto unappreciated effect of seminal fluid on immune parameters in the human cervix. Through inducing expression of cytokines and chemokines, which, in turn, recruit and activate DCs, macrophages, and T lymphocytes, seminal fluid elicits an environment competent to initiate and boost adaptive immune responses required for tolerance of male Ags expressed in pregnancy by the semiallogeneic conceptus. More broadly, this response would be expected to impact all of the physiological and pathophysiological events to which cervical leukocytes contribute: the control of immune responses to sperm Ags and bacterial and viral pathogens, clearance of excess sperm and seminal debris, and tissue remodeling processes important at parturition and menstruation. Because the epithelial microenvironment is a critical factor in cervical metaplasia (21, 61), exposure to seminal fluids could also influence the incidence and progression of cervical cancer. Cytokines influencing the numbers and phenotypes of APCs would be expected to interact with seminal plasma-induced expression of tumorigenic and angiogenic factors (22) to favor carcinoma growth. Further studies are required to examine the duration of the response and whether seminal fluid signaling extends to the higher regions of the uterus to exert direct effects on embryo development and implantation, as well as to investigate the downstream effects of seminal fluid-induced immune responses on pathways affecting fertility and infertility, infection, and disease.

Disclosures

The authors have no financial conflicts of interest.

References

- Robertson, S. A. 2005. Seminal plasma and male factor signalling in the female reproductive tract. *Cell Tissue Res.* 322: 43–52.
- Robertson, S. A., L. R. Guerin, L. M. Moldenhauer, and J. D. Hayball. 2009. Activating T regulatory cells for tolerance in early pregnancy - the contribution of seminal fluid. *J. Reprod. Immunol.* 83: 109–116.
- De, M., R. Choudhuri, and G. W. Wood. 1991. Determination of the number and distribution of macrophages, lymphocytes, and granulocytes in the mouse uterus from mating through implantation. *J. Leukoc. Biol.* 50: 252–262.
- Robertson, S. A., V. J. Mau, K. P. Tremellen, and R. F. Seamark. 1996. Role of high molecular weight seminal vesicle proteins in eliciting the uterine inflammatory response to semen in mice. *J. Reprod. Fertil.* 107: 265–277.
- Phillips, D. M., and S. Mahler. 1977. Leukocyte emigration and migration in the vagina following mating in the rabbit. *Anat. Rec.* 189: 45–59.
- Lovell, J. W., and R. Getty. 1968. Fate of semen in the uterus of the sow: histologic study of endometrium during the 27 hours after natural service. *Am. J. Vet. Res.* 29: 609–625.
- O'Leary, S., M. J. Jasper, G. M. Warnes, D. T. Armstrong, and S. A. Robertson. 2004. Seminal plasma regulates endometrial cytokine expression, leukocyte recruitment and embryo development in the pig. *Reproduction* 128: 237–247.
- Scott, J. L., N. Ketheesan, and P. M. Summers. 2006. Leucocyte population changes in the reproductive tract of the ewe in response to insemination. *Reprod. Fertil. Dev.* 18: 627–634.
- Findlay, G. D., X. Yi, M. J. Maccoss, and W. J. Swanson. 2008. Proteomics reveals novel Drosophila seminal fluid proteins transferred at mating. *PLoS Biol.* 6: e178.
- Kachkache, M., G. M. Acker, G. Chaouat, A. Noun, and M. Garabédian. 1991. Hormonal and local factors control the immunohistochemical distribution of immunocytes in the rat uterus before conceptus implantation: effects of ovariectomy, fallopian tube section, and injection. *Biol. Reprod.* 45: 860–868.
- McMaster, M. T., R. C. Newton, S. K. Dey, and G. K. Andrews. 1992. Activation and distribution of inflammatory cells in the mouse uterus during the preimplantation period. *J. Immunol.* 148: 1699–1705.
- Robertson, S. A., L. R. Guerin, J. J. Bromfield, K. M. Branson, A. C. Ahlström, and A. S. Care. 2009. Seminal fluid drives expansion of the CD4+CD25+ T regulatory cell pool and induces tolerance to paternal alloantigens in mice. *Biol. Reprod.* 80: 1036–1045.
