CD8+ Lymphocyte Counts and the Risk of Death in Advanced HIV Infection

Jay M. Schlumpberger, MD; Girma Wolde-Tsadik, PhD; Janis F. F. Yao, MS; and Jimmy Hara, MD
Los Angeles, California

Background. Mortality related to human immunodeficiency virus (HIV) infection occurs predominantly in patients with CD4+ lymphocyte counts of less than 50 cells/mm^3. We followed 133 HIV-infected patients with enrollment CD4 counts of less than 50 cells/mm^3 to determine if the risk of death during a 1-year period could be predicted by a single enrollment CD8+ lymphocyte count.

Methods. Enrollment data including age, sex, T-cell subset counts, p24 antigen status, antiretroviral use, and preexisting HIV-related illnesses were collected on a cohort of 133 consecutive patients with enrollment CD4 counts of less than 50 cells/mm^3. The cohort was followed for 1 year, and survival data were analyzed in relation to enrollment variables.

Results. The mean enrollment CD8 count of those patients alive at 1 year was 600 cells/mm^3, compared with a mean enrollment CD8 count of only 370 cells/mm^3 in patients who had died prior to 1 year ($P < .001$). For every 100-cell decline in the enrollment CD8 count, the risk of death increased by 16% (95% confidence interval [CI], 5% to 22%), independent of other enrollment variables, including CD4 counts and p24 antigen status. A significant CD8 count warning level of 415 cells/mm^3, irrespective of the presence of other enrollment variables, was associated with death within 1 year. The Kaplan-Meier estimated chance of death within 1 year was 54% (95% CI, 42% to 66%) for patients with CD8 counts of less than 415 cells/mm^3 compared with only 25% (95% CI, 14% to 36%) for patients with CD8 counts greater than 415 cells/mm^3.

Conclusions. This study finds that a single CD8 count has important prognostic significance in patients with advanced HIV infection and suggests that potential therapies to enhance CD8 counts might be beneficial to patients with advanced HIV infection.

Key words. AIDS; HIV infections; antigens, CD8; survival rate. (*J Fam Pract 1994; 38:33-38*)

The effect of human immunodeficiency virus (HIV) infection on T-cell subsets was noted by early investigators of the acquired immunodeficiency syndrome (AIDS).[1,2] Subsequently, much attention has been focused on the CD4+ T-lymphocyte subset in patients infected with HIV.

The number of CD4+ T lymphocytes (CD4 count) tends to decline in patients infected with HIV. This decline in CD4 counts is associated with increasing clinical manifestations of immune deficiency and an increased risk of progression to AIDS.[3–7] Recent studies have found that the risk of death in patients infected with HIV rises dramatically when the CD4 count falls below 50 cells/mm^3, and mortality related to HIV infection occurs predominantly in patients with fewer than 50 CD4 cells/mm^3.[8–10]

Less attention has been focused on the CD8+ T-lymphocyte subset in patients with HIV infection. The

Submitted, revised, October 8, 1993.

Portions of this research were presented at the Mead Johnson 3rd annual Family Medicine Research Forum, December 1992, Fort Lauderdale, Florida, and at the 9th annual UCLA Multi-Campus Family Medicine Research Forum, May 1993, Northridge, California.

From the Department of Family Practice (J.M.S., J.H.) and the Department of Research and Evaluation (G.W.-T., J.F.F.Y.), Kaiser Permanente Medical Center, 4747 Sunset Blvd, Los Angeles, CA 90027. Requests for reprints should be addressed to Jay Schlumpberger, MD, Department of Family Practice, Kaiser Permanente, 4747 Sunset Blvd, 4th floor, Los Angeles, CA 90027.

 ISSN 0094-3509

number of CD8+ lymphocytes (CD8 count) rises dramatically shortly after HIV infection and remains elevated. CD8 counts rise dramatically shortly after HIV infection and remain elevated through most of the long asymptomatic phase of HIV infection.[11,12] In addition, CD8+ T lymphocytes have been reported to suppress replication of HIV in vitro.[13–15] Based on these observations, it has been postulated that changes in CD8 counts may reflect an important immunologic response to HIV infection.[11–16] This article is a report of survival among 133 HIV-infected patients with less than 50 CD4 cells/mm^3. Of particular interest is the relation between a single CD8 count and the risk of death in patients with advanced HIV infection.

