JOURNAL OF CLINICAL MICROBIOLOGY, June 2002, p. 2089–2094 Vol. 40, No. 6
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.6.2089–2094.2002
Copyright © 2002, American Society for Microbiology. All Rights Reserved.

Detection of Replication-Competent Human Immunodeficiency Virus Type 1 (HIV-1) in Cultures from Patients with Levels of HIV-1 RNA in Plasma Suppressed to Less Than 500 or 50 Copies Per Milliliter†

Lisa M. Demeter,[1]* Ronald J. Bosch,[2] Robert W. Coombs,[3] Susan Fiscus,[4] James Bremer,[5] Victoria A. Johnson,[6] Alejo Erice,[7] J. Brooks Jackson,[8] Stephen A. Spector,[9] Kathleen M. Squires,[6]‡ Margaret A. Fischl,[10] Michael D. Hughes,[2] and Scott M. Hammer[11]

University of Rochester School of Medicine and Dentistry, Rochester,[1] and Columbia University, New York,[11] New York; Harvard School of Public Health, Boston, Massachusetts[2]; University of Washington, Seattle, Washington[3]; University of North Carolina, Chapel Hill, North Carolina[4]; Rush Medical College, Chicago, Illinois[5]; University of Alabama at Birmingham School of Medicine and Birmingham Veterans Affairs Medical Center, Birmingham, Alabama[6]; University of Minnesota, Minneapolis, Minnesota[7]; Johns Hopkins University, Baltimore, Maryland[8]; University of California at San Diego, San Diego, California[9]; and University of Miami School of Medicine, Miami, Florida[10]

Received 17 August 2001/Returned for modification 29 November 2001/Accepted 12 March 2002

We determined the frequency with which human immunodeficiency virus (HIV) peripheral blood mononuclear cell cultures convert from positive to negative in subjects enrolled in a substudy of AIDS Clinical Trials Group (ACTG) 320, which compared the efficacy of treatment with a combination of indinavir, zidovudine, and lamivudine (indinavir arm) to that of a combination of zidovudine and lamivudine (dual-nucleoside arm). All subjects included for study had positive baseline HIV cultures. Cultures were performed in real time with 10^7 fresh patient peripheral blood mononuclear cells, using the ACTG consensus method. We found lower rates of positive HIV cultures in the indinavir treatment arm than in the dual-nucleoside treatment arm (64 versus 96% at week 24, $P < 0.001$). Within the indinavir arm of the study, we found that positive cultures were less likely to occur in samples with a plasma HIV type 1 (HIV-1) RNA level of <500 copies/ml than in those with a level of ≥500 copies/ml (44 versus 90%, $P < 0.001$). In addition, HIV cultures from samples with HIV-1 RNA levels of ≥500 copies/ml turned positive 8.5 days earlier, on average, than those from samples with levels of <500 copies/ml ($P < 0.001$). However, 38% of samples with plasma RNA levels of <50 copies/ml still were positive for HIV by culture. Thus, the rates of HIV isolation by standard culture procedures decrease as the plasma viral load decreases below 1,000 copies/ml; however, HIV isolates were still obtained from a substantial proportion of subjects with RNA levels of <50 copies/ml. The delay in the time required for HIV cultures to turn positive should be considered when attempting to obtain an HIV isolate from patients with suppression of plasma viral load.

Currently available combination antiretroviral regimens can suppress human immunodeficiency virus type 1 (HIV-1) viral load in plasma to below the limits of detection in a significant proportion of patients (8, 9, 15). Initial reports of the inability, with standard culture techniques, to culture HIV-1 from patients with viral load suppression raised the question of whether circulating peripheral reservoirs of replication-competent HIV-1 could be eradicated (13, 14, 16, 17). Subsequent studies demonstrated that HIV-1 could be cultured from HIV-infected patients with viral load suppression by using more sensitive, labor-intensive techniques that included CD8 cell depletion (2, 5, 13, 17). We performed real-time peripheral blood mononuclear cell (PBMC) cultures using the AIDS Clinical Trials Group (ACTG) consensus method in a subset of subjects enrolled in a phase III clinical trial of indinavir, zidovudine, and lamivudine. We found that this method, which does not utilize CD8 depletion, resulted in the isolation of HIV-1 in 38% of samples with plasma HIV-1 RNA levels that were <50 copies/ml. We have studied the impact of viral load on the yield of HIV-1 cultures and have explored the ability of a positive HIV culture to predict the subsequent viral load responses.

