Motility and Tumoricidal Activity of Interleukin-2-stimulated Lymphocytes

Stuart Ratner and Gloria H. Heppner

E. Walter Albachten Department of Immunology, Michigan Cancer Foundation, Detroit, Michigan 48201

ABSTRACT

The motility of murine splenic lymphocytes stimulated nonspecifically by recombinant interleukin 2 (RIL-2) was studied in a three-dimensional collagen-gel system. Nonadherent BALB/c splenic lymphocytes were cultured in medium containing Cetus RIL-2 (700 to 1000 units/ml) or excipient control. They were then allowed to locomote randomly for 16 to 18 h into slabs of type 1 rat tail collagen gel. The gels were digested with collagenase, and total lymphocyte populations and motile subpopulations were collected and compared with respect to their lymphokine-activated killer activity (measured as 4-h cytotoxicity against the natural killer-resistant mammary adenocarcinoma line 410.A), their natural killer activity (measured as 4-h cytotoxicity versus lymphoma YAC-1), and their subset distribution (defined by immunofluorescence). Some of the slabs were not digested but fixed for measurement of leading-front distance. RIL-2-stimulated lymphocyte populations displayed greater motility than unstimulated populations; the mean leading front distance was 2.4 times greater, and the percentage of cells exhibiting motility was approximately doubled. The most motile RIL-2-stimulated cells, however, were not the most tumoricidal. Motile subpopulations displayed approximately 25 to 60% lower lymphokine-activated killer activity than did the total populations from which they were derived. Natural killer activity followed a similar pattern. Motile subpopulations contained a lower proportion of asialo-GM1* and T-null cells than did total populations and a higher proportion of IJ'T4* cells. Chemokinetic stimulation with α-interferon increased overall motility, but the lymphokine-activated killer activity of the motile subpopulation was still lower than that of the total population. Lymphocyte motility is important in the infiltration of tumors and other inflammatory lesions. The results indicate that the most tumoricidal lymphocytes in RIL-2-stimulated populations may not be the best tumor infiltrators, and that the tumoricidal activity of circulating lymphocytes may be a misleading indicator of the effectiveness of immunotherapy.

INTRODUCTION

Lymphocyte populations nonspecifically stimulated in vitro with high titers of RIL-2 acquire tumoricidal capabilities (1). NK activity is usually present in these populations (2, 3), but the most distinctive property is LAK activity: the ability to lyse a variety of NK-resistant targets, including fresh, autologous solid tumor cells, without previous sensitization (4). RIL-2-stimulated lymphocytes have proven to be effective antitumor effectors in a variety of animal immunotherapy models (5–8), and clinical trials are in progress (9, 10).

In adoptive immunotherapy, lymphocytes infused into a tumor-bearing host must escape from trapping in irrelevant capillary beds, extravasate at appropriate points, and penetrate into primary or metastatic tumor masses. Since these steps require random or directed movement (11–13), the efficiency of adoptive immunotherapy must depend in part upon the motility of the infused lymphocytes. It is therefore important to determine how RIL-2 stimulation affects lymphocyte motility.

RIL-2-stimulated lymphocyte populations are heterogeneous with respect to phenotype and tumoricidal activity (14–17). This heterogeneity complicates the measurement of motility; the collective motility of the total population may not accurately reflect that of the tumoricidal subpopulation. In this study, we measured the random motility of RIL-2-stimulated murine splenocytes in a collagen-gel system that permits retrieval of cells for analysis of phenotype and function. We found tumoricidal capability and motility to be independently, and perhaps inversely, regulated.

