Is HIV Becoming More Virulent? Initial CD4 Cell Counts among HIV Seroconverters during the Course of the HIV Epidemic: 1985–2007

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(See the editorial commentary by Dorrucci and Phillips, on pages 1293–5)

Background. Whether human immunodeficiency virus (HIV) seroconverters have been presenting with progressively lower CD4 cell counts over the course of the HIV epidemic is controversial. Additional data on whether HIV might have become more virulent on a population level (measured by post-seroconversion CD4 cell counts) may provide important insights regarding HIV pathogenesis.

Methods. To determine whether post-seroconversion CD4 cell counts have changed over time, we evaluated 2174 HIV seroconverters as part of a large cohort study during the period 1985–2007. Participants were documented antiretroviral-naive HIV seroconverters who had a CD4 cell count measured within 6 months after receiving a diagnosis of HIV infection. Multiple linear regression models were used to assess trends in initial CD4 cell counts.

Results. The mean initial CD4 cell count decreased during the study period from 632 cells/mm^3 in 1985–1990 to 553 cells/mm^3 in 1991–1995, 493 cells/mm^3 in 1996–2001, and 514 cells/mm^3 in 2002–2007. During those periods, the percentages of seroconverters with an initial CD4 cell count <350 cells/mm^3 were 12%, 21%, 26%, and 25%, respectively. In the multiple linear model, the mean decrease in CD4 cell count from 1985–1990 was 65 cells/mm^3 in 1991–1995 ($P < .001$), 107 cells/mm^3 in 1996–2001 ($P < .001$), and 102 cells/mm^3 in 2002–2007 ($P < .001$). Similar trends occurred with regard to CD4 cell percentage and total lymphocyte count. Similar decreases in initial CD4 cell counts were observed among African American and white persons during the epidemic.

Discussion. A significant decrease in initial CD4 cell counts among HIV seroconverters in the United States has occurred during the HIV epidemic. These data provide an important clinical correlate to suggestions that HIV may have adapted to the host, resulting in a more virulent infection.

CD4 cell counts are the most important clinical marker for immune competence and disease progression among HIV-infected persons [1] and are key determinants of the initiation of HAART [2]. Most clinicians expect a several-year window between HIV seroconversion and the need for antiretroviral therapy. However, some reports have suggested that HIV-infected persons are presenting with lower CD4 cell counts in recent years and are experiencing rapid HIV progression, which requires the introduction of HAART shortly after diagnosis [3–5]. Some of these data may be confounded, because patients may have been infected with HIV for several years before actually receiving the diagnosis.

Studies examining trends in post-seroconversion CD4 cell counts among documented HIV seroconverters during the HIV epidemic are conflicting. A recent study that examined a cohort of HIV seroconverters from Europe, Australia, and Canada suggested

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that initial CD4 cell counts have decreased from 1985 to 2002 [4, 6]. However, several previous studies found no significant trends in post-seroconversion CD4 cell counts over various time periods during the HIV epidemic [7–12], or a decreasing trend has been observed only among certain HIV transmission groups [13]. Some studies have had methodological difficulties or short periods of evaluation [11, 14–17].

Whether HIV-infected patients have presented with progressively lower CD4 cell counts over the course of the HIV epidemic remains unclear, and no recent study examining the subject has been conducted in the United States. If initial CD4 cell counts are decreasing, this could suggest that HIV has become more virulent, providing valuable information to clinicians as well as important insights regarding HIV pathogenesis on a population level. We evaluated racially diverse documented HIV seroconverters from various geographic areas in the United States to evaluate whether CD4 cell counts at diagnosis have changed during the course of the HIV epidemic.