MATERIALS AND METHODS

Study designs of ACTG 320 and ACTG 867. ACTG 320 was a phase III trial of subjects with >3 months of prior experience with zidovudine and CD4 cell counts of ≤200 cells/mm³, and it demonstrated that treatment with the combination of indinavir, zidovudine, and lamivudine (indinavir arm) was superior to treatment with zidovudine and lamivudine (dual-nucleoside arm) in delaying clinical progression (9). A total of 1,178 subjects had been randomized by 21 February 1997, the date that the study was terminated and the subjects were unblinded, following an interim review that demonstrated an improved clinical outcome in the indinavir arm of the study.

The primary goal of ACTG 867, the virology substudy of ACTG 320, was to evaluate the correlations between HIV-1 RNA in plasma, qualitative PBMC coculture, and syncytium-inducing (SI) phenotype. ACTG 867 was designed to enroll a minimum of 200 subjects. Enrollment was limited to 8 of the 40 AIDS Clinical Trials Units that participated in ACTG 320. Entry criteria for ACTG 867 were the same as those for ACTG 320 (9), except that subjects were asked to give consent for the collection of additional blood samples for HIV-1 culture. Informed consent was obtained from all subjects, approval of study procedures was obtained from all appropriate institutional review boards, and studies were

* Corresponding author. Mailing address: University of Rochester Infectious Diseases Unit, 601 Elmwood Ave., Box 689, Rochester, NY 14642. Phone: (585) 275-4764. Fax: (585) 442-9328. E-mail: Lisa_Demeter@urmc.rochester.edu.

† AIDS Clinical Trials Group Protocol 867.

‡ Present address: LAC+USC Medical Center, Rand Schrader Clinic, Los Angeles, CA 90033.

conducted in compliance with the appropriate federal guidelines. ACTG 867 enrolled 225 subjects between January and June 1996. Twenty-four subjects were excluded from further analysis because no baseline culture result was available, nine were excluded because they had a negative baseline culture, and two were excluded because the baseline culture was indeterminate. An additional 15 patients were excluded because no follow-up HIV culture result was available at any of the visits at weeks 8, 24, or 40. The remaining 175 subjects who had a positive baseline culture and at least one follow-up PBMC culture (either positive or negative) were selected for further study. Follow-up was restricted to 40 weeks due to the early closure of the study, and the length of follow-up varied among subjects according to the date of enrollment.

Virology studies. The plasma HIV-1 RNA concentration was determined by using the AMPLICOR MONITOR assay (Roche Diagnostics, Branchburg, N.J.). At the time these studies were conducted, preliminary data had demonstrated a lower limit of quantification of 500 copies/ml for the standard AMPLICOR MONITOR assay. For this reason, all standard AMPLICOR results lower than this limit were considered to be <500 copies/ml. All samples with HIV-1 RNA concentrations of ≤2,000 copies/ml according to the standard AMPLICOR MONITOR assay were retested using the Roche ultrasensitive assay, which has a lower limit of quantification of 50 copies/ml. Six ACTG laboratories performed batch assays after the conclusion of the clinical trial.

HIV-1 PBMC cultures were performed in real time in accordance with the ACTG consensus assay (10). PBMCs from patients and HIV-negative donors were isolated within 30 h of collection by Ficoll-Hypaque gradient centrifugation. Before cocultivation with patient PBMCs, PBMCs from HIV-negative donors were stimulated by culturing in RPMI 1640 with 20% heat-inactivated fetal bovine serum, 100 U of penicillin per ml, 50 μg of gentamicin per ml, 5% interleukin-2, and 5 μg of phytohemaglutinin (Difco) per ml at a concentration of 2×10^6 cells/ml for 1 to 3 days at 37°C in 5% CO_2. Patient and stimulated donor PBMCs (10^7 cells each) were then cocultivated in RPMI 1640 supplemented with 20% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U of penicillin per ml, 50 μg of gentamicin per ml, and 5% interleukin-2 (final volume, 10 ml) at 37°C in 5% CO_2. Patient and donor PBMCs used in this assay were not CD8 cell depleted. Cultures were sampled for p24 antigen every 3 to 4 days and supplemented each week with fresh phytohemagglutinin- and interleukin-2-stimulated PBMCs from HIV-negative donors. Negative cultures were defined as those held for at least 18 days that had a supernatant p24 antigen concentration of <30 pg/ml. Cultures were defined as positive if at least one of the following three criteria was met: two consecutive p24 values of greater than 30 pg/ml, of which the second value was at least four times greater than the first value or had an optical density reading of ≥2.0; two consecutive HIV p24 assay values with an optical density reading of ≥2.0; or three consecutive increasing HIV p24 antigen values of >30 pg/ml, where neither consecutive value was greater than four times that of the previous samples but where the third value was at least four times greater than the first. Cultures that had detectable p24 antigen without evidence for a sustained rise in concentration over time were classified as indeterminate.

