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Enhanced Shedding of Cytomegalovirus in Semen of Human Immunodeficiency Virus-Seropositive Homosexual Men

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Site-specific shedding of cytomegalovirus (CMV) was assessed in a longitudinal study of homosexual and bisexual men. At initial testing, CMV was cultured from the semen of 33% (19 of 58) of asymptomatic and mildly symptomatic men who were seropositive for human immunodeficiency virus (HIV) at the time of entry into the study, whereas it was cultured from the semen of 17% (10 of 58) of the men who were HIV seronegative. CMV was isolated much more frequently from semen than from urine or throat washing specimens, and it was rarely recovered from stool or blood, regardless of the subject's HIV serostatus. CMV was cultured from the semen of 31% (16 of 52) of the men relatively early after seroconversion to HIV (mean, 12.8 months). CMV was persistently isolated from the semen of a greater proportion of the HIV-seropositive men than from the semen of the HIV-seronegative men during a 4.5-year follow-up period (52 of 110 - [47%] and 15 of 58 [26%] men, respectively). There was an increased relative risk for shedding of CMV in semen in association with decreased CD4$^+$ cell numbers and increased levels of serum immunoglobulin A. However, there was no association of CMV shedding with an increased risk for the development of AIDS.

Cytomegalovirus (CMV) has been associated with AIDS since the first reports of the epidemic in homosexual men (18, 25, 33, 45, 51). This herpesvirus is now the most common opportunistic viral infection in patients with AIDS (45). CMV has been identified as a cause of pneumonia, gastrointestinal disease, and retinitis in patients with AIDS (26).

CMV has several properties that support its potential role as a cofactor in the progression of human immunodeficiency virus (HIV) infection, the causative agent of AIDS, particularly in homosexual men. The prevalence of CMV infection in homosexual men is quite high (95%), as defined by antibody seropositivity (45). Moreover, there is frequent reactivation or reinfection with CMV in homosexual men, as evidenced by the presence of anti-CMV immunoglobulin M (IgM) in blood (5, 38) and excretion of the virus (12, 36). CMV infection may alter the course of HIV infection by enhancing HIV replication and cytopathic effects in dually infected cells (6, 35, 52), suppressing T-cell immunity (47) and transactivating expression of proviral HIV (30, 37, 42).

We have previously reported (48) that CMV is isolated more frequently from the semen of HIV-seropositive subjects in a small cohort of homosexual men who were asymptomatic or who had persistent lymphadenopathy (LAD) than from HIV-seronegative homosexual men. We confirmed and extended that work in the prospective study of CMV shedding in a larger group of HIV-seropositive and HIV-seronegative homosexual men described here.

MATERIALS AND METHODS

Subjects. The study cohort consisted of 191 homosexual or bisexual men who were part of the Pittsburgh portion of the Multicenter AIDS Cohort Study, a longitudinal investigation of the natural history of HIV infection (27). The average age of the cohort was 32.3 years (range, 18 to 61 years); and there were 180 white men, 10 black men, and 1 Hispanic man. An epidemiologic questionnaire, clinical examination, and clinical sampling for laboratory tests were done at the time of entry into the study (from April 1984 through May 1985) and at approximately 6-month intervals thereafter. Sixty-seven study participants were CMV seropositive (IgG antibody; FIAX; Whittaker Bioproducts, Walkersville, Md.) and were persistently HIV seronegative during the investigation (group 1). Seropositivity for HIV was defined as positivity for HIV antibodies by enzyme immunoassay (LAV-EIA; Genetic Systems, Seattle, Wash.) and was confirmed by Western blotting (immunoblotting; Novapath Immunoblot; Bio-Rad Laboratories, Hercules, Calif.) (44). Of the 67 men in group 1, baseline CD4$^+$ blood lymphocyte counts were $\leq$400/mm^3 in 4 men, 401 to 700/mm^3 in 15 men, and $>$700/mm^3 in 48 men. Fifty-eight of the men in group 1 were asymptomatic at baseline, whereas nine men reported either symptoms of LAD (tender or enlarged, noninguinal lymph nodes present for at least 3 consecutive days) or minor constitutional symptoms. Sixty-eight men were HIV and CMV seropositive at the time of entry into the study (group 2). Baseline CD4$^+$ cell numbers for men in group 2 were $\leq$400/mm^3 in 31 men, 401 to 700/mm^3 in 19 men, and $>$700/mm^3 in 18 men. Eighteen of the men in group 2 were asymptomatic at the time of entry into the study (Centers for Disease Control [CDC] group II) (7), 24 reported symptoms of LAD, and 22 had symptoms of either constitutional disease (CDC group IV.A.) or secondary, non-AIDS-defining infections (CDC group IV.C-2). Data on symptoms were unavailable for four subjects in group 2 at baseline testing. Fifty-six men who were seronegative for HIV and seropositive for CMV at the time of entry into the natural history study subsequently seroconverted to HIV at a later study visit (group 3). Upon identification of such HIV seroconverters, the men were entered into this study of CMV excretion. Four men in group 3 had CD4$^+$ cell counts of $\leq$400/mm^3, 21

