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Interleukin-2-Inducible Natural Immune (Lymphokine-Activated Killer Cell) Responses as a Functional Correlate of Progression to AIDS

BLUMA G. BRENNER,[1,2]* MERVYN GORNITSKY,[3] AND MARK A. WAINBERG[1,4]

McGill AIDS Centre, Lady Davis Institute—Jewish General Hospital,[1] and Departments of Experimental Surgery,[2] Dentistry,[3] and Medicine,[4] McGill University, Montreal, Quebec, Canada H3T 1E2

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The functions of natural killer (NK) cells and their interleukin-2-inducible counterparts, lymphokine-activated killer (LAK) cells, are often impaired in human immunodeficiency virus (HIV)-infected individuals. A statistical approach was used to establish if changes in LAK activity were associated with antiviral drug therapy, HIV-1 burden, or lymphocyte subset alterations. Our study group included 61 HIV-positive subjects without any opportunistic infections (OI^-), 16 of whom received zidovudine (AZT), and 97 HIV-positive individuals with AIDS-related infection (OI^+), 50 of whom received AZT. As expected, there was a stepwise decrease in total lymphocyte numbers in OI^+ groups as a result of the selective loss of $CD4^+$ cells. The groups receiving AZT therapy had fewer $CD4^+$ cells but lower circulating p24 antigen levels than corresponding untreated groups did. No significant changes in the relative proportions or absolute numbers of $CD56^+$ subsets in HIV-positive groups could be ascribed to OI status or AZT intervention. LAK cell cytotoxic responses, measured as LU_{20} values (which give a measure of 20% cytolysis of target cells), lysis per unit $CD56^+$ NK cell, or lysis per unit blood volume, declined in OI^+ groups. No main or interactive effects of AZT therapy on LAK activities were observed. Multivariate general linear models were used to determine the interactive effects of NK- and T-cell subsets on measured LAK cell responses against K-562 and U-937 targets. $CD56^+$ and $CD4^+$ cell numbers were the principal variables that predicted U-937 or K-562 target cell lysis ($r = 0.8$). p24 antigenemia and $CD57^+$ LAK cell numbers were added negative and positive predictors of LAK activity, respectively. These findings indicate that declines in NK-mediated LAK cell responses serve as functional correlates of progression in HIV-infected individuals.

Natural killer (NK) cells and their interleukin-2 (IL-2) inducible counterparts, lymphokine-activated killer (LAK) cells, play an important role in viral and tumor immunosurveillance (29). This heterogeneous family of cytotoxic cells ($CD56^+$, $CD57^{+/-}$, $CD16^{+/-}$, and $CD8^{+/-}$) differs serologically from T cells and macrophages. NK cells lack CD3 (the T-cell receptor) and CD4 (the human immunodeficiency virus type 1 [HIV-1] receptor on helper T cells and macrophages) (24, 29). Functionally, these cells make up a distinct branch of the immune network mediating multiple non-major histocompatibility complex cytotoxic responses that include NK and LAK activities and antibody-dependent cellular cytotoxicity (24, 29). In the wake of marked declines in the $CD4^+$ cell-orchestrated responses in patients with AIDS, natural immunity may play an increasingly important role in sustaining immunocompetence in patients with AIDS. Alternately, any compromise of the NK cell network may contribute separately to the immunopathogenesis of AIDS.

Conflicting data concerning NK, antibody-dependent cellular cytotoxicity, and LAK cell responses in AIDS have been reported (reviewed in references 3 and 6). Some groups have found normal or elevated activities in HIV-positive individuals; other studies show progressive declines in NK cell-mediated responses with disease progression. These observed discrepancies have arisen, in part, from differences in HIV-positive patient populations, choice of targets, and assay conditions. With regard to NK cell numbers in HIV-positive individuals, no elevations or decreases in NK cell subsets have been reported. Many of those early studies showing increases in NK cell numbers used $CD57^+$ cells as the marker for NK cell numbers. $CD57^+$ cells have now been shown to be markers of $CD8^+$ T-cell subsets that emerge in HIV-positive subgroups (15, 17).

The cumulative findings suggest the presence of defective NK cell functions in seropositive individuals. The underlying mechanisms for these defects, however, remain unclear. Although NK cells do not express surface CD4 or CD4 mRNA, they can be susceptible to in vitro HIV-1 infection (3, 9). HIV-1 DNA or RNA virus-specific sequences have not, however, been isolated from purified NK cells obtained from HIV-infected individuals (26). Studies with peripheral blood leukocytes (PBL) and LAK cells derived from HIV-seronegative individuals have shown that HIV-1 proteins directly inhibit in vitro NK and LAK activities (7, 8). The numbers of NK cells conjugating to targets in HIV-positive groups have been shown to be reduced or within the range of controls (1, 9, 14, 26, 27). Postbinding lytic processes are qualitatively impaired by using PBL, LAK cells, cloned NK cells, or NK cell lines isolated from HIV-positive donors (1, 3, 9, 14, 27, 30). It is well established that cytolytic processes are modulated by cytokines and mediated by lytic factors (24, 29). HIV-infected individuals show reductions in production and release of lymphokines and cytotoxic factors, including IL-2, NK cell-stimulating factor (IL-12), NK cell cytotoxic factor, tumor necrosis factor alpha, and interferon (1, 22, 26). Addition of IL-2 could only partially restore NK cell functional responses in HIV-positive populations (6, 25).