For analyses comparing plasma HIV-1 RNA concentrations with PBMC culture results, only results from the same date were used. Assays for SI phenotype were performed at preentry and at week 40 by measuring the cytopathic effect of each clinical HIV isolate in MT2 cells, according to previously published procedures (11).

Statistical methods. The baseline HIV-1 RNA concentration was defined as the geometric mean of the two measurements of HIV-1 RNA concentration obtained prior to starting the study treatment. If either of these measurements was outside the assay's range of quantification, then an imputed value was used: if a value of <500 copies/ml was obtained (which occurred in 1% of subjects), then a value of 500 copies was used, and if a value of >750,000 copies/ml was obtained (which occurred in 9% of subjects), then a value of 750,000 copies was used. The baseline CD4 cell count was defined as the mean of the preentry and entry measurements and excluded the screening value, which had to be ≤200 cells/mm^3. Analyses were based on subjects with available data, regardless of whether they discontinued their randomized treatment. Fisher's exact test and the Cochran-Mantel-Haenszel (CMH) test (stratified by a screening CD4 cell count of <50 versus 51 to 200 cells/mm^3) were used for comparison of proportions. The Wilcoxon rank sum test was used for the comparison of ordered categorical and continuously measured baseline variables. For tests involving the number of days of culture, the generalized estimating equations (GEE) approach was used to account for the possible correlation between multiple observations for the same subject (12). Robust variance estimates with an exchangeable working correlation were used. When treatment was tested, a covariate for the stratification factor (screening CD4 cell count, <50 versus 51 to 200 cells/mm^3) was included in the model. All analyses presented are based on randomized treatment assignment. However, similar results were obtained when subjects were analyzed in an as-treated fashion. Logistic regression analysis was used to examine predictors of virologic suppression. Significance was assessed with the likelihood ratio. All reported *P* values represent two-sided tests and are unadjusted for multiple comparisons.

TABLE 1. Baseline characteristics of the patient population

Characteristic	Value for patients		
	ZDV+3TC arm[a] (n = 90)	IDV+ZDV+3TC arm[a] (n = 85)	Total (n = 175)
Sex, no. (%)			
Male	77 (86)	70 (82)	147 (84)
Female	13 (14)	15 (18)	28 (16)
Age (yr), mean (SD)	39.4 (8.8)	38.6 (7.5)	39.0 (8.2)
Race and ethnicity, no. (%)			
White and non-Hispanic	54 (60)	46 (54)	100 (57)
Black and non-Hispanic	20 (22)	18 (21)	38 (22)
Hispanic	16 (18)	17 (20)	33 (19)
Other	0 (0)	4 (5)	4 (2)
Intravenous drug use, no. (%)			
Never	77 (86)	71 (84)	148 (85)
Currently	0 (0)	0 (0)	0 (0)
Previously	13 (14)	14 (16)	27 (15)
Hemophiliac			
Yes	2 (2)	0 (0)	2 (1)
No	88 (98)	85 (100)	173 (99)
Karnofsky score, mean (SD)	91.9 (8.3)	89.4 (8.2)	90.7 (8.3)
CD4 cell count (per mm^3), mean (SD)	77.4 (57.1)	89.3 (66.6)	83.2 (62.0)
No. of mo of prior ZDV treatment, median (25th–75th %ile)	20 (8–39)	22 (9–42)	21 (8–41)
HIV-1 RNA ($\log_{10}$ copies/ml), mean (SD)	4.95 (0.68)	4.94 (0.63)	4.95 (0.66)

[a] Groups were treated with zidovudine and lamivudine (ZDV+3TC) or with indinavir, zidovudine, and lamivudine (IDV+ZDV+3TC).

RESULTS

Patient population. The baseline characteristics of the study subjects are shown in Table 1. The mean baseline CD4 cell count was 83 cells/mm^3; the baseline plasma HIV-1 RNA concentration was 4.95 $\log_{10}$ copies/ml. The baseline characteristics of subjects assigned to each of the two treatment arms were similar, with the exception of a lower mean Karnofsky score for subjects in the indinavir arm (89.4 versus 91.9, P = 0.026, Wilcoxon rank sum test). Overall, the baseline characteristics of study subjects were similar to those of the subjects enrolled in the parent study, ACTG 320 (9).