METHODS

Study cohort. We evaluated documented HIV seroconverters from 1985 through 2007 as part of a large cohort study to determine if post-seroconversion CD4 cell counts have changed over time. The TriService AIDS Clinical Consortium HIV Natural History Study is an ongoing, prospective observational cohort of HIV-infected persons who are Department of Defense beneficiaries (active duty members, retirees, and dependents) at 7 US military medical centers. All active duty US military personnel are confirmed to be HIV-negative prior to enlistment and undergo routine HIV screening every 1–5 years. Since the study initiation in 1985, the Natural History Study has enrolled over 4900 participants. HIV-infected participants in the study mainly acquired HIV via sexual transmission; <1% of our cohort is estimated to have used illegal drugs [18], because of military drug policies and testing procedures.

All participants in this study were documented HIV seroconverters, with a ⩽4-year interval (mean interval [±SD], 1.5 ± 0.9 years) between the last negative and first positive HIV test result. HIV infection status was assessed using ELISA, and positive results were confirmed by Western Blot. All included participants were antiretroviral naive and had a CD4 cell count measured within 6 months after receiving a diagnosis of HIV infection. This substudy involving 2174 participants was approved by the governing central institutional review board.

Data collection. Following enrollment, data collected from participants included demographic information (age, sex, and self-reported race/ethnicity); body mass index (calculated as weight in kilograms divided by the square of height in meters); medical histories, including medication use; and clinical laboratory studies, including WBC counts, total lymphocyte counts, CD8 cell counts, CD4 cell counts, and CD4 cell percentages (determined by flow cytometry); and HIV load (Amplicor; Roche). Viral load testing became available for our study cohort in ~1996; viral loads measured within 6 months after HIV diagnosis were used when available. All laboratory tests were performed at the participating military medical centers, which are accredited by the Clinical Laboratory Improvement Amendments.

Statistical methods. Statistical analyses included summaries of baseline characteristics and immune-related cell counts for the study population. Data were recorded as mean values (±SD) for continuous variables and as percentages for categorical variables. The study period of 1985–2007 was a priori divided into 4 periods according to time of HIV diagnosis: 2 periods in the pre-HAART era (1985–1990 and 1991–1995) and 2 periods in the HAART era (1996–2001 and 2002–2007). The χ^2 test was used to compare characteristics among patients from the 4 calendar periods.

Linear regression models were used to assess trends in initial CD4 cell counts across calendar periods. Models were adjusted for length of seroconversion window (i.e., time from last negative HIV test result to first positive HIV test result), time from positive HIV test result to initial CD4 cell count measurement, age, sex, race, body mass index, enrollment site, and HIV load, which was categorized into standard groupings when available (1267 participants) and categorized as "missing" when not available (907 participants); this will be referred to as the fully adjusted model. These same fully adjusted regression models were repeated for CD4 cell percentage and CD8 cell, total lymphocyte, and WBC counts. Predicted means of CD4 cell counts were computed by time period with use of marginal weighting to reflect the actual distribution of adjusting characteristics in this cohort; tests of pairwise differences used the Tukey-Kramer adjustment for multiple comparisons. In addition to adjusting for race in our fully adjusted models, a time period by race interaction was added to the fully adjusted model and tested, and separate models by race were performed. Similarly, we considered a time period by enrollment site interaction. All analyses were conducted using SAS, version 9 (SAS Institute).

RESULTS

Baseline characteristics. The study population included 2174 documented HIV seroconverters. The participants had a mean (±SD) age of 29 ± 7 years and 96% were men; 44% were white/non-Hispanic, 45% were African American, and 11% were other races (table 1). Thirty-five percent of participants had a seroconversion window of <1 year, 41% had a window of 1–2 years, 17% had a window of 2–3 years, and 7% had a window of 3–4 years. Ninety-three percent of the study cohort had a CD4 cell count measured within 3 months after receiving a diagnosis of HIV infection.

The baseline characteristics of HIV seroconverters during the

Table 1. Baseline clinical and laboratory characteristics of HIV-seroconverters by time period, 1985–2007.

