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DISSECTION OF THE LYMPHOKINE-ACTIVATED KILLER PHENOMENON

Relative Contribution of Peripheral Blood Natural Killer Cells and T Lymphocytes to Cytolysis

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The in vitro culture of human peripheral blood lymphocytes in IL-2 results in the generation of cytotoxic cells that lyse NK-resistant tumor cell targets without MHC restriction. This has been referred to as the LAK (lymphokine-activated killer)[1] phenomenon (1–3). There has been considerable controversy regarding the types of effector cells mediating this activity, particularly concerning the relative contribution of natural killer cells versus cytotoxic T cells. Grimm and colleagues (1) have reported that the effectors of the LAK phenomenon express the pan T cell antigens CD3 and CD5. However, in subsequent studies (2), these investigators characterized the precursor population as a lymphoid cell lacking CD3 and CD5.

In contrast, we (4) and others (5, 6) have shown that $CD3^-$ NK cells that express the CD16 (Leu-11) antigen can lyse NK-resistant hematopoietic tumor cell targets after culture in vitro with IL-2. These studies however, did not exclude the possibility that a subset of $CD3^+$ T lymphocytes may also contribute to the LAK phenomenon. Here, we determine the relative contribution of NK cells and T lymphocytes to the LAK phenomenon, examining both the precursor and effector phase of this response.

Materials and Methods

Human Peripheral Blood Leukocytes. Human peripheral blood from normal, random donors was obtained from the Stanford Blood Center, Palo Alto, CA. The mononuclear cells were isolated using Ficoll/Hypaque. After passage through nylon wool (7), nonadherent lymphocytes were fractionated by centrifugation on discontinuous gradients consisting of 30% and 40% Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) in PBS containing 10% FCS (7). Low bouyant density and high bouyant density lymphocytes were isolated from the interface and bottom of the Percoll gradients, respectively. Total cell recovery was ~85%. ~25% of lymphocytes were in the low bouyant density fraction, depending on the individual donor. The population of lymphocytes in the interface fraction were highly enriched for cells with large granular lymphocyte (LGL) morphology.

Monoclonal Antibodies. Leu series mAbs were prepared at the Becton Dickinson Monoclonal Center. The CD nomenclature of the differentiation antigens identified by these mAbs is summarized in Table I. Anti-Leu-11 reacts with CD16, the Fc receptor for IgG expressed on NK cells and neutrophils (9). Anti-Leu-19 reacts with the Leu-19 (NKH-

[1] *Abbreviations used in this paper:* LAK, lymphokine-activated killer; LGL, large granular lymphocytes; PE, phycoerythrin.

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TABLE I
CD Nomenclature for Differentiation Antigens Identified by mAb Used in This Study

CD cluster	mAb	Major cell reactivity
CD3	Leu-4	T cells (antigen receptor complex)
CD4	Leu-3	T subset and monocytes
CD5	Leu-1	T cells and B cell subset
CD8	Leu-2	T subset and NK subset
CD16	Leu-11	NK cells and neutrophils (Fc receptor)
ND	Leu-19 (NKH-1)	NK cells and T subset

1) antigen, an ~220 kD protein of unknown function expressed on NK cells and a subset of T lymphocytes (10, 11).

Immunofluorescence and Flow Cytometry. Methods of immunofluorescence, flow cytometry, and data analysis have been described (9, 12, 13). A FACS 440 flow cytometer and Consort 40 data analysis system (Becton Dickinson Immunocytometry Systems, Mountain View, CA) were used. Fluorochrome-conjugated, isotype-matched mAbs that do not react with human leukocytes were used to control for nonspecific binding in the immunofluorescence assays.

Cell Lines. All tumor cell lines were obtained from the American Type Culture Collection, Rockville, MD and were tested monthly to ensure against mycoplasma infection. Freshly isolated human tumors were generously provided by Drs. Anthony Rayner and Brett Gemlo (Department of Surgery, University of California Medical School, San Francisco, CA).

Cytotoxicity Assays. ^{51}Cr-labeled tumor cells were used as targets in a 4-h radioisotope-release assay (9).

