Cancer Immunol Immunother (1989) 29: 155–166

Cancer Immunology Immunotherapy

Heterogeneous lymphokine-activated killer cell precursor populations

Development of a monoclonal antibody that separates two populations of precursors with distinct culture requirements and separate target-recognition repertoires

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Summary. We developed a monoclonal antibody (mAb) 211, which recognizes the precursors in peripheral blood of lymphokine-activated killer cells (LAK) induced by recombinant interleukin-2 (rIL-2). In conjunction with complement mAb 211 also eliminates natural killer cells (NK) and a majority of the cytotoxic T lymphocytes. B cells and monocytes do not express the 211 antigen. Since mAb 211 recognized such a large percentage of peripheral blood lymphocytes we examined which 211^+ subpopulation was the predominant precursor of rIL-2-induced LAK cells using two-color fluoresence-activated cell sorting (fluorescein-conjugated 211 mAb plus phycoerythrin-CD11b). This method identified the $211^+/ CD11b^+$ population as the predominant phenotype of the rIL-2-induced LAK precursor. In addition, we directly compared the phenotype of the LAK precursor induced by delectinated T-cell growth factor (TCGF) to that induced by rIL-2. The 211-depleted population, which was devoid of NK cells and LAK precursors (inducible by rIL-2), was capable of generating LAK activity when TCGF was used as the source of lymphokine. LAK cells induced by TCGF from the 211-depleted population lysed a fresh sarcoma and an NK-resistant cultured melanoma tumor target but not the Daudi cell line, which was lysed by rIL-2-induced LAK cells. Lymphoid subpopulations, depleted using NKH1a mAb, behaved similarly, generating high levels of lysis against the two solid tumor targets when cultured with TCGF but not with rIL-2. CD 3-depleted populations showed enrichment for LAK precursors using either rIL-2 or TCGF. These results indicate that while rIL-2-induced LAK precursors cannot be separated from cells with NK activity, TCGF-induced LAK cells can be generated from populations of peripheral blood mononuclear cells without NK activity.

Introduction

We first reported that naive lymphoid cells cultured in T-cell growth factor (TCGF) became highly cytotoxic for fresh tumor cells but not for normal splenocytes [29]. Subsequent reports further characterized this lymphokine-activated killer (LAK) phenomenon and the precursor and effector populations mediating this activity in both mouse and man [5, 6, 11, 21]. These studies demonstrated that LAK activity was mediated by a broadly reactive, cytolytic cell, non-restricted by major histocompatibility complex (MHC). The initial reports suggested that the LAK precursor in mouse and man was a subpopulation of T cells [5, 6, 11, 21]. Subsequent studies by our laboratory and by others reported that null (non-T non-B) or natural killer (NK) cells were the predominant precursor of LAK activity [2, 7–9, 14–18, 23, 26, 28]. These apparent discrepancies appear to have been due to the source of interleukin-2 (IL-2) utilized for LAK generation. Initial LAK studies were performed with delectinated TCGF, then with the availability of recombinant IL-2 (rIL-2) most laboratories studying this phenomenon utilized this pure source of nonglycosylated lymphokine, which appeared to be functionally identical to the native material [19].

TCGF used in early human studies was obtained from supernatants of human peripheral blood mononuclear cells (PBMC) stimulated with phytohemagglutinin alone or in combination with phorbol myristate acetate. These supernatants were generally delectinated and sometimes further purified to produce a TCGF-enriched fraction [4]. In murine studies, essentially all TCGF employed in early reports was from culture supernatants of concanavalin-A–stimulated Balb/c spleen cells, also known to contain many other lymphokines. Recently, Mule et al. [12] demonstrated that mouse rIL-4 (BSF-1) alone, a component of crude supernatants, is also capable of generating LAK activity from mouse splenocytes when tested against fresh sarcoma targets. They also demonstrated that rIL-4 augmented splenic LAK activity when combined with rIL-2. Widmer et al. [27] have demonstrated a suppressive activity of human rIL-4 in the generation of rIL-2-induced LAK cells from human PBMC. These two recent reports demonstrate new mechanisms to regulate the generation and expression of LAK cells with a lymphokine other than IL-2.

The purpose of the current study was to develop new mAbs, which might better define the population(s) containing precursors of LAK activity in man, and to further define the LAK-inducing activities of rIL-2 versus TCGF. Utilizing the cascade technique of Springer [24], preparations of PBMC membranes were depleted of many defined antigens and were used in immunizations. Resulting hybrids were screened for inhibition of LAK generation in functional assays. One resulting hybrid produced an antibody that efficiently eliminated the precursor of LAK activity in a complement-dependent cytotoxicity assay and is

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identified as mAb 211. This mAb also eliminated NK activity and substantially reduced both mixed lymphocyte response and cytotoxic T lymphocyte allo-reactivity. Using two-color immunofluorescence (FACS) this antibody identified an antigen on NK cells and some T cells but not on B cells or monocytes. Utilizing mAb 211 alone or in combination with antibodies recognizing CD11b, PBMC enriched for LAK precursors were positively selected using flow cytometry into 211^+ or 211^- populations and cultured in rIL-2. In nine determinations the majority of LAK activity was present in the 211^+ population. Interestingly peripheral blood lymphocytes, depleted with the mAb 211 and complement, which subsequently failed to generate LAK activity with rIL-2, generated substantial LAK activity against both a fresh sarcoma and a cultured NK-resistant melanoma cell line when delectinated TCGF was utilized.

Materials and methods

Media. Cells were cultured in complete medium, which consisted of RPMI 1640 (Biofluids, Rockville, Md) supplemented with 0.1 mM non-essential amino acids (Biofluids), 0.1 μM sodium pyruvate (Biofluids), 100 μg/ml gentamicin sulfate (Gibco, Grand Island, NY), 2 mM L-glutamine (NIH media production section), 50 μM 2-mercaptoethanol (Aldrich, Milwaukee, Wis) and 10% human AB serum (Biocel, Carson, Calif, or KC Biological, Kansas City, Mo).

Interleukin-2. Human recombinant IL-2 (rIL-2) was kindly supplied by the Cetus Corp., Emeryville, Calif. The biological and biochemical properties of rIL-2 have been described in detail [19]. Natural human, delectinated serum-free TCGF was obtained from Cellular Products Inc., Buffalo, NY, in 1983 and stored at −20° C. This product was prepared by the stimulation of human peripheral blood lymphocyte cultures with 0.08% phytohemagglutinin P and TPA (12-*O*-tetradcanoylphorbol 13-acetate) in serum-free medium. Following 2 days of culture, supernatants were harvested, concentrated and passed over a DEAE-cellulose column. The column was washed with TRIS to remove interferon and the TCGF was eluted with 0.1 M NaCl and 0.1 M TRIS. The TCGF lots used in these studies had more than 98% of the phytohemagglutinin and TPA removed (Cellular Products Inc. specifications).

