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Increased LAK activity against HIV-infected cell lines in HIV-1$^+$ individuals

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SUMMARY

The role of natural killer (NK) cells and their inducible counterparts, lymphokine-activated killer (LAK) cells in AIDS with regard to HIV-1 viral immunosurveillance and the control of secondary opportunistic disease has yet to be established. In this study, we have demonstrated that LAK cells derived from all HIV-1$^+$ groups showed striking increases in their capacity to lyse HIV-1-infected U-937 cells relative to their uninfected U-937 counterparts. Surprisingly, similarly derived LAK cells from healthy seronegative controls showed no differences in their lysis of HIV-1-infected *versus* uninfected U-937 cells. The differential ability of LAK effectors from seropositive donors to lyse HIV-1-infected targets was demonstrable using a number of U-937 subclones and their HIV-1-infected counterparts. Again, no differences in LAK cell-mediated lysis of HIV-1-infected and uninfected U-937 subclones were observed in seronegative individuals. Our findings that HIV-1$^+$ individuals show selective expansion of non-MHC restricted, HIV-1-directed cytotoxic LAK cells indicate that natural immunity may indeed play a role in HIV-1 viral immunosurveillance.

Keywords lymphokine-activated killer cells natural killer cells AIDS U-937 HIV

INTRODUCTION

The immunopathogenesis of AIDS has been primarily associated with a selective depletion of CD4-expressing T cells due to their susceptibility to HIV-1 [1]. The clinical course of AIDS following HIV-1 infection has been shown to include a long asymptomatic period (typically 3–5 years). Cellular and humoral immune mechanisms may play a role in limiting viral replication and opportunistic diseases. Elucidation of HIV-specific immune responses in early phases of AIDS and their associated changes with progressive immunocompromise is crucial.

Natural killer (NK) cells and their inducible counterparts, lymphokine-activated killer (LAK) cells have been shown to elicit a number of broadly reactive and non-MHC-restricted cytolytic responses to virally infected and tumour targets [2–4]. These responses include NK activity, antibody-dependent cellular cytotoxicity (ADCC), and LAK activity. Phenotypically, NK cells express an array of NK-associated surface markers (CD56$^+$, CD16$^+$, CD57$^\pm$) while lacking T-associated markers, including CD3, the T cell receptor, and CD4, the HIV-1 viral receptor [2–4].

Patterns of decreased peripheral blood NK activity, ADCC responses and circulating CD16$^+$ and CD56$^+$ NK subsets have been observed in HIV-1$^+$ individuals with AIDS and persistent generalized lymphadenopathy (PGL) when compared with healthy seronegative and asymptomatic HIV-1$^+$ populations [5–12]. The effects of changes in natural immunity on disease course have yet to be established. Observed decreases may render seropositive individuals more susceptible to opportunistic diseases. Conversely, NK cell responses may be detrimental to seropositive populations. NK cells may potentially recognize and destroy virally infected CD4 cells or virally coated bystander uninfected cells [13–15].

IL-2 has been shown to play a central role in stimulating NK cell proliferation, differentiation and cytolytic activities. IL-2 in HIV-1$^+$ individuals has been observed to restore partially *in vitro* NK and ADCC functions [5,6,16]. A novel class of effectors, LAK cells, have been generated via *ex vivo* incubation of peripheral blood lymphocytes (PBL) with IL-2 [17,18]. These LAK cells have been shown to mediate unique cytolytic activities killing both NK-sensitive and NK-resistant targets. NK cells have been demonstrated to be the primary progenitors and effectors of inducible LAK cell responses [19–21]. LAK cells, expressing NK antigenic phenotype, have also been induced *in vivo* following IL-2 therapy [22–25].

The relative abilities of inducible LAK cells to evoke anti-tumour and anti-viral cytolytic responses in seropositive populations have not been identified to date. Recent investigations in our laboratory have shown a diminished efficacy of PBL derived from HIV-1$^+$ individuals to generate cytotoxic LAK cells against K562, RAJI and U-937 targets [13,26]. In this study we

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have contrasted the ability of HIV-1$^+$ and HIV-1$^-$ individuals to generate LAK cell effectors cytotoxic to the U-937 cell line, isolated U-937 subclones, and their HIV-1-infected counterparts. LAK cells derived from all HIV-1$^+$ subgroups showed significantly increased cytolysis of HIV-1-infected U-937 targets relative to their uninfected counterparts. It should be emphasized that these enhanced non-MHC-restricted, HIV-directed lytic responses were restricted to seropositive individuals.

PATIENTS AND METHODS

Study subjects

Blood samples were obtained upon informed written consent from HIV-1$^+$ individuals receiving treatment at our dental clinic. Our study group consisted primarily of seropositive homosexuals. Clinical status of these individuals was established according to standardized guidelines established by the Centres for Disease Control (CDC) [27]. The CDC2 subgroup included HIV-1$^+$ individuals with no signs or symptoms of AIDS. The CDC4D subgroup consisted of seropositive individuals with Kaposi's sarcoma who were otherwise asymptomatic. The CDC4CD subgroup included overt AIDS patients with two or more AIDS-associated secondary opportunistic infections (OI$^+$, CDCC1 and C2) and/or cancers (CDC4D) (e.g. Kaposi's sarcoma (KS), *Pneumocystis carinii* pneumonia, oral candidiasis, oral hairy leukoplakia, and/or disseminated herpes). The CDC0 subgroup was comprised of age-matched healthy, HIV-1$^-$ individuals recruited from hospital personnel.