- Moldenhauer, L. M., K. R. Diener, D. M. Thring, M. P. Brown, J. D. Hayball, and S. A. Robertson. 2009. Cross-presentation of male seminal fluid antigens elicits T cell activation to initiate the female immune response to pregnancy. *J. Immunol.* 182: 8080–8093.
- Guerin, L. R., L. M. Moldenhauer, J. R. Prins, J. J. Bromfield, J. D. Hayball, and S. A. Robertson. 2011. Seminal fluid regulates accumulation of FOXP3+ regulatory T cells in the preimplantation mouse uterus through expanding the FOXP3+ cell pool and CCL19-mediated recruitment. *Biol. Reprod.* 85: 397–408.
- Tremellen, K. P., R. F. Seamark, and S. A. Robertson. 1998. Seminal transforming growth factor beta1 stimulates granulocyte-macrophage colony-stimulating factor production and inflammatory cell recruitment in the murine uterus. *Biol. Reprod.* 58: 1217–1225.
- Pandya, I. J., and J. Cohen. 1985. The leukocytic reaction of the human uterine cervix to spermatozoa. *Fertil. Steril.* 43: 417–421.
- Thompson, L. A., C. L. Barratt, A. E. Bolton, and I. D. Cooke. 1992. The leukocytic reaction of the human uterine cervix. *Am. J. Reprod. Immunol.* 28: 85–89.
- Robertson, S. A. 2010. Immune regulation of conception and embryo implantation—all about quality control? *J. Reprod. Immunol.* 85: 51–57.
- Pudney, J., A. J. Quayle, and D. J. Anderson. 2005. Immunological microenvironments in the human vagina and cervix: mediators of cellular immunity are concentrated in the cervical transformation zone. *Biol. Reprod.* 73: 1253–1263.
- Kelly, R. W., and H. O. Critchley. 1997. Immunomodulation by human seminal plasma: a benefit for spermatozoon and pathogen? *Hum. Reprod.* 12: 2200–2207.
- Delvenne, P., P. Hubert, and N. Jacobs. 2004. Epithelial metaplasia: an inadequate environment for antitumour immunity? *Trends Immunol.* 25: 169–173.
- Muller, M., K. J. Sales, A. A. Katz, and H. N. Jabbour. 2006. Seminal plasma promotes the expression of tumorigenic and angiogenic genes in cervical adenocarcinoma cells via the E-series prostanoid 4 receptor. *Endocrinology* 147: 3356–3365.
- Jasper, M. J., K. P. Tremellen, and S. A. Robertson. 2006. Primary unexplained infertility is associated with reduced expression of the T-regulatory cell transcription factor Foxp3 in endometrial tissue. *Mol. Hum. Reprod.* 12: 301–308.
- Edgar, R., M. Domrachev, and A. E. Lash. 2002. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res.* 30: 207–210.
- Robertson, S. A., G. Mayrhofer, and R. F. Seamark. 1992. Uterine epithelial cells synthesize granulocyte-macrophage colony-stimulating factor and interleukin-6 in pregnant and nonpregnant mice. *Biol. Reprod.* 46: 1069–1079.
- Sharkey, D. J., A. M. Macpherson, K. P. Tremellen, and S. A. Robertson. 2007. Seminal plasma differentially regulates inflammatory cytokine gene expression in human cervical and vaginal epithelial cells. *Mol. Hum. Reprod.* 13: 491–501.
- Poppe, W. A., M. Drijkoningen, P. S. Ide, J. M. Lauweryns, and F. A. Van Assche. 1998. Lymphocytes and dendritic cells in the normal uterine cervix. An immunohistochemical study. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 81: 277–282.
- Prakash, M., S. Patterson, and M. S. Kapembwa. 2001. Macrophages are increased in cervical epithelium of women with cervicitis. *Sex. Transm. Infect.* 77: 366–369.
- Morelli, A. E., C. Sananes, G. Di Paola, A. Paredes, and L. Fainboim. 1993. Relationship between types of human papillomavirus and Langerhans' cells in cervical condyloma and intraepithelial neoplasia. *Am. J. Clin. Pathol.* 99: 200–206.
- Manicassamy, S., and B. Pulendran. 2011. Dendritic cell control of tolerogenic responses. *Immunol. Rev.* 241: 206–227.
