AIDS-Related Burkitt's-Type Lymphomas Are a Target for Lymphokine-Activated Killers Induced by Interleukin-2 and Prolactin (44051)

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Abstract. **The development of non-Hodgkin's lymphomas (NHL) is one of the major complications of AIDS. Although several biologic aspects of AIDS-related NHL have been clarified, their sensitivity to immune system cytotoxic effectors has not been tested. In this study, we have investigated the susceptibility of one major AIDS-related NHL type, Burkitt's-type lymphoma (BL), to the cytotoxic activity of lymphokine-activated killers (LAK) and prolactin-activated killers (PAK), which were generated from peripheral blood mononuclear cells upon stimulation with interleukin-2 (in the case of LAK cells) and prolactin (in the case of PAK cells). The sensitivity of AIDS-related BL to *in vitro* raised cytotoxic effectors was compared with that of BL variants of the general population, including sporadic BL and endemic BL. The data show that AIDS-related BL is susceptible to cytolysis by LAK cells, whereas both LAK and PAK cells can efficiently kill endemic BL. In contrast, sporadic BL showed resistance to all cytotoxic effectors tested. Intriguingly, in the case of AIDS-related and endemic BL suboptimal doses of interleukin-2 in combination with prolactin displayed a cytotoxic effect similar to that of LAK cells, suggesting a synergistic activity of the two agents. Overall, these data corroborate the notion that the distinct BL variants differ in their biologic features despite their morphologic and genetic similarity.** [P.S.E.B.M. 1996, Vol 213]

The frequency of acquired immune deficiency syndrome (AIDS)–related non-Hodgkin's lymphomas (AIDS-NHL) is steadily increasing, and at present AIDS-NHL represent the most common malignancy in some AIDS risk groups (1–3). AIDS-NHL are virtually in all cases high-grade B-cell lymphomas, which display a number of peculiarities when compared with NHL of similar histology arising in the general population, including frequency of systemic clinical symptoms, widespread extent of disease at presentation, poor prognosis, and the frequent involvement of extranodal sites (1–4).

Burkitt's-type lymphoma (BL) represents one major histologic category of AIDS-NHL (1–3, 5). This AIDS-related BL variant has also been defined as epidemic BL in order to distinguish it from the BL variants affecting the general population, namely sporadic BL (in the United States and Europe) and endemic BL (in equatorial Africa and Papua New Guinea) (5). The pathogenesis of AIDS-related BL has been elucidated to a certain extent (2, 3, 6, 7). On the one hand, host factors, including disrupted immunosurveillance, chronic immune stimulation, and deregulation of cytokine loops, are thought to lead to B-cell oligoclonal

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expansions (6, 7). On the other hand, accumulation of genetic lesions of both oncogenes and tumor suppressor genes as well as viral infection of the tumor clone would cause the emergence of a truly monoclonal AIDS-NHL (8). Despite these observations, several aspects of AIDS-related lymphomagenesis remain to be determined. In particular, studies requiring a pure tumor population, such as the analysis of the cellular interactions between the lymphoma and the host's residual immune function, have been hampered by the marked cytologic heterogeneity of AIDS-related BL and in general AIDS-NHL biopsies (4).

Natural killer (NK) cells and their inducible counterparts, namely lymphokine-activated killers (LAK) induced by interleukin-2 (IL-2), comprise a distinct branch of the immune network that elicits broadly reactive and non–MHC-restricted cytolytic responses to virally infected and tumor cells (9–12). Notably, tumors that are resistant to MHC-restricted cytotoxic activity can be killed by LAK effectors. In addition to being consistently observed following the intravenous injection of IL-2 into cancer patients, the LAK phenomenon may be part of the immune amplification pathway. LAK activity has in fact been reported in lymphnodes of experimentally immunized mice (13). In addition to classical (i.e., IL-2–induced) LAK effectors, prolactin-activated killers (PAK), derived from peripheral blood mononuclear cells (PBMC) after incubation with the endopituitary hormone prolactin (PRL), represent a novel member of this immune compartment sharing many of the LAK activities (14). Although NK function is overall decreased in the AIDS context (15–20), LAK activity may be generated at high levels against specific targets in human immunodeficiency virus (HIV)–infected individuals (21, 22).

In this study, we have therefore investigated the susceptibility of AIDS-related BL to be lysed by LAK and PAK cells and we have compared it with that of lymphomas of similar histology arising in the general population, including both sporadic and endemic BL. We report that the pattern of cytotoxic susceptibility varies among BL variants. AIDS-related BL may be more efficiently lysed by effectors which had been incubated with IL-2, whereas PRL had no effect on basal cytotoxicity. Endemic BL, already susceptible to unstimulated killers, is lysed with higher efficiency by both LAK and PAK effectors, whereas sporadic BL is resistant to the action of all the cytotoxic effectors tested. It is notable that, with respect to AIDS-related and endemic BL, activated killers may also be generated by the synergistic action of suboptimal doses of IL-2 in combination with PRL.