Effector cells

Venous blood was collected in heparinized tubes and PBL were isolated by Ficoll–Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation [28]. LAK cells were generated by *ex vivo* incubation of PBL (10^6 cells/ml) in RPMI-1640 (GIBCO, Grand Island, NY) complete medium containing 10% decomplemented fetal calf serum (FCS), 15 U/ml IL-2 (Boehringer-Mannheim, Mannheim, West Germany), 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10 mM HEPES for 5–6 days at 37°C. On the day of assay, LAK cells were washed twice in complete media.

Target cells

The U-937 cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD). UC subclones were derived by a two time limiting serial dilution, seeding 0·15–0·5 cells/well in 96-well microtitre plates [29]. The III_B strain of HIV-1 (kindly supplied by R. C. Gallo, NIH, Bethesda, MD) was used to infect the parental U-937 and UC subclones as previously described using a multiplicity of infection of three ED_{50}/cell [29,30]. Murine MoAbs to HIV-1 viral proteins p17 and p24 (supplied by R. C. Gallo) and fluorescein-labelled goat anti-mouse immunoglobulin (Miles Laboratories, Ekhart, IN) were used to quantify the percentage of HIV-1-infected cells [30]. Virtually all chronically infected cells expressed HIV-1. All cell lines were passaged twice weekly.

Cytotoxicity assays

LAK activity was monitored using 18 h chromium release assays with LAK cells as effectors (E) and ^{51}Cr-labelled tumour cell lines as targets (T). Target cells were labelled with 150 μC sodium ^{51}Cr-chromate (ICN Biochemicals, Montreal, Canada). LAK cells were adjusted to give appropriate E:T ratios in triplicate wells of U-bottomed 96-well microdilution plates. Spontaneous isotope release was determined from control wells containing only target cells, and maximum release was determined by the addition of 2% Triton-X-100 to appropriate cells. This spontaneous release represented approximately 20% total release. The percentage of cytotoxicity was calculated according to the formula: % cytotoxicity = (test sample release − spontaneous release)/(maximum release − spontaneous release).

Exponential regression analysis of subjects' cytolytic activities monitored at 5–6 E:T cell ratios was performed using computer software kindly provided by Dr H. F. Pross [31]. Cytolysis was calculated according to equation $y = A\ (1 - e^{-kx})$ where y = fractional chromium release, x = E:T ratio, k = negative slope constant derived from plotting ln $(A - y)$ (i.e. target survival) *versus* x, and A = asymptote of the curve. A and k are independent parameters with A representing the maximal amount of cell-mediated lysis and k representing the relative lytic potential of lymphocytes. Using A and k, the equations were solved for 20% cytolysis of 10 000 targets, inverted, and multiplied by 100 to yield LU_{20} values.

When indicated, lymphocyte counts and LAK cell recoveries were incorporated with LU_{20} values to yield absolute LU_{20} values, that represented the overall LAK cell lytic potential per millilitre of blood. Absolute LU_{20} = (lymphocyte count (cells/ml blood) × lymphocyte recovery (ratio of lymphocytes day 6/day 0) × observed LU_{20})/100 [26].

Depletion of NK cells from PBL

Prior to LAK cell generation, PBL were depleted of CD56$^+$ NK cell subsets using Leu-19 (Becton Dickinson, Mountain View, CA) and goat anti-mouse immunoglobulin. Briefly, PBL ($8–10 \times 10^6$ cells/ml) were incubated in RPMI-1640 media containing 1% FCS and Leu-19 (20 μl/10^6 cells) for 30 min at 4°C. After washing twice in HBSS (GIBCO), an excess (40 beads/cell) of magnetic goat anti-mouse-coated IgG beads (Fc specific, Advanced Magnetics, Cambridge, MA) was added. Following a 30 min incubation on ice, those cells bound to magnetic beads were removed using a magnetic separator (Advanced Magnetics). This depletion was then serially repeated using 20 beads/cell. Depleted PBL were then used to generate LAK cells as previously described.

Flow cytometric analysis

Distributions of CD4, CD8, CD57 and CD56 antigens on PBL and LAK cells were ascertained by cytofluorometric analysis using an EPICs analyser (Coulter Electronics, Burlington, Ontario). Cells were stained with relevant FITC or PE-conjugated MoAbs (Coulter Electronics, Burlington, Ontario and Becton Dickinson).