MATERIALS AND METHODS

Experimental Protocol. Fig. 1 summarizes the experimental protocol. Briefly, nonadherent BALB/c splenocytes were freed of erythrocytes and nonviable cells and cultured 72 to 80 h in the presence or absence of RIL-2. Viable cells were then purified and allowed to locomote randomly for 16 to 18 h in a three-dimensional collagen-gel motility assay in the presence or absence of 20 units/ml of RIL-2. In this system, the lymphocytes locomoted into hydrated gels of type I collagen. In some assay wells, the gels were fixed, and the distance traveled by the leading front cell was measured. In other wells, the gels were removed and enzymatically dissociated to yield either total populations (all lymphocytes in the system) or motile subpopulations (lymphocytes which had locomoted into the gel). After removal of nonviable cells, the total populations and motile subpopulations were compared with respect to tumoricidal activity and subset distribution. All techniques are described in detail below.

Lymphokines. RIL-2 was a gift of Cetus, Emeryville, CA. rIFN-α/α- D was donated by Hoffman-LaRoche, Nutley, NJ. This preparation has been shown to exert immunostimulatory effects on murine lymphocytes (18).

Lymphocyte Culture. Murine splenic lymphocytes were stimulated with RIL-2 by a procedure modified from that of Lafreniere and Rosenberg (19). Female BALB/c mice 2 to 5 mo of age were sacrificed by cervical dislocation, and their spleens were removed aseptically and pressed through sterile stainless-steel mesh into dishes of PBS-CS. Debris was removed by sedimentation, and the resulting cell suspension was depleted of erythrocytes and nonviable cells by centrifugation at 400 × g over 2-cm cushions of Ficoll-Hypaque (Histopaque 1077, Sigma, St. Louis, MO). The cells were washed twice and incubated 1 h at 37°C in disposable tissue culture dishes (Corning, Corning, NY). Nonadherent cells were cultured at an initial density of 2.5 to 4.0 × 10^6/ml in what will be referred to as “complete DME” (DME containing 10% FCS, 1 mm sodium pyruvate, 50 μM 2-ME, modified Eagle’s nonessential amino acid mixture, 2 μm L-glutamine, 100 μg/ml of streptomycin, 100 units/ml of penicillin, and 0.5% Fungizone [all components purchased from Grand Island Biological Corporation, Grand Island, NY, except 2-ME (Kodak, Rochester, NY) and FCS (KC Biological, Lenexa, KY)]). The cultures also received either 700 to 1000 units/ml of RIL-2 or Cetus excipient control. Culture was at 37°C, 10% CO2 in supine 75-cm2 tissue culture flasks (Corning), with each flask containing 30 to 60 ml of lymphocyte suspension. At the end of the culture period (72 to 80 h), viability was approximately 40% in the RIL-2-stimulated cultures and 10% in the control cultures.

Lymphocyte Motility Assay. This assay has been described in detail (20). In the following brief description, capital letters refer to elements of Fig. 1. The assay was performed in tissue culture wells 25 mm in
LYMPHOCYTE MOTILITY AND TUMORICIDAL ACTIVITY

Fig. 1. Schematic of experimental protocol. Key to collagen-based motility assay: A, tissue culture well, 25-mm diameter; B, base layer of type I rat tail collagen gel; C, monolayer of lymphocytes (5 to 7.5 x 10^5 cells) covered with a thin coating of gel; D, collagen-gel overlay, prepolymerized in an 18-mm tissue culture well, then lowered onto lymphocyte monolayer; E, sealing layer of collagen gel which binds components together after saturation with medium.

Overlays removed, fixed. Leading-front distance measured.

Overlays minced, gel digested (Collagenase III - DNAse). Lymphocytes recovered.

Base-overlay combinations minced. Gel digested. Lymphocytes recovered.

Differential toxin release (LAK assay).

Phenotypic distribution analyzed by immunofluorescence, flow cytometry - DR - cytotoxicity assayed by isotope release.

% motile = 2L/T x 100

where L is the mean number of viable cells recovered per overlay. It is multiplied by 2 to account for the approximately equal number of cells which locomote down into the base. T is the mean number of viable cells recovered per unseparated base-overlay combination. After enumeration, total populations and motile subpopulations were enumerated, and the percentage of viable cells exhibiting motility was estimated according to the formula.

