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Prostaglandin E_2, a seminal constituent, facilitates the replication of acquired immune deficiency syndrome virus *in vitro*

(plaque-forming assay)

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ABSTRACT **Acquired immune deficiency syndrome (AIDS)-associated virus is thought to be transmitted effectively through semen during sexual activities from male to male or from male to female. Prostaglandin (PG) E_2 is one of the immunosuppressive compounds present in high concentrations in human semen. We, therefore, investigated direct effects of PGE_2 and other PGs on AIDS-associated virus infection and replication *in vitro*. First, type III human T-lymphotropic virus (HTLV-III) was used to infect a T-cell line (MT-4) in culture. PGE_2 (10 nM to 10 μM) added to the culture medium enhanced the production of infectious virus in a dose-dependent fashion. In the presence of 5 μM PGE_2, 2.5-fold more virus were released from the infected MT-4 cells as compared to untreated control cells on day 3 after infection. Second, when we used an HTLV-III continuous-producer cell line (Molt-4/HTLV-III), PGE_2 and PGD_2 added to the culture medium increased the number of viruses released from Molt-4/HTLV-III cells. Other PGs such as $PGF_{2\alpha}$ and 13,14-dihydro-15-keto PGE_2 did not affect the replication of HTLV-III in this system. These results indicate that some PGs including seminal PGs enhance the AIDS-associated virus replication *in vitro*. We propose that PGE_2 in human semen might directly facilitate the infection of AIDS-associated virus and cause the efficient transmission of the virus during sexual activities.**

Acquired immune deficiency syndrome (AIDS) was reported first in 1981 (1, 2). As a transmissible agent of the disease, lymphadenopathy-associated virus (LAV) in 1983 (3), type III human T-lymphotropic virus (HTLV-III) (4), and AIDS-associated retrovirus (5) in 1984 were independently isolated. These retroviruses have so far been accepted to be a primary cause of immunological disorders leading to AIDS or AIDS-related complex. Epidemiological observations accumulated during past several years has enabled us to predict that the infusion of semen is the most effective route for the horizontal transmission of AIDS-causative virus, except for the direct transmission through blood or blood product transfusion (6). Human semen contains a large number of lymphocytes (7), and furthermore HTLV-III was isolated from T lymphocytes in semen of patients with AIDS (8). Receptive homosexual males, the highest risk group for AIDS, were repeatedly exposed to semen during the anal–genital intercourse of their sexual activities. We have proposed as a possible pathogenesis of AIDS in homosexual males that prostaglandin (PG) E_2, highly enriched in human semen, induces immunosuppression in semen recipients, which can be one of the underlying factors facilitating the AIDS-causative virus infection or leading to the development of the disease (9). The concentration of PGE_2 in human semen is more than 100 μM (9–11), while the concentration of other PGs such as PGD_2 and $PGF_{2\alpha}$ is at least 10 times lower.

In this paper, we examine the direct effects of PGs, including seminal PGs *in vitro*, on the infection and replication of AIDS-causative virus in certain human T-cell lines. The quantitative analysis of infectious viruses was performed by a sensitive plaque-forming assay established by Harada *et al.* (12–14).

MATERIALS AND METHODS

PGs. PGE_2, PGD_2, and $PGF_{2\alpha}$ were kindly supplied by Ono Central Research Institute (Osaka, Japan). 13,14-Dihydro-15-keto PGE_2 was purchased from Cayman Chemical (Ann Arbor, MI).

T-Cell Lines. HTLV-I-infected MT-4 was established by cocultivating leukocytes from an adult T-cell leukemia patient with cord blood leukocytes (15). A producer cell line of HTLV-III, Molt-4/HTLV-III, was established (16) from HTLV-III-infected Molt-4 cells. Molt-4 is HTLV-I-negative and was obtained from a patient with acute lymphocytic leukemia (17). All Molt-4/HTLV-III cells were positive for HTLV-III-specific antigens. These two cell lines were maintained at 37°C in a humidified CO_2 incubator in RPMI 1640 medium (GIBCO) supplemented with 10% (vol/vol) fetal calf serum, 100 units of penicillin per ml, and 100 μg of streptomycin per ml (the complete medium).

Virus Preparation. Molt-4/HTLV-III cell suspension was cultured for 4 days at 37°C. After cells were collected by centrifugation at 1500 × *g* for 10 min, the supernatant was filtered through a 0.22-μm pore size Millipore membrane. This virus preparation, the titer of which was 6×10^4 plaque-forming units (pfu)/ml, was stored in a small volume at −80°C until use.

