Seminal Fluid Enhances Replication of Human T-Cell Leukemia Virus Type 1: Implications for Sexual Transmission

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Seminal fluid enhanced human T-cell leukemia virus type 1 (HTLV-1) infection by transactivating the HTLV-1 long terminal repeat promoter, which is chromosomally integrated in a cell-type-dependent manner. Our data may indicate a potential role for seminal fluid in the sexual transmission of HTLV-1 and imply complex features of regulation of HTLV-1 expression.

Human T-cell leukemia virus type 1 (HTLV-1), the causative agent of adult T-cell leukemia and HTLV-associated myelopathy, is transmitted vertically via breastfeeding and horizontally via sexual intercourse. Male-to-female transmission occurs exceedingly more frequently than female-to-male transmission (15). Given such a disproportion between genders in susceptibility to sexual transmission of HTLV-1, it is possible that a semen-derived factor(s) facilitates male-to-female transmission (10–12). We show here that seminal fluid enhances in vitro HTLV-1 infection. We also report that the seminal fluid-mediated effect on HTLV-1 expression requires its chromosomal integration and is cell type specific.

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Seminal fluid enhances HTLV-1 replication and transmission. Seminal fluid was prepared from healthy male volunteers as described previously (1). Peripheral blood mononuclear cells (PBMC) obtained from asymptomatic HTLV-1 carriers were cultured in the presence or absence of seminal fluid.

HTLV-1 p19 antigen levels were measured as described previously (10). Cellular DNA was extracted by a QIAamp blood DNA kit (QIAGEN K.K., Tokyo, Japan) and subjected to PCR with a QuantiTect SYBR Green PCR kit (QIAGEN). The upstream and downstream primer sequences in the HTLV-1 tax gene that were selected for PCR analysis were 5’-CCCACCTTCCAGGTTTGGACAGAG-3’ and 5’-CTG TAGAGCTGAGCCGATAACGCG-3’, respectively. Quantitative determination of the amplified products was done with the iCycler iQ Real-Time Detection System (Bio-Rad Laboratories, Inc., Hercules, Calif.). Heat activation (15 min at 95°C) of hot-start Taq polymerase was followed by 50 cycles of denaturation (30 s at 95°C), annealing (30 s at 50°C), and extension (30 s at 72°C). The cell numbers used for PCR analysis were verified using those of tubulin mRNA.

Donor Seminal fluid Proviral load (copies/100 PBMC) (Tax mRNA/tubulin mRNA) × 100 p19 antigen (pg/ml)
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\textsuperscript{a} PBMC from six asymptomatic HTLV-1 carriers were propagated, and 3 million PBMC each were cultured in the presence or absence of seminal fluid (1%). Cell-free supernatants and cell pellets were collected on day 5. DNA and RNA were purified from the cell pellets, and real-time PCR and real-time RT-PCR were performed to estimate proviral loads and Tax mRNA levels, respectively. HTLV-1 p19 antigen levels in cell-free supernatants were determined by enzyme-linked immunosorbent assay.

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HTLV-1 infection, HTLV-1 replication in the coculture largely depends on viral transmission to PBMC from HTLV-1-uninfected individuals (4, 10). Seminal fluid increased HTLV-1-infected cell numbers in this coculture system but had little effect on infected cell numbers in MMC-treated PBMC alone (Fig. 1A), suggesting that seminal fluid facilitated de novo HTLV-1 infection in PBMC derived from uninfected donors.

**Seminal fluid upregulates expression from the HTLV-1 LTR.** Since we have previously demonstrated that certain seminal fluid-derived factors can transactivate the HTLV-1 long terminal repeat (LTR) (10–12), we investigated the effects of seminal fluid on the HTLV-1 LTR. Plasmid pHTLV-luc, provided by K.-T. Jeang (National Institute of Allergy and Infectious Diseases, Bethesda, Md.) (7), was transfected into PBMC with a Human T-Cell Nucleofector kit (Amaxa Biosystems) as described previously (13). Transfections of HeLa (cervical carcinoma) cells, CHOK1 cells, HeLa-luc cells, or CHOK1-luc cells (containing a chromosomally integrated HTLV-1 LTR-driven luciferase gene), also gifts of K.-T. Jeang (14), were performed by a modified calcium phosphate method (9). Transfection efficiency in these adherent cells was tested by cotransfection with pMACS14.1 (Miltenyi Biotec, Bergisch Gladbach, Germany), followed by flow cytometry for CD14 expression, and was found to be comparable (data not shown).

Unexpectedly, seminal fluid downregulated HTLV-1 LTR activity in HeLa cells (Fig. 1B). Interestingly, however, it enhanced HTLV-1 LTR activity in HeLa-luc cells (Fig. 1B), indicating that seminal fluid has opposing effects on the HTLV-1 LTR, depending on whether it exists episomally or is chromosomally integrated. The effects mediated by seminal fluid appear to be cell type dependent, because it had no effect on HTLV-1 LTR activity in CHO or CHO-luc cells (Fig. 1B). Trypan blue staining demonstrated that the seminal fluid concentrations used in these experiments were not toxic to those cell lines (data not shown). To clarify how seminal fluid influences HTLV-1 LTR activity in PBMC, we transfected carriers’

![FIG. 1. Seminal fluid-mediated effects on HTLV-1 infection. (A)](image)