Plasma HIV-1 RNA responses according to treatment arm. Suppression of plasma HIV-1 RNA levels to <500 copies/ml was achieved in 41% (34 of 83), 53% (41 of 77), and 56% (38 of 68) of subjects in the indinavir arm at weeks 8, 24, and 40, respectively. In contrast, only 4% (4 of 89), 6% (5 of 81), and 15% (10 of 68) of subjects in the dual-nucleoside arm achieved suppression of plasma HIV-1 RNA levels to <500 copies/ml at weeks 8, 24, and 40, respectively (P < 0.001, CMH test at each time point for comparisons between the two treatment arms).

TABLE 2. Frequency of positive HIV PBMC cultures, by randomized treatment arm[a]

Study wk	No. of positive cultures/total no. of cultures (%)[a]		*P* value[b]
	ZDV+3TC arm	IDV+ZDV+3TC arm	
0	90/90 (100)	85/85 (100)	
8	81/85 (95)	60/81[c] (74)	<0.001
24	75/78 (96)	46/72 (64)	<0.001
40	55/65 (85)	38/65 (58)	<0.001

[a] Subjects were treated with a combination of zidovudine and lamivudine (ZDV+3TC arm) or indinavir, zidovudine, and lamivudine (IDV+ZDV+3TC arm). Analyzed samples were obtained from each subject who had a positive baseline PBMC culture and at least one follow-up culture (either positive or negative).
[b] Determined by use of the CMH test for the comparison between arms, stratified by the screening CD4 cell count.
[c] Excludes one indeterminate culture.

Suppression of plasma HIV-1 RNA levels to <50 copies/ml was achieved in 22% (18 of 83), 36% (27 of 76), and 46% (31 of 68) of subjects in the indinavir arm at weeks 8, 24, and 40, respectively. Fewer than 7% of subjects in the dual-nucleoside arm achieved suppression of plasma HIV-1 RNA levels to <50 copies/ml at any time point ($P < 0.001$, CMH test).

Frequency of positive PBMC cultures according to treatment arm. There were significantly lower rates of positive PBMC cultures after the initiation of therapy among subjects in the indinavir arm than among subjects in the dual-nucleoside arm ($P < 0.001$ at weeks 8, 24, and 40, CMH test) (Table 2). Although the duration of culture incubation beyond 18 days was determined at the site level, this should not have led to differences in the length of incubation of cultures between the two arms of the study, since the sites and laboratories were blinded as to treatment assignment and viral load responses. We confirmed that duration of incubation for negative cultures did not differ between the two treatment arms ($P = 0.78$, GEE test, including the CD4 cell count as a covariate).

TABLE 3. Frequency of positive HIV-1 PBMC cultures in subjects randomized to receive triple therapy (including indinavir), according to week of therapy and plasma HIV-1 RNA concentration

Study wk	Frequency of positive PBMC culture in subjects with indicated results[a]			
	Standard AMPLICOR assay[b]		Ultrasensitive AMPLICOR assay[c]	
	<500 copies/ml	≥500 copies/ml	<50 copies/ml	≥50 copies/ml
8	18/34 (53)	39/44 (89)[d]	9/18 (50)	21/33 (64)
24	16/38 (42)	30/33 (91)	7/24 (29)	11/15 (73)
40	14/37 (38)	23/25 (92)	12/31 (39)	2/7 (29)
Total	48/109 (44)	92/102 (90)	28/73 (38)	34/55 (62)

[a] Number of subjects with positive HIV-1 PBMC cultures relative to the total number of subjects. Values in parentheses are percentages.
[b] Results from seven cultures were excluded, due to missing plasma HIV-1 RNA results. The difference in the proportions of subjects who had positive PBMC cultures was significant when comparing those with HIV-1 RNA levels of <500 copies/ml and those with HIV-1 RNA levels of ≥500 copies/ml at each week ($P < 0.001$, Fisher's exact test).
[c] The ultrasensitive assay was performed only on samples with a standard AMPLICOR result of ≤2,000 copies/ml.
[d] Excludes one culture which had indeterminate results.

TABLE 4. Frequency of positive cultures in subjects randomized to receive triple therapy (indinavir arm) according to plasma HIV-1 RNA concentration (standard AMPLICOR assay)[a]

HIV-1 RNA concn (copies/ml)	No. of positive cultures/ total no. of cultures from samples (%)
<500	48/109 (44)
500–999	11/17 (65)
1,000–4,999	12/13 (92)
5,000–50,000	37/40 (93)
≥50,000	32/32 (100)

[a] Contains only results obtained after baseline (week 8, 24, or 40).