Lymphocyte Activation. Lymphocytes (~10^6 cells/ml) were cultured in RPMI-1640 (M. A. Bioproducts, Walkersville, MD) containing 5% horse serum (JR Scientific, Woodland, CA), 1 mM glutamine (Gibco Laboratories, Grand Island, NY), and 100 μg/ml gentamicin (Gibco). rIL-2 (4) was added to the cultures at 1,000 U/ml. Preliminary studies established that this concentration of rIL-2 induced optimal cytotoxicity, although lower concentrations were sufficient to initiate the response.

Results and Discussion

Characterization of Effector Cells. We recently showed that two distinct subsets of peripheral blood lymphocytes can lyse NK-sensitive tumor targets without deliberate immunization or MHC-restriction (10). These cytotoxic subsets were identified by two-color immunofluorescence using antibodies against CD3, a component of the T cell antigen receptor and the Leu-19 (NKH-1) antigen (10). Both $CD3^-$,Leu-19^+ NK cells and the $CD3^+$,Leu-19^+ T cells isolated directly from peripheral blood mediated non-MHC restricted cytotoxicity (10). Within the $CD3^+$,Leu-19^+ T cell subset, most cells expressed the CD2, CD5, and CD8 differentiation regions, but did not express CD16 (10). In contrast, ~90% of the $CD3^-$,Leu-19^+ NK cell subset coexpressed CD16, and none expressed the pan T cell antigen CD5 (10). The minor population of $CD3^-$,$CD16^-$,Leu-19^+ lymphocytes also mediated non-MHC restricted cytotoxicity, and were phenotypically identical to $CD3^-$,$CD16^+$,Leu-19^+ NK cells, with the exception of lacking CD16 expression (10).

In the present studies, we examined the relative contribution of $CD3^-$,Leu-19^+ NK cells (referred to as NK cells), $CD3^+$,Leu-19^+ non-MHC restricted

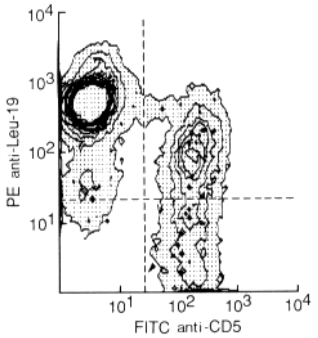

FIGURE 1. Coexpression of CD5 and Leu 19 on low bouyant density lymphocytes. Low bouyant density lymphocytes were stained with FITC-conjugated anti-Leu-1 (CD5) and biotin-conjugated anti-Leu-19, followed by PE-conjugated streptavidin. Cells were analyzed by flow cytometry, and data were displayed as contour plots of FITC and PE fluorescence. The contour map was divided into quadrants to represent: cells stained only with PE (*upper left*), cells stained with FITC and PE (*upper right*), cells stained only with FITC (*lower right*) and unstained cells (*lower left*). In the control sample (cells stained with FITC- and PE-conjugated IgG control antibodies), >98% of lymphocytes were in the lower right quadrant. In subsequent figures, $CD5^-$,Leu-19^+ lymphocytes are referred to as NK cells, $CD5^+$,Leu-19^+ lymphocytes are referred to as Leu-19^+ T cells, and $CD5^+$,Leu-19^- lymphocytes are referred to as Leu-19^- T cells.