Materials and Methods

Cell Lines. Cell lines used as targets in cytotoxicity assays included both AIDS-related BL cell lines and cell lines derived from BL of the general population. AIDS-related BL cell lines ($n = 5$) used in this study included the following: HBL-3, PA682, AS283A, ES3, and LAM. All these cell lines had been characterized in detail in previous studies (23–27). Lymphoma cell lines established from BL of the general population were representative of both the sporadic ($n = 4$) and the endemic ($n = 5$) BL variants, and included CD46 (sporadic), ST486 (sporadic), RAMOS (sporadic), JD38 (sporadic), DAUDI (endemic), RAJI (endemic), NAMALWA (endemic), P3HR1 (endemic) and EB3 (endemic). The characterization of these cell lines has been previously reported (28, 29). All cell lines used in this study were expanded in RPMI 1640 medium containing 10% fetal calf serum at 37°C in a humidified 5% CO_2 atmosphere. PA682, AS283A, CA46, ST486, RAMOS, JD38, P3HR1, and EB3 were the kind gift of Dr. R. Dalla-Favera (Columbia University, New York). DAUDI, RAJI, and NAMALWA were the kind gift of Dr. G. Bellone (Università di Torino, Italy). ES3 and LAM were the kind gift of Dr. A. Ganser (University of Frankfurt, Germany) and Dr. M. Ferrarini (Università di Genova, Italy), respectively.

Hormones and Cytokines. Recombinant human PRL (rPRL) was generously provided by Genzyme Corporation (Framingham, MA). rIL-2 was kindly provided by Glaxo IMB SA (Geneva, Switzerland).

Generation of LAK and PAK Activities. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood of blood bank donors by Ficoll-Hypaque gradient centrifugation, as previously reported (14). To avoid the contribution of exogeneous growth factors, LAK and PAK progenitors (1×10^6) were cultured in serum-free RPMI 1640 medium containing 0.25% bovine serum albumin as an aspecific source of proteins. rIL-2 and rPRL were added as specified below in Results and cultures were carried out in tubes or flasks at 37°C in a humidified 5% CO_2 atmosphere for 6 days.

Cytotoxicity Assay. Effectors derived from three distinct donors were used against all but one target cell line, for which only two donors were used. Cytotoxicity was measured in a 4-hr assay in 96-well microtiter plates with 4000 $Na_2{}^{51}CrO_4$ (NEN-Dupont, Milan, Italy)–labeled target cells per well, effector:target ratios of 24:1, 12:1, 6:1, and 3:1, and three replicates per condition. Percentage specific lysis was calculated as 100 × (Experimental − Spontaneous Release)/(Total − Spontaneous Release), with standard deviation (SD) usually <10%. LAK activity was also expressed as both Lytic Units (LU)/10^8 PBMC, which reduce ^{51}Cr-release dose-response data to linearity (30), and as Area Under Curve (AUC) (31). A LU is defined as the number of effector cells required to produce 30% specific cytotoxicity of 4×10^3 target cells (30). The

AUC is calculated by integrating the log[effector]/response curve over a standard dilution range (31). The percent spontaneous ^{51}Cr release (calculated as S/M × 100) was <10%. LAK cytotoxicity against a standard LAK target cell line (HL60) ranged from 690 to 850 LU/10^8 PBMC.

Statistical Analysis. LU values in treated and control cultures were logarythmically transformed to reach normal distribution and compared using the Wilcoxon's test for nonparametric data. AUC values in treated and control cultures were also compared using the Wilcoxon's test.

Results

Characteristics of AIDS-Related and Non–AIDS-related BL Cell Lines. The phenotypic and molecular features of the BL cell lines used in this study have been described in detail elsewhere (23–29). Cell lines were representative of the different clinical variants of BL (5), including both AIDS-related BL (n = 5) and BL of the general population, namely sporadic BL (n = 4) and endemic BL (n = 5). All the cell lines carried an activated c-*myc* gene as a consequence of chromosomal translocation. Infection by EBV was present in 3/5 AIDS-related BL and 5/5 endemic BL, whereas it was absent in all sporadic cases. In addition, 3/5 AIDS-related BL, 3/4 sporadic BL, and 4/5 endemic BL carried a p53 mutation.

General Experimental Strategy. All BL cell lines (AIDS-related, sporadic, and endemic BL) used as targets were challenged by cytotoxic effectors represented by unstimulated PBMC, LAK cells, PAK cells, or cytotoxic effectors generated by the combination of rIL-2 and rPRL. Each experimental condition was assessed by using cytotoxic effectors derived from three distinct individuals, with the exception of experiments performed on one AIDS-related BL (LAM), for which cytotoxic effectors were derived from only two individuals. The cytotoxic activity of the effectors on the target cell lines was calculated in each case by different methods, including percentage cytotoxicity, lytic units (LU)/10^8 PBMC (30), and area under the curve (AUC) (31). The results of cytotoxic activity are reported as (i) individual cytotoxic sensitivity of each BL cell line to the cytotoxic effectors generated from each single donor (e.g., Fig. 1); (ii) mean cytotoxic sensitivity of each BL cell line to the cytotoxic effectors generated by a given treatment (e.g., Fig. 2); (iii) mean cytotoxic sensitivity of each BL variant (AIDS-related, sporadic, endemic) to the cytotoxic effectors generated by a given treatment (e.g., Tables I–III and Figs. 3 and 4).