Association of HIV-1 RNA concentration and PBMC culture result. Because so few cultures from subjects in the dual-nucleoside arm were negative, analysis of the association between plasma HIV-1 RNA concentration and PBMC culture results was limited to samples obtained from subjects in the indinavir arm. At each week, the proportion of subjects with HIV-1 RNA concentrations of <500 copies/ml who had positive PBMC cultures was lower than the proportion of subjects with HIV-1 RNA concentrations of ≥500 copies/ml ($P < 0.001$, Fisher's exact test) (Table 3).

Lower plasma HIV-1 RNA concentrations were associated with a decreased likelihood of a positive PBMC culture. For example, 95% of samples (81 of 85) with a plasma HIV-1 RNA concentration of ≥1,000 copies/ml by the standard AMPLICOR assay had a positive PBMC culture, compared with 65% of those with 500 to 999 copies/ml and 44% of those with <500 copies/ml (Table 4). This trend was also seen with the subset of samples that were tested by the ultrasensitive assay (data not shown).

Correlation of plasma HIV-1 RNA concentration with the time required for PBMC cultures to become positive. We evaluated whether the apparently lower rate of culture isolation from samples with low viral loads was due to these cultures being incubated for a shorter period of time, leading to false-negative results. Table 5 shows the duration of incubation of the negative cultures obtained from subjects in the indinavir arm, broken down according to plasma HIV-1 RNA concentration. There was no significant difference in the incubation times by plasma HIV-1 RNA concentration ($P = 0.32$, GEE test).

However, there were differences in the times required for

TABLE 5. Distribution of negative cultures among ranges of total incubation time, according to plasma HIV-1 RNA concentration in subjects[a]

No. of days of incubation	No. of negative cultures (cumulative %) at HIV-1 RNA level of[b]:	
	<500 copies/ml	≥500 copies/ml
18–21	14 (23)	5 (50)
22–28	33 (77)	3 (80)
>28	14 (100)	2 (100)

[a] Subjects were randomized to receive combination therapy with indinavir. Results from three cultures are excluded because no plasma HIV-1 RNA result was available for the same time at which the culture was performed.
[b] There was no significant difference in the incubation times between the two groups ($P = 0.32$, GEE test).

TABLE 6. Distribution of positive culture results by the first day on which a culture was determined to be positive according to plasma HIV-1 RNA concentration (indinavir arm only)[a]

Days on which culture turned positive	No. of positive cultures (cumulative %) at plasma HIV-1 RNA concn of[b]:	
	<500 copies/ml	≥500 copies/ml
4–7	1 (2)	80 (45)
8–10	6 (15)	63 (81)
11–14	14 (44)	19 (92)
15–17	3 (50)	6 (95)
18–21	11 (73)	2 (97)
22–28	11 (96)	5 (99)
>28	2 (100)	1 (100)
Total[c]	48	176

[a] Results of the standard AMPLICOR MONITOR assay.
[b] There were significant differences in the days on which cultures turned positive between samples with different plasma HIV-1 concentrations ($P <$ 0.001, GEE test).
[c] Excludes five samples for which no plasma HIV-1 RNA result was available for the same time at which the culture was obtained.

cultures to turn positive, depending on the plasma HIV-1 RNA concentration of the sample used (Table 6). For example, 92% of cultures from samples with plasma HIV-1 RNA concentrations of ≥500 copies/ml turned positive within the first 2 weeks of incubation, compared to only 44% from samples with <500 copies/ml. HIV-1 cultures from samples with plasma HIV-1 RNA concentrations of ≥500 copies/ml turned positive 8.5 days earlier, on average, than cultures from samples with RNA concentrations of <500 copies/ml ($P < 0.001$, GEE test) (Table 6). Of the 103 cultures still negative at day 18, 32 (31%) subsequently turned positive. All but 3 of these 32 cultures were positive by day 28.

Predictors of virologic response. Our previous analyses of the ACTG 320 cohort had shown that greater age, lower RNA concentrations (baseline and week 8), and higher CD4 cell counts (baseline and week 8) were independent predictors of the virologic outcome at week 24 (3). Neither race and ethnicity nor duration of prior zidovudine use were predictors of the virologic outcome in those analyses. Similar associations were seen in the subset of subjects described here (data not shown).