Methods

A cohort of 133 patients was enrolled between August 1, 1991, and October 31, 1991. All patients with a CD4 count of less than 50 cells/mm^3 measured at the Kaiser–Los Angeles Regional B and T-Cell Laboratory were enrolled. In the case of patients with more than one qualifying CD4 count measured during the enrollment period, only the earliest T-cell subset data and date were used for enrollment. No distinction was made between patients whose CD4 count had recently fallen below 50 cells/mm^3 and patients whose CD4 counts had been less than 50 cells/mm^3 for several months or years. The enrollment demographic and clinical characteristics of the cohort (mean age, mean T-cell subset counts, sex, ethnicity, most recent p24 antigen status within the 3 months preceding enrollment, antiretroviral agent or agents in use at the time of enrollment, and prior history of opportunistic illnesses) are listed on Tables 1 and 2.

Survival status was determined for all patients at the end of 1 year following the dates of their enrollment, which were the same as those of their qualifying T-cell subsets. This status was obtained by review of hospital records, outpatient clinic records, death certificates processed through the medical center, and telephone interview of area hospices.

T-Lymphocyte Subset Analysis

All blood samples were processed on the day of collection at the same immunology laboratory using the same technique throughout the enrollment period. Anticoagulated whole blood samples were incubated at room temperature with single and combined murine monoclonal antibodies directly conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (RD1): anti-CD2 (T11-FITC, Coulter Corp, Miami, Fla), combined anti-CD4 and anti-CD8 (T4-RD1 and T8-FITC, Coulter Corp). The samples were counted on a flow cytometer (Profile One, Coulter Corp). Cytotrol (Coulter Corp) patient pool control cells were used as quality controls.

Statistical Methods

The main patient survival analyses were based on the Cox proportional hazards model with both univariate and multivariate independent variables. Significant univariate findings were used to create a pool of baseline variables, which were subsequently employed in a forward stepwise selection of independently significant risk factors that predispose patients to early death. An iterative procedure similar to that recently described by LeBlanc and Crowley and based on the log-rank test[17] was used to identify a CD8-count cutoff value that can singly define a warning level for possible clinical application. This was achieved by using median CD8 count values for deaths and survivors as the respective lower and upper limits of iteration, and the identification of an iterated value that revealed the highest and near-equivalent univariate and multivariate significance. The highest significance ensures the best discrimination between deaths and survivors, whereas the near-equivalence represents adjustment for factors other than CD8 counts. Mortality curves were constructed using the product-limit method (Kaplan-Meier) and two-group survival comparisons were based on the log-rank test.

Results

Tables 1 and 2 present enrollment characteristics of the cohort according to patient survival status at the end of the postenrollment year. Of the 133 study patients, 63 (47.4%) were alive 1 year following the date of their enrollment, 53 (39.8%) had died before the end of the postenrollment year, and 17 (12.8%) were lost to follow-up during the study period. The cohort was predominantly male (98%) with a mean age of 40 (±9) years. Forty-seven percent of the patients were white, 26% were Hispanic, and 15% were black.

The entries in Table 1 represent the number of persons with specific enrollment characteristics, by end-of-year status. The subgroups of enrollment antiretroviral therapy and prior history of opportunistic illnesses are not mutually exclusive since a patient could have a history of one or more opportunistic illnesses or be treated with one or a combination of antiretroviral agents. The entries in Table 2 represent the mean and standard deviation of enrollment age and enrollment T-cell subsets, by end-of-year status.

Table 1. Demographic and Clinical Characteristics at Enrollment in a Cohort of Patients with Advanced HIV Infection, by End-of-Year Status