Virus Infection. Infection of MT-4 cells with HTLV-III was made at a multiplicity of infection of 0.0006. After adsorption of HTLV-III (37°C, 1 hr), MT-4 cells, adjusted to 3×10^5 cells per ml, were cultured in the complete medium in the presence or absence of various concentrations of PGs. Successive culture of the HTLV-III-infected MT-4 cells was carried out at 37°C in a CO_2 incubator. Efficiency of viral infection was analyzed by the decrease in number of viable cells and by an indirect immunofluorescent method. Viable cells were detected by trypan blue dye exclusion method. On the third and fifth days after infection, the number of viruses released into the medium was determined by the plaque-forming assay.

Indirect Immunofluorecence Study. Labeling of HTLV-III-specific antigens was performed by the indirect immunofluorescence method described (14). Briefly, HTLV-III-infected cells were smeared on microscope slides, dried, and

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Abbreviations: AIDS, acquired immune deficiency syndrome; PG, prostaglandin; pfu, plaque-forming units; HTLV, human T-lymphotropic virus.

fixed for 3 min with cold methanol. Methanol-fixed samples were incubated with serum from a patient with hemophilia diluted 1:1000 (for HTLV-III serum was diluted 1:4096) at 37°C for 30 min in a humidified chamber. After washing twice with phosphate-buffered saline (PBS; 137 mM NaCl, 2.68 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.4), samples were incubated with fluorescein isothiocyanate-conjugated anti-human IgG (Dakopatts, Copenhagen) for 30 min and washed twice with PBS. Fluorescent cells were counted using a microscope, and the percentage of positive cells was calculated.

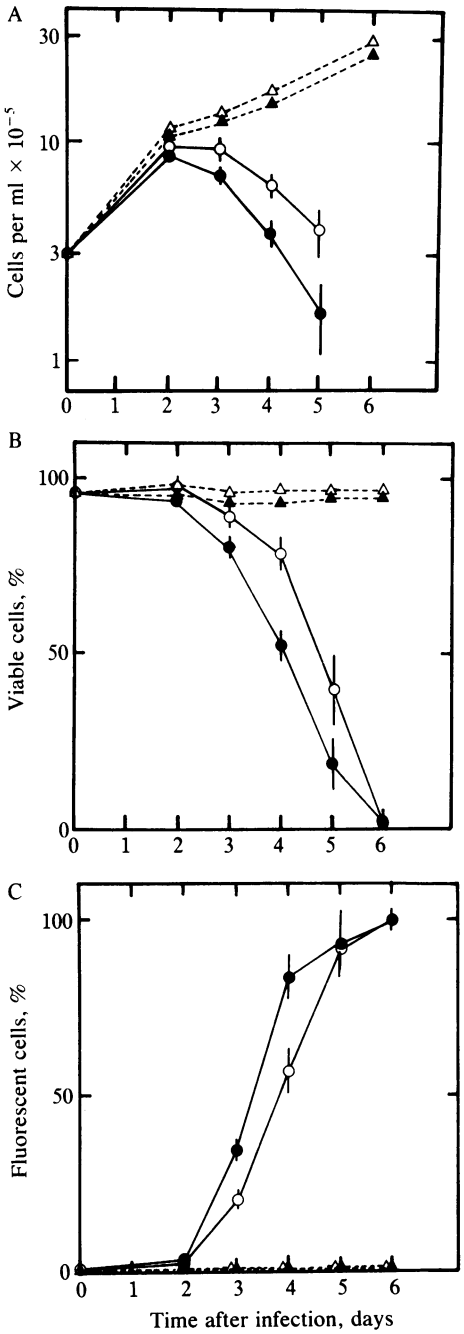

FIG. 1. Effects of PGE_2 on HTLV-III infection in MT-4 cells. After the adsorption of HTLV-III, MT-4 cells (3×10^5 cells per ml) were cultured to 37°C in the complete medium (6 ml) with (●) or without (○) 10 μM PGE_2. Uninfected MT-4 cells were also cultured in the presence (▲) or absence (△) of 10 μM PGE_2. On day 3, the culture medium was changed to the complete medium with or without 10 μM PGE_2. Cell growth (*A*) and viability of cells (*B*) were monitored each day from the second day. At the same time, the percentage of HTLV-III-antigen positive cells (*C*) was determined by the immunofluorescence method. The medium containing 10 μM PGE_2 was prepared as follows: PGE_2 was dissolved in pure ethanol at a concentration of 10 mM and stored at −20°C until use. This ethanol solution was diluted 1:1000 with the complete medium shortly before starting culture. The medium for the control contained 0.1% ethanol. Each point in the figures represents the mean ± SD of three experiments.