Isolation of peripheral blood mononuclear cells. Buffy coats or leukophereses were obtained from both normal volunteers and cancer patients through the Department of Transfusion Medicine, Clinical Center, NIH. PBMC were isolated by density gradient centrifugation over lymphocyte separation medium (Organon Tetnika Corp., Durham, NC). Gradient interfaces were aspirated and washed thrice prior to being resuspended in complete medium. Cells were counted and used fresh or cryopreserved in 90% human AB serum and 10% dimethylsulfoxide. PBMC used in cell-sorting studies were enriched for LAK precursors. PBMC were isolated as noted above, counted and incubated in polystyrene flasks for 2–15 h. Nonadherent cells were obtained from the flasks and transferred to a nylon-wool column, previously washed and warmed with 37° C complete medium. Columns were then incubated at 37° C for 1 h. Cells were gently eluted by passage of complete medium over the column. Nylon-nonadherent cells were then mixed with a 100-fold excess of washed sheep red blood cells, which had been depleted of leukocytes by separation over a lymphocyte separation medium gradient, repeated washings and irradiation with 3500 rad. The PBMC/sheep red blood cell mixture was incubated in 90% human AB serum at 37° C for 15 min, then centrifuged at 1500 rpm for 10 min and incubated on ice for 1 h. The cell pellet was gently resuspended and overlayed onto a lymphocyte separation medium gradient, which was centrifuged for 20 min at 2000 rpm, the resulting interface cells (designated as E^-) were aspirated and washed. PBMC remaining in the sheep red blood cell pellet were hypotonically lysed with ammonium chloride/potassium buffer (ACK, NIH media stores), and washed (designated as E^+). E^- cells were used as a source of enriched LAK precursors for immunization and in cell-sorting experiments

Immunization and screening protocol. E^-, nylon-nonadherent cells were treated with an appropriate dilution of anti-T3 and anti-DR for 45 min at 4° C. Cells were then washed and incubated with a 1:4 dilution of screened baby rabbit complement for 60 min. Viable cells were obtained by separation over lymphocyte separation medium and then extracted with a lysing buffer consisting of 0.5% NP40, 100 ml phosphate-buffered saline, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 0.2 unit/ml aprotinin at pH 7.4. Nuclei and endoplasmic reticulum were removed by centrifugation and the mAbs against CD2, CD3, CD4, CD5, CD7, CD8, CD11, CD38, Leu7, Leu8, Hu leucocyte, LeuM4, and DR were reacted with the cell membrane extract. mAbs were obtained from Becton Dickinson (Sunnyvale, Calif). Following a 2-h incubation at 4° C on a shaking platform, the suspension was repeatedly passed over a rat anti-(mouse IgG) column. The resulting effluent was then used as an immunogen and processed with Freund's complete adjuvant and injected i.m. into the limbs of Balb/c mice. Second and third immunizations were performed in Freund's incomplete adjuvant at 12–17-day intervals. Spleens and lymph nodes of immunized mice were harvested 3 days after their last injection of antigen and fused with the SP20 myeloma line (kindly provided by Dr. J Schlom, NCI). Hybrids were tested for production of antibody against LAK precursors in the following two screens. PBMC [$(1.8–2.5) \times 10^5$ cells] in 50 μl complete medium were transferred into each well of a round-bottom 96-well tissue-culture plate (Costar, Cambridge, Mass). Plates were set up in duplicate and 50 μl hybrid supernatant was added to wells of each plate and incubated at 4° C 1 h. One series of replicate plates were then washed in a sterile fashion and a 1:4 dilution of complement was added to each well. Following an incubation at 37° C for 45 min, cells were washed and incubated in 200 μl/well complete medium containing 1000 U/ml rIL-2. To the replicate plate, not treated with complement and containing 50 μl cells and 50 μl of hybrid supernatant, 100 μl complete medium with 2000 U/ml rIL-2 was added. Plates were then cultured at 37° C in a humidified atmosphere of 95% air and 5% CO_2 for 3–4 days. Plates were vibrated on a plate shaker to resuspend effector cells prior to the addition of ^{51}Cr-labeled tumor target cells to the wells. These were incubated at 37° C for an additional 4 h and then supernatants harvested and counted. Determination of percentage cytotoxicity was calculated as reported

below. Hybrid supernatants that inhibited LAK generation or activity were identified and subjected to repeated analyses. One hybrid, designated as 211, was recloned and used to produce the 211 mAb used in these studies.

Antibody and complement depletion studies. Human PBMC, isolated as noted above, were incubated with anti-CD3, NKH1a (kind gift of Dr. J. Ritz, Dana-Farber Cancer Institute), or mAb 211 for 45 min at 4° C. Cells were washed and resuspended in a 1:4 dilution of baby rabbit complement (lot 1277, Pel Freeze, Madison, Wis). This lot of complement was selected for its efficient lysis of the Jurkat cell line with the mAb T101 and low inherent toxicity towards LAK precursors, and was used in all complement depletion studies reported here. This lot of baby rabbit complement (lot 1277) was the only one of six tested which efficiently lysed the target cell line at a concentration that was not toxic to LAK precursors.

Fluoresence-activated cell sorting and analysis. PBMC or E^- populations were isolated as described and incubated with the appropriate antibody. Cells labeled with mAb 211 were subsequently incubated with goat anti-[mouse IgG $F(ab')_2$] fluorescein(FITC)- or phycoerythrin(PE)-conjugated reagent. Cells labeled with control mAb and the goat anti-(mouse IgG)$F(ab')_2$ always showed background staining but never any bright fluoresence. Cells staining positively with mAb 211 always exhibited a high degree of fluoresence with a fairly homogeneous profile. For double-labeling studies, cells treated with mAb 211 and second-step reagent, as described above, were subsequently labeled with antibodies specified in Results. Prior labeling with mAb 211 did not alter the percentage of cells staining with the subsequent antibody. All analysis and sorting was performed on a FACS 440 flow microfluorometer (Becton Dickinson, Mountain View, Calif), interfaced with a PDP 11/24 computer (Digital Equipment Corp., Maynard, Mass). Preparations of sorted cells were better than 95% pure on reanalysis. Aliquots of cells labeled only with 211-FITC or CD11-PE were used to set parameters on the FACS 440 to allow separation of cells expressing either or both markers. Appropriate FITC- and PE-labeled IG controls were used to determine background fluorescence. Stained cells were resuspended in ice-cold Ca^{2+}, Mg^{2+}-free Hanks basic salt solution (HBSS) supplemented with 4% human serum albumin and 100 μg/ml gentamicin. Cells were sorted onto a cushion of human serum albumin and were kept on ice until all sorting was completed.

Fresh tumor targets from surgical specimens. Freshly resected tumor was collected from surgery and transported in HBSS at 4° C until processing. Necrotic tumor and connective tissue were removed, and the remaining specimen was minced, using scissors or scalpels, into 1-cm fragments. The tumor was suspended in HBSS containing 5 U/ml hyaluronidase (Sigma, St Louis, Miss), 2 mg/ml collagenase (C-5138, Sigma) and 0.2–0.3 mg/ml deoxyribonuclease (Sigma). This mixture was transferred to a flask and digested with mechanical stirring for 8–12 h. The preparation was filtered through stainless-steel screens or nylon mesh (Nytex, Lawshe Instrument Co. Inc., Rockville, Md) to remove undigested clumps of tumor and debris and was washed three times in HBSS. Cells were transferred to 50-ml tubes, underlayed with 10 ml lymphocyte separation medium and centrifuged at 2000 rpm for 20 min. Cells located at the interface were harvested and washed three times, and their viability was determined by counting with trypan blue. An aliquot was sent for cytological analysis and the remainder cryopreserved in 90% human AB serum and 10% dimethylsulfoxide (Sigma).