Sensitivity of AIDS-Related and Non–AIDS-Related BL to LAK Effectors. LAK effectors were generated from PBMC after a 6-day culture in the presence of either rIL-2 25 U/mol (0.7 n*M*) or rIL-2 100 U/ml (3.0 n*M*). rIL-2 100 U/ml is the rIL-2 concentration routinely used in our laboratory to attain optimal cytotoxic response against conventional LAK targets (14). The individual sensitivity of each BL cell line to the cytotoxic effectors from each single donor is represented in Figure 1. As for other cell targets (12, 14), the LAK activity directed against some BL cases displayed a certain degree of variability among the different PBMC donors. Overall, when pooling the results obtained from the different donors tested against each target, most AIDS-related and endemic BL showed increased LU values after incubation with LAK cells compared with unstimulated PBMC (Fig. 2).

For each BL variant, the mean cytotolysis value was calculated by pooling all the PBMC donors stimulated at a given rIL-2 concentration and tested against all the cell lines belonging to the BL variant. The mean value of the LAK cytotoxic activity against each BL variant, together with the results of statistical analysis, is summarized in Table I, Table II, and Table III for AIDS-related, sporadic, and endemic BL, respectively. A graphical representation of the results is shown in Figure 3. A marked difference was observed in the sensitivity of each BL variant to cytotoxicity by unstimulated PBMC, with sporadic BL being virtually resistant and AIDS-related BL and endemic BL displaying moderate and high susceptibility, respectively. Both AIDS-related and endemic BL were significantly more susceptible to cell lysis by LAK cells generated by rIL-2 100 U/ml compared with unstimulated PBMC, whereas no similar effect was observed in sporadic BL. PBMC stimulation with rIL-2 25 U/ml failed to attain significantly different cytotoxicity against all BL variants. When considering AIDS-related BL, PBMC stimulation with rIL-2 100 U/ml led to the highest percentage increase in cytotoxicity observed among the different stimulation treatments tested (Fig. 4).

Sensitivity of AIDS-Related and non–AIDS-Related BL to PAK Effectors. PAK effectors were generated from PBMC after a 6-day culture in the presence of rPRL, as previously reported (14). Because of the wide range of individual variability in the optimal induction of cytotoxic effectors by rPRL (32), three rPRL concentrations (12, 25, or 50 ng/ml; 0.6, 1.2, or 2.4 n*M*) were tested in each single experiment. Figure 1 represents the cytotoxic sensitivity of each BL cell line to PAK cells obtained from different donors. For each effector/target combination, the optimal rPRL concentration (i.e., the rPRL concentration yielding the highest cytotoxicity value) is shown. As for LAK cells, also for PAK cells a certain degree of variability in cytotoxic activity was detected among different PBMC donors challenged against the same cell line. In some cases, rPRL stimulation of PBMC apparently induced a cytotoxic inhibitory effect compared with