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men had 401 to 700 cells per mm^3, and 31 men had >700 cells per mm^3 at baseline. At the time of this study, 34 men were asymptomatic and 14 men were symptomatic; i.e., 4 men had LAD and 10 men were either CDC group IV.A. or IV.C-2.; there were insufficient data from the other 8 subjects for clinical classification at baseline. The data presented in this analysis were primarily from samples obtained prior to 1988, when an insignificant proportion (only 7%) of our HIV-seropositive cohorts were receiving zidovudine.

Clinical samples and viral cultures. To obtain throat washings, the study subjects gargled with 10 ml of Hanks' balanced salt solution (GIBCO, Grand Island, N.Y.) supplemented with 5% (wt/vol) gelatin (Fisher, Pittsburgh, Pa.). Throat washings were treated with antibiotics (penicillin, 200 U/ml; gentamicin, 10 μg/ml; nystatin, 10 U/ml) for 30 min at 4°C. Urine samples were centrifuged at 550 × *g* for 10 min at 4°C, adjusted to pH 7 with sodium bicarbonate solution (7.5% [wt/vol]), and treated with antibiotics for 30 min at 4°C. Semen specimens were obtained in sterile containers by the volunteer at home, held at room temperature, and delivered to the laboratory within several hours of ejaculation. The samples were then diluted 1:10 with the gelatin-salt solution described above. Blood leukocytes were derived from 10 ml of anticoagulated blood (5 U of preservative-free heparin per ml of blood) by sedimentation of erythrocytes with dextran (1.5 ml of 6% [wt/vol] dextran; molecular weight, 500,000; Pharmacia, Uppsala, Sweden). The upper, leukocyte layer was removed and treated with deionized water for 10 s to lyse residual erythrocytes. Stool samples were suspended in salt solution (approximately 5 g of stool per 20-ml solution) and centrifuged at 550 × *g* for 30 min at 4°C, and the supernatants were treated with antibiotics for 60 min at 4°C. Specimens were held at 4°C for up to 1 day prior to processing and culture for virus.

For isolation of virus, 0.5 ml of specimen (as well as a 1:10 dilution of leukocytes) was diluted with 2.5 ml of Eagle's minimum essential medium (Whittaker Bioproducts); supplemented with 2% heat-inactivated fetal bovine serum (Sigma, St. Louis, Mo.), 25 U of nystatin per ml, and 50 μg of gentamicin per ml; and added to monolayers of human foreskin fibroblasts and the A-549 continuous line of human lung carcinoma cells (Bartels Immunodiagnostic Supplies, Bellevue, Wash.). The cultures were incubated at 37°C and were observed for 28 days for a cytopathic effect characteristic of CMV, herpes simplex virus (HSV), and adenovirus (ADV). Results from cultures with microbial contamination were not accepted. The foreskin fibroblasts were consistently permissive for replication of wild-type CMV, HSV, and ADV.

Lymphocyte phenotyping. Peripheral blood mononuclear cells were phenotyped for the number of $CD4^+$ and $CD8^+$ T lymphocytes as described previously (44) by using monoclonal antibodies anti-Leu 3 and anti-Leu 2a (Becton-Dickinson, Mt. View, Calif.).

CMV antibody and serum immunoglobulins. Serum IgG antibodies specific for CMV were quantitated from a dilution of sample by a solid-phase, indirect fluorescence immunoassay (FIAX; Whittaker Bioproducts). The units of IgG antibody were interpolated from a calibration curve. A level of <20 U was interpreted as seronegative for CMV antibodies, 20 to 30 U was interpreted as equivocal, and >30 U was interpreted as seropositive for CMV antibodies. The concentrations (in milligrams per deciliter) of IgG, IgA, and IgM were determined in sera by indirect immunofluorescence (FIAX).

Statistical analysis. Contingency table data were analyzed by standard chi-square methods for the comparisons of CMV, HSV, and ADV shedding in different sites among the three groups. Log-linear models were applied for the analysis of the relationships between shedding of CMV in semen and other sites. Kruskal-Wallis tests (chi-square approximation) were used for comparisons of baseline sexual behavior and laboratory variables among the various CMV shedding and HIV groups.