Cell-mediated cytotoxicity assay. A 4-h ^{51}Cr-release assay was performed to determine cytotoxicity of effector cells. Tumor cells were thawed and labeled with 200–400 μCi Na $^{51}CrO_4$ (Amersham Corp., Arlington Heights, Ill) for 90 min at 37° C in 0.4–0.8 ml complete medium. Cells were washed once and resuspended in 15 ml complete medium and incubated at 37° C on a rocking platform for 30–60 min. Cells were washed twice with complete medium, counted and added at 5×10^3 cells/well to various numbers of effector cells in round-bottom microtiter plates (Costar). Plates were next centrifuged at 1000 rpm for 5 min and incubated at 37° C for 4 h. The culture supernatants were harvested with SCS harvesting frames (Skatron, Norway), transferred to macrowell tube strips (Skatron) and counted in a gamma counter. Maximum isotope release was determined by incubation of targets in 2% sodium dodecyl sulfate (Sigma). Spontaneous release was determined by incubation of targets in complete medium without effectors. The percentage of specific lysis was calculated by the formula:

$100 \times$ (experimental ^{51}Cr release (cpm) − spontaneous release) / (maximal release − spontaneous release). The fresh tumors utilized in these studies were; leiomyosarcoma (Tu348), malignant fibrous histiocytoma (Tu466), melanoma (Tu470), and sarcoma (Tu507). K562, Daudi and FEMX are cultured cell lines maintained in this laboratory and used from frozen stocks or directly from culture.

Cytotoxic T lymphocyte and mixed lymphocyte reaction (MLR). Mixed lymphocyte reaction assays were prepared in 96-well round-bottom plates (Costar) with 1×10^5 responding PBMC and 4×10^4 irradiated Z2B stimulator cells (kind gift of Dr. R. Quinones) in 0.2 ml complete medium according to a modification of Sharp et al. [22]. Cultures were incubated in a 37° C, 5% CO_2, humidified incubator for 6 or 7 days before harvest. Wells were pulsed with 2 μCi/well [^{3}H]thymidine (New England Nuclear, Boston, Mass) 24 h before processing on a PHD cell harvester (Cambridge Technologies Inc., Cambridge, Mass). Controls included both stimulator cells, and responder cells from each treatment group, cultured alone for determination of spontaneous blastogenesis. Three replicate cultures were plated for each time point and results are presented as the percentage relative response, calculated as $100 \times$ (experimental MLR ^3H − experimental spontaneous ^3H) / (control MLR ^3H − control spontaneous ^3H) where ^3H is measured as cpm. Cytotoxic T lymphocytes were generated in upright 25-cm^2 flasks (Costar) with 5×10^6 responder PBMC and 1.7×10^6 irradiated (12000 rad) Z2B stimulator cells in 12 ml complete medium. Cells were harvested after 6 days of culture and tested in triplicate for cytotoxic activity against Z2B-labeled target cells. Results are presented as percentage specific cytotoxicity calculated by the formula: $100 \times$ (experimental ^3H − spontaneous ^3H) / (maximal ^3H − spontaneous ^3H) where ^3H is measured in cpm.

Table 1. FACS analysis of peripheral blood mononuclear cell populations

Primary mAb	Cells in PBMC positive for primary mAb (%)		Cells staining with primary mAb which also bind 211 (%)	
	Mean[a]	SD[b]	Mean	SD
211	48.9	(20.4)		
CD3	61.3	(5.0)	56.8	(8.3)
CD4	45.8	(2.7)	42.4	(7.7)
CD5	57.8	(7.5)	61.8	(12.5)
CD8	25.2	(1.9)	78.5	(5.4)
CD11b	19.6	(10.2)	36.9	(30.4)
CD14	20.4	(3.0)	3.8	(2.2)
CD20	18.4	(9.8)	1.3	(0.9)
DR	30.1	(1.3)	2.6	(1.4)
Leu19	14.2	(12.1)	85.5	(12.0)

[a] Mean percentage of positive-staining cells determined by two to five experiments performed on normal blood donors
[b] Standard deviation of the mean percentage positive-staining cells

Results

Generation and cellular reactivity of monoclonal antibody 211

The mAb anti-211 was generated from a fusion with murine spleen cells immunized with NP40-solubilized membrane determinants from human PBMC as described in Materials and methods. Of 2700 hybrid supernatants screened for inhibition of LAK generation in both complement-dependent and independent functional assays, only one reproducibly eliminated the LAK cell precursor. This mAb 211 effectively depleted the precursor of LAK cells only in complement-dependent assays. Reactivity of anti-211 for PBMC was tested using two-color FACS analysis. These data identified that anti-211 mAb recognized a determinant expressed on a majority of T cells and null cells, but not on B cells or monocytes (Table 1). The determinant recognized by mAb 211 was not uniquely distributed with mAb against CD3, CD4, CD5, CD8, CD11b, CD14, CD20, or DR.

Functional characterization of the 211$^+$ subset in peripheral blood

Human PBMC, depleted of 211$^+$ cells with mAb plus complement, were cultivated with rIL-2 for 3–5 days and were assayed for the capacity to lyse fresh and cultured tumor targets. Figure 1 shows a representative experiment. In this example, treatment of PBMC with mAb 211 and complement resulted in the virtual elimination of LAK activity against the fresh tumor target 470, while the LAK activity of the complement-treated group was essentially identical to non-treated control cells. Cytotoxicity against the cultured targets Daudi and K562 was substantially decreased (>125–13.5 $LU_{30}/10^6$ effectors for Daudi and >125–3.2 $LU_{30}/10^6$ effectors for K562) in the 211-depleted population. An aliquot of these same 211-depleted PBMC was also assayed for NK activity in a 6-h ^{51}Cr-release assay immediately after the complement-depletion step (Fig. 2). Complete abrogation of NK activity is seen with mAb 211 and complement, as compared to controls.

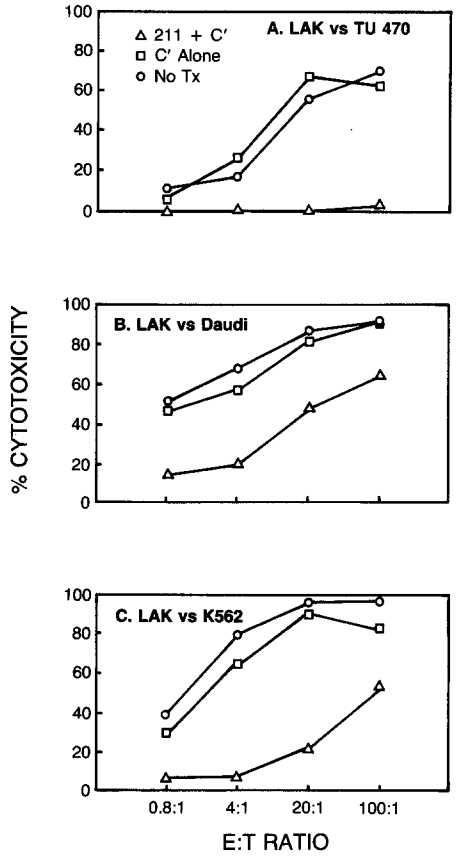


Fig. 1. LAK lysis of three targets in a 4-h ^{51}Cr-release assay. Effector cells were non-adherent PBMC treated with either media (*No Tx*), complement (*C'*) alone, or mAb 211 plus complement, washed, and incubated in complete medium containing 1000 U/ml rIL-2 for 4 days. Cells were then harvested and tested for lytic activity against a fresh human melanoma (**A**), Daudi cells (**B**) and K562 (**C**)

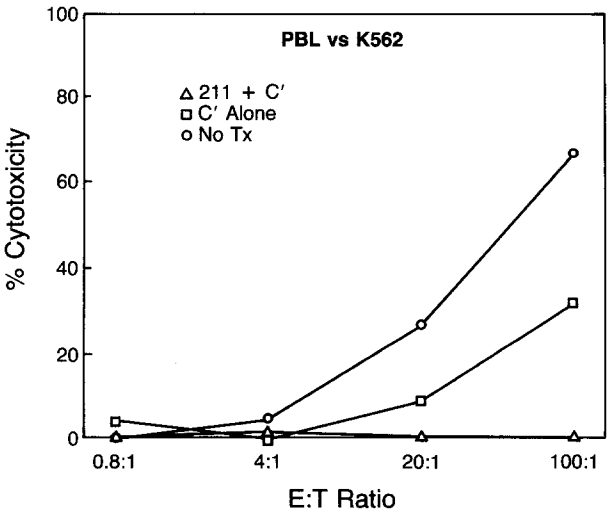


Fig. 2. Depletion of NK cells from PBMC using mAb 211 plus complement (*C'*). PBMC were treated with either media (*No Tx*), complement alone, or mAb 211 plus complement, washed, and assayed for lysis of K562 target cells in a 6-h ^{51}Cr-release assay. These same populations were used in experiments presented in Fig. 1 and Table 2