* Corresponding author. Mailing address: Lady Davis Institute—Jewish General Hospital, 3755 Cote Ste-Catherine Rd., Montreal, Quebec, Canada H3T 1E2. Phone: (514) 340-8260. Fax: (514) 340-7502.

The impact of sustained antiviral drug therapy on measured NK and LAK activities has not been ascertained. Zidovudine (3′-azido-2′,3′-dideoxythymidine [AZT]), an antagonist of HIV-1 reverse transcriptase, has been shown to initially increase NK cell and T-cell proliferation and function (10, 11, 19). Sustained AZT treatment, however, may cause significant leukopenia and depletion of bone marrow reserves (20, 23). Studies with PBL from HIV-negative individuals have shown that AZT inhibits in vitro NK and LAK activities (28).

During our studies of natural immunity in AIDS, we have monitored many immunological parameters in a cross-section of HIV-positive individuals (4, 5, 12, 13). LAK activities in HIV-positive individuals with AIDS-related secondary infections were shown to be reduced when compared with those in asymptomatic HIV-positive and HIV-negative subjects. Since many individuals within both HIV-positive groups had received ongoing AZT therapy, we wished to establish whether observed declines in LAK cell responses could be associated with antiviral drug therapy or HIV-1 burden. To this end, we determined the independent and interactive effects of opportunistic infection (OI) status and AZT therapy on LAK cell function, as well as on numbers and percentages of NK cell and T-cell subsets. We also used multivariate general linear models to establish how NK and T-cell subsets predict inducible LAK cell responses. Our findings show that LAK activities per unit $CD56^+$ LAK cell decline in symptomatic HIV-positive groups. This LAK cell dysfunction could not be ascribed to AZT intervention. $CD56^+$ and $CD4^+$ cell numbers, as well as HIV burden, predict NK cell-mediated LAK cell responses. This indicates a close association between the natural (NK) and adaptive (CD4) limbs of the immune network, with reductions in LAK activity representing a functional correlate of immunocompromise in AIDS.

MATERIALS AND METHODS

Study subjects. From 1987 to 1993, HIV-1-seropositive individuals receiving treatment at the dental clinic of our hospital donated 10 to 20 ml of blood with informed written consent. Their clinical files provided information on dates of HIV-1 testing, clinical histories, and drug therapies. Most patients fell into discernible Centers for Disease Control and Prevention classification groups (8a). The CDC-2 opportunistic infection-negative (OI^-) group included subjects with no clinical disease manifestations beyond HIV-1 seropositivity. The OI^+ group comprised HIV-positive individuals with one or more AIDS-defining diseases (CDC-4C or CDC-4D), such as Kaposi's sarcoma, *Pneumocystis carinii* pneumonia, or neurologic symptoms. Alternately, OI^+ individuals had multiple chronic AIDS-associated infections, including oral hairy leukoplakia, disseminated herpes simplex, or shingles (herpes zoster). Both OI^- and OI^+ groups were substratified according to AZT therapy (300 to 500 mg/day) (AZT^- or AZT^+). No specific length of AZT administration was a requirement for inclusion in this study. All patients receiving immunomodulatory treatments (i.e., interferon or leukovorin), anti-retrovirus drugs other than AZT (e.g., dideoxyinosine [ddI]) or with questionable OI status were excluded from analysis. Independent values for LAK activity, resting and inducible lymphocyte subset numbers, and HIV antigenemia were obtained from 158 HIV-1-positive subjects. In total, there were 45 OI^-AZT^-, 16 OI^-AZT^+, 47 OI^+AZT^-, and 50 OI^+AZT^+ subjects. HIV-seronegative individuals were recruited from hospital personnel and formed the CDC-0 control group.