A logistic regression approach was used to examine the associations between shedding of CMV in semen and selected laboratory covariates on the basis of the univariate analysis results (1). A total of 517 complete datum points (CMV shedding in semen, HIV status, $CD4^+$ and $CD8^+$ cell counts, serum IgG and IgA concentrations, and CMV IgG levels at the same visit) were included in the analysis. The final model included HIV status, serum IgA concentration, and $CD4^+$ cell counts as variables. The relative risks were estimated for different situations in relation to CMV shedding in semen.

RESULTS

Detection of CMV in cross-sectional samples. Data from the first study visit indicated that CMV was the most frequently isolated virus from all three groups of men examined in this study (Table 1). HSV and ADV were only occasionally isolated from the specimens. CMV was detected 1.9- to 4.9-fold more frequently in semen than in urine or throat washings, with overall average prevalences of 27, 8, and 7%, respectively. CMV was rarely isolated from stool specimens from the three cohorts and was not cultured from any peripheral blood leukocyte specimen obtained at visit one.

The cross-sectional results from the first study visit also showed that CMV was more frequently isolated from men in groups 2 and 3 (HIV seropositive) than from men in group 1 (HIV seronegative) (Table 1). In particular, shedding of CMV in semen was 1.4- to 2.0-fold greater in HIV-seropositive men than it was in HIV-seronegative men. CMV excretion profiles in group 2 men (HIV seropositive at the time of entry into the study) and group 3 men (with documented time of seroconversion to HIV) were comparable.

A similar pattern of virus isolation was observed in the cumulative data for all study subjects at all available samplings during the 4.5-year follow-up period (Table 2). CMV was the most frequently isolated virus and was detected more often in the semen, urine, and throat washing specimens from the two groups of HIV-seropositive men than it was in the specimens from the HIV-seronegative men. Cumulative results for stool and blood samples were not available because they were not routinely obtained after the first study visit because of the low levels of virus isolated from the initial specimens (Table 1).

The last column of Table 2 presents the prevalence of positivity for each virus at any site for the three groups of subjects throughout the study period. These rates of "ever shedders" were about twofold higher than the baseline prevalence estimates for CMV isolation from semen only (Table 1). Furthermore, the greater prevalence of CMV shedding was evident in the two groups of HIV-seropositive men compared with that in the HIV-seronegative group by this parameter, whereas there were no differences in the excretion of HSV or ADV among the three groups of study subjects.

It should be noted that seropositivities for HSV IgG antibody in groups 1, 2, and 3 were 69, 88, and 86%, respectively. All of the men who shed HSV were antibody

TABLE 1. Isolation of CMV, HSV, and ADV from HIV-seronegative and HIV seropositive men at the first clinical evaluation

Study group	Virus isolated	No. of men positive for virus from the following sites/total no. tested (%):				
		Semen	Urine	Throat	Stool	Blood
Group 1 (HIV seronegative)	CMV	10/58 (17.2)[a]	6/65 (9.2)	4/66 (6.0)	0/37 (0.0)	0/48 (0.0)
	HSV	0/58 (0.0)	0/65 (0.0)	2/66 (3.0)	0/37 (0.0)	0/48 (0.0)
	ADV	1/58 (1.7)	1/65 (1.5)	1/66 (1.5)	1/37 (2.7)	0/48 (0.0)
Group 2 (HIV seropositive)[b]	CMV	19/58 (32.8)	5/68 (7.4)	7/67 (10.4)	3/39 (7.7)	0/54 (0.0)
	HSV	0/58 (0.0)	1/68 (1.5)	1/67 (1.5)	0/39 (0.0)	0/54 (0.0)
	ADV	0/58 (0.0)	0/68 (0.0)	0/67 (0.0)	2/39 (5.1)	0/54 (0.0)
Group 3 (HIV seropositive)[c]	CMV	16/52 (30.8)	4/56 (7.1)	3/55 (5.5)	0/3 (0.0)	0/18 (0.0)
	HSV	0/52 (0.0)	0/56 (0.0)	1/55 (1.8)	0/3 (0.0)	0/18 (0.0)
	ADV	0/52 (0.0)	0/56 (0.0)	0/55 (0.0)	0/3 (0.0)	0/18 (0.0)

[a] $P = 0.068$ ($\chi^2 = 3.32$) compared with group 2 men.
[b] Men with indeterminate duration of HIV infection.
[c] Specimens were tested at the first clinic visit after documented seroconversion to HIV.

positive for this herpesvirus by the indirect immunofluorescence assay (FIAX). ADV antibody levels were not determined in the cohorts described here.

In this study, men who shed CMV in semen were more likely to shed the virus in urine or throat washings, regardless of their HIV serostatus. The estimated relative risk of shedding CMV in urine if they shed in semen was 10.1-fold (95% confidence limits, 4.96, 20.72) and was 6.1-fold (95% confidence limits, 2.91, 12.69) for shedding of CMV in throat washings if they shed in semen.