Table 2. Allogenic response of 211-depleted human peripheral blood mononuclear cells[a]

Treatment	Cytolytic response (% lysis)[b] for E:T ratio of				Proliferative response[b]	
	80 : 1	16 : 1	3 : 1	0.6 : 1	Day 6	Day 7
Control	50	46	25	17	100	100
Complement alone	70	62	28	10	42	41
211 + complement	7.9	8	4.7	2.6	10	8.5

[a] Normal PBMC were treated with mAb 211 and/or complement washed, and then incubated alone, or with irradiated (12 000 rad) Z2B stimulator cells at a ratio of 3:1. Populations were tested for cytotoxic T lymphocyte activity on day 6, and for proliferation in the mixed lymphocyte reaction on days 6 and 7

[b] Percentage specific cytotoxicity and percentage relative response were calculated as described in Materials and methods

In this experiment, cells treated with complement alone generated substantial lysis of K562, albeit lower than that seen in the nontreated group.

211-depleted cells were also tested in the one-way mixed-lymphocyte reaction for both proliferation and cytotoxicity (Table 2). In [^{3}H]thymidine-uptake studies, the group treated with complement alone consistently showed a major reduction in proliferative response to alloantigen, while simultaneously demonstrating increased cytotoxicity to the allogeneic target. It should be noted that the complement used in all studies reported here was from a single lot and was chosen because it had little effect on LAK generation. In these studies treatment with anti-211 plus complement resulted in substantial elimination of the proliferative response as compared to the non-treated or complement-treated controls. Likewise, this same treatment was effective in blocking allogeneic cytotoxic T lymphocyte generation, resulting in only 8% lysis at an effector-to-target ratio of 80:1 compared to 50% and 70% for non-treated cells and cells treated with complement alone respectively. These complement-depletion studies suggest that a 211$^+$ cell is the principle precursor of LAK activity in PBL using rIL-2 and demonstrate its expression on diverse and functionally distinct populations of PBL.

Flow-cytofluorometric analysis of PBMC treated with mAb 211 and complement demonstrated substantial depletion of the 211$^+$ population. Few of the cells staining brightly for 211 remained following such treatment, as compared to control groups (Fig. 3). Phenotypic analysis of the cells remaining following depletion with mAb 211 and complement revealed a relatively large number of CD2$^+$ and some Leu7$^+$ cells (Fig. 4), Leu11 expression, which can be variable, was low for this particular donor.

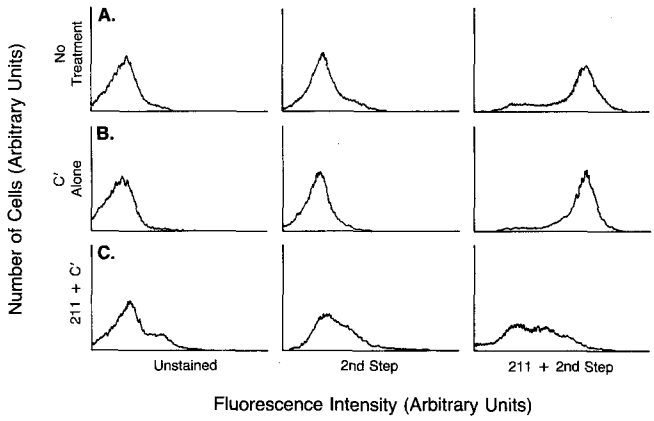


Fig. 3. Reactivity pattern of 211-depleted (*211 + C'*), non-treated (*No Tx*), and complement-alone-treated PBMC after staining with mAb 211 and FITC-labeled F(ab')$_2$ goat anti-(mouse IgG) (*211 + 2nd Step*), FITC-labeled F(ab')$_2$ goat anti-(mouse IgG) (*2nd Step*). Fluoresence intensity was determined using a FACS 440

Positive selection of 211$^+$ cells by FACS

To confirm the results of the experiments with cells depleted of mAb and complement, which demonstrated that a 211$^+$ cell was the principle precursor of LAK activity with

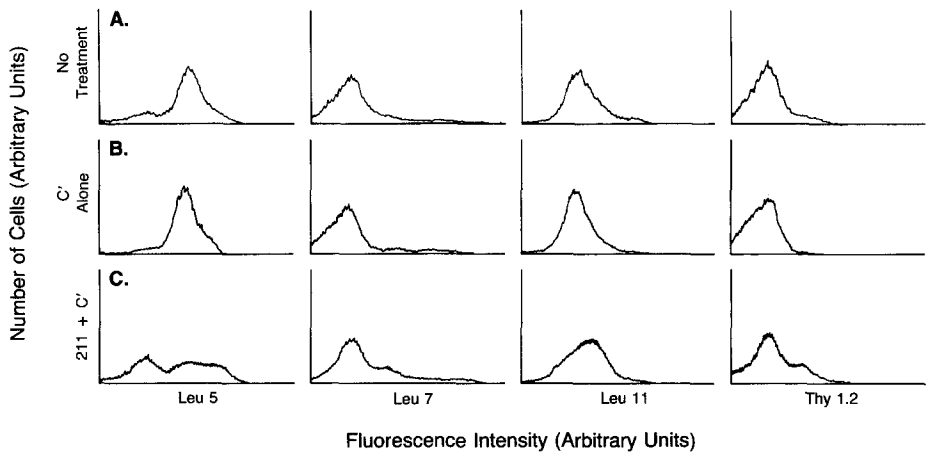


Fig. 4. Reactivity pattern of control PBMC and PBMC treated with mAb 211 + complement after staining with various mAb. Fluoresence intensity was determined using a FACS 440. mAb utilized in this analysis were Leu5, Leu7, Leu11 and, as a control, mAb Thy1.2

Table 3. Using flow cytometry LAK activity is generated primarily from 211-positive lymphocytes[a]

Expt.	Target	Cytotoxicity/10^6 cells (LU_{30})	
		211^-	211^+
1	K562	16	127
	FEMX	0.16	80
2	TU348	<0.1	9.8
	TU466	<0.1	5.1