Statistical analysis

Cytolytic parameters, lymphocyte subset distributions, and lymphocyte counts were monitored in individual subjects with a single determination for any individual in any given population. Throughout the text and tables, data are represented as mean values ± s.e.m. Levels of activities and subset distributions were compared using one way ANOVA. Where a significant overall F value was obtained, *post hoc* comparisons were done using Tukey and Newman–Keuls tests. Comparisons of cytolytic parameters (LU_{20} values) of U-937 targets *versus* their HIV-

infected counterparts were monitored using paired *t*-tests. LAK activities measured over a range of E:T ratios were compared in control and HIV-1$^+$ populations using two-way ANOVA. Similarly, LAK activities monitored in parallel against HIV-1-infected and uninfected targets were contrasted using two-way ANOVA. Statistical comparisons were calculated using software obtained from Systat Inc. (Evanston, IL) and Lionheart Press (Alburg, VT).

RESULTS

Cytotoxic lymphocyte distribution in AIDS

The relative numbers and subset distributions of PBL and LAK cells from HIV-1$^-$ (CDC0) and HIV-1$^+$ CDC2 (asymptomatic), CDC4D (KS), and CDC4CD (AIDS, OI$^+$) subgroups are shown in Table 1. PBL numbers declined with increasing duration of HIV-1 infection ($F=7{\cdot}1$, df$=80$, $P<0{\cdot}001$). LAK cell recoveries on a per cell basis, however, were similar in all subgroups ($F=1{\cdot}7$, df$=80$). Flow cytometric analysis indicated that the proportion of CD4-expressing cells progressively declined with advancing disease for PBL ($F=66{\cdot}7$, df$=41$, $P<0{\cdot}001$) and LAK cells ($F=33{\cdot}8$, df$=41$, $P<0{\cdot}001$). Conversely, significant increases in the proportions of CD8$^+$ PBL ($F=23{\cdot}0$, df$=41$, $P<0{\cdot}001$), CD8$^+$ LAK cells ($F=3{\cdot}6$, df$=41$, $P<0{\cdot}05$), CD57$^+$ PBL ($F=12{\cdot}5$, df$=41$, $P<0{\cdot}001$), and CD57$^+$ LAK cells ($F=6{\cdot}3$, df$=41$, $P<0{\cdot}001$) were observed in seropositive subgroups. CD57 was not representative of NK cells since only $11\pm1\%$ of CD57$^+$ LAK cells co-expressed CD56 whereas $42\pm3\%$ of CD57$^+$ LAK cells co-expressed CD8. It should be emphasized that corresponding proportions of CD56-expressing PBL ($F=0{\cdot}93$, df$=41$) and CD56-expressing LAK cells ($F=0{\cdot}63$, df$=41$) from seronegative and seropositive subgroups showed no significant differences.

LAK cell-mediated cytolysis of parental U-937 targets

As depicted in Fig. 1, LAK cell-mediated cytotoxicity of uninfected U-937 targets progressively declined with disease progression ($F=4{\cdot}7$, df$=84$, $P<0{\cdot}01$). Cytolysis of U-937 targets was significantly reduced in seropositive individuals with overt AIDS (CDC4CD) when compared with asymptomatic seropositive (CDC2) and seronegative (CDC0) controls ($P=0{\cdot}005$ and $0{\cdot}037$, respectively, Tukey tests). As shown in Fig. 1, the ability of seropositive individuals to lyse HIV-1-infected U-937 cells was significantly enhanced relative to uninfected U-937 cells ($P=0{\cdot}001$, $0{\cdot}038$, and $0{\cdot}001$ for CDC2, CDC4D and CDC4CD groups, respectively, paired *t*-tests). In direct contrast, there was no differential susceptibility of HIV-1-infected and uninfected U-937 targets to lysis by LAK effectors isolated from healthy seronegative controls. There were significant differences in the overall ability to lyse HIV-1-infected targets among groups ($F=4{\cdot}9$, df$=84$, $P<0{\cdot}01$). *Post hoc* Tukey tests ($P<0{\cdot}05$) yielded significant differences between CDC2 (asymptomatic) and CDC4CD (AIDS, OI$^+$) groups.

The absolute lytic potentials of LAK cells from seropositive and seronegative individuals were estimated by incorporating PBL lymphocyte counts and LAK cell recoveries into the calculated LU_{20} cytolytic values (Fig. 2). Absolute lysis of uninfected U-937 cells declined progressively with increasing duration of infection ($F=6{\cdot}7$, df$=84$, $P<0{\cdot}001$). The CDC4CD (AIDS) group showed significantly less absolute U-937 lysis than CDC2 (asymptomatic) and seronegative (CDC0) groups ($P=0{\cdot}043$ and $0{\cdot}001$, respectively, Tukey tests). The overall susceptibility of HIV-1-infected relative to uninfected U-937 targets was significantly increased in all seropositive individuals (paired *t*-tests). There were significant differences in absolute lysis of HIV-1-infected U-937 targets among groups ($F=3{\cdot}6$, df$=84$, $P<0{\cdot}01$). Absolute lysis of HIV-1-infected targets in the CDC4CD (OI$^+$) group was significantly less than the CDC2 (asymptomatic) group ($P=0{\cdot}014$, Tukey test).