Characteristic	All (n = 2174)	Time period 1985–1990 (n = 562)	1991–1995 (n = 630)	1996–2001 (n = 553)	2002–2007 (n = 429)	P
Age at HIV diagnosis, mean years ± SD	28.7 ± 6.7	27.3 ± 5.9	28.6 ± 6.4	29.4 ± 6.9	30.0 ± 7.6	<.001
Female sex	92 (4.2)	23 (4.1)	38 (6.0)	20 (3.6)	11 (2.6)	.04
Race						
White/non-Hispanic	948 (43.6)	286 (50.9)	264 (41.9)	203 (36.7)	195 (45.5)	<.001
African American	971 (44.7)	229 (40.8)	298 (47.3)	274 (49.6)	170 (39.6)	
Other	255 (11.7)	47 (8.4)	68 (10.8)	76 (13.7)	64 (14.9)	
BMI[a]						
Mean value ± SD	25.0 ± 3.5	23.5 ± 2.6	24.7 ± 3.3	25.3 ± 3.5	25.9 ± 3.9	<.001
Data missing	1048 (48.2)	414 (73.7)	290 (46.0)	177 (32.0)	167 (38.9)	
<24.9	594 (27.3)	111 (19.8)	185 (29.4)	180 (32.6)	118 (27.5)	
25–29.9	436 (20.1)	35 (6.2)	137 (21.8)	158 (28.6)	106 (24.7)	
⩾30	96 (4.4)	2 (0.4)	18 (2.9)	38 (6.9)	38 (8.9)	
Seroconversion window						
Mean years ± SD	1.45 ± 0.88	1.42 ± 0.77	1.62 ± 0.95	1.39 ± 0.89	1.35 ± 0.84	<.001
<1 year	769 (35.4)	176 (31.3)	194 (30.8)	225 (40.7)	174 (40.6)	
1 to <2 years	880 (40.5)	264 (47.0)	237 (37.6)	210 (38.0)	169 (39.4)	
2 to <3 years	374 (17.2)	101 (18.0)	131 (20.8)	79 (14.3)	63 (14.7)	
3–4 years	151 (7.0)	21 (3.7)	68 (10.8)	39 (7.1)	23 (5.4)	
Time from HIV diagnosis to first CD4 cell count						
Mean days ± SD	42.8 ± 30.2	47.9 ± 32.7	44.8 ± 31.7	39.7 ± 27.9	37.5 ± 25.8	
−30 to 29 days	799 (36.8)	170 (30.3)	212 (33.7)	226 (40.9)	191 (44.5)	<.001
30–89 days	1212 (55.8)	336 (59.8)	358 (56.8)	297 (53.7)	221 (51.5)	
90–149 days	135 (6.2)	47 (8.4)	50 (7.9)	24 (4.3)	14 (3.3)	
150–182 days	28 (1.3)	9 (1.6)	10 (1.6)	6 (1.1)	3 (0.7)	
Baseline HIV load[b]						
Mean copies/mL ± SD	4.3 ± 0.9	4.2 ± 0.8	4.2 ± 0.8	4.3 ± 0.9	4.2 ± 1.0	<.001
Data missing	907 (41.7)	468 (83.3)	427 (67.8)	12 (2.2)	0 (0)	
⩽1000 copies/mL	126 (5.8)	10 (1.8)	19 (3.0)	48 (8.7)	49 (11.4)	
1001–4000 copies/mL	128 (5.9)	9 (1.6)	23 (3.7)	57 (10.3)	39 (9.1)	
4001–10,000 copies/mL	150 (6.9)	12 (2.1)	27 (4.3)	66 (11.9)	45 (10.5)	
10,001–50,000 copies/mL	434 (20.0)	37 (6.6)	85 (13.5)	170 (30.7)	142 (33.1)	
50,001–100,000 copies/mL	207 (9.5)	11 (2.0)	31 (4.9)	99 (17.9)	66 (15.4)	
>100,000 copies/mL	222 (10.2)	15 (2.7)	18 (2.9)	101 (18.3)	88 (20.5)	

NOTE. Data are no. (%) of patients, unless otherwise indicated. BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

[a] BMI at HIV diagnosis was categorized with ⩽24.9 kg/m^2 as underweight or normal weight, 25–29.9 kg/m^2 as overweight, and ⩾30 kg/m^2 as obese.