(A) Seminal fluid facilitates de novo HTLV-1 infection. PBMC were isolated from three asymptomatic HTLV-1 carriers and treated with MMC as described previously (2). MMC-treated, HTLV-1-infected PBMC were cultured either alone or with PBMC derived from healthy uninfected donors at a ratio of 1:1. Where indicated, seminal fluid (1%) was added to the cultures. On day 7, whole cultures were harvested for DNA purification and proviral loads were determined by real-time PCR. Proviral loads in MMC-treated, HTLV-1-infected PBMC in the absence of seminal fluid were between 0.82 and 1.3 copies per 100 PBMC, and the results shown are means ± standard errors shown as proviral loads relative to them. Student t tests were performed for statistical significance (*, P < 0.05; **, P ≥ 0.05). (B) Differential effects of seminal fluid on HTLV-1 transcription. HeLa and CHOK1 cells were transfected with pHTLV-luc and pMT-Tax, while HeLa-luc and CHOK1-luc cells were transfected with pMT-Tax alone. The transfected cells were left untreated or treated with the indicated concentrations of seminal fluid and harvested for luciferase assays 2 days after transfection. Data are means ± standard errors from six independent experiments, and results are shown as fold induction relative to the luciferase activity in untreated (control) cells. Student t tests were performed for statistical significance (*, P < 0.05; **, P ≥ 0.05). (C) Seminal fluid increases expression from integrated provirus but decreases expression from transfected plasmid in PBMC. PBMC derived from asymptomatic HTLV-1 carriers were transfected with pHTLV-luc and either left untreated or treated with the indicated concentrations of seminal fluid. The transfected cells were harvested 2 days later, cell lysates were subjected to luciferase assays, and RNA purified from the cells was subjected to real-time RT-PCR for Tax and tubulin mRNAs. The data shown are means ± standard errors from six independent experiments, and results are shown as fold induction relative to those in untreated (control) cells. Student t tests were performed for statistical significance (*, P < 0.05; **, P ≥ 0.05).
PBMC with pHTLV-luc and treated the transfected cells with seminal fluid. LTR activity from the episomal plasmid was determined by luciferase assay, and LTR activity from the integrated proviral DNA was inferred on the basis of Tax mRNA levels. As shown in Fig. 1C, seminal fluid downregulated the activity of the episomal HTLV-1 LTR while upregulating expression from the integrated HTLV-1 LTR. These results suggest that seminal fluid can enhance the transcriptional activation of proviral DNA in carriers' PBMC, probably contributing to seminal fluid-induced HTLV-1 replication and transmission.

Sexual, particularly male-to-female, transmission has been critical for the coexistence of HTLV-1 with the host because infected females subsequently transmit the virus to the next generation. Male-to-female transmission is exceedingly more efficient than female-to-male transmission, at least in part because this virus is highly cell associated (15), although involvement of cell-free virus in sexual transmission was not ruled out. Furthermore, male-to-female transmission may also be potenti ated by the fact that the target tissue in the female genital tract is greater in size than that in the male genital tract.

This study suggests that seminal fluid-derived factors may play a role in sexual transmission. We have previously demonstrated that prostaglandin E2 (10), lactoferrin (11), and transforming growth factor β (12), all of which are major constituents of seminal fluid, could enhance in vitro HTLV-1 replication. However, while they upregulated HTLV-1 LTR activity in transient-expression assays (10–12), seminal fluid-mediated activity upregulated chromosomally integrated HTLV-1 LTR but not transiently transfected pHTLV-luc. Therefore, the effect of crude seminal fluid may not be simple addition of those factors but has more complex features. Our preliminary studies, including size fractionation and treatment with RNase A or proteinase K, indicate that not a single factor but a combination of several factors is involved in the effects of seminal fluid on the HTLV-1 LTR (data not shown).

Differential requirements for activation of the integrated and transiently transfected HTLV-1 LTR (14) and the human immunodeficiency virus type 1 LTR (3, 8) have been reported. The HeLa luc cells used in this study were a pool of three independent HeLa clones with integration of two to four copies of pHTLV-luc, to minimize biases stemming from particular cellular integration sites (14). We also used PBMC from several different HTLV-1-infected donors to perform the experiments whose results are shown in Fig. 1B. Therefore, it is unlikely that the discrepancy in seminal fluid-mediated effects between the integrated and transiently transfected HTLV-1 LTR depends on integration sites.

Since seminal fluid-mediated transactivation of the HTLV-1 LTR was observed in PBMC and HeLa cells but not in CHOK cells, cell-type-specific mechanisms must be considered. Interestingly, induction of the expression of certain genes in cervical epithelial cells by seminal fluid has also been reported (5, 6). We confirmed this observation and extended the targets of this seminal fluid activity to PBMC. It is of note that seminal fluid induces expression of heat shock protein 70 (Hsp70) in cervical epithelial cells (5) and that HTLV-1 expression is enhanced following the cellular stress response that results in production of Hsp70 family proteins (2). Therefore, Hsp70 may play a role in the cell-type-dependent effect of seminal fluid on HTLV-1 infection.

Our preliminary studies suggested that the observed activity of seminal fluid on HTLV-1 LTR transactivation appears to result not from a single factor but from a combination of several factors. This complex feature of the effect of seminal fluid was not unexpected, considering the fact that seminal fluid contains a number of factors, including nucleases, proteases, and many other bioactive factors. Further studies are necessary to determine by what mechanisms and which factor(s) in seminal fluid mediates chromosomal integration-dependent transactivation of the HTLV-1 LTR.

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REFERENCES