Interleukin 15 Induction of Lymphokine-activated Killer Cell Function against Autologous Tumor Cells in Melanoma Patient Lymphocytes by a CD18-Dependent, Perforin-related Mechanism

Ana M. Gamero, Dewayne Ussery, Douglas S. Reintgen, Christopher A. Puleo, and Julie Y. Djeu

ABSTRACT

Interleukin 15 (IL-15) is a novel cytokine that shares no homology with IL-2, but it requires the use of β and γ chains of the IL-2 receptor complex for binding and signaling. In vitro studies have shown induction of CTL and lymphokine-activated killer (LAK) cell activity in peripheral blood mononuclear cells (PBMCs) from normal donors by IL-15 against known tumor targets. The present study attempts to define the role of IL-15 in generating LAK activity from melanoma patient lymphocytes. PBMCs of patients newly diagnosed with metastatic melanoma were incubated with different doses of recombinant human IL-15 and tested against autologous tumor cells, LAK sensitive cell lines (i.e., FMEX and Daudi), as well as the natural killer-sensitive cell line K562, in a 15-h 51Cr release assay. The effect of IL-15 was found to be both time and dose dependent, with peak activity detected after 2 or 3 days of culture with 100 ng/ml of this cytokine. LAK and not CTL activity in patient PBMCs was detected by the inability of mAbs against CD4, CD8, and MHC class I to effectively block lysis of autologous tumor and FMEX melanoma cells. In addition, interaction via the CD18 adhesion molecule was shown to be critical in IL-15-induced LAK-mediated lysis of autologous tumor cells. Finally, incubation of patient PBMCs with IL-15 for 6 h resulted in the up-regulation of perforin mRNA transcription. These findings suggest that LAK activity can be generated from melanoma patient PBMCs in the presence of IL-15 to lyse autologous tumor cells in a non-MHC-restricted manner. This new cytokine may play an important role in antitumor immunity with a possible use for cancer immunotherapy.

INTRODUCTION

Melanoma is one form of cancer with new cases being diagnosed every year in the United States, and, despite extensive therapy, the survival rate after 5 years is only 2% (1). Cancer patient lymphocytes have been shown to have an impaired immune response, which might in turn contribute to the development of tumor growth. Some of the contributing factors include decreased production of cytokines (2), tumor-suppressing factors (3), suppressor T cells, and macrophages (4, 5). Improved forms of therapies to enhance the antitumor response and overcome the immunosuppressive environment are thus necessary. LAK cells may play an important role in immune surveillance against NK-resistant solid tumor cells without MHC restriction (6). LAK cells are NK cell-like lymphocytes of phenotype CD16+CD3+ with a small population of CD16+CD3+ CTLs (7). IL-2 can drive the activation of human peripheral blood lymphocytes to become LAK cells (8) and induce tumor regression in numerous animal models (see review in Ref. 9). Peripheral blood lymphocytes from patients with melanoma when incubated with IL-2 have been shown to generate LAK activity to kill fresh and cultured autologous tumors (10, 11). Adoptive immunotherapy with IL-2 alone or in combination with LAK has been used extensively in the treatment of adult renal cell carcinoma, melanoma, and colon and lung carcinoma (12–14). The efficacy of this therapy, however, is less than optimal. For example, IL-2 treatment of malignant melanoma patients has resulted in 20–30% regression but with few long-term complete remissions (14, 15). Furthermore, in vivo IL-2 administration has resulted in severe toxic side effects in these patients (12), which has restricted the usefulness of this cytokine, and at present only the treatment of a few cancer types (e.g., renal cell carcinoma) is approved. The search for other cytokines that can augment antitumor response with less clinical toxicity is currently being pursued; among these are IL-12 (16), granulocyte-macrophage colony-stimulating factor (17), and IFN-γ (18).