Cytotoxicity assays. Venous blood was collected in heparinized tubes, and PBL were isolated by Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) density gradient centrifugation (2). LAK cells were generated by ex vivo incubation of 2×10^6 PBL per ml for 5 or 6 days at 37°C in RPMI-1640 (Gibco, Grand Island, N.Y.) complete medium containing 10% decomplemented fetal calf serum, 30 U of IL-2 (Boehringer GmbH, Mannheim, Germany) per ml, 2 mM glutamine, 100 U of penicillin per ml, 100 mg of streptomycin per ml, and 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES). On the day of the assay, LAK cells were washed twice in complete medium. LAK activity was monitored as previously described by using ^{51}Cr release assays with LAK cells as effectors and ^{51}Cr-labeled U-937 or K-562 cells (American Type Culture Collection, Rockville, Md.) as targets (13). Exponential regression analysis of cytolytic activities monitored at five to six different effector/target ratios was performed with computer software kindly provided by H. F. Pross (21). By using this formula, equations were solved for 20% cytolysis of 10,000 targets, inverted, and multiplied by 100 to yield LU_{20} values. LAK activities (LU_{20} values) in individual patients were also calculated per unit $CD56^+$ LAK cell by multiplying lytic activity by the relative proportion of $CD56^+$ cells in LAK cells. Lymphocyte counts and LAK cell recoveries were incorporated with LU_{20} values, where indicated, to yield LU_{20} values per unit blood volume. LU_{20} per milliliter of blood volume = PBL count (10^{-6} cells per milliliter of blood) × LAK cell recovery (ratio of recovered LAK cells to starting PBL number) × observed LU_{20}. No cytotoxic LAK activity was detectable if IL-2 was omitted. Similar LAK recoveries and cytolytic responses were obtained when 15 to 250 U of IL-2 per ml was used to generate LAK cells (unpublished results).

Flow-cytometric analysis. Distributions of CD4, CD8, CD57, and CD56 antigens on PBL and LAK cells were ascertained by cytofluorometric analysis with an EPIC Profile 2 analyzer (Coulter Electronics, Burlington, Ontario, Canada). Cells were stained with relevant fluorescein isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies (Coulter Electronics; Becton Dickinson, Mississauga, Ontario, Canada). Lymphocyte subsets were measured as percentages of positively stained cells divided by total lymphocyte numbers. The numbers of lymphocyte subsets in PBL (cells per milliliter) were determined by multiplying the ratio of the relevant lymphocyte subset by the PBL count. The number of inducible $CD56^+$, $CD8^+$, and $CD57^+$ LAK cells per milliliter of blood was determined by multiplying the percentage of these subsets in LAK cells by the PBL number (cells per milliliter) and LAK cell recovery.

HIV-1 burden. The HIV-1 burden was assessed by measuring p24 and acid-dissociable p24 antigen in plasma and in LAK cell culture fluids harvested on day 6, by using commercially available kits (Coulter Corp., Hialeah, Fla.) as specified by the manufacturer. Only acid-dissociable p24 values are shown.

Statistical analysis. Statistical analyses and graphical representations were performed with a statistical software package (Systat, Evanston, Ill.). Frequency distributions for measured lymphocyte subsets and LAK activities often showed the presence of outlying values. Since these "severe outliers" can bias statistical tests, data were subjected to logarithmic transformation to give equal weight to all individual measured values. For ease of interpretation, data in text are presented as mean antilog values. A 2×2 analysis of variance assessed the independent and interactive associations of AZT therapy and OI status on measured functional activities and lymphocyte subset distributions.

Multivariate general linear models were used to determine how measured NK cell and T-cell subsets, as well as viral burden (measured as acid-dissociable p24 viral antigen in LAK

TABLE 1. Average numbers and percentages of $CD4^+$, $CD8^+$, $CD56^+$, and $CD57^+$ subsets in PBL and LAK cells derived from HIV-negative and HIV-positive populations, categorized by groups

Parameter[a]	Value in HIV^- controls	Value in HIV^+ Subgroup:			
		OI^-AZT^-	OI^-AZT^+	OI^+AZT^-	OI^+AZT^+
n	44	45	16	47	50
PBL no. (cells/ml)	1,600	1,268	1,048	1,117	776
CD4 cell no. (cells/ml)	852	385	264	112	60
Plasma p24 (pg/ml)		7	11	39	17
LAK fluid p24 (pg/ml)		22	7	46	27
LAK recovery (%)	64	54	54	53	56
PBL distribution (%)					
CD4	50 (648)	27 (294)	18 (186)	7 (52)	6 (34)
CD8	24 (319)	45 (538)	54 (581)	54 (532)	56 (397)
CD56	6 (78)	5 (61)	5 (53)	5 (44)	6 (43)
LAK distribution (%)					
CD4	54	21	20	7	5
CD8	28	41	44	34	46
CD57	4	15	19	18	20
CD56	7	7	6	7	9

[a] Total lymphocyte and $CD4^+$ cell numbers were determined by using whole blood. The levels of viral p24 antigen in acid-dissociated plasma and LAK culture fluids are presented. LAK cell recovery is expressed as a percentage of starting PBL number. The flow-cytometric distribution of lymphocyte subsets are represented as percentages of subsets within PBL or LAK cells. The total recovered numbers (cells per microliter) of each subset in PBL are shown in parentheses. Values for the HIV-negative control group are shown for comparative purposes, although this group was not entered into 2 × 2 analysis of variance comparisons.