Because culture data for these subjects were also available, we examined whether having a baseline SI virus isolate or a positive PBMC culture at week 8 predicted the virologic outcome at week 24. A baseline SI phenotype was not a significant predictor of suppression of plasma HIV-1 RNA levels to <500 copies/ml at week 24 or 40, although the small sample size may have limited our ability to detect a significant association. For example, in the 46 subjects who had SI virus at baseline, 57% had HIV-1 RNA levels of <500 copies/ml at week 24, compared with 54% of the 28 subjects who had non-SI virus at baseline.

Among the 74 subjects in the indinavir arm who had an HIV-1 RNA measurement at week 24, the week 8 PBMC culture result was significantly associated with the probability of suppression of HIV-1 RNA levels to <500 copies/ml at week 24 ($P < 0.001$) (Table 7). However, there was no apparent predictive value of the culture result among subjects with a concentration of HIV-1 RNA at week 8 of <50 copies/ml (Table 7). Among subjects with a concentration of HIV RNA of ≥50 copies/ml at week 8, a higher proportion of those with a negative week 8 culture had subsequent viral load suppression at week 24 than of those with a positive week 8 culture (Table 7). However, when adjustment was made for the actual HIV-1 RNA level at week 8, the culture result was no longer a significant predictor of subsequent virologic outcome in this subgroup of patients ($P = 0.31$).

DISCUSSION

We studied the frequency of positive PBMC cultures in a subset of subjects enrolled in a phase III clinical trial which compared triple therapy with indinavir and two nucleosides to dual-nucleoside therapy alone. In this study, we limited our analyses to subjects who had positive PBMC cultures at baseline. Cultures were performed in real time with freshly isolated patient PBMCs according to a standard procedure in which cultures were defined as positive by using uniform criteria (10). Of note is that the culture method used did not include CD8 cell depletion. Cultures were held for a minimum of 18 days before they were determined to be negative, although there was some variability in the length of time beyond 18 days that negative cultures were incubated. This study was prompted in part by reports of negative PBMC culture results, determined by using standard PBMC culture methods, for subjects with HIV-1 RNA suppression. Those studies demonstrated that depletion of CD8 cells from patient and donor PBMCs dramatically increased the positive culture rate (13, 17).

Surprisingly, we found that approximately 40% of samples with a plasma HIV-1 RNA concentration of <50 copies/ml yielded a positive PBMC culture. Since cultures with negative results were not incubated for 28 days at all sites, we believe that our isolation rate of 40% in these samples is an underestimate. We suspect that the higher rate of HIV isolation in our study than in previously published work is related both to differences in the patient populations studied and to technical differences in the standard culture method used. Samples from patients in our study were obtained for HIV-1 culture between 8 and 40 wks after initiation of treatment, whereas patients who were previously studied usually had achieved virologic suppression for longer periods of time. Technical differences in the culture methods that could have led to higher isolation rates in our study included the number of patient PBMCs used, the duration of culture incubation, and our performance of cultures in real time with freshly isolated patient PBMCs. Previously published studies have demonstrated that freshly isolated PBMCs give higher culture yields than methods using

TABLE 7. Relationship of suppression of HIV-1 RNA levels to <500 copies/ml in subjects at week 24 in indinavir arm with week 8 HIV-1 RNA concentration and PBMC culture result

PMBC culture result	% of subjects (no. achieving suppression/total no. tested) with indicated HIV-1 RNA concn		
	<50 copies/ml	≥50 copies/ml[a]	Both
Negative	89 (8/9)	83 (10/12)	86 (18/21)
Positive	88 (7/8)	36 (16/45)	43 (23/53)

[a] $P = 0.31$, for testing week 8 culture result (negative versus positive) among subjects with week 8 RNA level of ≥50 copies/ml, after adjusting for week 8 $\log_{10}$ HIV-1 RNA concentration.

cryopreserved PBMCs (1, 4, 10). We do not have data on whether adding a CD8 cell depletion step would further increase the sensitivity of the ACTG culture assay used in our study. Since few cultures were incubated for more than 28 days, we also do not know whether the rate of HIV isolation would be significantly increased by a further increase in the duration of culture incubation.

We studied in detail the association of treatment and viral load with culture yield. We found that there were significantly lower rates of positive cultures in the triple-therapy arm than in the dual-nucleoside arm. Because the two treatment arms differed in the frequency of suppression of plasma HIV-1 RNA levels, we reasoned that samples with lower plasma HIV-1 RNA levels would have a lower rate of positive cultures. We found that, when our analysis was limited to subjects in the indinavir triple-therapy arm, the frequency of positive PBMC cultures was strongly correlated with plasma HIV-1 RNA concentration but that a substantial proportion of subjects with suppressed HIV-1 RNA in plasma still had positive PBMC cultures.