Table 1. Lymphocyte counts, lymphokine-activated killer (LAK) cell recoveries, times following HIV-1 seroconversion, and subset distributions in HIV-1$^+$ and HIV-1$^-$ populations

	HIV$^-$	CDC classification groups HIV$^+$		
	CDC0	CDC2	CDC4D	CDC4CD
	(19)	(25)	(7)	(33)
PBL count	17 ± 1	15 ± 1	14 ± 3	$10\pm1^{1,2}$
LAK recovery	65 ± 4	52 ± 5	48 ± 8	51 ± 5
Time HIV$^+$(years)	NA	$2{\cdot}6\pm0{\cdot}3^4$	$3{\cdot}0\pm0{\cdot}9$	$3{\cdot}9\pm0{\cdot}4$
Subsets (%)	(10)	(11)	(5)	(19)
CD4 PBL	45 ± 2	24 ± 2^1	$12\pm4^{1,2}$	$9\pm2^{1,2}$
CD56 PBL	6 ± 1	5 ± 2	7 ± 3	8 ± 2
CD56 LAK	9 ± 1	7 ± 2	11 ± 4	10 ± 1
CD8 LAK	28 ± 2^3	40 ± 3	51 ± 8	36 ± 4
CD57 LAK	$4\pm1^{3,4}$	15 ± 2^4	24 ± 7	28 ± 4

PBL count (cells/ml blood $\times 10^{-5}$), LAK cell recovery (% day 6/day 1), and selected lymphocyte subset distributions (%) in PBL or LAK cells are given. Number of subjects are indicated in parentheses.

[1,2,3,4] Values are significantly less than healthy controls[1] (CDC0), HIV-1$^+$, CDC2[2], CDC4D[3], or CDC4CD[4].

NA, not applicable; PBL, peripheral blood lymphocytes.

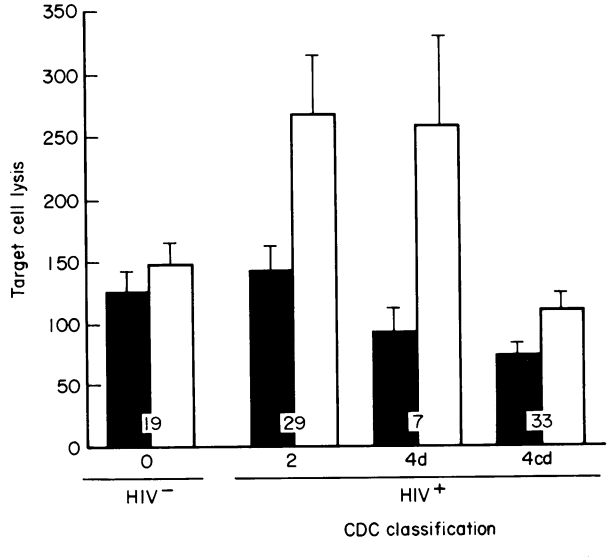

Fig. 1. Lymphokine-activated killer (LAK) cell activity (LU_{20} values) against the uninfected (■) and HIV-1-infected (□) U-937 cell lines using LAK cells from healthy control (CDC0) and HIV-1$^+$ individuals (CDC2, CDC4D and CDC4CD).

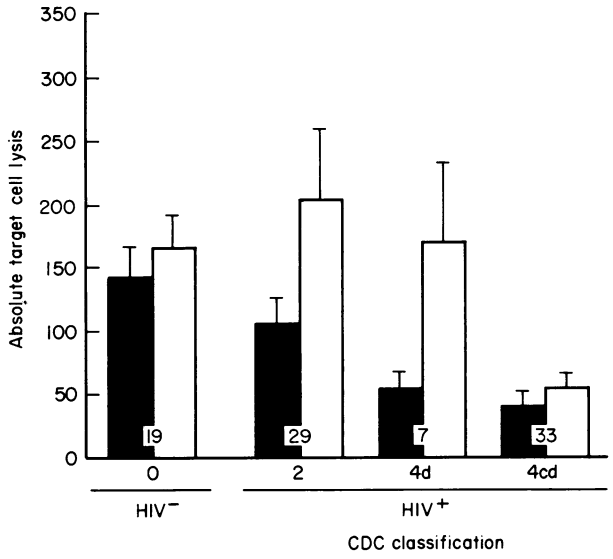

Fig. 2. Absolute target cell lysis of uninfected (■) and HIV-1-infected (□) U-937 targets in HIV-1$^-$ (CDC0) and HIV-1$^+$ individuals (CDC2, CDC4D, and CDC4CD). Absolute lysis incorporates peripheral blood lymphocyte (PBL) counts, lymphokine-activated killer (LAK) cell recoveries, and LU_{20} values as described in Patients and Methods.