Detection of CMV in longitudinal samples of semen. The men in this study were tested for CMV at approximately 6-month intervals for up to 4.5 years. Most of the data, however, were obtained from the subjects who were studied at five visits during a 2-year interval (Fig. 1). Testing of group 1 and 2 men began at the initial study visit, whereas most of the group 3 men were first tested at a study visit after confirmation of conversion to HIV seropositivity. The results indicate that CMV was isolated from the semen of the group 3 HIV-seropositive men at a relatively high frequency (31%) at the earliest time of testing after seroconversion to HIV (mean, 12.8 months) (Fig. 1). This level of CMV shedding in semen was comparable to that demonstrated in group 2 men with an indeterminate duration of HIV infection (33%) and was greater than the CMV shedding rate in the semen of HIV-seronegative subjects at the first visit (17%).

A majority (52 of 56) of the group 3 men were entered into this study of CMV shedding at a clinic visit subsequent to the first study visit when they were found to be HIV seropositive. We therefore examined whether the interval between seroconversion to HIV and the initial time of sampling for CMV could have biased our results. It was noted that this was not the case, because the average time from the estimated date of seroconversion (midpoint between the last seronegative date and the first seropositive date) and the date that the first sample for culture was taken was comparable between those seroconverters who shed CMV and those who were CMV negative, i.e., 12.8 and 10.7 months, respectively ($P > 0.05$, Student's t test).

Results for four group 3 subjects were available at study visits 6 to 36 months prior to HIV seroconversion. There was no distinct pattern of CMV excretion noted in those individuals. That is, CMV was not isolated prior to seroconversion in group 3 men, but it was detected in the semen of one of the four men at the initial visit, when the subject was found to be HIV seropositive, and 12 months later.

CMV was isolated from the semen of 8 to 17% (average, 13%) of HIV-seronegative group 1 men at the 6-month intervals during the 2-year follow-up period (Fig. 1). In contrast, CMV was isolated from 19 to 40% (average, 31%) of group 2 HIV-seropositive subjects and 26 to 46% (aver-

TABLE 2. Number of subjects who were ever positive for CMV, HSV, and ADV in semen and other sites

Study group	Virus	No. of men positive for virus from the following sites/total no. tested positive (%):			
		Semen	Urine	Throat	Any site[a]
Group 1 (HIV seronegative; $n = 67$)	CMV	15/58 (25.9)[b]	8/65 (12.3)	8/66 (12.1)	22/67 (32.8)[c]
	HSV	0/58 (0.0)	0/65 (0.0)	8/66 (12.1)	8/67 (11.9)
	ADV	1/58 (1.7)	1/65 (1.5)	1/66 (1.5)	3/67 (4.5)
Group 2 (HIV seropositive; $n = 68$)	CMV	29/58 (50.0)	15/68 (22.1)	16/67 (23.9)	35/68 (51.5)
	HSV	1/58 (1.7)	1/68 (1.5)	4/67 (6.0)	6/68 (8.8)
	ADV	1/58 (1.7)	0/68 (0.0)	0/67 (0.0)	3/68 (4.4)
Group 3 (HIV seropositive; $n = 56$)	CMV	23/52 (44.2)	10/56 (17.9)	7/55 (12.7)	26/56 (46.4)
	HSV	0/52 (0.0)	0/56 (0.0)	4/55 (7.3)	4/56 (7.1)
	ADV	0/52 (0.0)	0/56 (0.0)	0/55 (0.0)	0/56 (0.0)

[a] Includes semen, urine, throat washings, stool, and blood buffy coat.
[b] $P < 0.05$ ($\chi^2 = 6.2$) compared with group 2 men.
[c] $P < 0.05$ ($\chi^2 = 4.07$) compared with group 2 men.