The finding that PBMC cultures are positive in approximately 40% of samples that have plasma HIV-1 RNA levels below the current limits of detection further supports growing evidence that reservoirs of replication-competent virus continue to exist despite the apparent suppression of HIV-1 replication (2, 5, 7, 17, 18). In addition, at a more pragmatic level, our findings suggest that obtaining an HIV isolate may be possible in a substantial proportion of patients with viral load suppression, without resorting to CD8 cell depletion. We have demonstrated that positive PBMC cultures from samples with lower plasma HIV-1 RNA concentrations turn positive at a later time than those from samples with higher plasma HIV-1 RNA concentrations. Based on the results from this study, the ACTG consensus protocol for HIV PBMC culture now recommends a minimum incubation time of 28 days, rather than the originally recommended 21 days, before a culture is declared negative (6).

Because not all patients in the indinavir arm of the study developed negative PBMC cultures or achieved virologic suppression, we postulated that we might be able to detect an association between the PBMC culture result and the subsequent virologic outcome. We did find that a negative PBMC culture result at week 8 was associated with an increased likelihood of virologic suppression at week 24. However, the PBMC culture result did not provide additional predictive value regarding those subjects with a week 8 HIV-1 RNA concentration of $<$50 copies/ml. This finding suggests that PBMC culture does not provide predictive information in addition to that provided by the viral load, although the small sample sizes limited our power to evaluate this question.

In summary, we have demonstrated that HIV-1 can be isolated from a substantial proportion of patients with viral loads below the limits of detection of currently available assays, even when a standard PBMC culture technique is used. Lower viral loads result in delays in a detectable rise of p24 antigen, and it is likely that prolonging the incubation time for negative cultures to 28 days will improve the sensitivity of PBMC cultures to some extent. A negative PBMC culture at 8 weeks of therapy appears to provide some predictive power for subsequent virologic suppression, although this finding did not persist when controlling for the week 8 plasma HIV-1 RNA concentration.

ACKNOWLEDGMENTS

This work was supported in part by the ACTG and by grants (AI-27658, AI-38855, AI-38858 [subcontracts 96VC001, 96VC006, 96VC009, 96VC010, 96VC012, 200VC001, 200VC007], AI-27664, AI-30731, AI-40876, and RR-00044) from the National Institutes of Health. Supplemental support for some virology studies was provided by Merck & Company and Roche Molecular Systems. Study medications were provided by Merck & Company and Glaxo Wellcome Company.

We gratefully acknowledge the subjects and investigators at the eight AIDS Clinical Trials Units, whose participation made this study possible. We thank Luis Berrios, Michael Chiulli, and Robin Shepard for performance of HIV-1 RNA assays and Angela Dexter, Melissa Kerkau, Derlene Manfredi, Theresa Mescan, Hans Nickstadt, Jana Parsons, Ruth A. Rhodes, Joan Dragavon, Reggie Sampoleo, Corey Scherrer, and Kenneth P. Zammit for performance of HIV cultures.

