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

David J. Sharkey,* Kelton P. Tremellen,**† Melinda J. Jasper,* Kristina Gemzell-Danielsson,‡ and Sarah A. Robertson*

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.

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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 tetrachloride; DC, dendritic cell; LH, luteinizing hormone; MHCII, MHC class II; MMP, matrix metalloproteinase; NHS, normal human serum; qRT-PCR, quantitative RT-PCR; VDA, video image analysis.

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...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 drenched with methanol 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.

Materials and Methods

Subjects 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 a non-smoker or who had smoked less than 15 cigarettes per day for longer than 1 mo were included 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 = 6) and 3) coitus without condom (n = 6). Clinical and cervical biopsies (1 cm from the transformation zone) were taken from adjacent sites in the ectocervix, ∼1 cm from the transformation zone. Couples then abstained 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 drenched with methanol 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.

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 Abs including CD1a (HE149), 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 monocyte 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 were then incubated with biotinylated goat anti-mouse secondary Ab (1:300 in PBS with 1% BSA and 10% normal horse 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 tetrachloride (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 xylene, 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 nm 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 (Scientific, RANDALLSCIENCE, North York, ON, Canada) and then extraction with 100 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...
allow amplicon quantification using the arithmetic equation $2^{\Delta\Delta C_{t}}$. PCR amplification was performed in either an ABI 7000 or 3000 PCR system. RT-PCR was performed using TaqMan gene expression assays (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**

DNAase-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, quantile coexpression 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 $= 2^{\Delta C_{t}}$. 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).

**Macrophase and DC recruitment after coitus**

Macrophages and DCs were the most abundant leukocytes in cervical tissue. CD11a (integrin $\alpha$-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 laminar propria at the epithelial interface (Fig. 2B, 2C).

**MHCII expression by activated macrophages and DCs**

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).

CD14+ 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).

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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 non-parametric 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-1β), CCL5 (RANTES), and CCL20 (MIP-3α). 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).
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

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**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.
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 expla-
nation 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 in-
creases in both CD14+ macrophages and CD1a+ 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 sub-
population of DCs, indicating a diversity of maturation phe-
notypes. CD86 was increased ~10-fold after coitus, which to-
gether with elevated MHCII suggests competent Ag-presenting 
function in at least a subset of cervical DCs (30). Substan-
tially smaller increases in macrophages were noted in superficial 
scrapings of the cervical epithelium after insemination (16, 17,
Elevated CD4+ T cells in the cervical mucus are also linked generally retained within the tissue and do not emigrate into the lumen after donor insemination (17), implying that recruited T cells are present in the superficial epithelium and cervical mucus after coitus, consistent with previous studies (27). Exposure to seminal fluids at coitus elicited a substantial increase in CD3+ T cells within the cervix, consistent with previous studies (27).

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 ~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 eflux 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 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 ×100; all other images, original magnification ×20. Scale bar, 100 μm. Ep, stratified epithelium; St, stromal tissue.
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.