A new cytokine that might enhance an antitumor response is IL-15. IL-15 is a novel M, 15,000 cytokine, the activity of which was first identified in the supernatants of a monkey epithelial cell line, CV-1/EBNA (19). The expression of IL-15 mRNA has been detected in the placenta, skeletal muscle, kidney, and activated monocytes/macrophages (19). IL-15 shares many of the biological activities of IL-2, including induction of proliferation of phytotohexagglutinin-stimulated normal PBMCs, NK cells, B cells (19–21), and generation of CTL and LAK cells in vitro (19, 20). Also, IL-15 can induce production of IFN-γ, tumor necrosis factor α, and granulocyte-macrophage colony-stimulating factor in activated NK cells from normal individuals and synergizes with IL-12 to enhance NK activity (20).

IL-15 has been shown in transfection studies to interact with the β and γ subunits of the IL-2R complex, which is required for binding and signaling (22). The bioactivity of lymphocytes in response to IL-15 requires the β but not the α subunit of IL-2R, making IL-15 the only cytokine other than IL-2 able to utilize the IL-2Rβ chain (19, 20, 22). Although IL-15 shares some bioactivities and receptors with IL-2, they share little homology in their sequences. Thus, the potential use of IL-15 for cancer immunotherapy needs to be studied further. The present study explores the ability of IL-15 to induce LAK activity in lymphocytes from metastatic melanoma patients to kill autologous tumor cells obtained from fresh tumor biopsies. This reactivity was compared with that against other LAK-sensitive tumor cells, including K562, FMEX, and Daudi. The lytic mechanism induced by IL-15 in patient LAK cells was demonstrated to involve augmentation of perforin gene transcription and requires the participation of the adhesion molecule CD18.

MATERIALS AND METHODS

Tumor Cell Preparation and Culture Conditions. Surgical specimens were obtained with informed consent from patients newly diagnosed with metastatic melanoma without prior treatment at H. Lee Moffitt Cancer Center (Tampa, FL). Briefly, tumor specimens from the primary tumor site or involved lymph nodes were washed in RPMI 1640 (GIBCO-BRL, Grand Island, NY) and dissociated manually into single cell suspensions with surgical scalpels (24). RBC and cell debris were removed by centrifugation over a layer
of Ficoll-Hypaque (Pharmacia, Piscaway, NJ). The interface layer containing viable tumor cells was collected, washed twice with PBS (M. A. Biologies, Walkersville, MD), and cultured in complete medium consisting of RPMI 1640 containing 10% FCS, 2 mM L-glutamine, 10 units/ml penicillin, 100 μg/ml streptomycin (GIBCO-BRL), 10 mM sodium pyruvate, and 10 mM nonessential amino acids (Sigma Chemical Co.). Cell cultures were maintained in monolayers and passaged by brief trypsinization with 500 μg/ml trypsin and 200 μg/ml EDTA (Sigma).

Preparation of Human PBMCs. Heparinized whole blood (30 ml) from patients newly diagnosed with active metastatic melanoma disease (stage II and III) at H. Lee Moffitt Cancer Center and Buffy coats from normal volunteers at the Southwest Florida Blood Bank were obtained with informed consent. Samples were diluted 1:2 in PBS and layered on 12 ml of Ficoll-Hypaque solution. After centrifugation at 400 × g for 20 min at room temperature, the interface band of PBMC was collected and washed twice with PBS. The collected PBMCs were then resuspended in RPMI 1640 containing 5% heat-inactivated human AB serum (Biocell Laboratories, Carson, CA), 2 mM L-glutamine, 10 units/ml penicillin, 100 μg/ml streptomycin, and 5 mM/liter HEPES buffer (GIBCO-BRL). Plasticware was purchased from Costar (Cambridge, MA). All media and reagents contained less than 0.1 ng/ml of endotoxin as determined by the Limulus Amoebocyte Lysate assay (M. A. Biologies) to avoid nonspecific activation of PBMC.