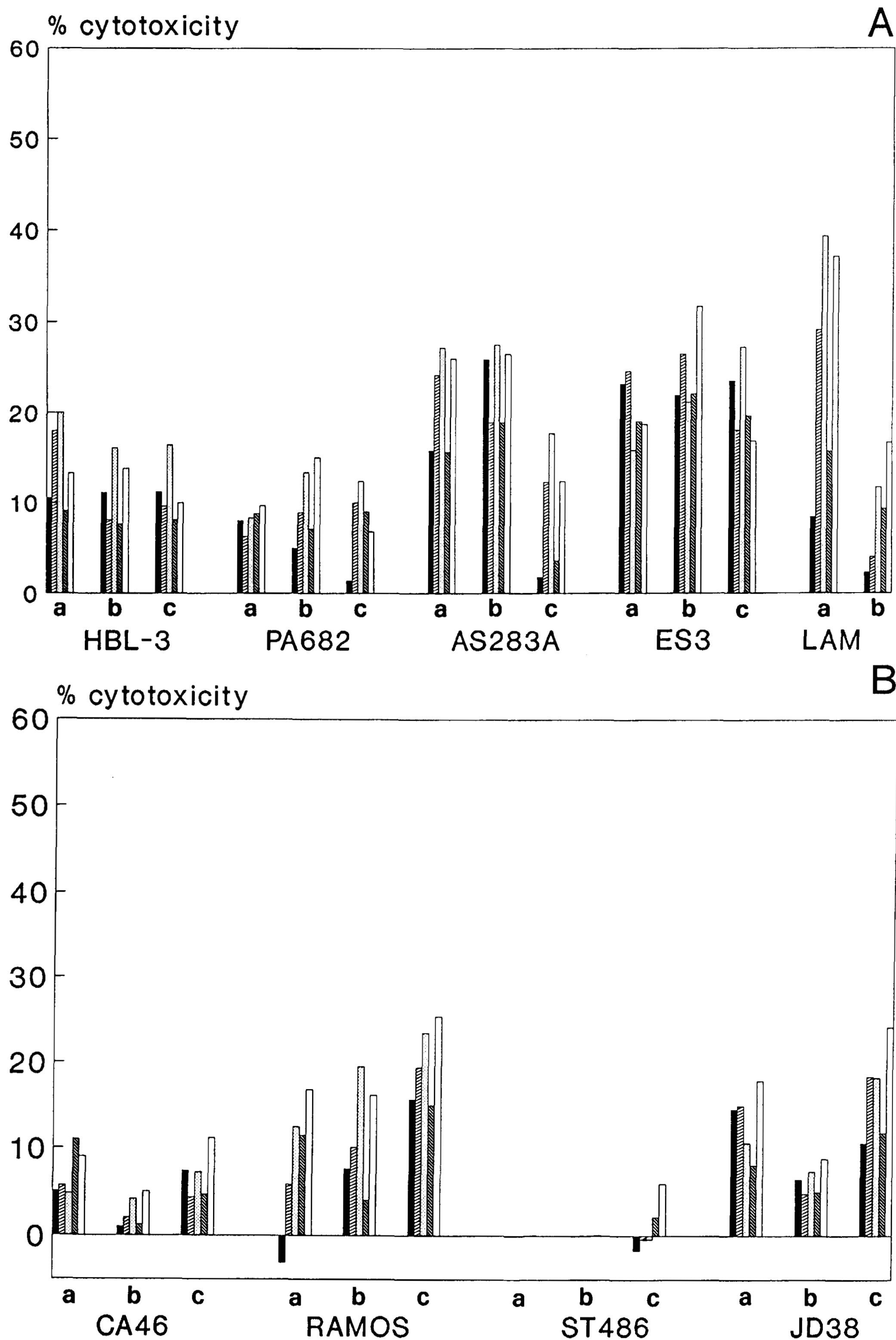
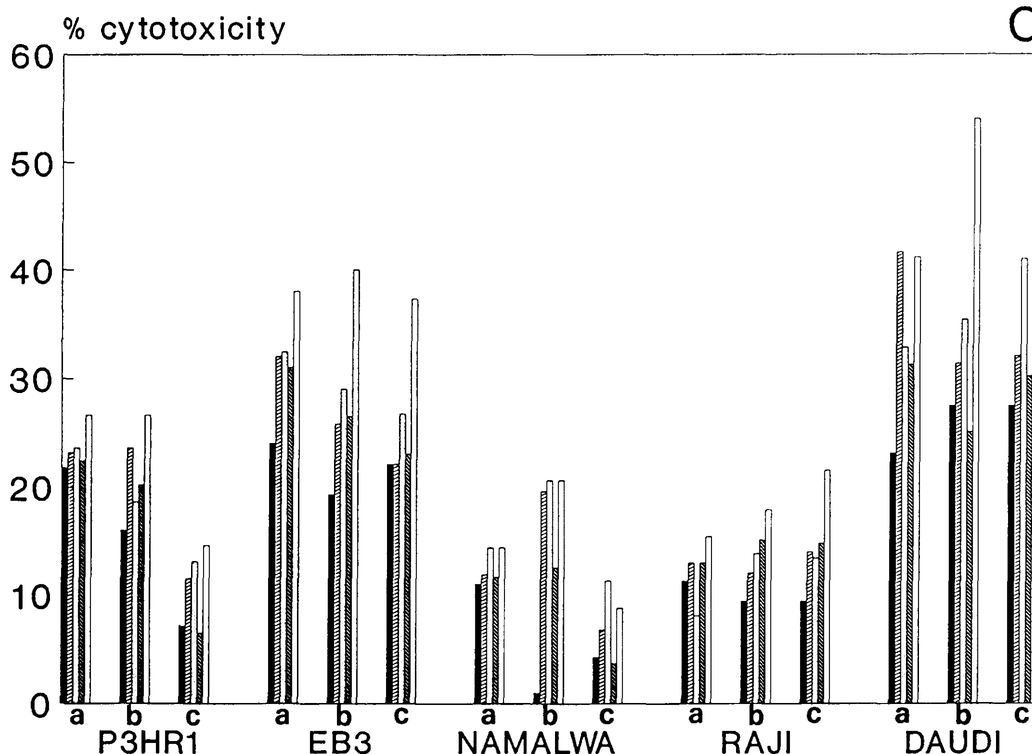


Figure 1. Analysis of cytotoxic activity of unstimulated and rIL-2– and/or rPRL-stimulated PBMC of different donors against BL cell lines. (A) AIDS-related BL (HBL-3, PA 682, AS283A, ES3, LAM). (B) Sporadic BL (CA46, RAMOS, ST486, JD38). (C) Endemic BL (P3HR1, EB3, NAMALWA, RAJI, DAUDI). Three PBMC donors (a, b, c) were used against each target cell line, with the exception of LAM (for which only two PBMC donors were used). For each donor (a, b, c), PBMC were cultured in medium (■), rIL-2 25 U/ml (□), rIL-2 100 U/ml (▤), rPRL (▧) 12-25-50 ng/ml as specified in the text), rIL-2 25 U/ml + rPRL (□ 12, 25, or 50 ng/ml as specified in the text). Cytotoxicity was determined in a 4-hr ^{51}Cr release assay as detailed in Materials and Methods. Results are expressed as percentage cytotoxicity at the 12:1 effector to target ratio.