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

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<td>NM_002993</td>
<td>CXL6</td>
<td>CXL6, GCP-2</td>
<td>↑ 1.04</td>
<td>↑ 1.81</td>
<td>↑ 2.90</td>
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<tr>
<td>NM_015725</td>
<td>IL17RB</td>
<td>IL17RB</td>
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<td>↑ 1.32</td>
<td>↑ 2.95</td>
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<tr>
<td>NM_000376</td>
<td>IL1B</td>
<td>IL-1β</td>
<td>↓ 2.62</td>
<td>↓ 2.62</td>
<td>↓ 2.24</td>
</tr>
<tr>
<td>NM_000877</td>
<td>ILR1</td>
<td>IL-1R1</td>
<td>↑ 4.34</td>
<td>↑ 4.34</td>
<td>↑ 2.24</td>
</tr>
<tr>
<td>NM_181078</td>
<td>IL4R</td>
<td>IL-4R</td>
<td>↑ 1.00</td>
<td>↑ 1.23</td>
<td>↑ 2.10</td>
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<tr>
<td>NM_000584</td>
<td>IL8</td>
<td>IL-8, CXL8</td>
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<td>↑ 1.82</td>
<td>↑ 6.93</td>
</tr>
<tr>
<td>NM_002310</td>
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<td>LIFR</td>
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<td>↓ 5.2</td>
<td>↓ 2.07</td>
</tr>
<tr>
<td>NM_002421</td>
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<td>↑ 1.71</td>
<td>↑ 1.30</td>
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</tr>
<tr>
<td>NM_004530</td>
<td>MMP2</td>
<td>MMP2</td>
<td>↓ 2.54</td>
<td>↓ 1.78</td>
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<tr>
<td>NM_002422</td>
<td>MMP3</td>
<td>MMP3</td>
<td>↑ 1.37</td>
<td>↑ 1.01</td>
<td>↑ 2.49</td>
</tr>
<tr>
<td>NM_002423</td>
<td>MMP7</td>
<td>MMP7</td>
<td>↓ 1.51</td>
<td>↓ 1.22</td>
<td>↓ 4.66</td>
</tr>
<tr>
<td>NM_002425</td>
<td>MMP10</td>
<td>MMP10</td>
<td>↓ 2.42</td>
<td>↓ 3.5</td>
<td>↓ 7.42</td>
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<tr>
<td>NM_000963</td>
<td>PTGS2</td>
<td>PTGS2, COX-2</td>
<td>↓ 4.05</td>
<td>↓ 3.81</td>
<td>↓ 6.28</td>
</tr>
<tr>
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<td>SOCS3</td>
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<td>NM_176823</td>
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<td>SI100A</td>
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<td>↑ 1.70</td>
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<tr>
<td>NM_024873</td>
<td>TNFAIP3</td>
<td>TNFAIP3</td>
<td>↓ 1.42</td>
<td>↓ 1.19</td>
<td>↓ 2.40</td>
</tr>
</tbody>
</table>

*Listed 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 cellular 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
Supplemental Table I. The antigen and cell lineage specificities of the mouse anti-human monoclonal antibodies used for immunohistochemical analysis of cervical tissues.

<table>
<thead>
<tr>
<th>Antigenic specificity</th>
<th>Clone</th>
<th>Reactive cell lineages</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1a</td>
<td>HI149</td>
<td>DCs, Langerhan cells</td>
</tr>
<tr>
<td>CD3</td>
<td>OKT3</td>
<td>pan T-lymphocytes</td>
</tr>
<tr>
<td>CD4</td>
<td>OKT4</td>
<td>T-lymphocytes</td>
</tr>
<tr>
<td>CD8</td>
<td>OKT8</td>
<td>T-lymphocytes</td>
</tr>
<tr>
<td>CD11a (LFA-1α)</td>
<td>HB202</td>
<td>macrophages, DCs, neutrophils and some lymphocytes</td>
</tr>
<tr>
<td>CD14</td>
<td>FMC17</td>
<td>monocyte / macrophages</td>
</tr>
<tr>
<td>CD15</td>
<td>FMC13</td>
<td>neutrophils</td>
</tr>
<tr>
<td>CD45 (LCA)</td>
<td>FMC51</td>
<td>all leukocytes</td>
</tr>
<tr>
<td>CD45RA</td>
<td>FMC71</td>
<td>naïve / virgin T-lymphocyte</td>
</tr>
<tr>
<td>CD45RO</td>
<td>UCHL1</td>
<td>activated / memory T-lymphocytes</td>
</tr>
<tr>
<td>CD57</td>
<td>TIB200</td>
<td>natural killer cells</td>
</tr>
<tr>
<td>CD80</td>
<td>L307.4</td>
<td>activated macrophages, DCs and B cells</td>
</tr>
<tr>
<td>CD86</td>
<td>IT2.2</td>
<td>activated DCs</td>
</tr>
<tr>
<td>FOXP3</td>
<td>236A/E7</td>
<td>regulatory T-lymphocytes</td>
</tr>
<tr>
<td>MHCII</td>
<td>FMC52</td>
<td>activated macrophages, DCs, some B cells</td>
</tr>
</tbody>
</table>