[b] HIV load testing became widely available in our study cohort in 1996. Mean HIV load is computed among participants for whom the value was available.

4 periods are shown in table 1. Comparisons among baseline characteristics showed a lower percentage of white/non-Hispanic participants and a higher percentage of other races in more-recent years (P < .001), as well as increases in age at diagnosis of HIV infection (P < .001) and body mass index (P < .001) over time. HIV loads varied among patients in different time periods when missing values were included; however, this was because 77% of participants in 1985–1995 were missing values, compared with 1% of participants in 1996–2007. Excluding missing values, there were no significant differences in baseline HIV loads over the study period (χ^2 = 13; P = .60). Finally, there were differences among the 4 periods with regard to the length of the seroconversion window (P < .001) and the time from diagnosis to measurement of the initial CD4 cell count (P = .001); both durations appeared to be shorter during recent years.

Descriptive trends and unadjusted regression analyses of CD4 cell counts during the HIV epidemic. The mean initial CD4 cell count was 632 cells/mm^3 in 1985–1990, 553 cells/mm^3 in 1991–1995, 493 cells/mm^3 in 1996–2001, and 514 cells/mm^3 in 2002–2007 (table 2). In the unadjusted regression models, compared with CD4 cell counts for participants in 1985–1990, the mean initial CD4 cell count was 79 cells/mm^3 less in 1991–1995 (P < .001), 140 cells/mm^3 less in 1996–2001 (P < .001), and 118 cells/mm^3 less in 2002–2007 (P < .001) (table 3). There was no significant difference in the mean initial CD4 cell count

Table 2. Laboratory values for immune-related cell counts by time period, 1985–2007.

Laboratory value	All	1985–1990	1991–1995	1996–2001	2002–2007
CD4 cell count, mean cells/mm^3 ± SD	551 ± 250	632 ± 276	553 ± 249	493 ± 214	514 ± 229
CD4 cell percentage, mean % ± SD	28 ± 9	30 ± 9	28 ± 9	27 ± 9	27 ± 9
CD8 cell count, mean cells/mm^3 ± SD	963 ± 564	997 ± 442	1010 ± 810	897 ± 429	935 ± 399
Total lymphocyte count, mean cells/mm^3 ± SD	2013 ± 792	2143 ± 675	2039 ± 1005	1868 ± 648	1926 ± 567
WBC count, mean cells/mL ± SD	5513 ± 2027	5870 ± 2059	5461 ± 2344	5257 ± 1815	5449 ± 1560

between 1996–2001 and 2002–2007 in the unadjusted model ($P = .52$). Similar trends were noted when examining individual years over the 23-year period (results not shown).

Trends in CD4 cell counts when categorized as <200, 200–349, 350–499, and ⩾500 cells/mm^3 were also significantly different over the 4 periods ($\chi^2 = 65.4$; $P < .001$). The percentage of HIV seroconverters with an initial CD4 cell count of <200 cells/mm^3 at the time of HIV diagnosis was 2% in 1985–1990, 4% in 1991–1995, 5% in 1996–2001, and 5% in 2002–2007. The percentage of participants with a CD4 cell count <350 cells/mm^3 was 12% in 1985–1990, 21% in 1991–1995, 26% in 1996–2001, and 25% in 2002–2007; CD4 cell counts of ⩾500 cells/mm^3 occurred at a rate of 65%, 54%, 45%, and 46%, respectively.