[a] E^- PBMC, isolated as described, were labeled with the 211 mAb and sorted into 211^+ and 211^- populations using FACS. 211^+ and 211^- populations were cultured in 1000 U/ml rIL-2 in complete medium for 3.5 days, then analyzed for lytic activity against both a fresh leiomyosarcoma (Tu348) and sarcoma (Tu466)/or K562 and FEMX

rIL-2, we undertook a series of positive-selection experiments by FACS. In two initial sorting experiments E^-, nonadherent PBMC were labeled and sorted into 211^- and 211^+ populations. These two populations were then cultured in 1000 U rIL-2 for 4 days and assayed for lytic activity against either cultured or fresh tumor targets. Data from these experiments are presented in Table 3. In the first experiment effectors were tested against the NK-sensitive, K562 cell line and an NK-resistant human melanoma cell line, FEMX. Two fresh human tumors were utilized as targets in the second experiment. In both studies the majority of IL-2-induced lytic activity (more than 98% for fresh tumor targets and 80% or 88% for K562 and FEMX respectively) was present in the 211^+ population. To define further the subset of 211^+ cells that were precursors for LAK activity, two-color sorting was performed utilizing both 211 and CD11b mAbs. PBMC, enriched for LAK precursors as described in Materials and methods, were reacted with both mAbs and were sorted into $211^+/CD\text{-}11b^+$, $211^+/CD11b^-$ or $211^-/CD11b^+$ populations. An example of a typical sorted population, further stained with LeuM3 to distinguish the monocytes in the $CD11b^+$ population, is shown in Fig. 5. These cells were cultured in complete medium with 1000 U rIL-2 for 3.5 days then assayed for cytotoxic activity against fresh tumor targets. In Fig. 6A, the sorted populations are compared to the unsorted E^- population for cytotoxicity against tumor 507, a fresh human sarcoma target. This result clearly identifies the $211^+/CD11b^+$ subset as the most predominant precursor of LAK activity, generating substantial lysis of tumor targets at effector-to-target ratios of 0.2:1, while the subset of $211^-/CD11b^-$ and $211^+/CD11b^-$ cells generated substantially less lysis against the same fresh tumor target. E^- cells are plotted in this figure to show that the $211^+/CD11b^+$ cells are further enriched for LAK precursors.

A summary of five determinations from three, dual-color sorting experiments are presented in Fig. 6B. In all cases the 211^+ precursor populations gave rise to the bulk of cytotoxic activity. However, some heterogeneity in phenotype of the LAK precursor was observed when in the third experiment the $211^+/CD11b^-$ subset of cells from one individual generated the highest lytic activity against both targets assayed. Data from the cytotoxicity assay from that sort are presented in Fig. 6C.

Here the E^-, and $211^+/CD11b^+$ generated similar lysis profiles against the fresh tumor 466, both of which exhibited substantially more lytic activity than $211^-/CD11b^+$ or E^+ populations. Lysis of another tumor target by these same effectors followed the same trend (data not shown). These findings extend the report of Skibber et al. [23], which in eight sorts identified a $CD11b^+/LeuM3^-/CD16^+$ cell as the predominant precursor of rIL-2-induced LAK activity, which the current studies demonstrate includes 211 in this phenotypic profile.

211^- cells generate LAK cells in response to delectinated TCGF

In the preceding series of experiments we demonstrated that mAb 211 and complement depleted both NK cells and the precursors of LAK activity generated with rIL-2.

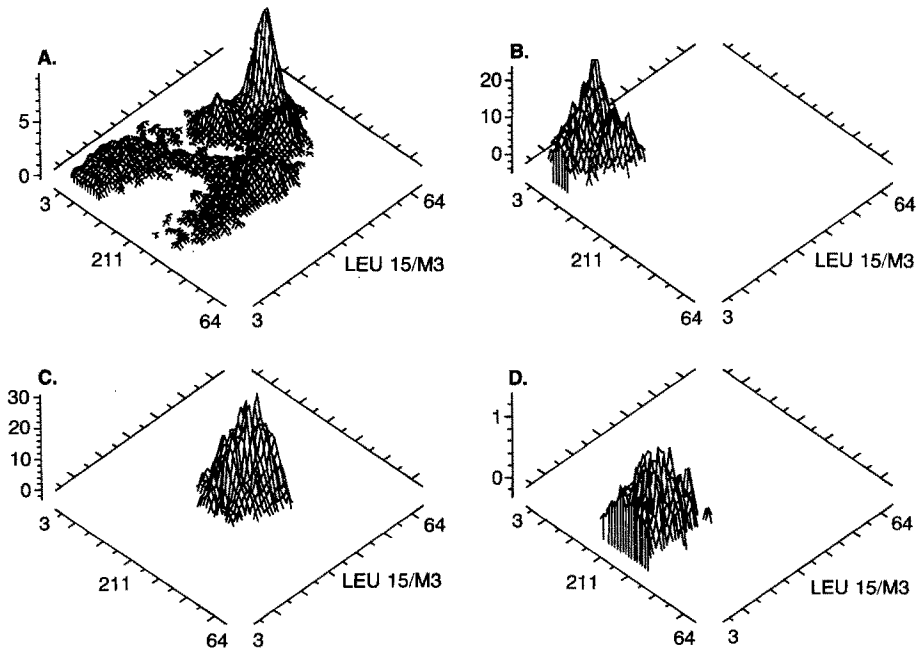
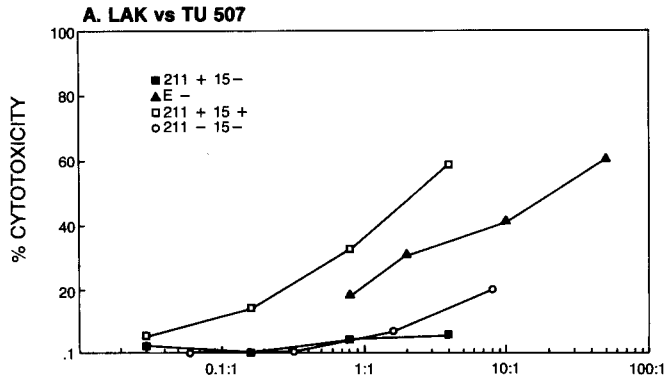


Fig. 5. FACS analysis of nonadherent PBMC and sorted populations used in a typical study. The mAb LeuM3, which is specific for monocytes and does not label any 211^+ cells, was added in this experiment to further distinguish the $211^-/Leu15^+$ $LeuM3^+$ population from the $211^+/Leu15^+$ cells, and demonstrate the brightly staining monocyte population. **A** FACS analysis of the stained unsorted population. **B** FACS analysis of the population sorted for $211^-/Leu15^-/LeuM3^-$. **C** Flow cytometric analysis of the population sorted for $211^+/Leu15^+$ cells. **D** FACS analysis of the population sorted for $211^+/Leu15^-$ $LeuM3^-$ cells



B.

Exp	Target	Lytic Units$_{30}$/10^6 Effectors			
		211−/15−	211−/15+	211+/15−	211+/15+
3	TU466	N.D.	<0.01	N.D.	111
4A	TU507	0.22	N.D.	<0.01	197
4B	TU466	0.81	N.D.	0.22	560
5A	TU507	0.02	N.D.	89	37
5B	TU466	<0.01	N.D.	128	42

TU466 Sarcoma
TU507 Osteogenic Sarcoma

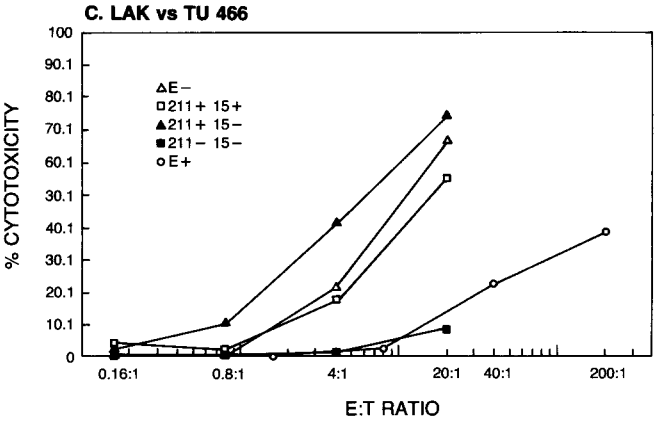


Fig. 6. Cytotoxicity mediated by precursors of rIL-2-induced LAK. Non-adherent PBMC were labeled with mAb-211 – FITC and Leu15-phycoerythrin (CD11) then sorted into $211^-/15^-$, $211^-/15^+$, $211^+/15^-$, or $211^+/15^+$ populations and cultured in 1000 U/ml rIL-2 for 3.5 days. **A** Precursors sorted as described were assayed for cytotoxicity against a fresh sarcoma tumor target (Tu507) following 3.5 days of culture in rIL-2. **B** Summary of three precursor sort experiments against fresh sarcoma tumor targets. **C** Cytotoxicity of effectors from experiment **B** assayed against the fresh sarcoma tumor target (Tu466). This is the only experiment in 11 separate precursor sorts to observe a $Leu15^-$ population mediating substantial LAK activity