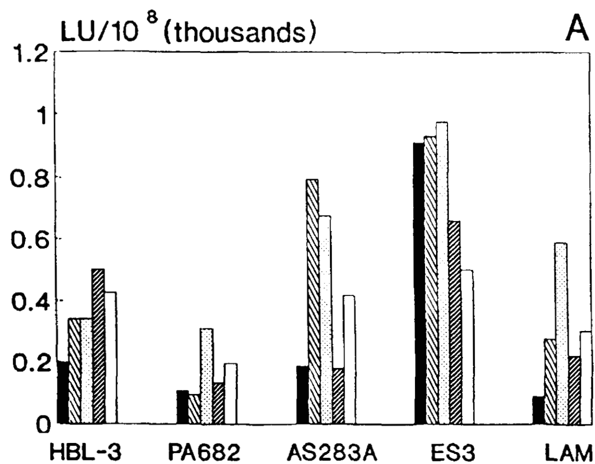


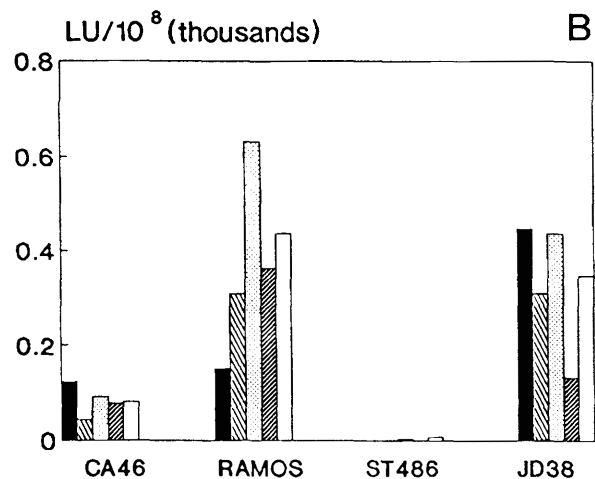
baseline cytotoxicity (i.e., the cytotoxicity of unstimulated PBMC; Figs. 1 and 2).

For each BL variant, statistical analysis was performed by calculating the mean cytotoxicity value obtained testing all cell lines belonging to the group with all the PBMC donors at all rPRL concentrations. The mean value of BL directed PAK cytotoxicity, as well as the results of the statistical analysis, is reported in Table I for AIDS-related BL, Table II for sporadic BL, and Table III for endemic BL. A graphical representation of the results is shown in Figure 3. Endemic BL was the only BL variant significantly more susceptible to lysis by PAK cells compared with unstimulated killers, whereas rPRL had virtually no effect in inducing a cytotoxic activity against AIDS-related or sporadic BL. Within the endemic BL variant, the percentage increase of cytotoxicity of PAK cells is lower than that of conventional LAK cells or that of cytotoxic effectors induced by suboptimal doses of rIL-2 + rPRL (Fig. 4).

Sensitivity of AIDS-Related and Non–AIDS-Related BL to Cytotoxic Effectors Generated by the Combination of rIL-2 + rPRL. The combination of rIL-2 25 U/ml and rPRL was used to generate cytotoxic effectors from PBMC, as previously reported (14). In our laboratory, rIL-2 25 U/ml fails to induce a significant cytotoxic effect against most cell targets, including BL cells (14; our unpublished observation). As described above for the generation of PAK cells, different rPRL concentrations (12, 25, or 50 ng/ml) were used in combination with rIL-2. The individual behavior of each target cell line after challenging with the cytotoxic effectors generated from each donor by the rIL-2 + rPRL combination is shown in Figure 1. For each effector/target combination, the data represent the rIL-2 + rPRL combination yielding the highest cytotoxic activity. The mean cytotoxic sensitivity of each BL cell line to the cytotoxic effectors generated by the rIL-2 + rPRL combination is shown in Figure 2.

For each BL variant, calculation of the mean activity of the cytotoxic effectors, either unstimulated or generated by the combination of rIL-2 + rPRL, was performed as described above in the case of LAK and PAK cells. The data, together with the results of statistical analysis, are summarized in Table I, Table II, and Table III for AIDS-related BL, sporadic BL, and endemic BL, respectively. A graphical representation of the results are shown in Figure 3. Both AIDS-related and endemic BL were significantly more sensitive to cytotoxic effectors generated by the rIL-2 + rPRL combination than to unstimulated PBMC. The synergistic role of rIL-2 + rPRL at the concentrations used was defined based on the observation that the two agents taken independently were unable to induce significant increase of cytotoxicity (in the case of AIDS-related BL) or, alternatively, generated a cytotoxic activity of significantly lower magnitude (in the case of endemic BL). Notably, PBMC stimulation with rIL-2 + rPRL was the most effective treatment