Multiple regression modeling of CD4 cell count trends during the HIV epidemic. Multiple linear regression models were created that adjusted for all covariates, to examine potential changes in the initial CD4 cell counts during the HIV epidemic (table 3). In the fully adjusted multiple regression model, compared with 1985–1990, the mean initial CD4 cell count of seroconverters was 65 cells/mm^3 lower in 1991–1995 ($P < .001$), 107 cells/mm^3 lower in 1996–2001 ($P < .001$), and 102 cells/mm^3 lower in 2002–2007 ($P < .001$). We also compared the mean CD4 cell counts among seroconverters during 2002–2007 and 1996–2001 and found no difference ($P = .98$). Significant predictors of a higher initial CD4 cell count in the adjusted model included a shorter seroconversion window, a lower initial HIV load, younger age, and white/non-Hispanic race (table 3). There were no significant relationships between the initial CD4 cell count and time to first CD4 cell count, sex, or body mass index category. In addition, we examined enrollment site–specific trends, and the trends were similar to the overall cohort for all sites (results not shown). Fully adjusted multiple regression models that limited the analysis to only participants with HIV seroconversion windows of ⩽2 years had similar results. In addition, we reanalyzed our data excluding participants with CD4 cell counts that were potentially measured during their acute seroconversion illness (i.e., a time from estimated date of seroconversion to first CD4 cell count of ⩽90 days) and found similar results (data not shown).

Descriptive trends and regression analyses of other immune-related cell measurements. The mean initial CD4 cell percentage decreased over the 4 periods, from 30% in 1985–1990 to 28% in 1991–1995, 27% in 1996–2001, and 27% in 2002–2007 (table 2). In the unadjusted regression models, compared with 1985–1990, the mean initial CD4 cell percentage was 2% lower in 1991–1995 ($P < .001$), 3% lower in 1996–2001 ($P < .001$), and 3% lower in 2002–2007 ($P < .001$) (table 4). In the fully adjusted multiple regression model, compared with 1985–1990, the mean initial CD4 cell percentage had significantly decreased by 1.5% in 1991–1995 ($P = .006$), by 1.7% in 1996–2001 ($P = .03$), and by 2.3% in 2002–2007 ($P = .003$). The mean initial CD8 cell count varied over time (table 2); however, there were no statistically significant differences in the adjusted model comparing values among seroconverters in 1985–1990 with the other 3 time periods (table 4).

The mean initial total lymphocyte count showed an overall decrease over the study period (table 2). In the final adjusted regression model, compared with 1985–1990, the mean total lymphocyte count was 46 cells/mm^3 less in 1991–1995 ($P = .35$), 172 cells/mm^3 less in 1996–2001 ($P = .02$), and 240 cells/mm^3 less in 2002–2007 ($P = .002$) (table 4). Finally, the mean initial WBC count fluctuated over the study period (table 2). In the final adjusted model, the WBC count was 258 cells/mL lower in 1991–1995 than in 1985–1990 ($P = .04$); there were no significant differences in the WBC counts between 1985–1990 and the 2 other periods.

Models including race-specific effects by time period. Adjusted linear regression models were repeated, including a time period by race interaction for CD4 cell counts. Overall interactions between race and time period were not significant ($P = .96$). We also created separate models to examine changes in the initial CD4 cell counts by race, because immune-related cell counts may differ among the races (typically lower in African Americans) and trends in CD4 cell counts over time may vary by race. The adjusted mean initial CD4 cell counts by race are shown in figure 1. Mean CD4 cell counts among white participants were significantly different across the periods; the largest difference was the decrease of 111 cells/mm^3 from 1985–1990 to 2002–2007 ($P = .001$). The mean initial CD4 cell counts for African Americans in each period were also examined (figure 1). There was a significant decrease of 111 cells/

Table 3. Unadjusted and Adjusted Linear Regression Models for post-seroconversion CD4 cell counts during the course of the HIV epidemic.