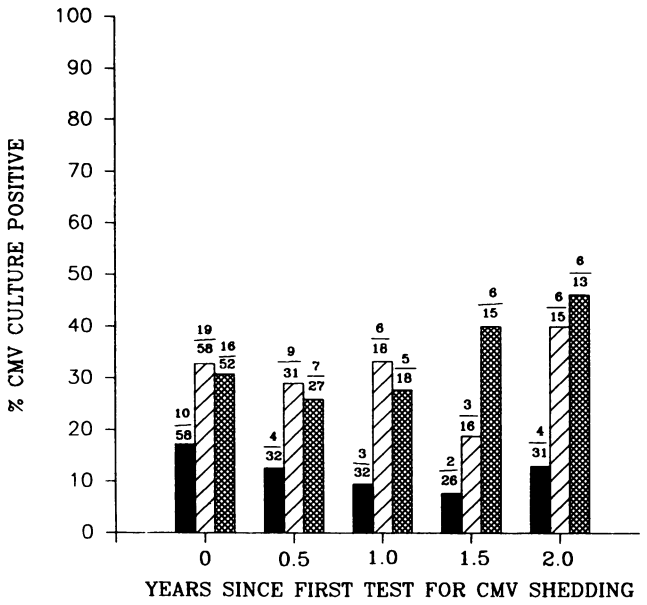

FIG. 1. Prevalence of CMV shedding in the semen of homosexual men who were HIV seronegative (group 1) (■), HIV seropositive with an unknown duration of infection (group 2) (▨), and HIV seropositive with a known time of seroconversion to HIV positivity (group 3) (▩) over a 2-year period of testing. Numbers above the bars are number of men who were CMV culture positive/total number of men tested.

age, 32%) of group 3 HIV-seropositive men during the 2-year period.

Additional analysis showed that, of the 74% of men who were tested at least twice over time, more men in the two HIV-seropositive groups (groups 2 and 3) shed CMV in their semen at one or more visits than HIV-seronegative men did. That is, 57% of group 2 men and 50% of group 3 men were positive for CMV in their semen at one or more study visits, whereas 26% of HIV-seronegative men were positive for CMV in their semen at one or more study visits ($\chi^2 = 9.99$; $P < 0.01$).

Relation of CMV excretion to sexual activity, laboratory parameters, and clinical symptoms. We examined the available laboratory covariates by univariate and logistic regression analyses to identify the relative risks for different biologic variables in relation to CMV excretion in semen. The results of the univariate analysis showed that there was a strong association between HIV serostatus and CMV excretion in semen in the total group of volunteers ($\chi^2 = 11.9$; $P < 0.001$). As expected, other laboratory parameters, including T-cell counts, CMV IgG antibody levels, and serum IgG and IgM concentrations, were significantly associated with HIV serostatus (Table 3). The only parameters that were significantly associated with CMV shedding, however, were CMV IgG levels and serum IgG concentrations in group 1 subjects and $CD8^+$ T-cell numbers in group 3 subjects.

Several measures of sexual exposure related to oral and anal intercourse were significantly associated with the isolation of CMV from semen in the HIV-seronegative group 1 men compared with the isolation of CMV from semen in group 2 and 3 men (Table 4). The only sexual practice, however, that was associated with CMV shedding in semen, independent of HIV serostatus, was receptive anal intercourse in the HIV-seronegative group 1 subjects. It should be noted that the number of partners with whom the group 1 subjects engaged in this activity was relatively few in both CMV shedders and nonshedders.

A logistic regression approach was used to determine the independent associations between shedding of CMV in semen and selected laboratory covariates based on the univariate analysis. The results indicate that, as expected, there was a significant association of shedding of CMV in semen with HIV seropositivity (relative risk, 2.78; coefficient ± standard error, 1.02 ± 0.30; $t = 4.87$; $P < 0.01$). Only $CD4^+$ T-cell counts and serum IgA concentrations were also significantly linked with shedding of CMV in semen (per a change of 100 $CD4^+$ cells, relative risk 1.07; coefficient ± standard error, −0.07 ± 0.04; $t = 1.87$; $0.05 < P < 0.10$; per a change of 100 mg/dl in the IgA concentration, relative risk, 1.22, coefficient ± standard error, 0.20 ± 0.06; $t = -3.59$; $0.05 < P < 0.10$). The estimated relative risk of CMV excretion in semen on the basis of this logistic regression analysis increased with incremental decreases in $CD4^+$ cell

TABLE 3. Laboratory parameters in HIV-seronegative and HIV-seropositive men in relation to shedding of CMV in semen[a]

Study group	Shedding of CMV in semen	No. of men	Median (range)					
			$CD4^+$ cells (no./mm³)	$CD8^+$ cells (no./mm³)	CMV IgG (U)	Serum IgG (mg/dl)	Serum IgA (mg/dl)	Serum IgM (mg/dl)
Group 1 (HIV seronegative)	Negative	48	883[b] (282–2,180)	658 (225–1,910)	196[c] (37–1,000)	1,360[b] (482–4,170)	259[c] (59–890)	146 (29–785)
	Positive	10	742[d] (543–1,479)	694 (421–5,372)	119 (88–244)	1,548[d] (989–3,200)	424 (150–1,015)	145 (89–491)
Group 2 (HIV seropositive)	Negative	39	442 (39–1,861)	779 (342–1,956)	247 (57–1,630)	2,070[e] (1,070–6,085)	269 (96–870)	162 (55–488)
	Positive	19	434[f] (197–835)	921 (180–1,581)	169 (60–1,060)	2,285[g] (835–5,046)	426 (65–969)	228 (49–553)
Group 3 (HIV seropositive)	Negative	34	738 (346–1,466)	640[h] (328–1,307)	231 (79–1,404)	1,403 (724–2,391)	280 (25–734)	185 (53–361)
	Positive	15	718 (228–3,015)	796 (521–1,691)	267 (60–631)	1,240 (745–3,011)	237 (92–600)	220 (70–384)