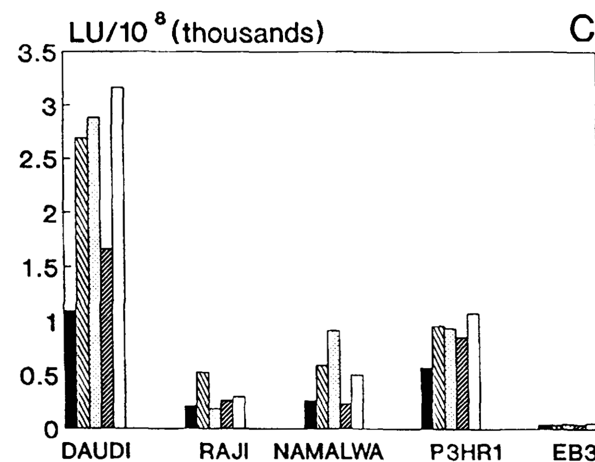
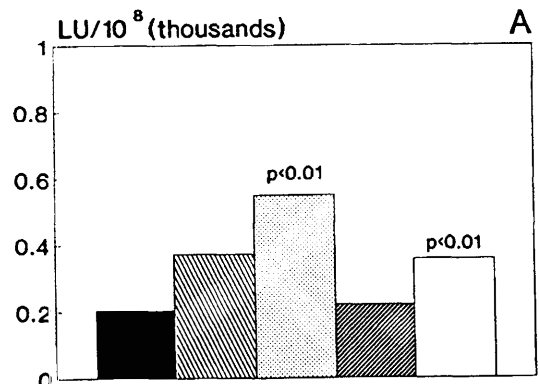
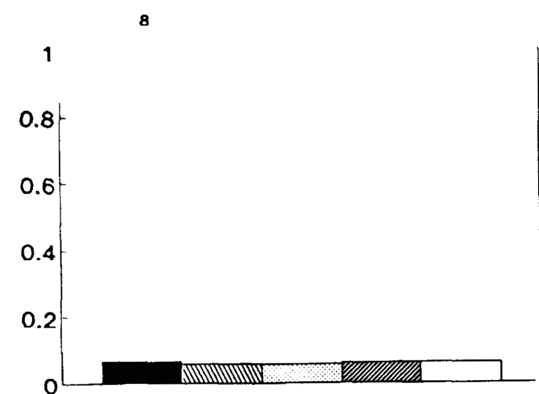


Figure 2. Individual susceptibility of AIDS-related BL (A), sporadic BL (B), and endemic BL (C) to unstimulated and rIL-2– and/or rPRL-induced cytotoxic effectors. Five cases of AIDS-related BL (HBL-3, PA682, AS283A, ES3, LAM), four cases of sporadic BL (CA46, RAMOS, ST486, JD38), and five cases of endemic BL (DAUDI, RAJI, NAMALWA, P3HR1, EB3) were used as targets. For each cell target, cytotoxic effectors were cultured with medium (■), rIL-2 25 U/ml (▧), rIL-2 100 U/ml (▒), rPRL (▨ the data shown are the mean of the values obtained by using rPRL 12, 25, or 50 ng/ml), or rIL-2 25 U/ml + rPRL (□; the data shown are the mean of the values obtained by using rPRL 12, 25, or 50 ng/ml). Results are geometrical means of lytic units (LU) obtained using PBMC from three donors (two donors in the case of LAM). SDs values ranged from 1.15 to 10.47 for AIDS-related BL; 1.58 to 10.72 for sporadic BL; and 1.10 to 2.29 for endemic BL.





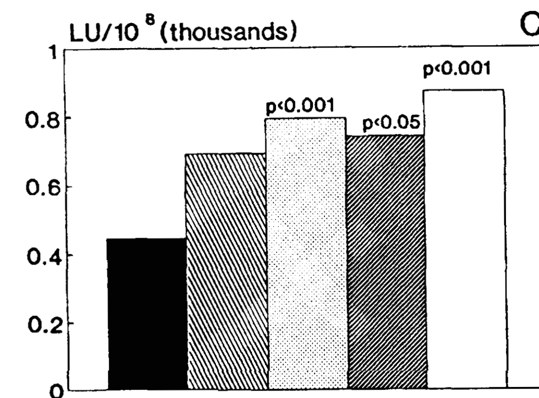


Figure 3. Differential susceptibility of BL variants (AIDS-related BL [A]; sporadic BL [B]; endemic BL [C]) to cytotoxic effectors induced by rIL-2 and/or rPRL. The figure represents the results of the statistical analysis performed using logarythmically transformed LU described in Figure 2. Results are given as geometrical means of LU and represent the susceptibility of each BL variant to unstimulated PBMC (■), or PBMC stimulated by rIL-2 25 U/ml (▧), rIL-2 100 U/ml (▒), rPRL 12, 25, or 50 ng/ml (▨), and rIL-2 25 U/ml + rPRL 12, 25, or 50 ng/ml (□). Statistical significancies are indicated by P values as derived from Wilcoxon's analysis. SDs values for each treatment are shown in Table I for AIDS-related BL, Table II for sporadic BL, and Table III for endemic BL.

inducing the cytolysis of endemic BL, being associated with the highest observed percentage increase in cytotoxicity (Fig. 4). In contrast to AIDS-related BL and endemic BL, the sporadic variant of BL did not

Table I. Single and Combined Effects of rIL-2 and rPRL on Development of LAK and PAK Cytotoxicity against AIDS-Related BL

Treatment of PBMC		LU		AUC	
rPRL	rIL-2	*g*Mean ± SD	P^a	*a*Mean ± SD	P^a
—	—	204.17 ± 2.40	—	19.34 ± 12.70	—
—	25 U/ml	371.53 ± 2.34	>0.05[b]	27.52 ± 10.17	<0.05
—	100 U/ml	549.54 ± 1.78	<0.01	32.34 ± 10.0	<0.05
+[c]	—	218.78 ± 2.34	>0.05	18.24 ± 6.99	>0.05
+[c]	25 U/ml	588.84 ± 1.78	<0.01	26.10 ± 8.77	<0.05

Note. BL, Burkitt's-type lymphoma; PBMC, peripheral blood mononuclear cells; LU, lytic unit; AUC, area under the curve; *g*Mean, geometrical mean; *a*Mean, arithmetical mean; SD, standard deviation.
[a] *P* was derived from Wilcoxon's test for nonparametric data.
[b] Statistical analysis was performed on logarythmically transformed lytic units.
[c] The values obtained using three concentrations of rPRL (12, 25, or 50 ng/ml) have been comprised for statistical analysis.