Measurement of Cytotoxicity. A 15-h 51Cr release assay, as described elsewhere (25), was used to measure the cytotoxicity of IL-15-activated patient PBMC against tumor cell targets because 4 h was inadequate to detect lysis, particularly of freshly isolated patient tumor cells. Briefly, 96-well, round-bottomed microtiter plates were set up in triplicate wells with 100 μl of PBMC at various concentrations to achieve E:T ratios of 100:1, 50:1, and 25:1. rhIL-15 (kindly provided by Immunex Corp., Seattle, WA) was added to PBMC in concentrations ranging from 3 to 100 ng/ml. The cells were then incubated at 37°C in 5% CO2 for 1, 2, or 3 days. Autologous tumor cells and 3 LAK target cells (K562, an erythroleukemia NK-sensitive tumor cell line; FMEX human melanoma; and DuAd B-cell lymphoma NK-resistant cell lines) were labeled with 100 μCi of Na251CrO4 (Amersham, Arlington Heights, IL) for 1 h at 37°C. The cells were washed twice with PBS, and 100 μl were added to effector cells at 5 × 103 cells/well. After 15-h incubation at 37°C, 100 μl of supernatant was collected, and radioactivity of the 51Cr released from target cells was measured by a γ counter. The percent of specific lysis was calculated by the formula: (Experimental cpm - spontaneous cpm/maximal cpm incorporated) × 100. Of the maximal label incorporated into the cells, the spontaneous release ranged from 10 to 16% for K562, DuAd, and FMEX cell lines and 15 to 25% for autologous tumor cells. Statistical analysis was performed using the Student's t test.

mAb-blocking Experiments. The mAbs used were anti-CD18, RH1-38 (IgG1, a gift of Dr. Robert Hall, Guthrie Research Institute, Sayre, PA), anti-CD4 (IgG1), anti-CD8 (IgG1), anti-MHC class I (IgG2a purchased from Immunotech, Westbrook, ME), and isotype control IgG2a (Sigma). The antibodies, each at a final concentration of 10 μg/ml, were added to the PBMC for 15 min before the addition of 51Cr-labeled target cells. The assay was completed as described earlier.

Total RNA Isolation and Northern Blot Analysis. Total cellular RNA from 2 × 107 PBMCs incubated with medium or with 50 ng/ml of rhIL-15 or rhIL-2 (Hoffman-LaRoche, Nutley, NJ; specific activity, 2 × 107 units/mg) was isolated according to the method of Chomczynski and Sacchi (26). Twenty μg of total RNA were fractionated on a 0.8% agarose gel and transferred to Nytran filters (Schleicher & Schuell, Keene, NH). Prehybridization was performed at 45°C in a 50% deionized formamide solution. Hybridization was done by overnight incubation using a 2.9-kb 32P-labeled human perforin cDNA (kindly provided by Dr. E. Podack, University of Miami, Miami, FL). After hybridization, the blots were washed at 60°C with 1X SSC (150 mM NaCl-15 mM sodium citrate), 0.2% SDS, and 1 mM EDTA, followed by exposure to Kodak XAR film at −70°C. The membrane was stripped and reprobed with [α-32P]glyceraldehyde 3-phosphate dehydrogenase cDNA (1.4 kb) from Clontech Laboratories (Palo Alto, CA) to check for equal loading of RNA.

RESULTS

To determine whether IL-15 could generate cytolytic activity in cancer patient lymphocytes, PBMCs obtained from metastatic melanoma patient blood were stimulated with increasing doses of rhIL-15 between 3 and 100 ng/ml, followed by an incubation period of 1, 2, or 3 days in endotoxin-free medium supplemented with 5% human AB serum to avoid nonspecific stimulation by FCS. The cytolytic activity and the optimal time for activation of these PBMCs was first tested against FMEX, a LAK-sensitive melanoma tumor cell line, at an E:T ratio of 25:1 in a 15-h 51Cr release assay. Fig. 1 shows data from a representative experiment of 2 that were performed with similar results. As shown, IL-15 activation of PBMC was detected as early as day 1, and IL-15 was able to activate patient PBMC in a dose-dependent fashion, with peak activity taking place between days 2 and 3. Lytic activity of PBMC after incubation with 3 ng/ml of IL-15 was not significantly different from that of medium control, but 10 ng/ml of this cytokine was able to induce a small but definite increase in cytotoxicity on all 3 days tested. When PBMC were stimulated from 10 to 30 ng/ml of IL-15, FMEX lysis increased from 7 ± 1.7% to 12 ± 1.4% at day 2 and 9.4 ± 0.7% to 17 ± 2.8% lysis at day 3, respectively. However, the greatest lysis was observed with 100 ng/ml of IL-15, resulting in 29 ± 3.5% and 30 ± 5.4% lysis of FMEX for days 2 and 3, respectively.