REFERENCES

1. **Balachandran, R., P. Thampatty, C. Rinaldo, and P. Gupta.** 1988. Use of cryopreserved normal peripheral blood lymphocytes for isolation of human immunodeficiency virus from seropositive men. J. Clin. Microbiol. **26:**595–597.
2. **Chun, T. W., L. Stuyver, S. B. Mizell, L. A. Ehler, J. A. Mican, M. Baseler, A. L. Lloyd, M. A. Nowak, and A. S. Fauci.** 1997. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc. Natl. Acad. Sci. USA **94:**13193–13197.
3. **Demeter, L. M., M. D. Hughes, R. W. Coombs, J. B. Jackson, J. Grimes, R. J. Bosch, S. A. Fiscus, S. A. Spector, K. E. Squires, M. A. Fischl, and S. M. Hammer.** 2001. Predictors of virologic and clinical outcomes in HIV-1 infected patients receiving concurrent treatment with indinavir, zidovudine and lamivudine (AIDS clinical trials group [ACTG] protocol 320). Ann. Intern. Med. **135:**954–964.
4. **Farzadegan, H., D. Imagawa, P. Gupta, M. H. Lee, L. Jacobson, A. Saah, K. Grovit, C. R. Rinaldo, Jr., and B. F. Polk.** 1990. The effect of fresh lymphocytes on increased sensitivity of HIV-1 isolation: a multicenter study. J. Acquir. Immune Defic. Syndr. **3:**981–986.
5. **Finzi, D., M. Hermankova, T. Pierson, L. M. Carruth, C. Buck, R. E. Chaisson, T. C. Quinn, K. Chadwick, J. Margolick, R. Brookmeyer, J. Gallant, M. Markowitz, D. D. Ho, D. D. Richman, and R. F. Siliciano.** 1997. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science **278:**1295–1300.
6. **Fiscus, S. A., S. L. Welles, S. A. Spector, and J. L. Lathey.** 1995. Length of incubation time for human immunodeficiency virus cultures. J. Clin. Microbiol. **33:**246–247.
7. **Furtado, M. R., D. S. Callaway, J. P. Phair, K. J. Kunstman, J. L. Stanton, C. A. Macken, A. S. Perelson, and S. M. Wolinsky.** 1999. Persistence of HIV-1 transcription in peripheral-blood mononuclear cells in patients receiving potent antiretroviral therapy. N. Engl. J. Med. **340:**1614–1622.
8. **Gulick, R. M., J. W. Mellors, D. Havlir, J. J. Eron, C. Gonzalez, D. McMahon, D. D. Richman, F. T. Valentine, L. Jonas, A. Meibohm, E. A. Emini, and J. A. Chodakewitz.** 1997. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. N. Engl. J. Med. **337:**734–739.
9. **Hammer, S. M., K. E. Squires, M. D. Hughes, J. M. Grimes, L. M. Demeter, J. S. Currier, J. J. Eron, Jr., J. E. Feinberg, H. H. Balfour, Jr., L. R. Deyton, J. A. Chodakewitz, and M. A. Fischl.** 1997. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. N. Engl. J. Med. **337:**725–733.
10. **Hollinger, F. B., J. W. Bremer, L. E. Myers, J. W. Gold, L. McQuay, and the NIH/NIAID/DAIDS/ACTG Virology Laboratories.** 1992. Standardization of sensitive human immunodeficiency virus coculture procedures and establishment of a multicenter quality assurance program for the AIDS Clinical Trials Group. J. Clin. Microbiol. **30:**1787–1794.
11. **Japour, A. J., S. A. Fiscus, J.-M. Arduino, D. L. Mayers, P. S. Reichelderfer, and D. R. Kuritzkes.** 1994. Standardized microtiter assay for determination of syncytium-inducing phenotypes of clinical human immunodeficiency virus type 1 isolates. J. Clin. Microbiol. **32:**2291–2294.
12. **Liang, K. Y., and S. L. Zeger.** 1986. Longitudinal data analysis using generalized linear models. Biometrika **73:**13–22.
13. **Markowitz, M., M. Vesanen, K. Tenner-Racz, Y. Cao, J. M. Binley, A. Talal, A. Hurley, X. Ji, M. R. Chaudhry, M. Yaman, S. Frankel, M. Heath-Chiozzi, J. M. Leonard, J. P. Moore, P. Racz, D. F. Nixon, and D. D. Ho.** 1999. The effect of commencing combination antiretroviral therapy soon after human

immunodeficiency virus type 1 infection on viral replication and antiviral immune responses. J. Infect. Dis. **179:**527–537.
14. **Perelson, A. S., P. Essunger, Y. Cao, M. Vesanen, A. Hurley, K. Saksela, M. Markowitz, and D. D. Ho.** 1997. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature **387:**188–191.
15. **Staszewski, S., J. Morales-Ramirez, K. T. Tashima, A. Rachlis, D. Skiest, J. Stanford, R. Stryker, P. Johnson, D. F. Labriola, D. Farina, D. J. Manion, N. M. Ruiz, and the Study 006 Team.** 1999. Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. N. Engl. J. Med. **341:** 1865–1873.
16. **Wong, J. K., H. F. Gunthard, D. V. Havlir, Z. Q. Zhang, A. T. Haase, C. C. Ignacio, S. Kwok, E. Emini, and D. D. Richman.** 1997. Reduction of HIV-1 in blood and lymph nodes following potent antiretroviral therapy and the virologic correlates of treatment failure. Proc. Natl. Acad. Sci. USA **94:** 12574–12579.
17. **Wong, J. K., M. Hezareh, H. F. Gunthard, D. V. Havlir, C. C. Ignacio, C. A. Spina, and D. D. Richman.** 1997. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science **278:**1291–1295.
18. **Zhang, L., B. Ramratnam, K. Tenner-Racz, Y. He, M. Vesanen, S. Lewin, A. Talal, P. Racz, A. S. Perelson, B. T. Korber, M. Markowitz, and D. D. Ho.** 1999. Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. N. Engl. J. Med. **340:**1605–1613.