Characteristic	Survivors (n = 63) No. (%)	Deaths (n = 53) No. (%)	Lost to Follow-up (n = 17) No. (%)	All (N = 133) No. (%)
Sex				
Female	2 (3)	1 (2)	0 (0)	3 (2)
Male	61 (97)	52 (98)	17 (100)	130 (98)
Ethnicity				
White	28 (44)	29 (55)	5 (29)	62 (47)
Black	10 (16)	7 (13)	3 (18)	20 (15)
Hispanic	18 (29)	13 (25)	4 (24)	35 (26)
Asian	2 (3)	0 (0)	0 (0)	2 (2)
Native American	1 (2)	0 (0)	0 (0)	1 (1)
Unknown	4 (6)	4 (8)	5 (29)	13 (10)
P24 antigen*				
Positive	13 (21)	21 (40)	4 (24)	38 (29)
Negative	29 (46)	16 (30)	7 (41)	52 (39)
Unknown	21 (33)	16 (30)	6 (35)	43 (32)
Antiretroviral				
Zidovudine	30 (48)	24 (45)	9 (53)	63 (47)
Didanosine	5 (8)	7 (13)	0 (0)	12 (9)
Zalcitabine	14 (22)	13 (25)	7 (41)	34 (26)
Acyclovir	8 (13)	11 (21)	3 (18)	22 (17)
None	14 (22)	10 (19)	1 (6)	25 (19)
Unknown	7 (11)	1 (2)	3 (18)	11 (8)
History of OI/OM				
Pneumocystic carinii pneumonia	17 (27)	18 (34)	6 (35)	41 (31)
Kaposi's sarcoma	11 (18)	15 (28)	3 (18)	29 (22)
Toxoplasmosis encephalitis	1 (2)	3 (2)	1 (1)	5 (4)
Cryptococcal meningitis	4 (6)	3 (6)	1 (6)	8 (6)
Mycobacterium avium complex*	3 (5)	9 (17)	3 (18)	15 (11)
Cytomegalovirus retinitis*	1 (2)	8 (15)	1 (6)	10 (8)
Lymphoma†	0 (0)	2 (4)	0 (0)	2 (2)
Candidal esophagitis‡	2 (3)	10 (19)	0 (0)	12 (9)
None	27 (43)	11 (21)	0 (0)	38 (29)

*P < .05.
†P < .01.
‡P < .001.
OI/OM denotes opportunistic infection/opportunistic malignancy.

Based on univariate analyses, the study revealed that age ($P = .038$), positive p24 antigen status ($P = .023$), prior histories of *Mycobacterium avium* complex (MAC) ($P = .022$), cytomegalovirus (CMV) retinitis ($P = .011$), lymphoma ($P = .009$), and candidal esophagitis ($P < .001$) were significantly associated with early death. Although the sizes of the groups with some of these characteristics were small, these baseline character-

Table 2. Age and T-Cell Characteristics at Enrollment in a Cohort of Patients with Advanced HIV Infection, by End-of-Year Status

Characteristic	Survivors (n = 63) (mean ± SD)	Deaths (n = 53) (mean ± SD)	Lost to Follow-Up (n = 17) (mean ± SD)	All (n = 133) (mean ± SD)
Age, y*	39 ± 8	42 ± 10	38 ± 10	40 ± 9
Total T cells/mm^3†	761 ± 440	493 ± 338	583 ± 423	631 ± 417
CD4+ T cells/mm^3*	24 ± 12	19 ± 13	21 ± 14	21 ± 13
CD8+ T cells/mm^3‡	600 ± 382	370 ± 276	448 ± 352	489 ± 354

*P < .05.
†P < .001.
‡P < .0001.

Table 3. Enrollment Characteristics Independently Associated with Relative Risk of Death After 1 Year in a Cohort of Patients with Advanced HIV Infection

Factor	Relative Risk (95% CI)	*P* Value
History		
Lymphoma	13.97 (2.86–68.55)	.009
Candidal esophagitis	3.92 (1.94–7.97)	<.001
Older Age	1.03 (1.01–1.06)	.024
Elevated CD8+ T cells/mm^3 counts*	0.998 (0.997–0.999)	.001

**The median enrollment CD8 counts for deaths and survivors of the 1-year postenrollment period were 275 and 484 cells/mm^3, respectively. For every increase of 100 cells in the enrollment CD8 count, the risk of death decreased by 16% (95% CI, 5% to 22%). CI denotes confidence interval.*

istics cannot be dismissed, since their presence revealed a significant difference in survival. For instance, the two patients with a prior history of lymphoma at the time of enrollment died within 60 days of enrollment, compared with the rest of the cohort, which had a median survival time of at least 1 year.

The enrollment total T-cell count ($P < .001$), the enrollment CD4 count ($P = .016$), and especially the enrollment CD8 count ($P < .001$) were found to be significantly associated with a favorable length of survival. The total T-cell count in this cohort was found to be correlated more with the CD8 count ($r = .98$, $P < .001$) than with the CD4 count ($r = .41$, $P < .001$). It should be noted that the T-cell subset counts for the patients who were lost to follow-up, on average, lie between the values for survivors and those for deaths.