cytotoxic T cells (referred to as Leu-19^+ T cells), and $CD3^+$,Leu-19^- T cells (referred to as Leu-19^- T cells) to the LAK phenomenon. Prior studies (2) indicated that precursors of the LAK phenomenon were low bouyant density lymphocytes that comigrated, on Percoll gradients, with NK cells. Therefore, low bouyant density lymphocytes (designated LGL) from normal peripheral blood were cultured in medium containing rIL-2. After 7 d, cells were harvested and stained with FITC-conjugated anti-Leu-1 (CD5) and biotin-conjugated anti-Leu-19, followed by PE (phycoerythrin)-conjugated streptavidin (Fig. 1). Anti-CD5 was used to identify and isolate T cells, since antibodies against CD3 have been shown to either inhibit or induce T cell–mediated cytotoxicity (14–16). NK cells ($CD5^-$,Leu-19^+, comprising 55% of total low bouyant density lymphocytes), Leu-19^+ T cells ($CD5^+$,Leu-19^+, 24%), and Leu-19^- T cells ($CD5^+$,Leu-19^-, 14%) were isolated by cell sorting using a FACS. Reanalysis indicated >95% purity in all populations. These rIL-2 activated cells were then tested for cytotoxicity against the NK-sensitive tumor cell line, K562, as well as an NK-resistant colon carcinoma cell line (COLO) (Fig. 2*B*). Both the rIL-2-activated NK cells and Leu-19^+ T cells lysed K562. However, significant lysis of the solid tumor cell line was predominately mediated by rIL-2-activated NK cells. rIL-2-activated Leu-19^+ T cells showed only minimal cytotoxic activity against COLO, while Leu-19^- T cells totally lacked cytotoxic function against either K562 or COLO (Fig. 2*B*). For comparison, we also isolated NK cells, Leu-19^+ T cells, and Leu-19^- T cells from the low bouyant density lymphocytes population, and tested these freshly isolated cells for cytotoxicity before culture in rIL-2. As shown in Fig. 2*A*, freshly isolated NK cells efficiently lysed K562, but did not lyse COLO. Freshly isolated Leu-19^+ T cells mediated low levels of cytotoxicity against K562, but not COLO. Leu-19^- T cells were not cytotoxic. Essentially

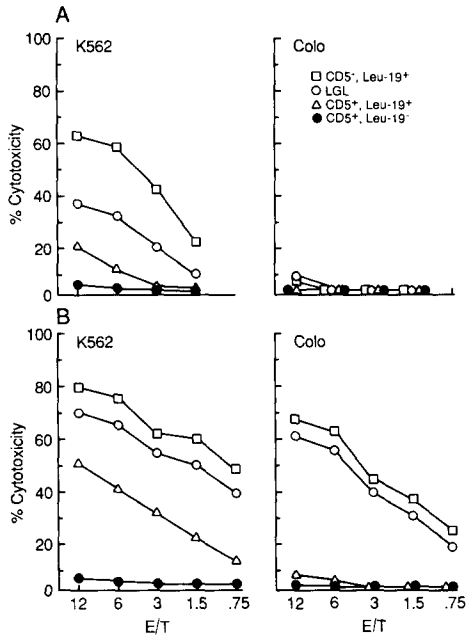

FIGURE 2. Cytotoxicity mediated by rIL-2-cultured low bouyant density lymphocyte subsets. *A:* Freshly isolated low bouyant density lymphocytes (LGL; ○), Leu-19$^+$ T cells (△), Leu-19$^-$ T cells (●), and NK cells (□) were assayed for cytotoxic activity against K562 (*left*) and COLO (*right*) before culture. *B*: Low bouyant density lymphocytes were cultured in medium containing rIL-2 for 7 d. Lymphocytes were harvested and stained with mAbs, as described in Fig. 1. rIL-2-activated Leu-19$^+$ T cells, Leu 19$^-$ T cells, and NK cells were isolated using the FACS and tested for cytotoxicity against K562 (*left*) and COLO (*right*).

identical results were obtained with lymphocytes isolated from three different blood donors.

Cytotoxicity against freshly isolated human tumors was also mediated exclusively by IL-2-activated NK cells, as shown in Fig. 3. In these experiments, low bouyant density lymphocytes were cultured in rIL-2, and the NK cell (CD5$^-$,Leu-19$^+$) and T cell (CD5$^+$,Leu-19$^+$ and CD5$^+$,Leu-19$^-$) subsets were isolated using the FACS. rIL-2-activated NK cells and T cells were assayed for cytotoxicity against K562, the colon carcinoma cell line (COLO), freshly isolated melanoma tumor cells, freshly isolated Wilms' tumor cells, and freshly isolated sarcoma tumor cells. Freshly isolated tumors, as well as the COLO cell line, were efficiently killed by rIL-2-activated NK cells, but not rIL-2-cultured T cells (Fig. 3). As seen previously, rIL-2-cultured T cells mediated low levels of cytotoxicity against K562. It should be noted that the magnitude of cytotoxicity against the three freshly isolated tumors was equivalent to the activity observed against COLO. COLO and the freshly isolated tumor cells were completely resistant to lysis mediated by unactivated peripheral blood NK cells (not shown). Since cytotoxicity against COLO and the fresh tumor cells was equivalent, COLO was used for most subsequent experiments.