- Prakash, M., S. Patterson, F. Gotch, and M. S. Kapembwa. 2003. Recruitment of CD4 T lymphocytes and macrophages into the cervical epithelium of women after coitus. *Am. J. Obstet. Gynecol.* 188: 376–381.
- Okamura, K., Y. Yonemoto, Y. Hamazaki, H. Higashiiwai, and A. Yajima. 1988. Nitroblue tetrazolium reduction by leukocytes in the cervix of pregnant women. *Am. J. Obstet. Gynecol.* 159: 417–420.
- McKenzie, J., A. King, J. Hare, T. Fulford, B. Wilson, and M. Stanley. 1991. Immunocytochemical characterization of large granular lymphocytes in normal cervix and HPV associated disease. *J. Pathol.* 165: 75–80.

34. Pace, D., M. Longfellow, and J. N. Bulmer. 1991. Characterization of intraepithelial lymphocytes in human endometrium. *J. Reprod. Fertil.* 91: 165–174.
35. Denison, F. C., V. E. Grant, A. A. Calder, and R. W. Kelly. 1999. Seminal plasma components stimulate interleukin-8 and interleukin-10 release. *Mol. Hum. Reprod.* 5: 220–226.
36. Scott, M. E., Y. Ma, S. Farhat, S. Shiboski, and A. B. Moscicki. 2006. Covariates of cervical cytokine mRNA expression by real-time PCR in adolescents and young women: effects of *Chlamydia trachomatis* infection, hormonal contraception, and smoking. *J. Clin. Immunol.* 26: 222–232.
37. Lieberman, J. A., A. B. Moscicki, J. L. Sumerel, Y. Ma, and M. E. Scott. 2008. Determination of cytokine protein levels in cervical mucus samples from young women by a multiplex immunoassay method and assessment of correlates. *Clin. Vaccine Immunol.* 15: 49–54.
38. Hubert, P., F. van den Br ule, S. L. Giannini, E. Franzen-Detrooz, J. Boniver, and P. Delvenne. 1999. Colonization of in vitro-formed cervical human papillomavirus-associated (pre)neoplastic lesions with dendritic cells: role of granulocyte/macrophage colony-stimulating factor. *Am. J. Pathol.* 154: 775–784.
39. Woodworth, C. D., and S. Simpson. 1993. Comparative lymphokine secretion by cultured normal human cervical keratinocytes, papillomavirus-immortalized, and carcinoma cell lines. *Am. J. Pathol.* 142: 1544–1555.
40. Fichorova, R. N., M. Bajpai, N. Chandra, J. G. Hsiu, M. Spangler, V. Ratnam, and G. F. Doncel. 2004. Interleukin (IL)-1, IL-6, and IL-8 predict mucosal toxicity of vaginal microbicide contraceptives. *Biol. Reprod.* 71: 761–769.
41. Barclay, C. G., J. E. Brennan, R. W. Kelly, and A. A. Calder. 1993. Interleukin-8 production by the human cervix. *Am. J. Obstet. Gynecol.* 169: 625–632.
42. Fichorova, R. N., and D. J. Anderson. 1999. Differential expression of immunobiological mediators by immortalized human cervical and vaginal epithelial cells. *Biol. Reprod.* 60: 508–514.
43. Robertson, S. A., M. Allanson, and V. J. Mau. 1998. Molecular regulation of uterine leukocyte recruitment during early pregnancy in the mouse. *Trophoblast Res.* 11 (Suppl. 1): 101–119.
44. Robertson, S. A., A. C. O'Connell, S. N. Hudson, and R. F. Seamark. 2000. Granulocyte-macrophage colony-stimulating factor (GM-CSF) targets myeloid leukocytes in the uterus during the post-mating inflammatory response in mice. *J. Reprod. Immunol.* 46: 131–154.
45. Moldenhauer, L. M., S. N. Keenihan, J. D. Hayball, and S. A. Robertson. 2010. GM-CSF is an essential regulator of T cell activation competence in uterine dendritic cells during early pregnancy in mice. *J. Immunol.* 185: 7085–7096.