Variable	Unadjusted Model		Adjusted Model[a]	
	Estimated CD4 count difference ± SE, cells/mm^3	*P*	Estimated CD4 count difference ± SE, cells/mm^3	*P*
Period				
1985–1990	Referent		Referent	
1991–1995	−79.0 ± 14.2	<.001	−65.0 ± 14.2	<.001
1996–2001	−139.5 ± 14.6	<.001	−106.9 ± 20.1	<.001
2002–2007	−118.0 ± 15.6	<.001	−101.5 ± 20.7	<.001
Seroconversion window				
<1 year	…		56.3 ± 21.0	.007
1 to <2 years	…		35.7 ± 20.7	.08
2 to <3 years	…		8.8 ± 22.3	.69
3–4 years	…		Referent	
Time from HIV diagnosis to CD4 cell count				
−30 to 29 days	…		63.5 ± 44.5	.15
30–89 days	…		49.3 ± 44.1	.26
90–149 days	…		12.4 ± 47.8	.80
150–182 days	…		Referent	
Baseline HIV load				
Data missing	…		133.0 ± 22.6	<.001
⩽1000 copies/mL	…		289.9 ± 26.0	<.001
1001–4000 copies/mL	…		228.3 ± 25.8	<.001
4001–10,000 copies/mL	…		142.1 ± 24.5	<.001
10,001–50,000 copies/mL	…		94.7 ± 19.2	<.001
50,001–100,000 copies/mL	…		56.9 ± 22.3	.01
>100,000 copies/mL	…		Referent	
Age per 10 years	…		−20.5 ± 7.8	.008
Race				
White/non-Hispanic	…		46.5 ± 16.4	.005
African American	…		6.8 ± 16.4	.68
Other	…		Referent	
Female sex	…		32.4 ± 24.9	.19
BMI category[b]				
Data missing	…		−7.2 ± 14.4	.62
⩽24.9	…		−19.5 ± 14.7	.18
25–29.9	…		Referent	
⩾30	…		−13.7 ± 26.1	.60

NOTE. BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

[a] Adjusted for all variables in the table as well as site of enrollment

[b] BMI at HIV diagnosis was categorized with ⩽24.9 kg/m^2 as underweight or normal weight, 25–29.9 kg/m^2 as overweight, and ⩾30 kg/m^2 as obese.

mm^3 from 1985–1990 to 1996–2001 ($P = .001$), similar to the decrease of 94 cells/mm^3 observed from 1985–1990 to 2002–2007 ($P = .03$). There were no significant differences in the initial CD4 cell counts between 1996–2001 and 2002–2007 among either white or African American participants. Among other races, a decrease in initial mean CD4 cell count of 97 cells/mm^3 was observed from 1985–1990 to 2002–2007 ($P = .64$), but this and other differences were not statistically significant because of the small sample size of this group.

DISCUSSION

Our study was conducted to determine whether HIV-infected persons have presented with progressively lower CD4 cell

Table 4. Unadjusted and adjusted linear regression models for post-seroconversion CD4 cell percentage, CD8 cell count, total lymphocyte count, and WBC count by time period, 1985–2007.

Factor, period (no. of participants)	Unadjusted Model		Adjusted Model[a]	
	Estimated difference ± SE	*P*	Estimate difference ± SE	*P*
CD4 cell percentage, % (n = 1981)				
1985–1990 (n = 428)	Referent		Referent	
1991–1995 (n = 574)	−1.9 ± 0.6	<.001	−1.5 ± 0.6	.006
1996–2001 (n = 545)	−2.8 ± 0.6	<.001	−1.7 ± 0.7	.03
2002–2007 (n = 229)	−3.1 ± 0.6	<.001	−2.3 ± 0.8	.003
CD8 cell count, cells/mm^3 (n = 2134)				
1985–1990 (n = 544)	Referent		Referent	
1991–1995 (n = 595)	12.9 ± 33.0	.70	30.1 ± 34.9	.39
1996–2001 (n = 538)	−99.5 ± 33.9	.003	−70.2 ± 49.0	.15
2002–2007 (n = 229)	−62.1 ± 36.2	.09	−52.8 ± 50.3	.29
Total lymphocyte count, cells/mm^3 (n = 1826				
1985–1990 (n = 535)	Referent		Referent	
1991–1995 (n = 613)	−104.3 ± 46.4	.02	−45.7 ± 48.9	.35
1996–2001 (n = 449)	−274.9 ± 50.0	<.001	−172.2 ± 71.2	.02
2002–2007 (n = 124)	−217.0 ± 62.8	<.001	−239.6 ± 78.3	.002
WBC count, cells/mL (n = 2058)				
1985–1990 (n = 452)	Referent		Referent	
1991–1995 (n = 572)	−409.6 ± 118.4	<.001	−257.9 ± 124.1	.04
1996–2001 (n = 537)	−613.5 ± 121.9	<.001	−235.8 ± 175.8	.18
2002–2007 (n = 224)	−421.1 ± 138.5	.002	−114.7 ± 186.1	.54