Activation of PBMCs by IL-15 was studied in 11 melanoma pa-
Patients to investigate the effectiveness of IL-15 (Fig. 2). PBMCs from all patients when cultured in the presence of 100 or 50 ng/ml of IL-15 for 3 days had enhanced lytic function, which is markedly above that of untreated lymphocytes. At the E:T ratio of 25:1, the specific lysis was highest in patient H (29.6 ± 2.39%) and lowest in patient C (5.6 ± 1.53%) after IL-15 exposure. We were able to retest the PBMCs from patient C on a different occasion with 50 ng/ml of IL-15, which also resulted in augmented cytolytic function with the specific lysis of 11 ± 1.2% above that of medium control.

Because IL-15 utilizes the β and γ subunits of the IL-2 receptor to stimulate lymphocytes, it was of interest to investigate whether IL-15 and IL-2 had comparable efficiency in activating PBMCs from melanoma patients. Fig. 3 shows data from one patient whose PBMCs were stimulated with increasing doses of IL-15 (Fig. 3a) or IL-2 (Fig. 3b), and their cytolytic function was determined by FMEX cell lysis at day 3, with E:T ratios that ranged from 100:1 to 12.5:1. As shown, PBMC activation by either cytokine was dose dependent; however, the level of lytic activity induced by IL-15 was only comparable to that of IL-2 at 100 ng/ml and not at the lower concentrations. This result was reproducible in a second set of experiments (data not shown).

The next step was to examine whether IL-15 could activate PBMCs from melanoma patients to lyse autologous melanoma tumor cells. The cytolytic function was evaluated at day 2 or 3 based on data obtained above with FMEX cell lysis, and the E:T ratios ranged from 25:1 to 100:1, depending on availability of patient PBMCs. PBMCs from two patients, stimulated with increasing doses of IL-15, showed autologous antitumor activity in a dose-dependent manner (Fig. 4).

Patients A and B had 2.1 ± 1.87% and 6 ± 3.7% lysis when cultured in medium alone. Exposure of PBMCs to 3 and 10 ng/ml of IL-15 did not increase the cytotoxicity against autologous tumor targets significantly above that of medium control at the E:T ratio of 25:1 or 50:1. However, increasing IL-15 from 30 to 100 ng/ml enhanced lysis of autologous tumor cells at day 2 in patient A from 16 ± 0.1% to 35 ± 2.7%, and at day 3, in patient B from 15 ± 1.1% to 21 ± 1.9% at the 50:1 E:T ratio. Increasing the E:T ratio to 100:1 resulted in higher lytic activity in patient B. On the basis of these findings, cytotoxicity in subsequent experiments was measured after 3 days of incubation in IL-15 at 50–100 ng/ml.

We next compared the level of killing of the autologous tumor cells and the standard laboratory cell line FMEX in the PBMCs from the same patient. The E:T ratios for autologous tumor cells and LAK-sensitive tumor cell lines were kept at 100:1 and 25:1, respectively, whenever possible, to obtain comparable levels of lysis. More ratios were performed when cell availability permitted. PBMCs from two pairs of patients were activated for 3 days at 37°C with 50 and 100 ng/ml of IL-15, respectively (Fig. 5). At the E:T ratio of 25:1, lysis of autologous tumor targets in PBMCs from patients C and D was barely
detectable, whether they were treated or untreated with 50 ng/ml of IL-15. Only in patient A, whose PBMCs were treated with 100 ng/ml of IL-15, was higher cytotoxicity detected above background. However, when PBMCs from patients were tested at the higher E:T of 100:1, marked elevation of lysis of autologous tumor cells was observed in all 4 patients. At the E:T ratio of 25:1, all 4 patients had detectable LAK activity against FMEX cells. It should be noted that the level of LAK activation against autologous cells does not usually coincide with the level against FMEX target cells in the same patient (e.g., patient B, who had the highest efficiency of LAK function ever, when PBMCs from patients were tested at the higher E:T of 100:1, marked elevation of lysis of autologous tumor cells was observed in all 4 patients. At the E:T ratio of 25:1, all 4 patients had detectable LAK activity against FMEX cells. It should be noted that the level of LAK activation against autologous cells does not usually coincide with the level against FMEX target cells in the same patient (e.g., patient B, who had the highest efficiency of LAK function against autologous tumor cells, did not show the highest level of killing of FMEX cells among the 4 patients tested).