[a] Data are from baseline visits for men in group 1 and from the first study visit when CMV was isolated from semen in men in groups 2 and 3; group 3 men were tested at clinic visits after documented seroconversion to HIV positivity.
[b] $\chi^2 = 26.9$ to 28.1, $P < 0.01$; group 1 men compared with group 2 and 3 men, negative shedders (Kruskal-Wallis test).
[c] $\chi^2 = 5.0$ to 5.6, $P < 0.05$; CMV negative versus positive shedders, group 1 men only.
[d] $\chi^2 = 6.2$ to 9.8, $P < 0.05$; group 1 men compared with group 2 and 3 men, positive shedders.
[e] $\chi^2 = 19.7$ to 20.6, $P < 0.01$; group 2 men compared with group 1 or 3 men, negative shedders.
[f] $\chi^2 = 5.4$ to 8.5, $P < 0.05$; group 2 men compared with group 1 or 3 men, positive shedders.
[g] $\chi^2 = 5.3$, $P < 0.05$; group 2 compared with group 3 men, positive shedders.
[h] $\chi^2 = 3.8$, $P = 0.05$; CMV negative versus positive shedders, group 3 men only.

TABLE 4. Association of sexual practices with shedding of CMV in semen in HIV-seronegative and HIV-seropositive men[a]

Study group	Shedding of CMV in semen	No. of men	Median no. (range) of male partners in previous 6 mo	Median no. (range) of male partners in previous 6 mo with whom subject engaged in:			
				Insertive oral intercourse	Insertive anal intercourse	Receptive oral intercourse	Receptive anal intercourse
Group 1 (HIV seronegative)	Negative	49	5 (0–100)[b]	3 (0–30)[b]	1 (0–15)[b]	2 (0–100)[b]	1 (0–3)[b,c,d]
	Positive	10	8 (2–60)[e,f]	6 (1–55)[e,f]	2 (0–30)	7 (0–52)[e]	2 (0–30)
Group 2 (HIV seropositive)	Negative	38	6 (0–400)[g]	6 (0–375)[g]	2 (0–40)[g]	4 (0–400)[g]	2 (0–200)[g]
	Positive	19	12 (1–100)[h]	11 (1–55)[h]	5 (0–25)	10 (0–80)[h]	2 (0–45)
Group 3 (HIV seropositive)	Negative	36	3 (1–192)	2 (0–21)	1 (0–10)	2 (0–45)	1 (0–11)
	Positive	14	2 (0–15)	1 (0–15)	1 (0–8)	1 (0–15)	1 (0–9)

[a] Data are from baseline visits for group 1 men and from the first study visit when CMV was isolated from semen in group 2 and 3 men; group 3 men were tested at clinic visits after documented seroconversion to HIV.
[b] $\chi^2 = 7.5$ to 19.5, $P \leq 0.03$; group 1 men compared with group 2 and 3 men, negative shedders (Kruskal-Wallis test).
[c] $\chi^2 = 5.7$, $P = 0.02$; CMV negative versus positive shedders, group 1 men only.
[d] $\chi^2 = 6.8$; $P < 0.01$; group 1 men compared with group 3 men, negative shedders.
[e] $\chi^2 = 7.6$ to 9.7, $P \leq 0.03$; group 1 men compared with group 2 and 3 men, positive shedders.
[f] $\chi^2 = 4.4$ to 4.8, $P < 0.05$; group 1 men compared with group 3 men, positive shedders.
[g] $\chi^2 = 3.7$ to 18.2, $P \leq 0.05$; group 2 men compared with group 1 or 3 men, negative shedders.
[h] $\chi^2 = 7.3$ to 9.0, $P \leq 0.01$; group 2 men compared with group 3 men, positive shedders.

numbers and increases in serum IgA concentrations (Table 5). The highest relative risk for shedding of CMV was 1.68, on the basis of a decrease of 300 $CD4^+$ T cells per mm^3 and an increase of 150 mg/dl in the serum IgA concentration.

There were no significant differences in CMV shedding patterns in relation to non-AIDS-defining, mild clinical symptoms (LAD and CDC groups IV.A. and IV.C-2) among the group 2 and 3 men at the time of entry into the study (29 and 33% prevalence of CMV shedding, respectively; $\chi^2 = 0.145$; $P > 0.05$). Shedding of CMV also was not linked to an enhanced risk for the development of AIDS (Table 6). That is, 23 of the 68 group 2 subjects and 9 of the 56 group 3 subjects developed AIDS during the 5-year follow-up period. There was no significant association of shedding of CMV in semen at baseline testing, ever shedding of CMV in semen, or isolation of CMV from any clinical specimen and the subsequent development of AIDS.