Table II. Single and Combined Effects of rIL-2 and rPRL on Development of LAK and PAK Cytotoxicity against Sporadic BL

Treatment of PBMC		LU		AUC	
rPRL	rIL-2	*g*Mean ± SD	P^a	*a*Mean ± SD	P^a
—	—	69.10 ± 14.79	—	10.81 ± 9.36	—
—	25 U/ml	60.25 ± 16.21	>0.05[b]	11.06 ± 9.05	>0.05
—	100 U/ml	58.88 ± 14.79	>0.05	14.71 ± 14.85	>0.05
+[c]	—	63.09 ± 19.05	>0.05	9.22 ± 7.06	>0.05
+[c]	25 U/ml	63.09 ± 15.13	>0.05	13.21 ± 12.69	>0.05

Note. BL, Burkitt's-type lymphoma; PBMC, peripheral blood mononuclear cells; LU, lytic unit; AUC, area under the curve; *g*Mean, geometrical mean; *a*Mean, arithmetical mean; SD, standard deviation.
[a] *P* was derived from Wilcoxon's test for nonparametric data.
[b] Statistical analysis was performed on logarythmically transformed lytic units.
[c] The values obtained using three concentrations of rPRL (12, 25, or 50 ng/ml) have been comprised for statistical analysis.

Table III. Single and Combined Effects of rIL-2 and rPRL on Development of LAK and PAK Cytotoxicity against Endemic BL

Treatment of PBMC		LU		AUC	
rPRL	rIL-2	*g*Mean ± SD	P^a	*a*Mean ± SD	P^a
—	—	446.68 ± 2.29	—	28.42 ± 13.24	—
—	25 U/ml	691.83 ± 3.47	>0.05[b]	36.49 ± 19.06	<0.05
—	100 U/ml	794.33 ± 3.16	<0.001	38.13 ± 19.34	<0.002
+[c]	—	741.31 ± 6.03	<0.05	32.48 ± 22.11	<0.005
+[c]	25 U/ml	870.96 ± 3.55	<0.001	41.88 ± 24.51	>0.001

Note. BL, Burkitt's-type lymphoma; PBMC, peripheral blood mononuclear cells; LU, lytic unit; AUC, area under the curve; *g*Mean, geometrical mean; *a*Mean, arithmetical mean; SD, standard deviation.
[a] *P* was derived from Wilcoxon's test for nonparametric data.
[b] Statistical analysis was performed on logarythmically transformed lytic units.
[c] The values obtained using three concentrations of rPRL (12, 25, or 50 ng/ml) have been comprised for statistical analysis.

show significant increase of cytolysis after challenging with PBMC incubated with rIL-2 + rPRL (Table II and Fig. 3).

Discussion

This study was aimed at investigating the cytotoxic sensitivity of AIDS-related BL and at comparing it to the sensitivity of other BL variants, including sporadic and endemic BL. Our data show that the different BL variants associate with distinct patterns of cytotoxic sensitivity. Indeed, AIDS-related BL, moderately susceptible to unstimulated PBMC, is lysed with markedly higher efficiency by LAK cells; endemic BL is highly sensitive to unstimulated PBMC and even more to both LAK and PAK cells; sporadic BL appears to be resistant to all types of cytotoxic effectors tested. The different behavior of AIDS-