cell culture fluid), predicted measured LAK cell responses against U-937 and K-562 targets in HIV-infected individuals. The regression hypothesis was as follows: ln LAK activity per milliliter of blood = Constant + (β_1 × ln $CD56^+$ LAK cell number) + (β_2 × ln $CD4^+$ LAK cell number) + (β_3 × ln $CD8^+$ LAK cell number) + (β_4 × ln $CD57^+$ LAK cell number) + (β_5 × ln p24 antigen in LAK cell culture fluids). Since NK cell and T-cell subsets can overlap, forward selection was used as a strategy to identify in increasing consecutive order variables that were statistically significant predictors of LAK activity. In forward stepping, the default begins with no variables in the equation, enters the most "significant" predictor at the first step, and continues adding and deleting variables until none can significantly improve the fit. The *F* values and correlation coefficients were determined when all significant variables were entered. Similar models were used to predict the relationship between LAK activity per milliliter of blood and numbers of $CD56^+$, $CD4^+$, $CD8^+$, and $CD57^+$ LAK cells derived from both HIV-positive and HIV-negative subjects. The final equation was as follows: ln LAK activity per milliliter of blood = constant + (β_1 × ln $CD56^+$ LAK cell number) + (β_2 × ln $CD4^+$ PBL number). Although not presented, a similar approach was used to predict LAK activity (measured as lytic units) against both targets as a function of the relative percentages of $CD56^+$, $CD4^+$, $CD8^+$, and $CD57^+$ subsets in LAK cells. Two- or three-dimensional graphical representations of LAK activity per milliliter of blood with $CD56^+$ LAK cell numbers and $CD4^+$ PBL numbers were subjected to linear smoothing.

RESULTS

Effects of HIV infection on lymphocyte subset numbers and distributions. HIV-infected subjects were categorized into four groups according to disease classification and antiviral drug therapy. The independent effects of AZT and OI on immunological variables were determined by using 2 × 2 analysis of variance. As expected, there were significant reductions of PBL and $CD4^+$ cell numbers in OI^+ groups (Table 1, $F = 14$ and 59, $P < 0.001$, respectively). AZT recipients had fewer PBL and CD4 subset numbers ($F = 8$ and 4, $P < 0.001$ and 0.05, respectively). There were significant changes in numbers and percentages of $CD8^+$ cells in all OI^+ and all AZT^+ groups, which paralleled declines in $CD4^+$ cell numbers ($F = 3.1$ and 4.2 for OI-associated effects on $CD8^+$ cell numbers and percentages in PBL; $F = 3.8$ for AZT-associated increases in the percentages of $CD8^+$ PBL). There were, however, no significant differences in numbers or percentages of $CD56^+$ subsets in PBL or isolated LAK cells among our HIV-positive subgroups. LAK cell recoveries were not significantly different among HIV-positive groups.

Levels of p24 viral antigen (picograms per milliliter) were relatively low in all HIV-positive groups (Table 1). Plasma from 64, 63, 53, and 44% of OI^-AZT^-, OI^-AZT^+, OI^+AZT^-, and OI^+AZT^+ subjects, respectively, showed no detectable levels of p24 antigen. LAK cell culture fluids, harvested on day 6, showed increases in levels of p24 antigen relative to levels in plasma, with detectable p24 viral antigen in 96, 83, 100, and 95% of samples from respective HIV-positive subgroups. The sensitivity of viral antigen detection was increased following acid dissociation of plasma and LAK cell culture fluids. Treated (AZT^+) and asymptomatic (OI^-) HIV-positive individuals showed significantly lower levels of p24 antigen in LAK cell culture fluids (Table 1, $F = 6.8$ and 4.4, $P < 0.05$ for OI and AZT effects on p24 antigen).

Inducible LAK activity in HIV-positive groups. There was a significant stepwise decline in LAK activity against U-937 targets in OI^+ groups (Fig. 1A, $F = 6.1$, $P < 0.02$). There were no changes, however, in LAK activity associated with AZT intervention. Since OI and AZT influenced numbers and distributions of lymphocyte subsets, values of LAK activity in individual subjects were recalculated to determine lysis per unit $CD56^+$ LAK cell (Fig. 1B). A significant reduction in lysis per unit $CD56^+$ LAK cell in OI^+ groups ($F = 7.2$, $P < 0.01$) was observed without any AZT-associated changes. Similarly, overall LAK activity per milliliter of blood was lower in OI^+ groups. These reductions could not be ascribed to AZT