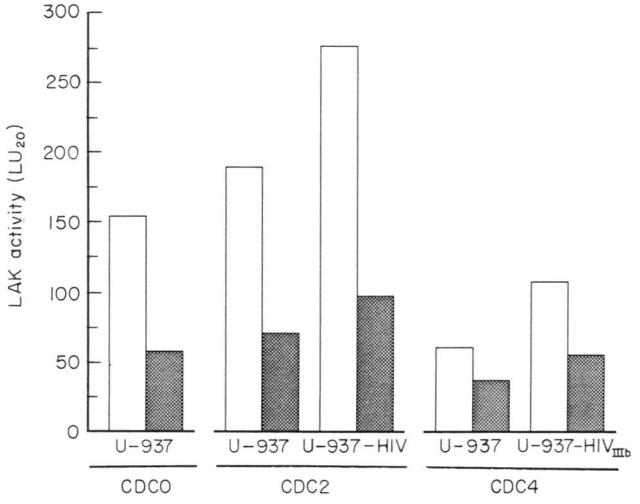

Fig. 3. Lymphokine-activated killer (LAK) cell activity (LU_{20} values) against uninfected and HIV-1_{IIIB}-infected U-937 targets by LAK cells derived from peripheral blood lymphocyte (PBL) or PBL depleted of CD56-expressing cells. Values represent the mean values for five CDC0, three CDC2 and five CDC4CD subjects. □, LAK; ▣, CD56-depleted LAK.

CD56$^+$ cells are the progenitors of cytotoxic LAK cells against U-937 and U-937 HIV targets

As depicted in Fig. 3, CD56 cell depletion diminished LAK cell function directed against both U-937 uninfected and HIV-1-infected U-937 targets in all subgroups. The relative proportion of CD56-expressing cells in residual LAK cells from CD56-depleted PBL still represented ~50% of the corresponding proportions of CD56-expressing LAK cells from undepleted PBL (unpublished results). This may explain the failure to obtain complete abrogation of LAK cell function upon CD56 cell depletion.

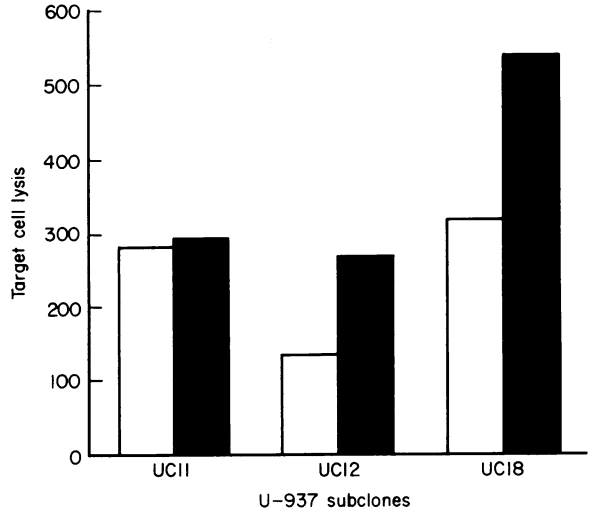

Fig. 4. Average target cell lysis (LU_{20} values) of uninfected (□) and HIV-1_{IIIB}-infected (■) U-937 subclones, UC11, UC12, UC18, using lymphokine-activated killer (LAK) cells derived from 10 HIV-1$^+$ individuals.

LAK cell-mediated cytolysis of U-937 subclones

LAK cells from eight healthy seronegative and 10 seropositive individuals were simultaneously assayed for their ability to lyse uninfected and HIV-1-infected UC11, UC12 and UC18 subclones (Fig. 4). Cytolysis of HIV-1-infected UC18 subclones was significantly greater than corresponding lysis of uninfected UC18 cells using LAK effectors from seropositive individuals ($F=6{\cdot}18$, df$=1{\cdot}36$, $P<0{\cdot}05$ for the CDC2 subgroup and $F=6{\cdot}68$, df$=1{\cdot}48$, $P<0{\cdot}05$ for the CDC4 subgroup). Cytolysis of HIV-1-infected UC12 subclones relative to their uninfected counterparts was higher in asymptomatic seropositives ($F=3{\cdot}26$, df$=1{\cdot}24$, $P=0{\cdot}08$). No significant differences, however, were observed in the corresponding lysis of uninfected and HIV-1-infected UC11 cells in these same seropositive groups ($F=0{\cdot}10$ and $0{\cdot}43$ for CDC2 and CDC4CD subgroups, respectively). There were no significant differences in the ability of seronegative controls (CDC0) to lyse HIV-1-infected UC11, UC12 and UC18 targets relative to their uninfected counterparts ($F=1{\cdot}00$, $0{\cdot}40$, $0{\cdot}65$, HIV-1-infected *versus* uninfected UC11, UC12 and UC18 targets, respectively).

Levels of cytolysis of uninfected UC12 cells were significantly less than corresponding levels of UC11 and UC18 cytolysis ($P<0{\cdot}01$, paired t-tests). In this regard, UC12 are considerably larger and show a significantly slower doubling time than parental U-937, UC11 and UC18 cell lines [29].

DISCUSSION

The ability of *ex vivo*-induced LAK cells to elicit potent antiviral and anti-tumour immune responses has generated interest as their prognostic relevance *in vivo* and their potential usefulness in immunotherapy. In this study, cytotoxic LAK cells from healthy control and HIV-1$^+$ populations were generated using 15 U/ml IL-2. This IL-2 concentration corresponds to *in vivo* steady state concentrations contained in the serum of patients receiving IL-2 therapy [22]. Low IL-2 concentrations, both *in vitro* and *in vivo*, have been shown to selectively augment NK cell responses in AIDS and healthy control populations with no concurrent enhancement of T cell responses [23,32–34].