Plaque-Forming Assay. The number of infectious viruses was determined by the method of Harada *et al.* (12, 14). This plaque-forming assay was carried out using MT-4 monolayer cells made on a poly(L-lysine) (M_r 120,000; Sigma)-coated culture dish (35 × 10 mm). We added 100 μl of variously diluted virus preparations to the monolayer cells, incubated them for 60 min at room temperature for the adsorption of viruses, and overlayed 1 ml of agarose-overlay medium containing the complete medium and 0.6% agarose (Sea-Plaque agarose, Marine Colloids, Rockland, ME). Dishes were incubated in a CO_2 incubator at 37°C for 5 or 6 days, and the number of plaques was counted. On day 3, 1 ml of agarose-overlay medium containing neutral red was added to each dish.

RESULTS

HTLV-III Infection of MT-4 Cells. After adsorption of HTLV-III, MT-4 cells were cultured in medium with or without 10 μM PGE_2. Cell growth and viability of cells were monitored each day from the second day after infection (Fig. 1 *A* and *B*). The expression of HTLV-III-specific antigens in host cells that accompanied viral infection was determined by the immunofluorescence method (Fig. 1*C*). Growth of HTLV-III infected MT-4 cells was inhibited from the third day of infection, and the viability of these cells decreased rapidly, indicating that the virus infection resulted in the cytolysis of MT-4 cells as described (12). Frequency of HTLV-III-antigen positive cells increased from the third day after infection and reached the maximum on the fifth day. In uninfected MT-4 cells, 10 μM PGE_2 had no effect on either growth or viability of the cells. However, in the case of HTLV-III-infected MT-4 cells, PGE_2 accelerated the growth inhibition and cytolysis (Fig. 1 *A* and *B*). Furthermore, frequency of HTLV-III-antigen positive cells was higher when cells were cultured in the presence of PGE_2 (Fig. 1*C*). All these findings suggest that PGE_2 added in the culture medium facilitates the virus infection.

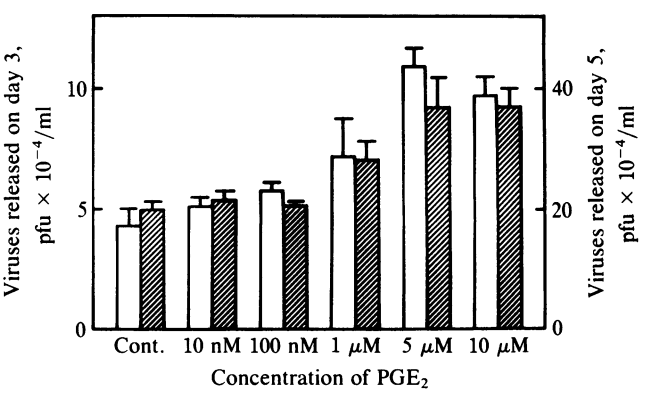

FIG. 2. Determination of infectious viruses released from HTLV-III-infected MT-4 cells into the culture medium containing various concentrations of PGE_2. We performed the infection and culture of MT-4 cells as described in Fig. 1, except for adding various concentrations of PGE_2 (10 nM to 10 μM). On days 3 (open bars) and 5 (hatched bars), an aliquot (3 ml) of each culture was centrifuged, and the supernatant, diluted with PBS, was used as a virus preparation. Plaque formation was measured on three plates per sample. The representative result of three experiments is shown in the above figure. Each column represents the mean ± SD (n = 3 plates).

Table 1. Effect of PGs on the replication of HTLV-III in Molt-4/HTLV-III cells

PG added, μM	HTLV-III released, pfu × 10^{-4} per ml				
	No added PG	PGE_2	PGD_2	$PGF_{2\alpha}$	13,14-Dihydro-15-keto-PGE_2
0	1.96 ± 0.64 (100)	—	—	—	—
1	—	3.40 ± 0.21 (173)	4.42 ± 0.27 (225)	1.65 ± 0.03 (84)	2.33 ± 0.06 (119)
10	—	4.85 ± 0.23 (247)	2.70 ± 0.22* (138)	2.60 ± 0.60 (132)	2.47 ± 0.23 (126)

Molt-4/HTLV-III cells, adjusted to 3 × 10^5 cells per ml, were cultured at 37°C in the complete medium with or without PGs. Each medium was prepared according to the method described in Fig. 1. After 4 days of culture, the supernatant of Molt-4/HTLV-III cell culture was used for the determination of released infectious viruses by the plaque-forming assay. Each value was the mean ± SD (n = 3 plates); values in parentheses are percent of control.

*Cell viability was markedly reduced (≈30%) in the medium containing 10 μM PGD_2, whereas it was not significantly affected (≧90%) in the cases of other PGs and 1 μM PGD_2.