To demonstrate that IL-15-induced lysis of tumor targets occurred in a non-MHC-restricted fashion, IL-15-activated PBMCs were tested against two standard LAK-sensitive cell lines (K562 and Daudi), as well as FMEX tumor cells for comparison. Fig. 6 shows lytic activity of three patient PBMCs stimulated with 50 ng/ml of IL-15 for 3 days at the E:T ratio of 25:1. Specific lysis of these PBMCs against K562 were 20 ± 2.2%, 13 ± 0.8%, and 14 ± 1.2%, respectively (Fig. 6a); against Daudi, 18 ± 3.0%, 11 ± 1.2%, and 23 ± 5.1%, respectively (Fig. 6b); and against FMEX, 12 ± 0.9%, 24 ± 4.1%, and 11 ± 1.2%, respectively (Fig. 6c). No significant lysis was found against these three tumor targets by unstimulated PBMCs.

Because FMEX is a LAK-sensitive melanoma cell line that expresses MHC class I antigens on its surface, it was necessary to distinguish that lysis of FMEX by activated PBMC was through LAK cells and not by existing specific CTLs already present in the patient blood to kill in a MHC-restricted fashion. To rule out this possibility, blocking mAbs against CD4, CD8, and MHC class I molecules were added to the lytic assay to examine their ability to inhibit cytotoxic function. Experiments from two melanoma patients demonstrate that killing of FMEX was not inhibited by these antibodies (Fig. 7a).

Additional experiments performed with autologous combinations indicated similar results, except for anti-MHC class I, which moderately inhibited lysis in patient C (Fig. 7b). Note that patient C was tested 3 months after he was assessed in Fig. 5 and still showed IL-15-enhanced lytic function. To demonstrate that IL-15 induction of LAK activity in patient PBMC parallels the response seen in normal PBMCs, two healthy donor PBMCs were also tested. Fig. 7c shows that IL-15 also activated normal PBMCs to lyse FMEX cells in a CD4- and CD8-dependent manner.

Cytolytic activity depends on the recognition and binding of the effector cell to the tumor target cell. The adhesion molecule CD18 has been shown by our group and others to play a critical role in target lysis by NK and LAK cells (23, 25, 27, 28). Thus, CD18 participation in the lysis of allogeneic and autologous tumor targets by IL-15-activated patient PBMCs was next examined. Addition of anti-CD18 antibody, RH1–38, at the start of the experiments markedly inhibited lysis of FMEX tumor cells (Fig. 7a) and autologous tumor cells (Fig. 7b). Killing of FMEX tumor cells decreased from 20 ± 2.4% to 10 ± 2.2% in patient F and from 34 ± 1.5% to 8 ± 0.4% in patient C. Similarly, lysis of autologous tumor cells decreased from 14 ± 1.2% to 6.3 ± 1.5% in patient F and 19.5 ± 5.0% to 1 ± 0.1% in patient C. Similar results were obtained with healthy donor PBMCs against FMEX target cells (Fig. 7c). mAbs, anti-CD4 IgGl, and anti-CD8 IgGl served as isotype-matched controls to RH1–38 IgGl against CD18.