To exclude the possibility that Percoll fractionation biased these results, unseparated peripheral blood mononuclear cells were also cultured in rIL-2. After 7 d of culture, cells were stained with FITC-conjugated anti-Leu 1 (CD5)

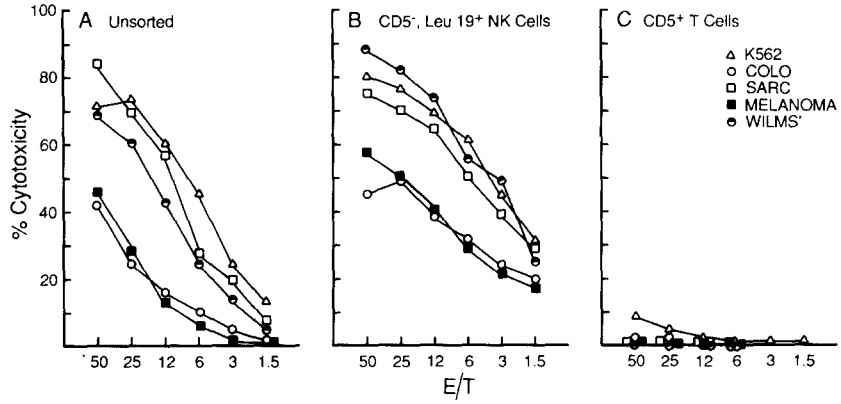

FIGURE 3. Cytotoxicity against freshly isolated tumor cells mediated by low bouyant density lymphocyte subsets. Low bouyant density lymphocytes were cultured in medium containing rIL-2 for 7 d. Lymphocytes were harvested and stained with mAbs, as described in Fig. 1. rIL-2-activated unsorted cells (*A*), $CD5^-$,Leu-19^+ NK cells (*B*), and T cells (pool of $CD5^+$,Leu-19^- and $CD5^+$,Leu-19^+) (*C*) were tested for cytotoxicity against K562 (△), COLO (○), a fresh melanoma tumor (■), fresh sarcoma tumor (□), and fresh Wilms' tumor (◓).

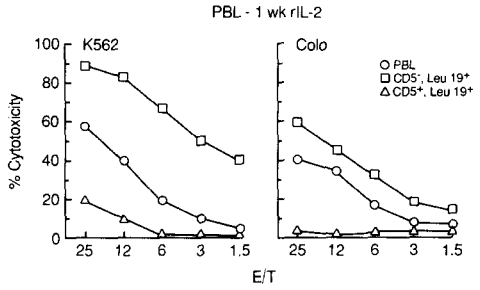

FIGURE 4. Cytotoxicity mediated by rIL-2-cultured peripheral blood mononuclear cell subsets. Peripheral blood mononuclear cells (○) isolated from Ficoll/Hypaque gradients were cultured in medium containing rIL-2 for 7 d. Lymphocytes were harvested and stained with mAbs, as described in Fig. 1. Leu-19^+ T cells (△) and NK cells (□) were isolated using the FACS and tested for cytotoxicity against K562 (*left*) and COLO (*right*).

and biotin–anti-Leu-19, followed by PE-conjugated streptavidin. Leu-19^+ T cells (comprising 21% of total lymphocytes), Leu-19^- T cells (66%), and NK cells (8%) were isolated to >95% purity using the FACS. Again, lysis of the NK-insensitive colon cell line, COLO, was predominately mediated by rIL-2-activated NK cells (Fig. 4). Although peripheral blood rIL-2-activated Leu-19^+ T cells showed low levels of killing against K562, these T cells did not substantially contribute to the lysis of COLO. Again, Leu-19^- T cells killed neither K562 nor COLO.