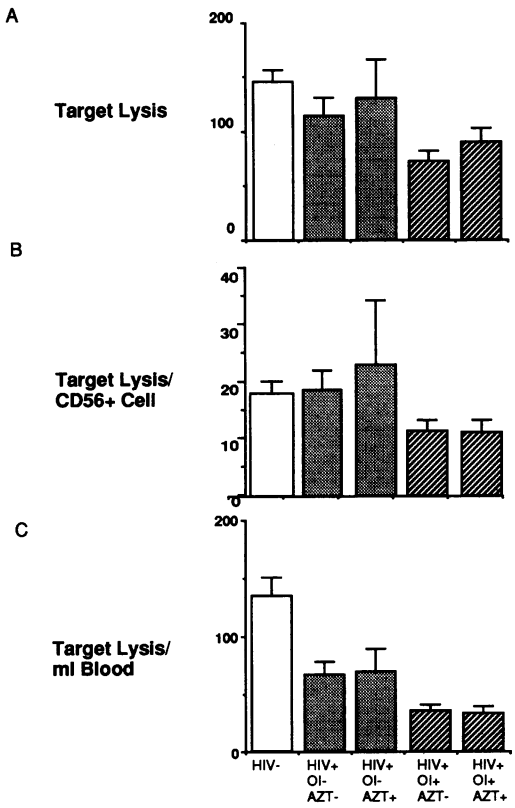

FIG. 1. LAK activity against U-937 targets in HIV-negative and HIV-positive populations. Shown are average values ± standard errors of the mean (error bars) for LAK activity against U-937 targets, expressed as LU_{20} values (A), lytic units per unit $CD56^+$ cell (B), and lytic units per milliliter of blood (C). HIV-infected individuals were subgrouped on the basis of OI status and AZT drug therapy.

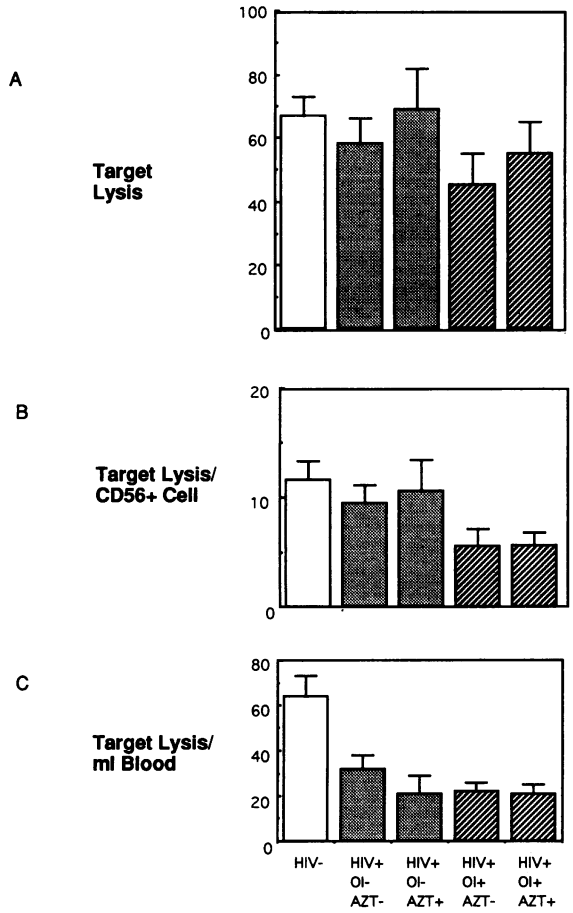

FIG. 2. LAK activity against K-562 targets in HIV-negative and HIV-positive populations. Shown are average values ± standard errors of the mean (error bars) for LAK activity against K-562 targets, expressed as LU_{20} values (A), lytic units per unit $CD56^+$ cell (B), and lytic units per milliliter of blood (C). HIV-infected individuals were subgrouped on the basis of OI status and AZT drug therapy.

intervention (Fig. 1C, $F = 11.6$ and 0.0 for OI and AZT effects, respectively). Similar patterns of declining K-562 target lysis with an OI^+ status were observed (Fig. 2).

Immunological parameters predictive of LAK activity in HIV-positive individuals. We wished to determine how numbers of inducible $CD56^+$, $CD4^+$, $CD8^+$, and $CD57^+$ LAK cells, as well as HIV-1 burden (measured as acid-dissociable p24 in LAK cell culture fluids), predicted measured levels of K-562 and U-937 target cell lysis per unit blood volume in HIV-positive individuals. Independently, these five parameters were predictive of U-937 (Pearson correlation coefficients, $r =$ 0.59, 0.49, 0.49, 0.57, and −0.20, respectively; $F = 57$, 33, 34, 49, and 4, respectively) and K-562 ($r = 0.71$, 0.52, 0.56, 0.68, and −0.18, respectively; $F = 48$, 18, 22, 40, and 2, respectively) target cell lysis per milliliter of blood.

Since the four tested NK cell and T-cell subsets represent overlapping and/or interacting cell populations, a computer-based multivariate general linear hypothesis with forward selection was used to predict how the five measured parameters, in combination, explained measured LAK activities per milliliter of blood in HIV-positive subjects. β Regression coefficients and partial correlation coefficients for each significant immunological variable are presented. The overall correlation coefficient (r) and significance (F test) of regression equations were determined once all significant parameters had been entered into the regression equation.