The antitumor immune response by which IL-15 induces cytolytic function in metastatic melanoma patient PBMCs might be mediated by pore formation involving perforin, a granular protein responsible in cell lysis in NK and CD8+ CTL cells (29). To study the effect of IL-15 on perforin mRNA expression, total RNA from patient PBMCs incubated with IL-15 was examined by Northern blot analysis. Basal levels of perforin mRNA could be detected in PBMCs incubated in medium alone in all three patients tested. However, culturing of PBMC in the presence of IL-15 for 6 h caused a dramatic increase in the expression of perforin gene transcription (Fig. 8). In addition, normal donor PBMCs incubated with IL-15 or IL-2 for 6 h also resulted in a comparable increase in perforin mRNA levels. This shows that IL-15, like IL-2, is able to augment perforin gene expression in PBMCs, wherein both cytokines share a similar lytic mechanism.
GENERATION OF LAK IN MELANOMA PATIENT PBMC BY IL-15

K562

DAUDI

FMEX

Fig. 6. Non-MHC-restricted lysis of tumor cells by IL-15-activated patient PBMCs. Melanoma patient PBMCs from 3 patients (PAT D, PAT E, and PAT C) were incubated with 50 ng/ml of IL-15 for 3 days and then tested against (A) K562, (B) Daudi, and (C) FMEX tumor cells in a 15-h 51Cr release assay at the E:T of 25:1. Columns, percent specific lysis of triplicate determinations; bars, SEM. *, significantly different from medium control (P < 0.05).

DISCUSSION

The results from these studies clearly demonstrate the ability of IL-15 to induce LAK activity in PBMCs from metastatic melanoma patients to kill LAK-sensitive tumor targets as defined by the lysis of K562, Daudi, and FMEX tumor cells. But more important, these activated PBMCs were able to lyse autologous melanoma tumor cells prepared from fresh surgical tumor biopsies by a lytic mechanism mediated by CD18 and perforin. Patient PBMCs incubated with 100 ng/ml of IL-15 resulted in maximal lytic activity, which was evident 2 and 3 days later when testing against the melanoma cell line FMEX at the E:T ratio of 25:1 and against autologous tumor targets at the E:T ratios of 100:1 and 50:1 as measured by a 15-h 51Cr release assay. The IL-15 response in these PBMCs appeared to be both time and dose dependent. This response could be shown in 11 patients wherein IL-15 stimulation resulted in enhanced cytolytic activity against FMEX at the E:T ratio of 25:1. PBMCs from one patient, patient C, when tested on two different occasions after stimulation with IL-15, also resulted in LAK activity against FMEX, as well as the autologous tumor targets.

Long-term established tumor cell lines represent a useful tool in the laboratory to measure NK/LAK activity in lymphocytes. We questioned whether the level of cytotoxicity detected against autologous tumor cells differed from that against the standard laboratory cell line, FMEX. All 4 patient PBMCs activated with IL-15 that we examined had significant LAK activity against FMEX tumor cells but variable LAK activity against the autologous tumor cells at the E:T ratio of 25:1. However, increasing the E:T ratio to 100:1 resulted in markedly significant lysis against the autologous tumor cells. Nevertheless, the level of efficiency against FMEX cells did not usually reflect that against autologous tumor cells in each individual, even when the E:T ratio was adjusted higher in the autologous combination. Thus, use of

Fig. 7. CD18-dependent lysis of tumor cells by IL-15-generated LAK cells in patient PBMCs. PBMCs from 2 melanoma patients (F and C) were incubated with 50 ng/ml of IL-15 for 3 days. Anti-CD4, anti-CD8, anti-CD18, and anti-MHC I were added at a final concentration of 10 μg/ml prior to the addition of tumor targets (A) FMEX and (B) autologous tumor cells, followed by a 15-h 51Cr release assay at the E:T of 100:1. C, the same experiment was performed using normal donor PBMCs against FMEX tumor cells. Columns, percent specific lysis of triplicate determinations; bars, SEM. *, significantly different from IL-15 alone (P < 0.05).
autologous tumor cells might provide a better assessment of antitumor effect in patient PBMCs induced by IL-15.