Characterization of Precursor Cells. Low bouyant density peripheral blood lymphocytes, enriched on Percoll gradients, were stained with FITC-conjugated anti-Leu 1 (CD5) and biotin-conjugated anti-Leu-19, followed by PE-streptavidin. Leu-19^+ T cells, Leu-19^- T cells, and NK cells were isolated to >95% purity using the FACS, and were placed in culture in medium containing rIL-2. After 7 d, lymphocytes were harvested and examined for antigenic phenotype and cytotoxic function. After culture in rIL-2, >95% of the Leu-19^+ T lymphocytes retained the phenotype $CD3^+$,$CD5^+$,Leu-19^+. Likewise, Leu-19^- T lymphocytes

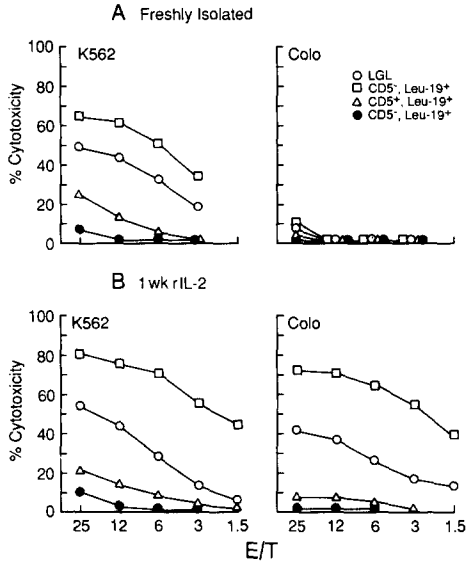

FIGURE 5. Precursors of rIL-2-activated cytotoxic cells. *A*: Freshly isolated low bouyant density lymphocytes (○) were stained with mAbs, as described in Fig. 1. Leu-19$^+$ T cells (△), Leu-19$^-$ T cells (●) and NK cells (□) were isolated using the FACS and assayed immediately for cytotoxic activity against K562 (*left*) and COLO (*right*). *B*: Leu-19$^+$ T cells, Leu-19$^-$ T cells and NK cells were isolated using the FACS and placed in culture with rIL-2. After 7 d, cells were harvested and assayed for cytotoxicity against K562 (*left*) and COLO (*right*).

remained CD3$^+$,CD5$^+$,Leu-19$^-$. >95% of rIL-2-cultured NK cells maintained the phenotype CD3$^-$,CD5$^-$,Leu-19$^+$, and as observed before culture, ~90% expressed the CD16 antigen. There was no evidence that NK cells ever acquired pan T cell antigens as a consequence of culture in rIL-2 or that T cells lost CD3 expression after culture.

The cytotoxic activity of these subsets was tested before culture (Fig. 5*A*) or after culture in rIL-2 for 7 d (Fig. 5*B*). Again, the rIL-2-cultured NK cells were potent mediators of lysis against both K562 and COLO; whereas the freshly isolated NK cells lysed K562, but not COLO (Fig. 5). In contrast, freshly isolated and rIL-2-activated Leu-19$^+$ T cells lysed K562, but showed only minimal cytotoxicity against COLO. In this individual, culture in rIL-2 did not significantly augment the cytotoxicity of Leu-19$^+$ T cells against K562; however, in several other individuals (e.g., Figs. 6 and 8), we did observe rIL-2-enhanced cytotoxicity against K562, but not COLO. Non–MHC restricted cytotoxic lymphocytes were not generated by culture of Leu-19$^-$ T cells in rIL-2.