Based on multivariate analyses, we found that a history of lymphoma, a history of candidal esophagitis, and patient age each were significant independent contributors to a higher risk of death. An elevated CD8 count at enrollment, in contrast, was found to be a significant independent contributor to a favorable survival prognosis, thus associated with a lower risk of death. Table 3 presents the significant relative-risk estimates of these independent factors associated with patient mortality. A diagnosis of lymphoma revealed the highest relative risk (14.0) but, as noted earlier, there were only two cases with a history of lymphoma, and this relative rarity is reflected in the large confidence interval. Survivors and deaths had candidal esophagitis diagnosis rates of 3.2% and 18.9%, respectively, with a highly significant ($P < .001$) relative risk (3.9) associated with the presence of this condition. For every increase of 100 cells in the enrollment CD8 count, the risk of death decreased by 16% (95% CI, 5% to 22%). This contribution of higher CD8 counts to favorable survival prognosis is independent of other enrollment characteristics, including CD4 counts, p24 antigen status, and antiretroviral use.

The median enrollment CD8 counts for deaths and survivors of the 1-year postenrollment period were 275 and 484 cells/mm^3, respectively. Increases by a cell count of 5, starting from the lower boundary of 275, and subsequent univariate and multivariate analyses resulted in a cell-count cutoff point of 415 that showed the maximum significance of the unadjusted ($P < .001$) and adjusted ($P < .001$) hazards ratio with the closest values. CD8 counts that were not within the median boundary points did not reveal significant relative risk attributable to CD8 baseline counts. Thus, the proximity and small size of the probability values suggests that, irrespective of the presence of other risk factors, the CD8 count of 415 cells/mm^3 can singly be used as a high-risk level in patients with a CD4 count <50 cells/mm^3. Based on this level, patients were divided into two groups: those with enrollment CD8 counts ≥415 cells/mm^3 and those with enrollment CD8 counts <415 cells/mm^3. The Figure shows the cumulative mortality curves for these two groups. The estimated chance of death within 1 year for patients with an enrollment CD8 count of <415 cells/mm^3 was 54% (95% CI, 42% to 66%), compared with only a 25% (95% CI, 14% to 36%) chance of death within 1 year for patients with an enrollment CD8 count ≥415 cells/mm^3.

Discussion

This study finds an association between a single CD8+ T-lymphocyte count and the risk of death in patients with advanced HIV infection. In particular, lower CD8 counts at enrollment were associated with higher risk of death within 1 year among patients with CD4 counts below 50 cells/mm^3. This study supports a previously identified association between the clinical status of HIV-infected patients and CD8+ T-lymphocyte anti-HIV activity.[18,19]

A study of patients at earlier stages of HIV infection than those in our study found no relation between initial CD8 counts and progression to AIDS.[20] Still another study of initial CD8 counts and progression to AIDS found a weak increase in the risk of progression to AIDS associated with higher CD8 counts.[21] However, this second study was not confined to patients with initial CD4 counts <50 cells/mm^3, and it analyzed progression to AIDS over a 60-month period following the initial CD8 count. For the immediate 6-month period before the diagnosis of AIDS, that study revealed that the risk of progression to AIDS was elevated by 10% for each 100-cell decline in the CD8 count, a finding that concurs