The lytic activity induced in melanoma patient PBMCs by IL-15 was non-MHC restricted and CD18 dependent for the cytolytic process. The non-MHC-restricted lysis was first demonstrated by examining killing of 3 unrelated LAK-sensitive tumor targets: K562, Daudi, and FMEX. All three patients tested exhibited significant lysis above background levels against these 3 targets cells. In addition, LAK activity was distinguished from CTL activity by measuring the killing of the autologous tumor cells and FMEX cells in the presence of anti-CD4, anti-CD8, and anti-MHC I mAbs, which failed to effectively inhibit lysis. It is worth mentioning that in one of the patients, the killing of autologous tumor cells appeared to be moderately reduced by anti-MHC class I. However, the same patient was able to kill K562 tumor cells, which lack expression of MHC class I molecules (data not shown). Induction of LAK activity was also confirmed by testing IL-15-treated healthy donor PBMCs to lyse FMEX cells, which resulted in similar data. Second, initiation of the cytolytic process requires contact of the tumor cells with the effector cells, and an important component is the interaction of ICAM-1 on target cells with CD11/CD18 on NK, IL2-induced LAK, and CTL (23, 27, 28, 30–32). We now show that CD18 is also critical in the cytolytic process against autologous and allogeneic melanoma tumor lines in the IL-15-induced LAK mechanism. Addition of the blocking antibody against CD18, RH1-38 (31), markedly reduced LAK lysis of both tumor cell types.

Once the effector cell recognizes its target, a lethal hit is delivered, which involves perforin. The key role of perforin in cell-mediated cytolysis is clearly demonstrated in perforin knock-out mice, which exhibit impaired CTL and NK functions (33). Perforin, when released from storage in the cytoplasmic granules in activated CTL, NK, and LAK cells, polymerizes in the target membrane via a Ca$^{2+}$-dependent pathway, causing membrane perforation and cell death (29). IL-2 and IL-12 are able to augment perforin mRNA expression in NK and CD8$^+$ T cells (34–36). Our results add yet another cytokine, IL-15, which can induce up-regulation of perforin mRNA transcription not only in normal PBMCs but also in patient PBMCs. These findings demonstrate that, like IL-2 and IL-12, IL-15 uses the perforin cytolytic system to eliminate tumor cells.

The identification of novel cytokines that might enhance antitumor immunity is of primary interest because of their potential use in immunotherapy of cancer patients. IL-15 shares a number of biological properties with IL-2, but differences exist that should be emphasized. IL-15 is expressed in a variety of tissues, including activated monocytes/macrophages (19), whereas IL-2 is expressed and produced only by T cells. IL-15 utilizes only the ß and γ subunits of the IL-2 receptor complex without requiring the use of the α subunit to exert its biological activities (22). In comparing IL-15 to IL-2 for activation of patient PBMCs against FMEX, however, we found that the level of killing obtained with IL-15 was not similar to that with IL-2 at equivalent concentrations tested. It has been suggested that IL-15 may also utilize an additional non-IL-2-related receptor subunit, as demonstrated by the inability of the murine IL2/IL-3-dependent 32D cell line and the triple negative (CD3−CD4−CD8−) thymocytes, both of which express the high affinity IL-2Ra,ß,γ complex, to respond to IL-15 (19, 22). Studies on the murine L929 cells stably expressing the human IL-2R α and ß chains also support the presence of another component of the IL-15R. L929 cells transfected with the αβ or the αβγ combinations of the IL-2R subunits can bind IL-15 with similar affinities (22). The combination of IL-15 and IL-2 when used at equal concentrations does not result in synergy, and the two cytokines are only additive when used at suboptimal amounts (20), indicating the usage of the same receptor. Our findings support that IL-15 is another cytokine that can drive PBMCs to acquire LAK activity. More important, IL-15 can generate an antitumor LAK activity in cancer patient lymphocytes to kill autologous melanoma tumor cells. Thus, IL-15 might represent a potential cytokine with future clinical applications for cancer immunotherapy.
REFERENCES


Interleukin 15 Induction of Lymphokine-activated Killer Cell Function against Autologous Tumor Cells in Melanoma Patient Lymphocytes by a CD18-Dependent, Perforin-related Mechanism

Ana M. Gamero, Dewayne Ussery, Douglas S. Reintgen, et al.


Updated version  Access the most recent version of this article at:  http://cancerres.aacrjournals.org/content/55/21/4988

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.