We therefore studied whether 211-depleted PBMC could generate LAK activity if delectinated supernatants were employed as a source of IL-2. Nonadherent PBMC were treated with complement, mAb 211+ complement, mAb T3+ complement, or a medium control. Half the cells from each treatment group were cultured in complete medium with 1000 U/ml rIL-2, the remaining cells were cultured in complete medium supplemented with 10% TCGF. Following 3.5 days in culture, effector cells were tested for lytic activity against both a fresh sarcoma target and the

Table 4. 211-depleted peripheral blood mononucler cells (PCMB) generate LAK with T-cell growth factor (TCGF) but not with rIL-2[a]

Treatment	Cytotoxicity/10^6 (LU$_{10}$)			
	Tu466		FEMX	
	rIL-2	TCGF	rIL-2	TCGF
No Tx	17.7	4.4	22.2	5.9
Complement alone	16.1	6.6	21.7	8.8
211 + C	<0.1	4.1	4.5	7.6
CD3 + C	48.7	8.8	38.4	17.6

[a] PBMC were separated into groups and treated with media (No Tx), complement (C), mAb 211 plus complement (211 + C), or mAb T3 plus complement (CD3 + C) washed and recounted. Cells treated with mAb 211 + C, but not with media or alone, were depleted of functional NK activity as defined by the 6-h ^{51}Cr-release assay against K562. Cells treated with mAb T3+C were enriched for NK activity in the same assay. These three groups of cells were each divided in half with part of each group cultured with 1000 U/ml rIL-2 in complete medium and the remainder cultured with 10% TCGF in complete medium. Groups were harvested 3.5 days later, counted, and tested for lytic activity against the NK-resistant cultured melanoma line (FEMX) and a fresh sarcoma tumor (Tu466) target

Table 5. 211-depleted peripheral blood mononucler cells generate LAK with TCGF but not with rIL-2[a]

Target	Cytotoxicity/10^6 effectors (LU$_{30}$)					
	rIL-2			TCGF		
	No Tx	C	211+C	No Tx	C	211+C
FEMX	11.9	17.5	1.7	6.6	10.6	11.9
Daudi	27.3	75.7	20.0	8.6	8.1	0.2

[a] PBMC were separated into groups and treated with media (No Tx), complement (C), or mAb 211 plus complement (211+C). Cells treated with mAb 211+C, but not with media or C alone, were depleted of functional NK activity as defined by the 6-h ^{51}Cr-release assay against K562. These three groups of cells were each divided in half. One half was cultured with 1000 U/ml rIL-2 in complete medium and the remainder was cultured with 10% TCGF in complete medium. Groups were harvested 3.5 days later, counted, and tested against the NK-resistant cultured melanoma line (FEMX) and Daudi

NK-resistant FEMX cell line. As seen in Table 4, cells treated with mAb 211+ complement were depleted of rIL-2-responsive LAK precursors generating less than 0.1 LU for Tu466 and 4.5 LU for FEMX compared to 17.7 LU and 22.2 LU for respective control effector populations. However, this same mAb-211-depleted population generated LAK activity essentially equivalent to the non-depleted population when cultured with TCGF and tested against the same two tumor targets. Interestingly this TCGF-induced LAK from 211-depleted PBMC appears to be more restricted in its recognition of tumor targets than the classic rIL-2-induced LAK from PBMC. An example of this is presented in Table 5. As above, nonadherent PBMC were depleted with mAb 211+ complement and, together with controls, were split into two groups, half being cultured in rIL-2 and the rest in TCGF. Three days

Table 6. LAK precursor phenotype is different depending on lymphokine preparation used for the generation of LAK and the type of target used to assay activity

Expt.	Target	Cytotoxicity (LU_{10})/10^6 effectors (% of control)[a]							
		rIL-2				TCGF			
		C	211 + C[b]	NKH1 + C[c]	T3 + C[d]	C	211 + C[b]	NKH1 + C[c]	T3 + C[d]
1–5	T466/FEMX	116 (85)	15 (13)	13 (11)	422 (343)	140 (44)	99 (26)	70 (40)	671 (742)
1–4	Daudi	83 (61)	21 (33)	4 (5)	459	110 (49)	13 (5)	22 (9)	16025

[a] Percentage of control cytotoxicity (LU_{10})/10^6 effectors calculated by the formula: 100 × (experimental cytotoxicity)/(cytotoxicity of non-treated control) where cytotoxicity was measured as LU_{10}/10^6 effectors

[b] 211 + C data is the mean of 5 determinations against solid tumor targets and 3 determinations against Daudi

[c] NKH1a + C data are the mean of three determinations against both the solid tumor targets and Daudi

[d] T3 + C data are the mean of three determinations against the solid tumor targets (Tu466 of FEMX) and a single determination against Daudi

later cells were harvested and tested for cytotoxic activity against Daudi cells and the NK-resistant FEMX cell line. Here again, non-treated groups or groups treated with complement only, generate good lytic activity against the FEMX target (11.9 and 17.5 $LU_{30}/10^6$ effectors, respectively), while the group treated with 211 + complement developed only 1.7 LU, representing between 9.7% (medium) and 14.2% (complement only) of the control responses. These same cells, treated with 211 + complement, when cultured in rIL-2 were much more efficient at lysing the Daudi cell line (20 LU), generating 73% of the non-treated control response. However, the same starting populations cultured in 10% TCGF and assayed simultaneously against the same targets differed markedly in their pattern of cytotoxicity. The group treated with 211 + complement, which generated little activity with rIL-2, generated good lysis of the NK-resistant melanoma cell line with TCGF (11.9 $LU_{30}/10^6$ effectors compared to 10.6 $LU_{30}/10^6$ effectors for the complement only control). This same effector population, which lysed FEMX, could not lyse the Daudi target (0.2 $LU_{30}/10^6$ effectors compared to 8.6 and 8.1 $LU_{30}/10^6$ effectors for media and complement treated controls respectively). Results of five consecutive experiments, summarized as the percentage of medium control response, are presented in Table 6. This summary demonstrates further that PBMC depleted of 211^+ cells and cultured in rIL-2 fail to generate high lysis of the FEMX or fresh sarcoma target (only 15% of control response) while the same cells cultured in 10% TCGF generated high LAK activity (99% of control for the same determinations). These data document that the 211-depleted population, cultured in TCGF and highly lytic for the FEMX or fresh tumor targets, was not effective at lysing the Daudi cell line (13 ± 5% of the control response). A similar but more variable trend was observed when NKH1a + complement was included in three out of five experiments. As expected, on the basis of positive selection studies [17], populations depleted in NKH1a and complement, cultured in 1000 U/ml rIL-2, generated little lysis of the three tumor targets tested (13 ± 11% for FEMX and TU466 and 4 ± 5% for Daudi). When these same NKH1a-depleted precursor populations were cultured in parallel with 10% TCGF, substantially higher lysis was observed of the two solid tumor targets (70% ± 40%) than was seen with Daudi cells (22 ± 9%). Treatment of precursor populations with anti-T3 + complement enriched LAK precursors regardless of lymphokine source, generating lytic activity ranging from 172% to 16025% of control responses.