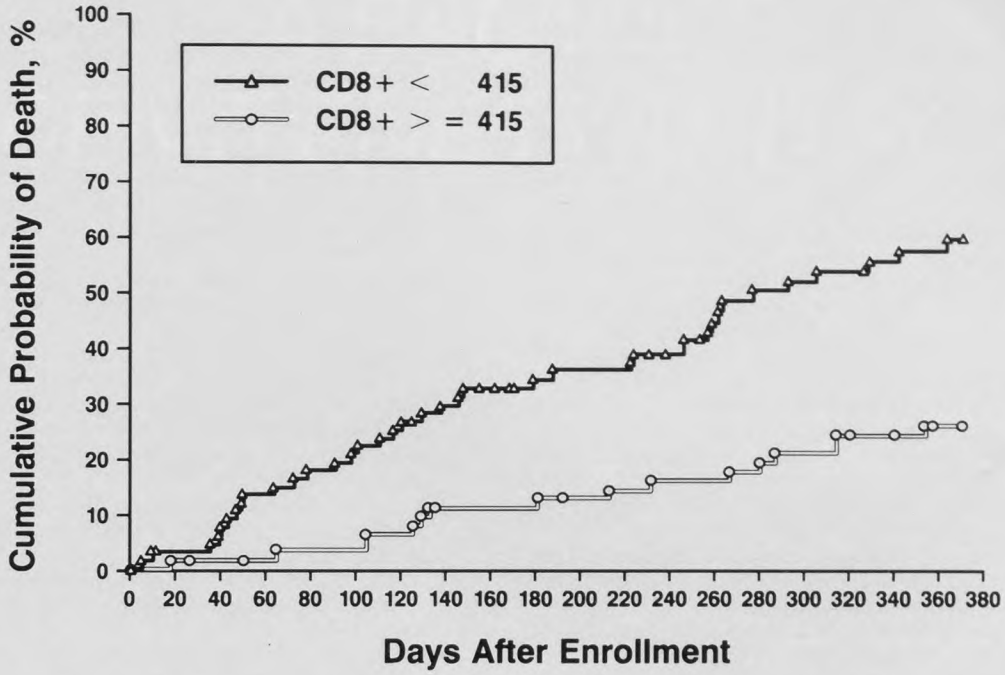

Figure. Kaplan-Meier estimates of the cumulative probability of dying according to the number of days after enrollment for two groups: those with CD8 counts >415 cells/mm^3 and those with counts <415 cells/mm^3 at the time of enrollment.

with those of our study regarding the risk of death within 1 year after the initial CD8 count. The combined findings suggest that CD8 counts may be a useful marker for disease progression only in later stages of HIV infection.

It has been postulated that the initial rise in CD8 counts following HIV infection represents an important immunologic response to HIV infection.[11–16] Long-term survivors of HIV infection also have been shown to possess strong CD8+ T-lymphocyte anti-HIV activity, compared with patients who demonstrate clinical progression of HIV infection.[22] This anti-HIV activity includes suppression of HIV replication mediated by a soluble antiviral factor produced by activated CD8+ T lymphocytes.[19,23,24] The findings of our study suggest that CD8+ T-lymphocyte-mediated anti-HIV activity is still present in varying degrees, even in patients with advanced HIV infection.

The study identified a clinically useful CD8 count warning level of 415 cells/mm^3; below that level the risk of death within 1 year is greater than 50%. This warning level is in agreement with findings that a decline in CD8 counts below 400 cells/mm^3 predicts dissemination of CMV and MAC in patients with HIV infection.[25] These combined findings suggest that patients with advanced HIV infection and CD8 counts below approximately 400 cells/mm^3 should be strongly considered for prophylaxis against CMV and MAC as such prophylactic therapies become available. Rifabutin was recently approved for the prevention of disseminated MAC disease in patients with HIV infection.[26]

Family physicians caring for patients with terminal illness frequently are asked, "How long will I live?" This study finds that CD8 counts may provide prognostic information about patients with advanced HIV infection. Such prognostic information would be useful to family physicians in counseling HIV-infected patients and their families, in initiating appropriate prophylaxis and monitoring for certain terminal conditions associated with AIDS, and in planning hospice care for patients with advanced HIV infection.

Finally, this study requires replication before the potential value of CD8 counts as a marker for disease progression in advanced HIV infection can be accepted. This study's results are limited in their general application to women because of the small number of women participating in the study. Our study suggests that the urgent need for new surrogate markers of AIDS progression for use in evaluating novel therapies[27] might be partially addressed by the use of CD8 counts as surrogate markers in patients with advanced HIV infection,[28] and that potential therapies directed at increasing CD8 counts might prove beneficial in patients with advanced HIV infection.[29–30]

References

1. Gottlieb MS, Schroff R, Schanker HM, et al. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med 1981; 305:1425–31.