To further examine the cytotoxic potential of these cells, NK cells and Leu-19$^+$ T cells were isolated from the low bouyant density lymphocyte population, were cultured for 1 wk in rIL-2, and were then assayed for cytolytic activity against K562, COLO, ZR (an NK-resistant breast carcinoma cell line), and JY (an NK-resistant B lymphoblastoid cell line) (Fig. 6). Freshly isolated NK cells and Leu-19$^+$ T cells lysed K562, although NK cells were more effective effectors. Against the hematopoietic cell targets (K562 and JY), rIL-2 enhanced the cytotoxicity of both the NK cells and Leu-19$^+$ T cells. In contrast, only the NK

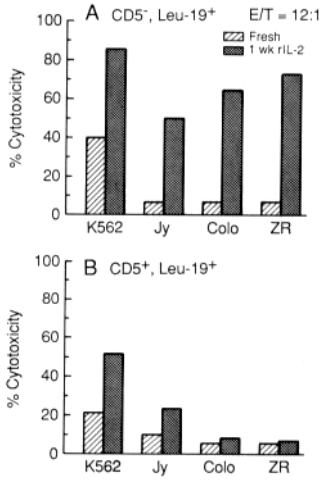

FIGURE 6. Cytotoxicity mediated by rIL-2 activated NK cells and Leu-19$^+$ T cells. NK cells (*A*) and Leu-19$^+$ T cells (*B*) were isolated using the FACS and placed in culture with rIL-2 for 7 d. Freshly isolated cells and rIL-2-activated cells were assayed for cytotoxicity against K562, JY, COLO, and ZR. E/T ratio is 12:1.

cells showed substantial rIL-2-induced cytotoxicity against the solid tumor cell lines. These results unequivocally demonstrate that rIL-2 provides the only signal necessary for NK cells to lyse solid tumor cell targets. T cells, accessory cells, or additional factors are apparently not required. Furthermore, these studies show that NK cells with the ability to efficiently lyse NK-insensitive solid tumor cell targets arise from a precursor population with the phenotype CD5$^-$,Leu-19$^+$. More extensive immunophenotyping has shown that ~90% of these precursor cells coexpress CD16, and all lack CD3. We have recently reported that this CD3$^-$,CD16$^+$ NK cell population does not rearrange T cell antigen receptor β genes (17), suggesting that NK cells are distinct in origin from the T cell lineage and do not use the T cell antigen receptor for target recognition.

Within the CD3$^-$,Leu-19$^+$ NK cell subset, ~10% of these cells lack expression of CD16. Freshly isolated CD3$^-$,CD16$^-$,Leu-19$^+$ lymphocytes killed NK-sensitive tumor cell targets, but not NK-insensitive targets (10). To determine whether this population responds to rIL-2, low bouyant density lymphocytes were stained with a combination of FITC-conjugated anti-Leu-4 (CD3), FITC-conjugated anti-Leu-11 (CD16), and biotin-conjugated anti-Leu-19, followed by PE-conjugated streptavidin. Lymphocytes displaying only red (PE) immunofluorescence would therefore possess the phenotype CD3$^-$,CD16$^-$,Leu-19$^+$ (10). This small population (<5% of peripheral blood, low bouyant density lymphocytes) was isolated to >95% purity using the FACS, and cultured in medium containing rIL-2 for 24 h, 1 wk, and 4 wk (Fig. 7). After rIL-2 activation, CD3$^-$,CD16$^-$,Leu-19$^+$ cells lysed both K562 and COLO (Fig. 7). Reexamination of the antigenic phenotype indicated that >95% of the cells remained CD3$^-$,CD16$^-$,Leu-19$^+$. These findings show that a small subset of Leu-19$^+$ lymphocytes, lacking expression of both the CD3 and CD16 antigens can respond to rIL-2 and lyse both K562 and COLO. The relationship of these CD3$^-$,CD16$^-$,Leu-19$^+$ lymphocytes to CD3$^-$,CD16$^+$,Leu-19$^+$ NK cells is unknown, although we have suggested that