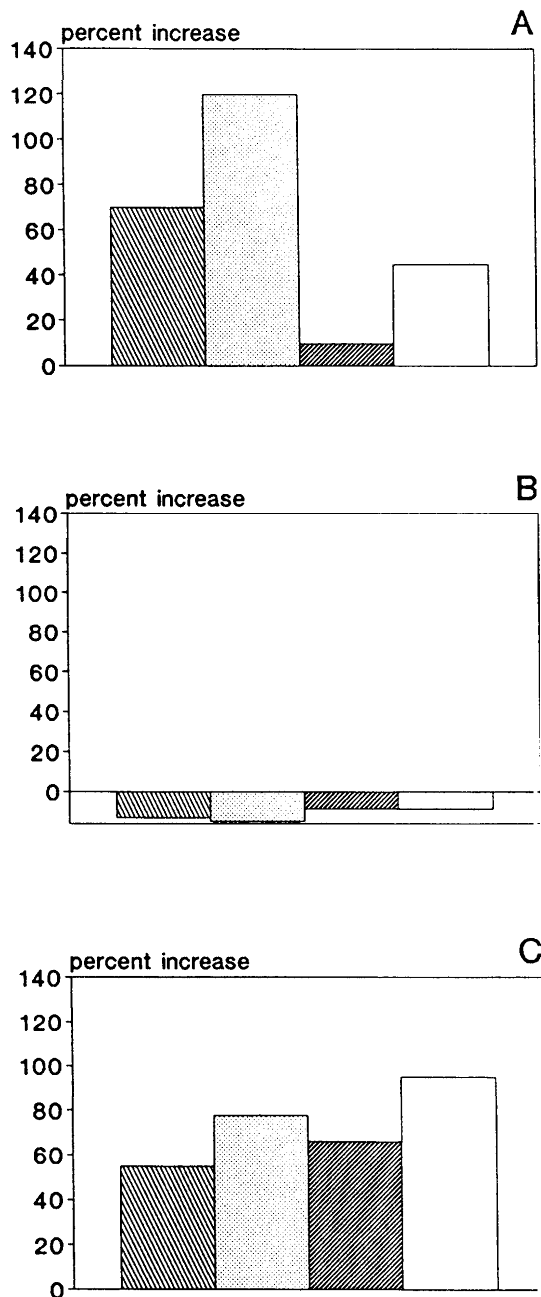


Figure 4. Percentage increase of cell lysis of AIDS-related BL (A), sporadic BL (B), and endemic BL (C) upon incubation of cell targets with PBMC stimulated with rIL-2 25 U/ml (▧), rIL-2 100 U/ml (▤), rPRL 12, 25, or 50 ng/ml (▨), and rIL-2 25 U/ml + rPRL 12, 25, or 50 ng/ml (□).

related BL, compared with sporadic BL, toward cytotoxic effectors is intriguing, since the biologic features of AIDS-related BL have been long thought to mimick closely those of sporadic, rather than endemic, BL (4–7). Recently, however, several reports have pointed to the morphologic and/or immunophenotypic peculiarities of AIDS-related versus sporadic BL (33–36). In this respect, the differences in cytotoxic susceptibility detected in this study may represent an additional factor distinguishing these two BL clinical variants.

The molecular basis for the observed differences in cytotoxic sensitivity among BL variants is presently obscure. Since all three variants harbor similar genetic lesions, including c-*myc* translocation and p53 disruption (8, 28, 29), it is unlikely that the variability in cytotoxic sensitivity may be directly ascribed to any known genetic alteration of the tumor. Rather, the phenotypic peculiarities of the distinct BL variants (4, 5, 33–36), or, alternatively, differences in the status of Epstein-Barr virus infection of the tumor clone (5, 8, 29, 34), may influence the cytotoxic sensitivity of BL cells through presently unclarified mechanisms. Studies on large panels of cases belonging to distinct BL variants are needed in order to assess definitively this issue.

Independent of the precise biologic mechanism regulating BL susceptibility to cytotoxic effectors, it is notable that PRL alone fails to increase significantly the lysis of both AIDS-related and sporadic BL. Indeed, previous studies have shown that the cytotoxic activity of PAK cells may be directed against a variety of hematopoietic cancers, including myeloid leukemias, T cell neoplasms, and some B cell malignancies, among which cases of endemic BL (14). Although it has been suggested that the spectrum of PAK targets would overlap with that of LAK cells, this study suggests that there might be tumors susceptible to one, but not the other, category of cytotoxic effectors.

Intriguingly, despite its ineffectiveness as a single agent, PRL may synergize with IL-2 in causing a significantly increased lysis of AIDS-related BL cells. The synergism between PRL and IL-2 is also detected in the case of endemic BL, which, though already sensitive to PAK cells, is killed with significantly higher efficiency by cytotoxic effectors generated by the combination of PRL and IL-2. In addition to AIDS-related and endemic BL, the synergism between PRL and IL-2 in stimulating the generation of cytotoxic effectors is also observed against other tumors (14). Several biologic mechanisms may be hypothesized to explain the synergism of PRL and IL-2. For example, the interaction between PRL and IL-2 may rest upon the activation of an integrated cellular pathway requiring multiple receptor-ligand signaling or, alternatively, may be due to mutual receptor regulation (37). Also, reciprocal upregulation of peptide secretion may be involved, since PRL is able to stimulate IL-2 synthesis in mouse lymphocytes (38). These hypothesis are the focus of current investigations.

LAK cells have been widely used as a therapeutic strategy *in vivo* against several types of human cancers, including hematopoietic tumors (39–41). The first prerequisite for the treatment of a given cancer with LAK cells is that the tumor itself be sensitive to LAK-mediated lysis. However, it is also essential that a sufficient cytotototoxic activity may be generated from the

LAK progenitors of the tumor host. This study shows that at least one type of AIDS-related tumor (i.e., AIDS-related BL) is indeed susceptible to LAK-mediated lysis. Although LAK cells directed against specific cellular targets have been successfully generated from the peripheral blood of HIV infected individuals (21, 22), it is not presently known whether AIDS patients can mount a significant LAK response against an autologous lymphoma. In this respect, it should be noticed that AIDS-related BL, in contrast to other types of AIDS-related lymphoma, tends to associate with a relatively well preserved cellular immunity of the patient, thus putatively increasing the chances of the generation of cytotoxic effectors (42–44). Experimental studies aimed at directly testing the activity of LAK and PAK cytotoxic effectors of AIDS patients against AIDS-related lymphomas are urgently needed.

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