46. Shen, L., J. V. Fahey, S. B. Hussey, S. N. Asin, C. R. Wira, and M. W. Fanger. 2004. Synergy between IL-8 and GM-CSF in reproductive tract epithelial cell secretions promotes enhanced neutrophil chemotaxis. *Cell. Immunol.* 230: 23–32.
47. Prabhala, R. H., and C. R. Wira. 1995. Sex hormone and IL-6 regulation of antigen presentation in the female reproductive tract mucosal tissues. *J. Immunol.* 155: 5566–5573.
48. Laflamme, J., A. Akoum, and P. Leclerc. 2005. Induction of human sperm capacitation and protein tyrosine phosphorylation by endometrial cells and interleukin-6. *Mol. Hum. Reprod.* 11: 141–150.
49. Naz, R. K., and P. Kaplan. 1994. Interleukin-6 enhances the fertilizing capacity of human sperm by increasing capacitation and acrosome reaction. *J. Androl.* 15: 228–233.
50. Robertson, S. A., W. V. Ingman, S. O'Leary, D. J. Sharkey, and K. P. Tremellen. 2002. Transforming growth factor beta—a mediator of immune deviation in seminal plasma. *J. Reprod. Immunol.* 57: 109–128.
51. Gutsche, S., M. von Wolff, T. Strowitzki, and C. J. Thaler. 2003. Seminal plasma induces mRNA expression of IL-1beta, IL-6 and LIF in endometrial epithelial cells in vitro. *Mol. Hum. Reprod.* 9: 785–791.
52. Maegawa, M., M. Kamada, M. Irahara, S. Yamamoto, S. Yoshikawa, Y. Kasai, Y. Ohmoto, H. Gima, C. J. Thaler, and T. Aono. 2002. A repertoire of cytokines in human seminal plasma. *J. Reprod. Immunol.* 54: 33–42.
53. Politch, J. A., L. Tucker, F. P. Bowman, and D. J. Anderson. 2007. Concentrations and significance of cytokines and other immunologic factors in semen of healthy fertile men. *Hum. Reprod.* 22: 2928–2935.
54. Bezold, G., J. A. Politch, N. B. Kiviat, J. M. Kuypers, H. Wolff, and D. J. Anderson. 2007. Prevalence of sexually transmissible pathogens in semen from asymptomatic male infertility patients with and without leukocytospermia. *Fertil. Steril.* 87: 1087–1097.
55. Tomlinson, M. J., A. White, C. L. Barratt, A. E. Bolton, and I. D. Cooke. 1992. The removal of morphologically abnormal sperm forms by phagocytes: a positive role for seminal leukocytes? *Hum. Reprod.* 7: 517–522.
56. White, H. D., G. R. Yeaman, A. L. Givan, and C. R. Wira. 1997. Mucosal immunity in the human female reproductive tract: cytotoxic T lymphocyte function in the cervix and vagina of premenopausal and postmenopausal women. *Am. J. Reprod. Immunol.* 37: 30–38.
57. Watson, J. G., J. Carroll, and S. Chaykin. 1983. Reproduction in mice: the fate of spermatozoa not involved in fertilization. *Gamete Res.* 7: 75–84.
58. Robertson, S. A., C. T. Roberts, K. L. Farr, A. R. Dunn, and R. F. Seamark. 1999. Fertility impairment in granulocyte-macrophage colony-stimulating factor-deficient mice. *Biol. Reprod.* 60: 251–261.
59. Peters, B., T. Whittall, K. Babaahmady, K. Gray, R. Vaughan, and T. Lehner. 2004. Effect of heterosexual intercourse on mucosal alloimmunisation and resistance to HIV-1 infection. *Lancet* 363: 518–524.
60. Kho, E. M., L. M. McCowan, R. A. North, C. T. Roberts, E. Chan, M. A. Black, R. S. Taylor, and G. A. Dekker; SCOPE Consortium. 2009. Duration of sexual relationship and its effect on preeclampsia and small for gestational age perinatal outcome. *J. Reprod. Immunol.* 82: 66–73.
61. Giannini, S. L., P. Hubert, J. Doyen, J. Boniver, and P. Delvenne. 2002. Influence of the mucosal epithelium microenvironment on Langerhans cells: implications for the development of squamous intraepithelial lesions of the cervix. *Int. J. Cancer* 97: 654–659.