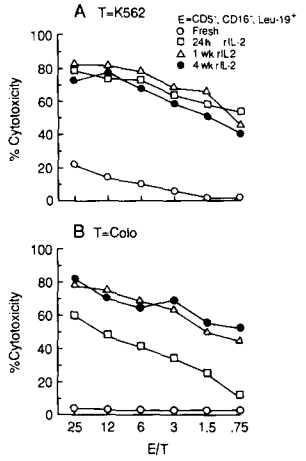

FIGURE 7. Cytotoxicity mediated by rIL-2-activated $CD3^-$,$CD16^-$,Leu-19^+ cells. Freshly isolated low bouyant density lymphocytes were stained with mAbs, as described in the text. $CD3^-$,$CD16^-$,Leu-19^+ lymphocytes were isolated using the FACS and placed in culture with rIL-2 for 24 h (□), 1 wk (△), and 4 wk (●). Freshly isolated (○) and rIL-2-activated cells were assayed for cytotoxicity against K562 (*A*) and COLO (*B*).

this population may represent the stage of NK cell differentiation before acquisition of CD16 Fc receptors (10).

Kinetics of the Response. Leu 19^+ T cells and NK cells were isolated from the low bouyant density lymphocyte population using the FACS, and cultured in medium containing rIL-2. Cells were harvested after 18 h and after 1 wk, then assayed for cytotoxicity against K562 and COLO. As shown in Fig. 8, killing of K562 was substantially enhanced in both the Leu-19^+ T cell and NK cell populations within 18 h. Maximal lysis of COLO by NK cells was also detectable after 18 h, and did not significantly increase upon longer culture. Leu-19^+ T cells mediated only minimal lysis against the solid tumor cell target at all time points examined.

Conclusions. These studies show that the LAK phenomenon is predominately mediated by rIL-2-activated NK cells. Both the precursor and effector of this activity were typical NK cells, with the phenotype $CD3^-$,Leu-19^+. There was no evidence for a unique LAK cell. Moreover, rIL-2 apparently provided the only signal necessary for NK cells to lyse fresh solid tumor cell targets; there was no evidence that accessory cells or T lymphocytes were required to generate this response. The mechanism whereby NK cells become competent to recognize and lyse solid tumor cell targets after brief exposure to rIL-2 is not understood. Overall, peripheral blood T lymphocytes contributed little to the LAK phenomenon. However, these Leu-19^+ T cells lysed hematopoietic targets without deliberate immunization or MHC restriction, and their cytotoxicity was usually significantly augmented by rIL-2. We are examining whether or not these Leu-19^+ T cells preferentially recognize antigens expressed on hematopoietic cells, and not other tissues. We observed that Leu-19^+ T cells apparently proliferated more rapidly in response to rIL-2 than did NK cells (unpublished observation). The practical implications of this finding are that long term culture of unseparated low bouyant density peripheral blood lymphocytes in IL-2 results predom-

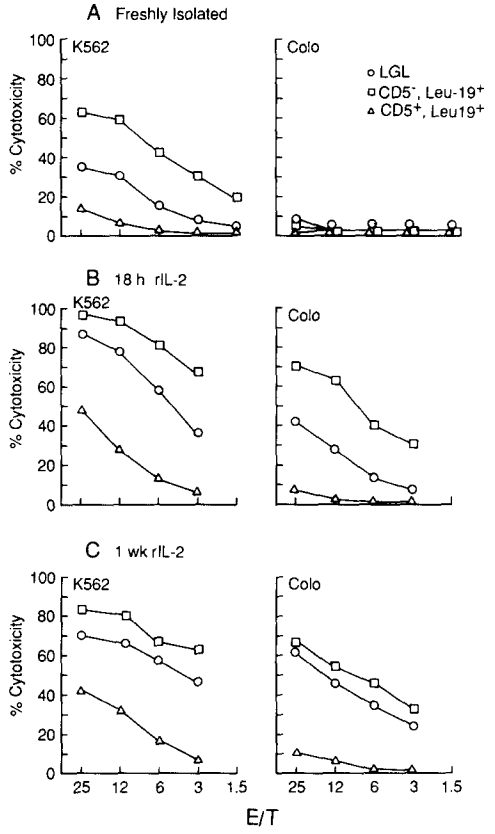

FIGURE 8. Kinetics of rIL-2-activated cytotoxicity. Freshly isolated low bouyant density lymphocytes (○) were stained with mAbs, as described in Fig. 1. Leu-19$^+$ T cells (△) and NK cells (□) were isolated using the FACS, and placed in culture with rIL-2. At the indicated time points (*A*, freshly isolated; *B*, 18 h; *C* 1 wk), cells were harvested and assayed for cytotoxicity against K562 (*left*) and COLO (*right*).

inately in T cell growth, with a relative loss of NK cells. If the primary objective is to optimize generation of effectors mediating solid tumor cell cytotoxicity, this differential growth rate should be taken into account.