The results of such analysis are summarized in Table 2. As expected, the numbers of $CD56^+$ LAK cells were the major significant predictors of either U-937 or K-562 target cell lysis per milliliter of blood. However, the consecutive addition of numbers of $CD4^+$ and $CD57^+$ LAK cells, as well as the presence of HIV-1 antigenemia, increased the predictive power of $CD56^+$ cells in determining measured LAK cell responses (raising r values from 0.59 to 0.66, 0.70, and 0.72, respectively, for U-937 cells and from 0.70 to 0.74, 0.77, and 0.80, respectively, for K-562 cells). HIV-1 antigenemia negatively influenced U-937 target cell lysis. Once inducible numbers of $CD56^+$ LAK cells were entered into regression equations, there was no significant additional influence of $CD8^+$ cell number. In a similar fashion, percentages of $CD56^+$, $CD4^+$, and $CD57^+$ subsets in LAK cells and of p24 viral antigen in LAK cell culture fluids could be combined into a hypothesis that could predict LAK activity, measured as lytic units, against both U-937 and K-562 targets (unpublished results).

Regression analysis also showed that addition of $CD4^+$ PBL numbers increased the predictive power of $CD56^+$ LAK cells in explaining U-937 and K-562 target cell lysis per unit blood volume, when the hypotheses incorporated data from all HIV-negative and HIV-positive subjects (Table 2). There was a remarkable similarity in β regression and partial correlation

TABLE 2. Determination of the variables that are predictors of measured LAK activity per milliliter of blood against U-937 and K-562 targets in HIV-positive subjects alone or HIV-negative and HIV-positive subjects[a]

Target	Variable	β regression coefficient	Correlation coefficient[b]
U-937 (HIV^+ subjects)	Constant	0.36	0
	$CD56^+$ LAK cells	0.41	0.33***
	$CD4^+$ LAK cells	0.22	0.31***
	$CD57^+$ LAK cells	0.4	0.29***
	p24 antigenemia	−0.17	−0.24**
			0.72 (df = 4, 107, F = 27)
U-937 (HIV^- and HIV^+ subjects)	Constant	0.35	0
	$CD56^+$ LAK cells	0.61	0.47***
	$CD4^+$ PBL	0.29	0.41***
			0.72 (df = 2, 162, F = 86)
K-562 (HIV^+ subjects)	Constant	0.53	0
	$CD56^+$ LAK cells	0.39	0.45***
	$CD4^+$ LAK cells	0.13	0.24**
	$CD57^+$ LAK cells	0.31	0.31*
	p24 antigenemia	−0.12	−0.20*
			0.80 (df = 2, 54, F = 22)
K-562 (HIV^- and HIV^+ subjects)	Constant	0.37	0
	$CD56^+$ LAK cells	0.61	0.62***
	$CD4^+$ PBL	0.22	0.36***
			0.79 (df = 2, 83, F = 69)

[a] Multivariate regression analysis with forward selection was used to determine the influence of variables on LAK activity. Only "significant" variables that improved the predictive power of $CD56^+$ LAK cells were entered into regression equations. Partial regression and correlation coefficients are shown for each variable. The F values and overall regression coefficient were determined when all the variables were entered into the equation.

[b] *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. df, degrees of freedom.

coefficients for $CD56^+$ LAK cell and $CD4^+$ T-cell participation in explaining measured LAK cell responses against both tested targets. There were no further improvements in predicting LAK cell responses to either target by addition of $CD8^+$ or $CD57^+$ LAK cell numbers as variables. The independent effects of numbers of $CD56^+$ LAK cells and $CD4^+$ PBL on U-937 target cell lysis are illustrated in Fig. 3. The combined effects of $CD56^+$ and $CD4^+$ cells in determining U-937 and K-562 lysis are graphically represented in Fig. 4.

DISCUSSION

This study shows that inducible LAK activity is an additional parameter with which to monitor immune and disease status in HIV-infected individuals. LAK responses showed stepwise declines in OI^- groups individuals compared with seronegative controls and in OI^+ subjects compared with OI^- groups. These reductions in LAK activities in HIV-positive subjects could not be directly ascribed to AZT intervention as had been previously suggested on the basis of in vitro effects of AZT on LAK activity (28).

Despite marked differences in CD4 cell numbers among our HIV-positive groups, there were no corresponding LAK cell recoveries or absolute numbers or relative proportions of $CD56^+$ subsets in PBL or LAK cells. Homeostatic mechanisms that conserve a CD56-CD3 balance in HIV-positive subjects may therefore exist (16, 31). There were, however, significant defects in LAK activity in OI^+ groups, particularly when measured as lytic activity per unit $CD56^+$ cell and lytic activity per milliliter of blood. This agrees with results of a number of studies showing functional impairments in NK function in HIV-infected individuals (1, 14, 27, 30). This suggests that reductions in NK and LAK activities in HIV-infected individuals may be related to the relative proportion of inducible and/or cytolytic NK effectors within the NK cell pool. This is important in that simply monitoring NK cell numbers, as is done in many clinical trials, may not accurately reflect levels of NK cell-mediated functional responses in HIV-positive subjects.