Confirming our initial observations [13,26], we have found that inducible LAK cell function against both uninfected and HIV-infected U-937 targets was severely impaired in HIV-1$^+$ individuals with overt AIDS (CDC4CD) when compared with healthy seronegative controls. In contrast to our earlier studies [10,26], the cytolytic potential of LAK cells derived from the asymptomatic (CDC2) seropositive individuals generally fell within the range of healthy controls. Since LU_{20} values from both the healthy seronegative and overt AIDS group have not significantly changed from our previously obtained values, our larger asymptomatic population may now have included individuals with improved clinical status by way of earlier detection and improved treatments. This is the first comprehensive study of inducible LAK cell function in seropositive individuals. We are currently aware of only one other study of LAK cell function in AIDS where no significant differences in LAK activity against K562 and RAJI targets were observed in nine patients with AIDS as compared with seronegative controls [35].

We have observed a differential enhancement of LAK cell-mediated lysis of HIV-1-infected targets relative to their uninfected counterparts in seropositive populations. Corresponding increases in lysis of HIV-1-infected cells were absent in healthy seronegative individuals. Other studies have demonstrated that NK cells from seronegative heterosexuals, seronegative and seropositive homosexuals lyse HIV-1-infected targets more effectively than corresponding uninfected targets [2,36–41]. The observation of elevated NK cell-mediated, HIV-1-directed lysis was dependent upon the target cell line and the HIV-1 strain [36,40,41]. Enhanced susceptibility of HIV-1-infected targets involved NK cells with or without accessory HLA-Dr-expressing cells [36]. These observations combined with our present findings strongly suggest that multiple cytolytic effector subsets and pathways mediate NK and LAK cell-mediated cytolysis of HIV-1-infected targets. Furthermore, this study has shown that HIV-1-directed LAK effectors were preferentially activated in HIV-1$^+$ individuals.

It should be emphasized that the absence of HLA-matched targets strongly negates the involvement of classic viral specific cytotoxic T lymphocytes (CTL) in the HIV-1-directed lysis observed in this study. Increased lysis of HIV-1_{IIIB}-infected relative to uninfected targets was demonstrable in 79 HLA-unmatched seropositive individuals. Lysis of uninfected and HIV-1-infected targets was diminished in CDC2 and CDC4CD subgroups upon depletion of CD56-expressing NK cells. Recent studies in our laboratory have identified the emergence of novel CD8-expressing LAK cytotoxic cells in asymptomatic (CDC2) seropositive individuals (manuscript submitted for publication). These CD8 subsets did not, however, elicit conventional MHC-restricted CTL responses. Lysis of K562, a target lacking both class 1 and 2 MHC determinants, was reduced upon CD8 cell depletion (unpublished results). Thus both CD56 and CD8 non-MHC responses contributed to elevated LAK cell-mediated responses to HIV-1-infected targets in asymptomatic seropositives. Only CD56 responses mediated elevated HIV-1-directed responses in overt AIDS patients.

Our findings have shown that the enhanced susceptibility of HIV-1-infected U-937 targets was not universal. The same individuals that showed elevated lysis of HIV-1-infected UC12 and UC18 clones relative to their uninfected counterparts, showed no corresponding enhanced lysis of HIV-infected UC11 subclones. In this regard, UC11 is an interesting variant, showing less accumulated viral DNA, long latency to HIV-1 infection, and reduced levels of Sendai virus-inducible tumour necrosis factor-alpha (TNF-α) mRNA [29]. This target may be particularly useful in defining the target structures that mediate HIV-1 directed LAK cell-mediated cytolysis.

Reasons for the impairments of NK and inducible LAK activities in HIV-1$^+$ individuals remain unclear. It has been suggested that defective NK cytolytic machineries contribute to diminished NK cell function in AIDS subjects [11,12]. Other groups have reported that NK cells can be susceptible to *in vitro* HIV-1 infection despite their failure to express CD4 mRNA or surface CD4 [5,40,41]. Our findings confirm other studies that have indicated overall reductions in circulating CD56-expressing subsets in PBL of seropositive individuals [5,14,15]. We have, however, found no significant differences in the relative proportion of inducible CD56-expressing LAK cells in seropositive populations that could account for observed reductions in their LAK cell function.

A number of distinct cellular mechanisms have been implicated in HIV-1 immunosurveillance. These include cytotoxic function mediated by CTL [11,42,43], ADCC [6–8,44] and NK activity [5,22–24]. Our findings [6,13,21] indicate that inducible non-MHC-restricted LAK activity derived from NK cell progenitors can also elicit anti-HIV responses. The emergence of novel HIV-1-directed immune responses in seropositive individuals indicates that they can contribute to the disease process. Whether these HIV-1-directed responses are beneficial or detrimental to AIDS progression awaits further study. Using our U-937 subclones and their HIV-1-infected counterparts, we have demonstrated that the differential cytotoxicity of HIV-1-infected targets relative to their uninfected counterparts is dictated both by the particular HIV-1-infected target and the source and inducibility of the effector cell population. These subclones will be particularly useful in the further identification, characterization and expansion of HIV-1-directed cytolytic effectors.