Discussion

We have previously demonstrated that adoptive transfer of LAK cells in combination with rIL-2 can mediate complete and partial regressions of tumor in selected patients with advanced cancer [20]. The mechanism of this antitumor activity in humans is not understood. Purification studies have revealed that most (76%–97%) of the LAK activity generated with rIL-2, as measured by the ^{51}Cr-release assay, is generated by less than 20% (range 8%–19%) of the PBMC [18]. A reagent that identifies this precursor might, in concert with separation techniques, significantly reduce the cost of media and materials required to develop LAK cells for patient treatment. Towards this end we set out to define better the precursor population that mediates LAK activity in vitro.

Since LAK cells are a functionally defined population we screened monoclonal-antibody-producing hybridomas for an antibody that would block the generation of effector function of LAK or that would eliminate this cell in a complement-dependent fashion. In this study, we report on the development of mAb 211, which in the presence of complement, can eliminate virtually all precursors of rIL-2-induced LAK. Together with this loss in LAK activity, a parallel deficiency in NK and alloreactive T cells is observed in the remaining cells. Analysis of the 211 phenotype on different subpopulations of PBMC identified its expression on a majority of T cells but not on B cells or monocytes. Evidence that NK cells express 211 is provided by experiments that demonstrate depletion of cells lytic for the NK-sensitive erythroleukemia line K562, following treatment with mAb 211 + complement and FACS analysis of $Leu19^+$ cells double-labeled with mAb 211. These observations demonstrate that 211 is distributed on NK cells and on a majority of both CD4- and CD8-positive T cells. The evidence that demonstrated 211 on the LAK precursor cells was further strengthened by positive selection experiments performed using FACS. However, it was clear from earlier precursor frequency and phenotypic studies that 211 expression alone included too large a segment of lymphocytes to identify the precursor of LAK activity selectively.

Table 7. Characteristics of two compartments for precursors of LAK activity identified by differential expression of the 211 antigen[a]

Functional activity/phenotype	LAK precursor compartment	
	1	2
211 expression	+	−/dull
NK activity	+	−
rIL-2 -induced LAK		
lyses FEMX/Tu466	+	−
lyses Daudi	+	−
TCGF -induced LAK		
lyses FEMX/Tu466	+	+
lyses Daudi	+	−
NKH1a expression	+	−

[a] Characteristics of the two compartments for precursors of LAK activity identified by their differential expression of the 211 antigen

Our laboratory and others have previously shown that the predominant precursor of LAK activity generated with rIL-2 was an E^-, $CD3^-$, $CD11b^+$, $CD16^+$, $LeuM3^-$ PBL [2, 7–9, 14, 15, 17, 18, 23]. We have expanded this phenotypic description by double-labeling and sorting E^- precursor cells with mAb 211 and anti-CD11b. Results of these analyses clearly identified the 211^+ $CD11b^+$ cell as the principal precursor in two normal donors. In a third donor the 211^+ $CD11b^+$ population generated a high degree of lysis yet the 211^+ $CD11b^-$ cells were two or three times more cytotoxic. This one observation was the only result in ten separate FACS experiments, performed in this laboratory, which identified a $CD11b^-$ precursor cell as mediating substantial lysis of fresh tumor targets [23]. These findings support the view that the predominant precursor of LAK activity generated with rIL-2 and cultured in human serum is found in the classic null (non-T, non-B) population. This is consistent with most recent reports using rIL-2 for the generation of LAK cells (see Table 7).

Another finding of our current study was the response of the 211^- or 211^{dull} population to TCGF supernatants but not to rIL-2. This observation allows for the phenotypic segregation of non-MHC-restricted cytotoxic cells into two apparently distinct populations; (a) dependent on IL-2 and (b) dependent on some other factor in delectinated TCGF (possibly in concert with IL-2). Since the initial studies from this laboratory, which identified LAK cells generated with TCGF as being distinct from cytotoxic T lymphocytes or NK cells, there have been no direct comparisons of the LAK precursor phenotype when generating LAK cells with rIL-2 or TCGF. Here we have identified a mAb which dissects this phenomenon into at least two compartments (summarized in Table 7). The first compartment consists of cells with NK activity which respond to rIL-2 or TCGF to generate LAK effectors and lyse a wide selection of both cultured and fresh tumor targets. As described in these experiments the precursor(s) of the effectors in this first compartment are NKH1a and 211^+. Depletion of these populations with mAb and complement dismantles rIL-2-generated LAK activity. The second compartment, defined primarily by the absence or low density of the 211 antigen or NKH1a/Leu19, contains cells without NK activity, which do not generate LAK activity when cultured in rIL-2. However, cells from this compartment will generate "LAK" effector activity when cultured in TCGF. This TCGF-induced LAK effector lyses a more selective repertoire of targets than the standard rIL-2-induced LAK effector. In this context it should be emphasized that LAK is defined by its ability to lyse a fresh tumor target cell. The effectors in this second compartment lyse the NK-resistant melanoma cell line and a fresh tumor target but not the generally more sensitive Epstein-Barr-virus- transformed Daudi cell line.

Several observations warrant further experimentation. Why do these 211-depleted LAK effector cells lyse both of the solid tumor targets but not Daudi cells? One explanation, rendered improbable by the selective nature of the cytotoxic effector, is the possibility that the phytohemagglutinin used in generating the delectinated TCGF may mediate an apparent LAK effect through lectin-dependent cell-mediated cytotoxicity (LDCC). Although this mechanism is capable of mediating cytotoxicity in the presence of agglutinating concentrations of lectin, it is an unlikely candidate to explain T-cell-mediated cytotoxicity when only trace amounts of lectin remain and no bridging is observed [1]. Also, the selective failure of 211-depleted TCGF-induced LAK cells to lyse Daudi while lysing both solid tumor targets is additional evidence against this LDCC hypotheses.

An alternative explanation of this phenomenon is that trace amounts of contaminating lectin and/or phorbol ester may result in the activation of 211^{dull} or 211^- cells. However, additional studies using TCGF generated in the absence of phorbol myristate acetate have generated similar results to TCGF generated with phorbol myristate acetate (data not shown). Thus demonstrating that the phorbol ester plays no role in these observations. Further, the 211-depleted population is greatly reduced in cytotoxic T lymphocyte precursors based on alloresponse data (Table 2) and the selective cytolytic nature of the effector argues further against a promiscuous cytotoxic T cell as the mediator of this activity.

Recently, Mule et al. [12] have demonstrated that murine rIL-4 (formerly BSF-1) is capable of generating LAK cells from mouse spleen cells (but not from PBL). However, the breadth of cytolytic activity mediated by these LAK effectors was not reported. New experiments have shown that IL-4 can augment rIL-2-induced LAK cell lysis of fresh sarcoma targets but not the NK-sensitive YAC-1 target [13]. These observations open the possibility that precursors for IL-4-induced LAK exist that are distinct from those for IL-2. A similar phenomenon may exist for some cytokine in man, and may afford an explanation of these results. This role cannot be mediated by IL-4 in man, however, since Widmer et al. demonstrated that rIL-4 suppresses IL-2-induced LAK cells from PBMC and does not generate LAK cells on its own [27]. Tumor necrosis factor α (TNF-α) also appears to be an unlikely candidate for this role inasmuch as TNF can augment the lytic activity of LAK generated with suboptimal, but not optimal concentrations of IL-2 [16]. Further LAK augmented in this fashion appears to lyse both cultured and fresh targets equally well.