The present studies conflict with earlier reports (1–3) on the LAK phenomenon. The most notable discrepancy is that these investigators suggested that LAK was mediated partially by a CD3$^+$ T cell population (1–3). Recently, we have shown that a subset of low bouyant density peripheral blood T lymphocytes can lyse NK-resistant targets in the presence, but not in the absence, of mitogenic lectins (16). In these early studies of the LAK effector, lectin-containing supernatant was used as the source of IL-2 (1, 2). Perhaps the T cell–mediated cytotoxicity in these prior studies was actually lectin-dependent cellular cytotoxicity. We have also shown that maximal cytotoxicity is achieved within 18 h after exposure of NK cells to rIL-2. Earlier studies (1) suggested that this process required 3 d. Again, we believe that this difference in kinetics is due to the preparations of IL-2 used in the experiments. In the previous studies, the concentration of IL-2 used to generate LAK activity was <5 U/ml (3). We and

others (4, 5) have since determined that optimal generation of cytotoxicity requires >500 U/ml. Since the concentration of IL-2 is the rate-limiting factor in the induction of cytotoxicity, it seems quite likely that under suboptimal conditions that the generation of cytotoxicity would require a longer time. In accordance with the studies by Grimm et al. (2), our data confirm that the precursor of the LAK effector is a $CD3^-$ lymphocyte, and is in fact an NK cell.

The results from this study only apply to the LAK phenomenon using lymphocytes obtained from peripheral blood. Clearly, non–MHC restricted cytotoxic lymphocytes can be generated from lymphoid tissues devoid of NK cells, such as lymph nodes and thymus (2, 18). We have recently shown (unpublished observation) that culture of human thymocytes, but not peripheral blood T cells, in rIL-2 results in the generation of Leu-19^+,$CD3^+$ T lymphocytes that lyse solid tumor cell lines without MHC-restriction. Comparison of the thymic Leu-19^+ T cells and peripheral blood Leu-19^+ T cells is under investigation. These findings caution against direct extrapolation of results using animal models of the LAK phenomenon, where the effector cell populations are derived from the spleen, thymus, or lymph node, and not peripheral blood. It is important to remember that LAK is an activity, not a unique, homogeneous cell type. In blood, the effector of LAK is clearly an NK cells, whereas in other lymphoid tissues such as thymus, the effectors of LAK are probably $CD3^+$ T cells. Since peripheral blood has been the source for immunotherapeutic use of rIL-2-activated cytotoxic cells in cancer patients (19), our studies suggest that rIL-2-activated NK cells are probably the effectors in this situation.

Summary

In vitro culture of human peripheral blood lymphocytes in IL-2 results in the generation of cytotoxic cells that can lyse fresh and cultured solid tumor cells, as well as hematopoietic tumor cell lines, without deliberate immunization or MHC restriction. This has been referred to as the lymphokine activated killer (LAK) phenomenon. Here, we show that the majority of this activity is mediated by NK cells that express the Leu-19 (NKH-1) antigen, but do not express CD3. The precursor of this effector population also expressed the phenotype $CD3^-$,Leu-19^+. Peripheral blood $CD3^+$ T lymphocytes contributed little to the LAK phenomenon, although low levels of non–MHC restricted cytotoxicity against hematopoietic tumor cell targets were mediated by a subset of $CD3^+$ T lymphocytes that coexpressed the Leu-19 antigen. These studies clearly indicated that the LAK phenomenon is not mediated by a unique LAK cell, but is mediated mainly by IL-2-activated peripheral blood NK cells.

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