2. Detels R, Fahey JL, Schwartz K, et al. Relation between sexual practices and T cell subsets in homosexually active men. Lancet 1983; 1:609–11.
3. Melbye M, Biggar RJ, Ebbesen P, et al. Long-term seropositivity for human T-lymphotropic virus type III in homosexual men without the acquired immunodeficiency syndrome: development of immunologic and clinical abnormalities. Ann Intern Med 1986; 104:496–500.
4. Fahey JL, Taylor JMG, Detels R. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. N Engl J Med 1990; 322:166–72.
5. Moss AR, Bacchetti P, Osmond D, et al. Seropositivity for HIV and the development of AIDS or AIDS-related condition: three-year follow-up of the San Francisco General Hospital cohort. BMJ 1988; 296:745–50.
6. Goedert JJ, Kessler CM, Aledort LM, et al. A prospective study of human immunodeficiency virus type 1 infection and the development of AIDS in subjects with hemophilia. N Engl J Med 1989; 321:1141–8.
7. Phillips AN, Lee CA, Elford J, et al. Serial CD4 lymphocyte counts and development of AIDS. Lancet 1991; 337:389–92.
8. Yarchoan R, Venzon DJ, Pluda JM, et al. CD4 count and the risk for death in patients infected with HIV receiving antiretroviral therapy. Ann Intern Med 1991; 115:184–9.
9. Phillips AN, Elford J, Sabin C, et al. Immunodeficiency and the risk of death in HIV infection. JAMA 1992; 268:2662–6.
10. Nightingale SD, Jockusch JD, Haslaund I, et al. Logarithmic relationship of the CD4 count to survival in patients with human immunodeficiency virus infection. Arch Intern Med 1993; 153: 1313–8.
11. Lang W, Perkins H, Anderson RE, et al. Patterns of T-lymphocyte changes with human immunodeficiency virus infection: from seroconversion to the development of AIDS. J Acquir Immune Defic Syndr 1989; 2:63–9.
12. Gaines H, von Sydow MAE, von Stedingk LV, et al. Immunological changes in primary HIV-1 infection. AIDS 1990; 4:995–9.
13. Walker CM, Moody DJ, Stites DP, Levy JA. CD8+ lymphocytes can control HIV infection in vitro by suppressing viral replication. Science 1986; 234:1563–6.
14. Wiviott LD, Walker CM, Levy JA. CD8+ lymphocytes suppress HIV production by autologous CD4+ cells without eliminating the infected cells from culture. Cell Immunol 1990; 128:628–34.
15. Walker CM, Thomson-Honnebier GA, Hsueh FC, et al. CD8+ T cells from HIV-1 infected individuals inhibit acute infection by human and primate immunodeficiency viruses. Cell Immunol 1991; 137:420–8.
16. Levy JA. Mysteries of HIV: challenges for therapy and prevention. Nature 1988; 333:519–22.
17. LeBlanc M, Crowley J. Survival trees by goodness of split. J Am Stat Assoc 1993; 88:457–67.
18. Mackewicz CE, Ortega HW, Levy JA. CD8+ cell anti-HIV activity correlates with the clinical state of the infected individual. J Clin Invest 1991; 87:1462–6.
19. Brinchmann JE, Gaudernack G, Vartdal F. CD8+ T cells inhibit HIV replication in naturally infected CD4+ T cells. J Immunol 1990; 144:2961–6.
20. Fernandez-Cruz E, Desco M, Montes MG, et al. Immunological and serological markers predictive of progression to AIDS in a cohort of HIV-infected drug users. AIDS 1990; 4:987–94.
21. Anderson RE, Shiboski SC, Royce R, et al. CD8+ T lymphocytes and progression to AIDS in HIV-infected men: some observations. AIDS 1991; 5:213–5.
22. Levy JA. HIV pathogenesis and long-term survival [abstract PS-05–2]. Abstract presented at the IX International Conference on AIDS, Berlin, June 1993.
23. Walker C, Levy JA. A diffusible lymphokine produced by CD8+ T lymphocytes suppresses HIV replication. Immunology 1989; 66:628–30.
24. Walker CM. Non-cytolytic control of HIV replication by CD8+ T cells. Semin Immunol 1993; 5:195–201.
25. Fiala M, Kermani V, Gornbein J. Role of CD8+ in late opportunistic infections of patients with AIDS. Res Immunol 1992; 143: 903–7.
26. Product monograph. Dublin, Ohio: Adria Laboratories, 1993.
27. Cohen J. Searching for markers on the AIDS trail. Science 1992; 258:388–90.
28. Bogner JR, Goebel FD. Lymphocyte subsets as surrogate markers in antiretroviral therapy. Infection 1991; 19(suppl 2):103–8.
29. Armstrong JA, Ho M, Herberman R, et al. A phase I study of autologous CD8(+) lymphocytes expanded in vitro infused into patients with advanced ARC or AIDS (ACTG 080) [abstract SB 491]. Abstract presented at VI International Conference on AIDS, San Francisco, 1990.
30. Riddell SR, Watanabe KS, Goodrich JM, Li CR, et al. Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. Science 1992; 257:238–40.