Early studies from our laboratory using antibody and complement, or rosetting techniques, reported that the precursor for LAK activity was identified in a non-NK,

Table 8. LAK precursor studies

Ref.	Cells	Technique	IL-2	Culture	Time	Phenotype	Target/comments
11	PBL Ca pt		20% LF-TCGF	10% HuAB serum	7–21 days	–	Effectors lyse autologous fresh Tu, cultured Tu and cultured skin, but not fresh lymphocytes
5	PBL TDL Ca + N	Adherence Radiation	≈10% PP-TCGF	10% HuAB serum	3–7 days	Nonadherent Radiosensitive	TDL devoid of NK (K562), lyse fresh Tu after culture in TCGF
6	PBL Thymus BM Spleen LNC Ca + N	AB + C Rosette Percoll	10% PP-TCGF	10% HuAB serum	3–7 days	$CD3^-$, $CD11^-$ $CD5^-$, $Leu7^-$ 29° C $ERFC^-$ High density$^+$	CD3, Leu7 and CD5 + C increase LAK. CD11 + C eliminates NK but not LAK. Pre-LAK did not rosette with SRBC. Pre-LAK sedimented with NK
19	PBL		rIL-2 1–20 000 U/ml	10% HuAB serum	4 days		rIL-2 alone is sufficient to generate LAK
26	PBL Non-adh.	AB + C Indirect Rosette Leu1, CD16	rIL-2 5–400 ng/ml; 25–200 U/ml	10% FBS	18 h	$CD2^+$, $CD16^+$ $CD5^-$, DR^- $CD5^-$, $CD16^+$	Increasing dose of rIL-2 increases lysis of K562 and RDMC (NK-resistant). $CD5^+$ cells did not lyse RDMC. $CD16^+$ cells had high lysis of RDMC
4	PBL	Percoll Rosette	PP-TCGF	10% HuAB serum	6 days	Smaller than NK; larger than T; SRBC $ERFC^-$	Modified Percoll allowed some distinction between NK and pre-LAK $ERFC^-$, but no enrichment of LAK
9	PBL	FACS	rIL-2 50 U/ml	20% HuAB serum	18 h	$CD16+/Leu7^-$ $CD16+/Leu7^+$; mitomycin-C- and radioinsensitive; cyclohexamide-sensitive	$CD16^+$ and $Leu7^-$ or $Leu7^+$ both lysed K562, $CD16^-$ did not. CD 16$^+$ cultured with IL-2 generate increased lysis of three NK-resistant cell lines (CEM, RAJI, SB)
7	PBL Non-adh.	AB + C FACS	rIL-2 10 U/ml	10% autol. serum	3 days	$CD3^-$, $CD4^-$ $CD8^\pm$, $Leu7\pm$ $CD16^+$ $CD3^-$, $CD4^-$ $CD8^\pm$, $Leu7^\pm$ $CD16^+$	$Leu7^+$ (FACS) fresh NK but no LAK lysis against uncultured melanoma. Most lytic pre-LAK is $CD16^+/Leu7^-$
8	PBL	Percoll FACS	rIL-2 10–1000 U/ml	10% autol. serum	3 days	High density$^+$ Radiosensitive $CD16^+$ With IFN: (a) $Leu7^+$, $CD8^+$ (b) $CD3^-$, $CD4^-$	Pre-LAK found with NK in gradient. Lysis increases with increasing IL-2 concentration (fresh melanoma target). With IL-2 (10 U/ml) + IFN (200 U/ml) $Leu7^+$ and $CD8^+$ cells generate LAK in 3 days of culture
17	PBL Non-adh.	Percoll FACS	rIL-2 1000 U/ml	5% horse serum	7 days	$CD5^-/Leu19^+$	$CD5^-/Leu19^+$ predominant precursor of lytic activity against both K562 and (NK-resistant) COLO cell line
15	PBL Non-adh.	Percoll 29° C ERFC Panning LDA-LGL	rIL-2 500 U/ml	10% HuAB serum	3–5 days	High density$^+$ $CD16^+$ $CD3^-$	14% cells generated 69% total lytic activity against fresh ovarian, FEMX and MBL2 Tu targets. Limiting dilution analysis shows 10–50-fold increase in number of pre-LAK in the LGL population
2	PBL	FACS clone	rIL-2 100 U/ml	10% calf serum	4 days	$CD16^+$ Clones: $CD16^+$	Majority (61/72) cloned $CD16^+$ cells had LAK activity (fresh melanoma). Minority (5/86) cloned $CD16^-$ cells expressed LAK activity
25	PBL Non-adh.	FACS	rIL-2	10% calf serum		$CD5^-$	Sorted $CD5^+$ cells did not generate LAK. Yet $CD5^+$ LAK effectors were seen at end of culture (fresh melanoma)
23	PBL Non-adh.	Rosette FACS	rIL-2 1000 U/ml	10% HuAB serum	4 days	$CD11^+/CD16^+/$ $LeuM3^-$	Pre-LAK is primarily from $ERFC^-$, $CD11^+/CD16^+/LeuM3^-$ PBL
[a]	PBL Non-adh.	Rosette FACS AB + C	rIL-2 1000 U/ml	10% HuAB serum	3–4 days	$CD11^+$/mAb 211$^+$ With TCGF: mAB 211^-, $Leu19^-$ and also $T3^-$	With rIL-2 pre-LAK is $ERFC^-$, MAB 211$^+$ and primarily $CD11^+$. With LF-TCGF the pre-LAK is more broadly distributed; $T3^-$, 211^- or $Leu19^-$ all generate LAK

[a] This manuscript. *Abbrevations:* Ca pt, cells from cancer patient; Ca + N, cells from cancer patients and normal controls; TDL, thoracic duct lymphocytes; BM, bone marrow; LNC, lymph node cells; AB + C, antibody plus complement; ERFC, erythrocyte rosette forming cells; LDA-LGL, limiting dilution analysis of large granular lymphocytes; LF-, lectin free; PP-, partially purified

non-classic T cell population [5, 6, 11, 21]. Recent publications, including several from this laboratory, have disputed that observation and have proposed that the majority of activity resides in the non-T, non-B, null cell population, which also contains NK activity [2, 7–9, 14–18, 23, 26, 28]. Table 8 is an outline of published studies in chronological order that have attempted to define the LAK precursor. It is clear that some of the discrepancies between these studies can be attributed to the source of IL-2 used by those investigators. The early studies of Grimm et al. were performed with crude, delectinated supernatants or partially purified IL-2 [4]. These sources of IL-2 most probably contained additional lymphokines as well as trace amounts of lectin, while recent studies have all been performed with rIL-2. The use of fetal bovine serum or horse serum for such studies is also problematic since such additives have been shown to activate lysis of target cells without the addition of exogenous IL-2 [3].

In an attempt to define better the precursor population that mediates LAK activity we set out to identify a monoclonal antibody that might block or eliminate the functional activity defined as LAK. The work presented here further elucidates the precursor population responsible for LAK generation in rIL-2. The mAb 211, while not solely specific for LAK precursors, identifies and can deplete with complement up to 99% of the precursor population as determined by generation of LAK activity in a 3-day assay. In addition, positive selection of the 211-positive population always correlated with the great majority of lytic activity. While this establishes the expression of the antigen 211 on the predominant precursor of rIL-2-induced LAK cells, the double-labeled sorting studies extend our description of this cell to that subpopulation which coexpresses the CD11 determinant. The finding that cells treated with 211 plus complement, depleted of rIL-2-responsive LAK precursors, can generate substantial LAK activity with TCGF demonstrates that distinct populations of precursors for LAK activity exist, with distinct mechanisms of activation and spectrum of recognition.

Acknowledgements. The authors wish to thank Drs. James J. Mule and Jeffrey S. Weber for their critical review of the manuscript and Dr. Kevan Roberts for constructive discussions during the progress of these studies.

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Received October 5, 1988/Accepted January 18, 1989