To examine the extent to which PGE_2 enhances the replication of HTLV-III in the infected MT-4 cells, we measured the number of virus particles released into the medium by using the plaque-forming assay (Fig. 2). The concentrations of PGE_2 were varied from 10 nM to 10 μM. On the third day after infection, the amount of infectious virus released into the medium was increased by the addition of PGE_2. This effect was dose dependent from 10 nM to 5 μM PGE_2. When 5 μM or 10 μM PGE_2 was added to the culture medium, the yield of virus reached a value more than twice (2.5 and 2.3 times, respectively) that of the control. Essentially similar results were observed on the fifth day, although the number of infectious viruses had increased during the two additional days in culture. These results obtained by means of plaque-forming assays present direct evidence that PGE_2 enhances the replication of HTLV-III in the host cells.

To determine the specificity of PG on the stimulation of virus replication, we examined effects of $PGF_{2\alpha}$ and 13,14-dihydro-15-keto PGE_2, a metabolite of PGE_2. Neither of these PGs up to 10 μM had significant effects on the number of viruses released into the culture medium on the third day (data not shown).

An HTLV-III-Producer Cell Line, Molt-4/HTLV-III. We next investigated effects of PGs on viral replication in HTLV-III producer cells (Molt-4/HTLV-III) to determine whether PGE_2 affects processes after integration of the viral genome into the host genome. The addition of 10 μM PGE_2 increased the number of viruses released into the medium by 2.5 times (Table 1) while not affecting cell growth and viability of Molt-4/HTLV-III cells. Among PGs investigated, 1 μM PGD_2 also significantly increased the yield of virus. The stimulatory effect of PGD_2 was rather reduced at a higher concentration (10 μM), presumably due to the cytotoxic effect of PGD_2. On the other hand, the effects of $PGF_{2\alpha}$ and 13,14-dihydro-15-keto PGE_2 were insignificant (Table 1).

DISCUSSION

To investigate the *in vitro* effects of PGs—including PGE_2, a major PG in human semen—on AIDS-causative virus infection and replication, we chose MT-4 cell line as the target cell and HTLV-III as the AIDS-causative virus. This system has the following advantages as reported (12–14). First, MT-4, a HTLV-I-infected cell line, is one of the most susceptible cell lines to HTLV-III infection. Second, MT-4 cells are lysed by AIDS-causative virus infection, which could be a good *in vitro* model to study the clinical symptoms of AIDS. Third, although MT-4 cells are infected and transformed by HTLV-I, the plaque-forming activity was expressed only after HTLV-III infection. Our data presented here showed that PGE_2 enhanced the infection of HTLV-III in MT-4 cells. Furthermore, in the HTLV-III-producer cell line Molt-4/HTLV-III, concentrations of PGE_2 up to 10 μM increased the number of viruses released into the culture medium without any change of growth and viability of cells. These results indicate that PGE_2 or its metabolites may activate the transcription and/or translation of HTLV-III genes integrated in the host genome or the maturation leading to the production of infectious virus particles. It is not clear whether PGE_2 also participates in an early phase of infection before the integration of the viral genome into the host genome in the freshly infected MT-4 cells. The mechanism of action of PGE_2 has yet to be elucidated. The activity of the reverse transcriptase of HTLV-III was not directly affected by 10 μM PGE_2 in preliminary experiments (unpublished results).

PGE_2 and PGD_2 showed the stimulatory effect on virus replication, whereas $PGF_{2\alpha}$ and 13,14-dihydro-15-keto PGE_2 had no effect. PGE_2 (18, 19) and PGD_2 (11, 20) are well known to be immunosuppressive *in vivo* and *in vitro*. With regard to PGD_2 in the immune system, Park *et al.* (21) demonstrated that PGD_2-synthesizing activity is the highest among the three PG (PGE_2, PGD_2, and $PGF_{2\alpha}$)-synthesizing activities in thymus and spleen of adult rats. Therefore, the PGD_2-induced stimulation of virus replication in Molt-4/HTLV-III cells might be an undesirable action of PGD_2 on T lymphocytes. Although both PGE_2 and PGD_2 showed the stimulatory effects on AIDS-causative virus replication *in vitro* as described above, the role of PGD_2 appears insignificant because human semen is almost devoid of PGD_2 (9).

We proposed that seminal PGE_2 or its metabolites repeatedly introduced through the anus causes immunosuppression in the semen recipients, and the immunosuppressed people are more susceptible to AIDS-causative virus (9). Furthermore, in this paper, we present the direct *in vitro* evidence that PGE_2 facilitates the infection and replication of AIDS-causative virus. These findings may provide a reasonable explanation for the efficient transmission of AIDS-causative virus through semen.

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