DISCUSSION

In the present study, CMV was isolated more often from one or more sites in men with either a known or an indeterminate duration of HIV infection than from HIV-seronegative men tested at 6-month intervals over 4.5 years (cumulative rates of 46 and 52% compared with 33%, respectively). CMV was cultured approximately two- to fivefold more frequently from semen than from urine or throat washings, regardless of HIV serostatus. CMV was rarely recovered from the stool and was not isolated from blood of either HIV-seropositive or HIV-seronegative subjects.

Cumulative results from several studies conducted in the 1980s indicated that CMV was culturable from 24% of semen specimens from homosexual male cohorts with unknown or mixed HIV serostatus and without AIDS (4, 5, 10, 14, 24, 29, 36, 48), whereas CMV was culturable from an average of 27% of semen specimens at the baseline visit in our study. This is much higher than the reported levels of 0 to 3% of CMV shedding in the semen of heterosexual subjects (5, 10, 20, 34, 55). The overall prevalence of CMV in urine specimens and throat washings of men at the baseline visit in our investigation was 8 and 7%, respectively. This is less than the 17% prevalence noted in other studies in the urine of homosexual men without clinical symptoms of AIDS (5, 10, 12, 14, 19, 29, 36, 40, 48) and is comparable to the 8% level reported for throat washings or saliva (5, 10, 40, 48). As in the case of semen, CMV shedding is at low or undetectable levels in urine and throat washings of heterosexual males (5, 10, 12). Isolation of CMV from these three types of clinical specimens has been much higher in homosexual men with overt AIDS, with prevalence rates ranging from 68 to 100% (23, 32, 40).

The present investigation showed that the prevalence of shedding of CMV in semen is closely associated with HIV serostatus in homosexual and bisexual men who did not have AIDS. CMV was isolated from the semen of approximately 33% (19 of 58) of asymptomatic and mildly symptomatic, HIV-seropositive men with an unknown duration of HIV

TABLE 5. Relative risk of CMV shedding in semen in homosexual men

Decrement in no. of $CD4^+$ cells/mm^3	Relative risk (95% confidence interval) for the following increments in serum IgA concn (mg/dl)		
	50	100	150
100	1.19 (1.04, 1.35)	1.31 (1.09, 1.58)	1.45 (1.14, 1.85)
200	1.28 (1.04, 1.57)	1.41 (1.08, 1.83)	1.56 (1.14, 2.14)
300	1.37 (1.03, 1.82)	1.51 (1.08, 2.13)	1.68 (1.13, 2.48)

TABLE 6. Relation of CMV shedding with development of AIDS[a]

Study group	Clinical outcome	No. of men/total no. tested (%) who:		
		Shed CMV in semen at baseline	Ever shed CMV in semen	Ever shed CMV at any site
Group 2 (HIV seropositive)	AIDS	8/21 (38.1)	11/21 (52.4)	15/23 (65.2)
	Non-AIDS	11/37 (29.7)	18/37 (48.6)	20/45 (44.4)
Group 3 (HIV seropositive)[b]	AIDS	3/9 (33.3)	5/9 (55.6)	5/9 (55.6)
	Non-AIDS	11/43 (25.6)	18/43 (41.9)	18/44 (40.9)

[a] $\chi^2 < 0.2$; P was not significant for all comparisons.
[b] Specimens tested at clinic visits after documented seroconversion to HIV.

infection (group 2), whereas it was isolated from the semen of 17% (10 of 58) of HIV-seronegative subjects (group 1) at the first study visit. These results confirm and extend our previous study of a smaller cohort of homosexual men, in which 56% (5 of 9) of asymptomatic, HIV-seropositive men shed CMV in semen, whereas 12% (5 of 42) of HIV-seronegative men shed CMV in semen (48). The higher prevalence of CMV excretion in semen was noted as early as an average of 12.8 months after seroconversion to HIV positivity in the present study. That is, 31% of semen specimens from 52 men who seroconverted to HIV positivity during this study (group 3) were culture positive for CMV at the first postseroconversion testing. We also observed that CMV could be isolated more frequently over prolonged time periods from the semen of HIV-seropositive than from the semen of HIV-seronegative men. Our results are in contrast to those of other investigators, who reported no significant differences in CMV isolation rates from semen of asymptomatic, HIV-seropositive and HIV-seronegative homosexual men (5, 10). This could be related to differences in CMV culture procedures, sexual exposure variables, and the relative duration of HIV infection in those cohorts.