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REFERENCES

1 Fauci A, Schnittman SM, Poli G, Koenig S, Pantaleo, G. Immunopathogenic mechanisms in human immunodeficiency virus (HIV) infection. Ann Int Med 1991; **114**:678–93.
2 Trinchieri G. Biology of natural killer cells. Adv Immunol 1989; **47**:187–376.
3 Robertson MJ, Ritz J. Biology and clinical relevance of natural killer cells. Blood 1991; **76**:2421–38.
4 Ritz J, Schmidt RE, Michon J, Hercend T, Schlossman SF. Characterization of functional surface structures on human natural killer cells. Adv Immunol 1988; **42**:181–211.
5 Brenner BG, Dascal A, Margolese RG, Wainberg MA. Natural killer cell function in patients with acquired immunodeficiency syndrome and related diseases. J Leucocyte Biol 1989; **46**:75–83.
6 Brenner BG, Gryllis C, Wainberg MA. The role of antibody-dependent cellular cytotoxicity and lymphokine-activated killer cells in AIDS and related diseases. J Leucocyte Biol 1991; **50**:628–40.

7 Tyler DS, Stanley SD, Nastala CA *et al.* Alterations in antibody-dependent cellular cytotoxicity during the course of HIV-1 infection. Humoral and cellular defects. J Immunol 1990; **144**:3375–84.

8 Katz JD, Mitsuyasu R, Gottlieb MS, Lebow LT, Bonavida B. Mechanism of defective NK cell activity in patients with AIDS and AIDS-related complex. II Normal ADCC mediated by effector cells defective in NK cytotoxicity. J Immunol 1987; **139**:55–60.

9 Sirianni MC, Soddus S, Malorni W, Arancia G, Aiuti F. Mechanism of defective natural killer cell activity in patients with AIDS is associated with defective distribution of tubulin. J Immunol 1988; **140**:2565–8.

10 Brenner BG, Gryllis C, Gornitsky M, Cupples W, Wainberg M. Differential effects of chemotherapy-induced and HIV-1 induced immunocompromise on NK and LAK activities using breast cancer and HIV-1$^+$ patient populations. Anticancer Res 1991; **11**:969–74.

11 Vuillier F, Bianco NE, Montagnier L, Dighiero G. Selective depletion of low-density CD8$^+$, CD16$^+$ lymphocytes during HIV infection. AIDS Res Hum Retroviruses 1988; **4**:121–9.

12 Landay A, Ohlsson-Wilhelm B, Giorgi JV. Application of flow cytometry to the study of HIV function. AIDS 1990; **4**:479–97.

13 Weinhold KJ, Lyerly HK, Matthews TJ *et al.* Cellular anti-gp120 cytolytic reactivities in HIV-1$^+$ individuals. Lancet 1988; **i**:902–4.

14 Rivière Y, Tanneau-Salvadori FA, Regnault A *et al.* Human immunodeficiency virus-specific cytotoxic responses of seropositive individuals. Distinct types of effector cells mediate killing of targets expressing gag and env proteins. J Virol 1989; **63**:2270–7.

15 Weinhold KJ, Lyerly HK, Stanley DS, Austin AA, Matthews J, Bolognesi DP. HIV-1 gp120-mediated immune suppression and lymphocyte destruction in the absence of viral infection. J Immunol 1989; **142**:3091–7.

16 Schwartz DH, Merigan TC. Interleukin-2 in the treatment of HIV disease. Biotherapy 1990; **2**:119–36.

17 Ortaldo JR, Longo PL. Human natural lymphocyte effector cells. Definition, analysis of activity and clinical effectiveness. J Natl Cancer Inst 1988; **80**:999–1010.

18 Grimm EA. Human lymphokine-activated killer cells (LAK cells) as a potential immunotherapeutic modality. Biochim Biophys Acta 1986; **565**:267–79.

19 Skibber JM, Lotze MT, Muul LM, Uppenkamp IK, Ross W, Rosenberg SA. Human lymphokine-activated killer cells. Further characterization of the precursor and effector cell. Nat Immun Cell Growth Regul 1987; **6**:291–305.

20 Ortaldo JR, Mason A, Overton R. Lymphokine-activated killer cells. Analysis of progenitors and effectors. J Exp Med 1986; **164**:1193–205.

21 Phillips JH, Lanier LJ. Dissection of the lymphokine-activated phenomenon. Relative contribution of peripheral blood natural killer cells and T lymphocytes to cytolysis. J Exp Med 1986; **164**:814–25.

22 Weil-Hillman G, Fisch P, Prieve AF, Sosman JA, Hank JA, Sondel PM. Lymphokine-activated killer activity induced by *in vivo* interleukin-2 therapy: predominant role for lymphocytes with increased expression of CD2 and Leu19 antigens but negative expression of CD16 antigens. Cancer Res 1989; **49**:3680–8.

23 Gambacorti-Passerini C, Rivoltini L, Radrizzani F *et al.* Differences between *in vivo* and *in vitro* activation of cancer patient lymphocytes by recombinant interleukin-2: possible role of lymphokine-activated killer cell infusion in the *in vivo* induced activation. Cancer Res 1989; **49**:5230–4.