This cross-sectional study shows that variations in NK and CD4 cell numbers can predict fluctuations in measured LAK functional responses. Indeed, a multivariate linear regression model showed that $CD56^+$, $CD4^+$, and $CD57^+$ LAK cell numbers were independent immunological variables that combined to explain measured LAK cell responses in HIV-positive subjects. This statistical analysis provides a novel approach to infer NK functional immune responses from numbers of NK cell and T-cell subsets. This may be particularly relevant in clinical trials assessing drug treatments, in which only NK cell and T-cell phenotypic analysis are performed.

Although the finding that $CD56^+$ LAK cell numbers are the major predictors of measured LAK cell responses was to be expected, it was rather surprising that $CD4^+$ cell numbers improved the predictive power of $CD56^+$ cells in explaining measured LAK cell responses. Our previous studies negated a direct role of $CD4^+$ cells in LAK cell function, showing that depletion of CD4 subsets from PBL in HIV-negative and HIV-positive donors did not affect subsequent LAK activity (5). $CD4^+$ cell numbers may indirectly impact on measured LAK cell response. $CD56^+$ and $CD4^+$ cell proliferation, differentiation, and function may be comodulated by factors such as lymphokines (IL-2, IL-4, and IL-12) or by accessory cells such as monocytes and $HLA\text{-}Dr^+$ cells.

It is interesting that $CD8^+$ cell number did not influence the measured LAK cell response once the $CD56^+$ cell number was introduced into our multivariate regression models. We had previously reported the presence of $CD8^+$ cytotoxic LAK cells in asymptomatic HIV-infected subjects and their absence in HIV-negative and OI^+ individuals (5, 12). In recent unpub-

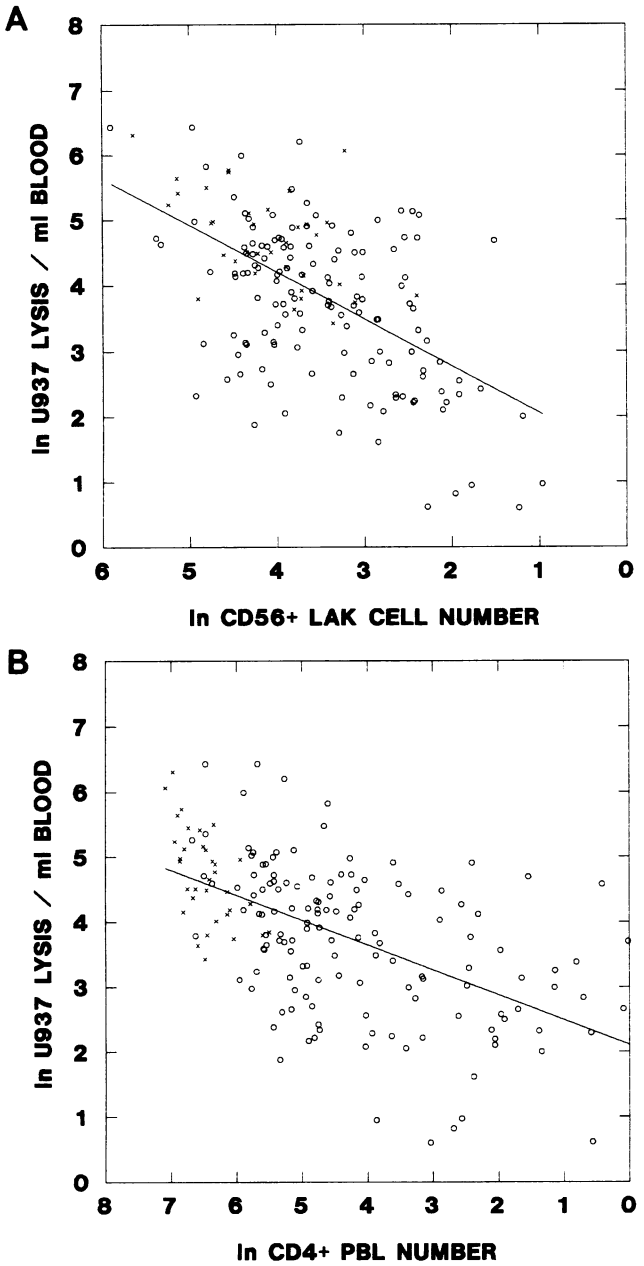

FIG. 3. (A) Relationship between LAK activity per milliliter of blood and inducible $CD56^+$ LAK cell numbers. (B) Corresponding relationship between LAK activity and total $CD4^+$ PBL numbers. Logarithmically transformed values are presented for HIV-negative (×) and HIV-positive (○) individuals. Data were subjected to linear smoothing.