It is unclear why CMV is so prevalent in the semen of homosexual men. The virus has been identified inside sperm by electron microscopy (24), and inclusion bodies have been noted within the cells of prostate and testicular tissues (31). Because CMV can infect blood mononuclear and polymorphonuclear leukocytes (43, 46), the virus presumably could infect inflammatory cells that are present in the semen of homosexual men (57). HIV has also been cultured from semen (57) and, conceivably, could reside in T cells, macrophages, and possibly, $CD4^+$ spermatocytes (2). Hence, these viruses may directly interact within the cells of the male genitalia or may indirectly interact via cytokines induced by either virus, leading to the transactivation of either CMV or HIV DNA (3, 8, 22).

The lack of isolation of CMV from blood leukocytes of HIV-seronegative and HIV-seropositive, asymptomatic men confirms earlier studies by several groups of investigators (16, 17, 40, 48). Those investigations differ from others which reported the isolation of CMV from the peripheral blood of asymptomatic HIV-seropositive (5, 49) and HIV-seronegative (5) subjects in groups at high risk for the development of AIDS. It is apparent, however, that the incidence of CMV viremia in HIV-seropositive individuals increases with a worsening clinical status that is associated with the development of AIDS. Evidence for this is that HIV-seropositive homosexual men with frank AIDS can have frequent and persistent CMV viremia (16, 17, 40). Furthermore, CMV is the most common viral pathogen in patients with AIDS and can be isolated from numerous anatomic sites (45). Therefore, active CMV infection, as determined by isolation of the virus from HIV-seropositive homosexual and bisexual men, appears to remain localized, particularly in semen, until the development of more severe immunosuppression and the associated clinical manifestations of AIDS.

In a similar study, we noted that another herpesvirus, Epstein-Barr virus (EBV), is excreted at greater frequencies in the throats of homosexual men beginning very early during HIV infection (15). Results obtained in the study described here show that a third herpesvirus, HSV, is infrequently cultured from HIV-seronegative or HIV-seropositive men, and there was no laboratory evidence of reactivation of varicella-zoster virus. Thus, the data from these studies suggest that the more lymphotropic herpesviruses, CMV and EBV (21), are more frequently reactivated relatively early in homosexual and bisexual men as a consequence of HIV infection than the more neurotropic herpesviruses are. This may be related to the profound immunosuppressive effects of HIV that could indirectly reactivate CMV and EBV, which are present in a latent form in approximately 95 and 98% of homosexual men, respectively (41, 45). Alternatively, these results may, at least in part, represent reinfection with CMV and EBV. Homosexual men at risk for HIV infection are likely to have multiple exposures to sexual partners who excrete CMV and EBV, which may cause reinfection in seroimmune individuals (9, 13, 28, 53, 54). Results from our cross-sectional analysis demonstrated a significant association among certain sexual practices (i.e., receptive anal intercourse) and CMV shedding in HIV-seronegative homosexual or bisexual men. This extends previous results which showed that CMV seropositivity in homosexual men correlated with a history of anal receptive intercourse (10, 36). The lack of such an association in our HIV-seropositive cohorts may be due to the close linkage of these same sexual practices with HIV infection, thereby obscuring relations with CMV infection.

There is no conclusive evidence yet that supports the role of CMV as a cofactor in the progression of HIV infection. Two studies have reported that elevated titers of CMV antibodies in the sera of HIV-seropositive homosexual men correlate with an enhanced risk for the development of AIDS (11, 39). Furthermore, Webster et al. (56) have shown that CMV-seropositive patients with hemophilia have a greater risk for the development of AIDS than do CMV-seronegative patients with hemophilia after seroconversion to HIV. In the present study, we found associations, by logistic regression analysis, of the isolation of CMV from semen and decreases in $CD4^+$ cell numbers and increases in serum IgA concentrations. This suggests that active CMV infection can result in more severe immunosuppression in HIV-seropositive, asymptomatic subjects. These laboratory variables have also been significantly associated with risk for the development of AIDS (39). No relationship, however, was discernible between CMV shedding and either the presence of symptoms of HIV infection or the development of AIDS in our cohort. Such an association may become evident as larger numbers of cases of AIDS develop in this study group.

In conclusion, latent CMV infection appears to be reactivated relatively early after seroconversion to HIV positivity in homosexual or bisexual men, as evidenced by the enhanced shedding of the herpesvirus in semen. It is unclear whether this is a marker of early immunosuppression and replication of HIV or whether CMV actually acts as a significant cofactor in seroconversion to HIV and progression to AIDS. Quantitative studies of CMV infection by such methods as measuring CMV DNA and RNA by using the newly developed polymerase chain reaction (17, 50) may be useful in further delineating the role of CMV in the natural history of HIV infection.

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