[a] Adjusted for length of seroconversion window, time from positive HIV test result to CD4 cell count, age, sex, race, body mass index, enrollment site, and HIV load. Some data for covariates were missing (detailed in table 1).

counts during the HIV epidemic. Our observations agree with those of other investigators, who have observed that patients starting HIV care more recently may be presenting with lower initial CD4 cell counts and requiring antiretroviral therapy initiation earlier in their disease course. Several reports have highlighted such occurrences [5] but were confounded by the lack of known HIV seroconversion dates. To avoid bias because of uncertain dates of HIV infection, we evaluated HIV-infected individuals who had documented windows during which seroconversion occurred.

We observed that the initial CD4 cell count among documented HIV seroconverters in the United States significantly decreased during the HIV epidemic. Our findings confirm results from the CASCADE collaboration, which was conducted among HIV-infected persons in Europe, Australia, and Canada [4]. Although some prior studies did not observe decreases in initial CD4 cell counts over time, most of these studies had methodological difficulties or short study intervals [7, 8, 11, 14–17]. Several studies examining the rate of HIV progression after diagnosis have also found that more-recent seroconverters have experienced a faster progression to low CD4 cell counts [4, 13].

Possible explanations for a decrease in the CD4 cell count at diagnosis include changes in the host, virus, or environment over time. Because of the faster replication cycle of the virus relative to the host and the lack of known significant environmental changes, the most plausible explanation may be that the virus evolved. Because early HIV-specific cytotoxic T lymphocyte responses mediated by human leukocyte antigen recognition are associated with initial CD4 cell counts [19], it is possible that the virus evolved with adaptation to the host, resulting in poorer cytotoxic T lymphocyte responses and early CD4 cell depletion [20, 21]. Other potential changes may include alterations in viral subtype, syncytium-induction, or the CXCR4 phenotype [12, 22, 23]; however, these viral characteristics are not known to have significantly changed among seroconverters during the HIV epidemic in the United States. Studies examining viral isolates for adaptations that correlate with the progressive decrease in initial CD4 cell counts are needed; prior studies have been limited by small sample size and narrow study periods [24].

The initial CD4 cell counts appeared to decrease early in the HIV epidemic, with more-recent periods demonstrating stabilization. Our findings suggest that if viral evolution was the cause of the CD4 cell count decreases early in the epidemic, it may have been inhibited by the introduction of HAART; perhaps HAART has caused an increase in the transmission of primary drug-resistant HIV strains with impaired virulence, or