DC = dendritic cell
Supplemental Table II. PCR primers used to quantify cytokine and chemokine mRNA expression.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Synonym</th>
<th>Nt position</th>
<th>Primer sequence</th>
<th>Product size</th>
<th>Genbank</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ACTB</strong></td>
<td>β-actin</td>
<td>1350 (fwd) 1511 (rev)</td>
<td>5’ TGTGATGGTGGTATGGTGGTGC 3’ ACACGCAGGCTATTGTA</td>
<td>162 bp</td>
<td>NM_001101</td>
</tr>
<tr>
<td><strong>CSF2</strong></td>
<td>GM-CSF</td>
<td>91 (fwd) 167 (rev)</td>
<td>5’ AGCCCTGGGAGCATGTGA 3’ ATCTCAGCAGGTGTCTCTACTC</td>
<td>77 bp</td>
<td>NM_000758</td>
</tr>
<tr>
<td><strong>FOXP3</strong></td>
<td></td>
<td>939 (fwd) 1026 (rev)</td>
<td>5’ AGATGCGAGGCACCGACTACTC 3’ AGGAGCCCTTTGTCGGATGAT</td>
<td>88 bp</td>
<td>NM_014009</td>
</tr>
<tr>
<td><strong>GATA3</strong></td>
<td></td>
<td>979 (fwd) 1070 (rev)</td>
<td>5’ GAGAAGGAGGTGTCCAGGATGAT</td>
<td>92 bp</td>
<td>X58072</td>
</tr>
<tr>
<td><strong>IL1A</strong></td>
<td>IL1α</td>
<td>263 (fwd) 453 (rev)</td>
<td>5’ CCAACGGGAACTTTTCTAAG 3’ GGCTTGTTCCTCACTACTC</td>
<td>191 bp</td>
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<tr>
<td><strong>IL6</strong></td>
<td>IL6</td>
<td>188 (fwd) 470 (rev)</td>
<td>5’ ACTACCTTCCTTCAGAAGC 3’ GGCTTGTTCCTCACTACTC</td>
<td>283 bp</td>
<td>NM_000600</td>
</tr>
<tr>
<td><strong>IL8</strong></td>
<td>CXCL8</td>
<td>131 (fwd) 285 (rev)</td>
<td>5’ GGGAGCATGATTCCCCTCTG 3’ CGCAGTGTGGTCTCCACTCTCA</td>
<td>155 bp</td>
<td>NM_000584</td>
</tr>
<tr>
<td><strong>LIF</strong></td>
<td>LIF</td>
<td>101 (fwd) 165 (rev)</td>
<td>5’ GTGACGGCCCAATAAAGGT 3’ TTCAGTGCAGGAGAATTCTA</td>
<td>65 bp</td>
<td>NM_002309</td>
</tr>
<tr>
<td><strong>CCL2</strong></td>
<td>MCP-1</td>
<td>64 (fwd) 130 (rev)</td>
<td>5’ CGCCTCCAGCATGAAATGCTC 3’ GGGAATGAGGTGGCTGACTA</td>
<td>67 bp</td>
<td>NM_0002982</td>
</tr>
<tr>
<td><strong>CCL20</strong></td>
<td>MIP-3α</td>
<td>83 (fwd) 171 (rev)</td>
<td>5’ CTGTCTTTGATATGTGGTCTCTG 3’ TCTGTGTATCCCAAGACAGCAGTCA</td>
<td>89 bp</td>
<td>NM_004591</td>
</tr>
<tr>
<td><strong>CCL4</strong></td>
<td>MIP-1β</td>
<td>139 (fwd) 205 (rev)</td>
<td>5’ AGCGCTCTCAACAGGAAATG 3’ CTCTTCCAGCGGTGTTAAAGAAA</td>
<td>67 bp</td>
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</tr>
<tr>
<td><strong>CCL5</strong></td>
<td>RANTES</td>
<td>90 (fwd) 161 (rev)</td>
<td>5’ CTGTGTCAGCATCCTTCAGATGCT 3’ TGGGTGTTCCAGGAATATGG</td>
<td>62 bp</td>
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<tr>
<td><strong>TBX21</strong></td>
<td>T-bet</td>
<td>725 (fwd) 837 (rev)</td>
<td>5’ AACACAGGACCGCAGCATTG 3’ TGGAGGACTGGAGCACAAT</td>
<td>113 bp</td>
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<tr>
<td><strong>TNFA</strong></td>
<td>TNFα</td>
<td>2531 (fwd) 2626 (rev)</td>
<td>5’ GACGACACTATTCTGGACCTTATGC 3’ GGAGGCGTTTGGAAGGT</td>
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