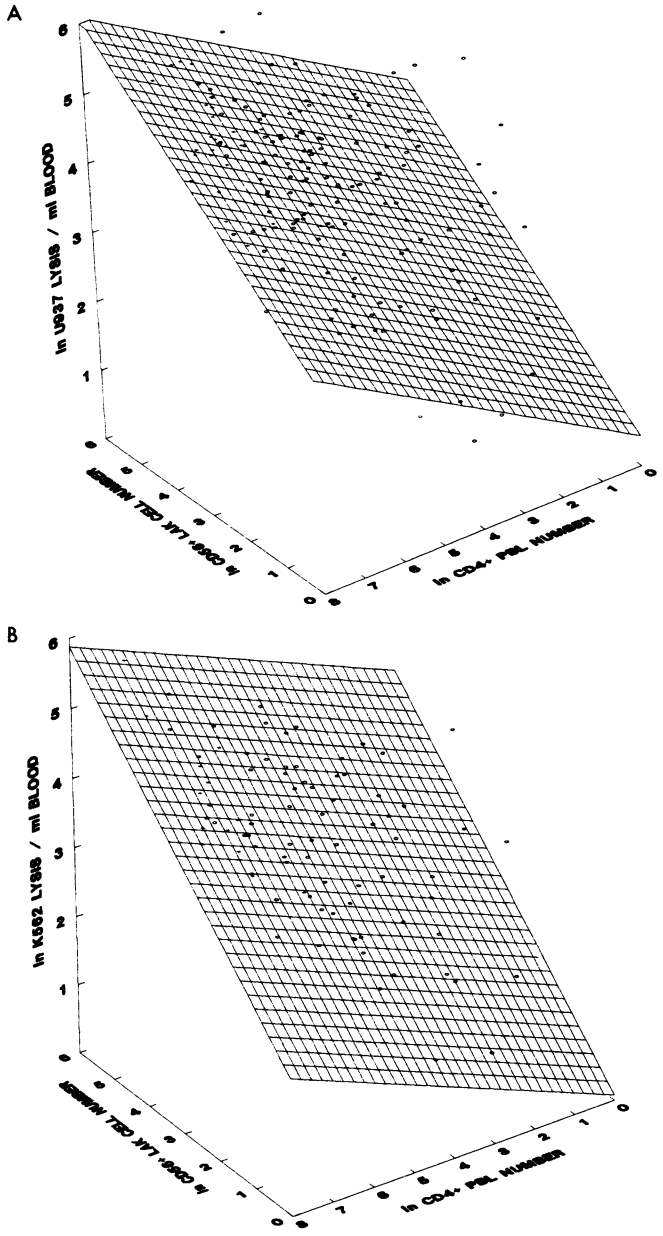

FIG. 4. Relationship between target cell lysis per milliliter of blood and numbers of $CD56^+$ and $CD4^+$ subsets for U-937 target lysis (A) and K-562 target lysis (B). Individual datum points from HIV-negative and HIV-positive subjects are presented. Data were subjected to linear smoothing.

lished studies involving positive sorting procedures, cytotoxic cells coexpressing CD8 and CD56 can be shown to account for a significant proportion of K-562 and U-937 target cell lysis in the OI^- individuals but not in HIV-negative or OI^+ groups. A suppressor role of CD8 cells in the induction of $CD56^+$ LAK cell responses was ruled out in these studies since the combined recovery of LAK activity from $CD8^-$ and $CD8^+$ LAK cells equaled the recovery from undepleted LAK cells. Thus, in our LAK cell assay system, there does not appear to be any role of $CD8^+$ T cells as either effectors or suppressors.

We measured LAK cell responses against both U-937 and K562 targets. Although LAK assays should ideally be measured against NK cell-resistant targets such as Raji and Daudi, these targets are much less susceptible than K562 and U-937 to LAK cell-mediated lysis in HIV-negative and HIV-positive subjects. Our previous studies showed that many HIV-positive subjects showed no lysis of Raji cells unless starting effector/target cell ratios of 20:1 or 50:1 were used. Because of limitations in blood sample sizes from HIV-positive patients, it is unfeasible to use Raji cells as a target.

That NK cell numbers and CD4 cell numbers independently predict LAK cell activity can perhaps explain the diversity in

clinical responses among HIV-positive individuals. Any functional compromise of individual NK cells associated with HIV infection can be compensated for if their relative numbers can be effectively increased. LAK activity may be an important functional parameter to monitor adaptive responses to HIV infection among individuals. The use of the LAK cell system may also help in the design of immunomodulatory strategies involving cytokines or ex vivo propagated LAK cells in reconstitution therapy (18, 25).

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