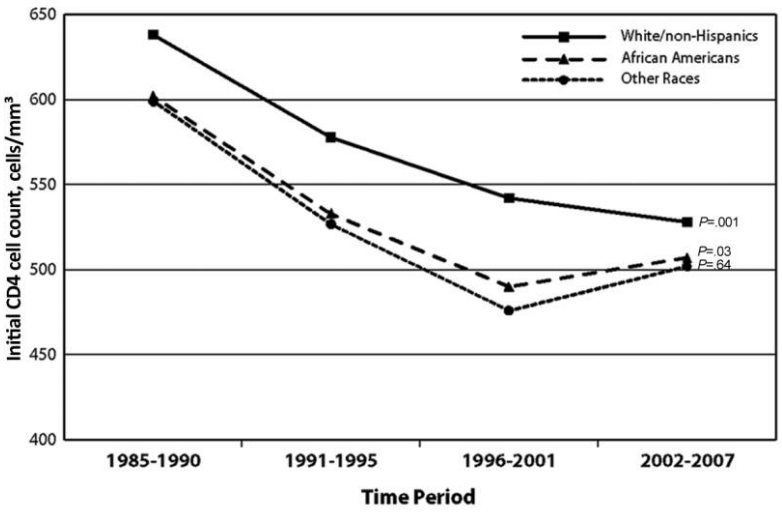

Figure 1. Adjusted mean initial CD4 cell counts, by race and time period. **P* values from linear regression models comparing initial CD4 cell counts from 1985–1990 to 2002–2007.

a loss of viral fitness and diversity attributable to antiretroviral use has occurred [25, 26].

Our study noted that the mean initial CD4 cell count decreased by 102 cells/mm^3 during the HIV epidemic; early CD4 cell counts after seroconversion have been demonstrated to be important predictors of HIV disease progression, which suggests that our findings may be important for HIV-infected persons and their physicians. In our study, 25% of currently presenting HIV-infected patients have CD4 cell counts of <350 cells/mm^3, which is the threshold for HAART initiation [2], and 5% present with a CD4 cell count <200 cells/mm^3, meeting the definition of AIDS. These data highlight the clinical importance of early HIV diagnosis and reconfirm the need for routine HIV testing.

Our study is the first, to our knowledge, to examine whether the decrease in initial CD4 cell count over time varies by race. Because of the racial diversity of our study population, we repeated our analyses stratified by race and noted that African Americans and white/non-Hispanic participants had similar decreases in initial CD4 cell counts. If the decrease in CD4 cell counts over time is a result of viral evolution, this suggests that the virus adapted to an immune determinant that is common among both racial groups.

CD4 cell percentages also decreased during the epidemic, confirming that HIV seroconverters are truly presenting with lower immune-related cell counts, because this measure is less dependent on the WBC count or the flow cytometry platform used. We also evaluated WBC counts over time to assure that changes in these values were not the reason for CD4 cell count decreases in our study; we found that patterns of change associated with WBC counts were different from that associated with CD4 cell counts. Lastly, we investigated the flow cytometry platforms at our clinical sites, and found no relationship between timing of changes in methodology and the observed CD4 cell count trends.

Limitations in our study include the predominantly male population that was evaluated. Although HIV-infected persons in the United States are predominantly men, which suggests that our findings may be generalizable, we could not evaluate changes in the initial CD4 cell count by sex. Second, HIV load measures were unavailable early in the epidemic; therefore, we were unable to determine whether viral loads increased during the same periods as the CD4 cell counts decreased. Third, although our findings may be attributable to a change in the characteristics of the study population over time, we carefully adjusted for all known factors that may have influenced CD4 cell counts. Regarding potential biases attributable to the exclusion of patients who initiated antiretroviral therapy before the initial CD4 cell counts, <1% of participants were excluded because of this criteria. Fourth, whereas other studies calculated the rate of CD4 cell decrease by year [4, 6], our data showed that the pattern of CD4 cell decrease was not linear; therefore, data are presented by periods. Finally, our cohort does not collect data on transmission risk factors or viral characteristics, such as baseline genotypes, replicative capacity, or coreceptor phenotype; of note, among those individuals with subtype data in our network, >90% have remained infected with HIV subtype B, suggesting that clade type does not account for the observed CD4 cell count changes.

In summary, the initial CD4 cell counts among HIV seroconverters in the United States have significantly decreased during the epidemic. The decrease in the post-seroconversion CD4

cell counts occurred early in the epidemic, with stabilization since the advent of HAART. These data may provide an important clinical correlate to studies suggesting that HIV may have adapted to the host, resulting in a more virulent infection.

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