24 McMannis JD, Fisher RI, Creekmore SP, Braun DP, Harris JE, Ellis TM. *In vivo* effects of recombinant IL-2. I. Isolation of circulating Leu19$^+$ lymphokine-activated killer effector cells from cancer patients receiving recombinant IL-2. J Immunol 1988; **140**:1335–40.

25 Phillips JH, Gemlo BT, Myers WW, Rayner AA, Lanier LL. *In vivo* and *in vitro* activation of natural killer cells in advanced cancer patients undergoing recombinant interleukin-2 and LAK cell therapy. J Clin Oncol 1987; **5**:1933–41.

26 Gryllis C, Wainberg MA, Gornitsky M, Brenner B. Diminution of inducible lymphokine-activated killer cell activity in individuals with AIDS-related disorders. AIDS 1990; **4**:1205–12.

27 Classification system for human T-lymphotropic virus type III/lymphadenopathy-associated virus infections. MMWR 1986; **35**:334–9.

28 Brenner BG, Benarrosh S, Margolese RG. Peripheral blood natural killer cell activity in human breast cancer patients and its modulation by T cell growth factor and autologous plasma. Cancer 1986; **58**:895–902.

29 Boulerice F, Geleziunas R, D'Addario M, Roulston A, Hiscott J, Wainberg MA. Differential susceptibilities of U-937 cell clones to infection by human immunodeficiency virus type 1. J Virol 1992; **66**:1183–7.

30 Tremblay M, Wainberg MA. Susceptibility to AZT of HIV-1 variants grown in Epstein–Barr virus transformed B cell lines. J Infect Dis 1989; **160**:31–36.

31 Pross HF, Baines MG, Rubin P, Shragge P, Patterson MS. Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX: The quantitation of natural killer cell activity. J Clin Immunol 1981; **1**:51–63.

32 Ozaki A, Nakano H, Minato K, Nakagawa K, Sasaki Y, Saijo N. The difference in surface phenotypes between cytotoxic lymphocytes induced *in vivo* by systemic administration of human recombinant interleukin-2 and lymphokine-activated killer cells induced *in vitro*. Eur J Cancer Clin Oncol 1988; **24**:1055–60.

33 Kedar E, Rezai AR, Giorgi JV, Gale RP, Champlin RE, Mitsuyasu RT, Fahey JL. Immunomodulating effects *in vitro* of interleukin-2 and interferon-gamma on human blood and bone marrow mononuclear cells. Nat Immun Cell Growth Regul 1988; **7**:13–30.

34 Eberlein TJ, Rodrick ML, Massaro AF, Jung SE, Mannick JA, Schoof DD. Immunomodulatory effects of systemic low-dose recombinant interleukin-2 and lymphokine-activated killer cells in humans. Cancer Immunol Immunother 1989; **30**:145–50.

35 Chin TW, Plaeger-Marshall S, Haas A, Ank BJ, Strehm ER. Lymphokine-activated killer cells in primary immunodeficiencies and acquired immunodeficiency syndrome. Clin Immunol Immunopathol 1989; **53**:449–59.

36 Bandyopadhyay S, Ziegner U, Campbell DE, Miller DS, Hoxie JA, Starr SE. Natural killer cell-mediated lysis of T cell lines chronically infected with HIV-1. Clin Exp Immunol 1990; **79**:430–5.

37 Rappocciolo G, Toso JF, Torpey DJ, Gupta P, Rinaldo CR. Association of alpha interferon production and natural killer cell lysis of U937 cells infected with human immunodeficiency virus. J Clin Microbiol 1989; **27**:41–48.

38 Ruscetti FW, Mikovits JA, Kalyanaraman VS *et al.* Analysis of effector mechanisms against HTLV-I and HTLV-III/LAV infected lymphoid cells. J Immunol 1986; **136**:3619–24.

39 Melder RJ, Balachandran R, Rinaldo CR, Gupta C, Whiteside TL, Herberman RB. Cytotoxic activity against HIV-infected monocytes by recombinant interleukin-2-activated natural killer cells. AIDS Res Hum Retroviruses 1990; **6**:1011–5.

40 Chehimi J, Bandyapodhyay S, Prakash K *et al.* *In vitro* infection of natural killer cells with different human immunodeficiency virus type 1 isolates. J Virology 1991; **65**:1812–22.

41 Robinson WE, Mitchell WM, Chambers WH, Schuffmann SS, Montefiori DC, Oeltmann TN. Natural killer cell infection and inactivation *in vitro* by the human immunodeficiency virus. Hum Pathol 1988; **19**:535–40.

42 Walker BD, Plata F. Cytotoxic lymphocytes against HIV. AIDS 1990; **4**:177–84.

43 Nixon DF, McMichael AJ. Cytotoxic T-cell recognition of HIV proteins and peptides. AIDS 1991; **5**:1049–59.

44 Ljunggren K, Karlson A, Fenyo EM, Jondal M. Natural and antibody-dependent cytotoxicity in different clinical stages of human immunodeficiency virus type 1 infection. Clin Exp Immunol 1989; **75**:184–9.