Overlays from the third group of wells were individually fixed in 1% PF in serum-free PBS, then stored in PBS. Each overlay was examined at x 200 on the mechanical stage of an inverted phase-contrast microscope (Biostar; American Optical, Buffalo, NY). Fields defined by a reticule eyepiece were selected at random in the central portion of the zone of locomoting cells. At each field, the distance between the bottom of the overlay and the leading cell front was determined by a calibrated fine-focus control. The leading cell front was defined as the first plane at which 3 cells were simultaneously in focus. Measurements were made at six fields in each overlay, with 5 or 6 replicate overlays per experimental condition. For the quantitation of chemotactic and chemokinetic effects, leading-front distance downward, into bases, was also measured. In the absence of exogenous modifiers of motility, upward and downward locomotion are approximately equal (20).

Tumor Cell Lines. The NK-resistant (22) murine mammary adenocarcinoma cell line 410.4 was derived from a spontaneous metastasis of a BALB/cfC3H mouse (23). It is routinely maintained in monolayer culture in Waymouth's medium containing 10% FCS, 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin (100 ¿g/ml). The line is regularly verified to be free of Mycoplasma and the following viruses: pneumovirus; reovirus type 3; Sendai; encephalomyelitis; K polyoma; minute; murine adenosivirus; murine hepatitis; lymphocytic choriomeningitis; and ectromelia (Microbiological Associates, Bethesda, MD). The Moloney virus-induced YAC-1 lymphoma of A/Sn origin (24) was obtained from the American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 medium supplemented with sodium pyruvate (100 nm) and FCS, L-glutamine, and antibiotics as above.

Assay of LAK Activity. LAK activity was measured as 4-h cytotoxicity exerted against the NK-resistant syngeneic mammary tumor line 410.4 in a [3H]proline release assay (25). This assay was chosen over the more commonly used chromium release assay, because the tumor line exhibits very high spontaneous chromium release. Exponentially growing cultures of line 410.4 were washed 6 times with proline-free DME (i.e., complete DME lacking Eagle's nonessential amino acid mixture). Sterile L-[2,3,4,5-3H]proline (approximately 100 µCi/mmol, New England Nuclear, Boston, MA) was added at 5 to 10 µCi/ml. After 24-h culture at 37°C, 10% CO₂, the tumor cells were detached by treatment with 0.25% trypsin-EDTA (Gibco), washed 3 times with PBS-CS, and resuspended at 10^5/ml of complete DME. Aliquots of
100 µl were dispensed into 96-well round-bottomed culture plates (Corning) which were centrifuged at 200 × g for 5 min and incubated overnight. Viable tumor cells attached firmly to the wells during this period. Effector cells suspended in complete DME were dispensed, 100 µl per well, to yield effectortarget ratios of 12.5:1 to 50:1. Control wells received only medium. After another centrifugation, the plates were incubated 4 h at 37°C, 5% CO2, then washed 3 times with PBS-CS to remove nonviable cells and debris. The remaining viable cells were solubilized with 100 µl of 0.3 N NaOH, and the solution was transferred to glass scintillation vials. Dioxane-based scintillation cocktail (RIA-Solv II; RPI Corp., Mt. Prospect, IL) was added, and 3H content was determined by scintillation counting (Packard Tricarb 4640; United Technologies, Downer’s Grove, IL). The percentage of cytotoxicity was calculated as follows.

\[
\frac{\text{mean cpm retained/test well}}{\text{mean cpm released/medium-control well}} \times 100
\]

Four or six replicate wells were prepared for each condition.

Assay of NK Activity. Exponentially growing YAC-1 target cells were washed and suspended at 10^7/ml of serum-free RPMI 1640 culture medium (pH 7.0) containing 100 µCi/ml of ^3H (as sodium carbonate, 200 to 900 Ci/g; New England Nuclear). After 90-min incubation at 37°C, 5% CO2, the cells were washed 3 times in PBS-CS, allowed to incubate an additional 30 min, washed twice more, and resuspended at 2 × 10^7/ml of complete RPMI 1640. The cells were dispensed into round-bottomed, 96-well plates, 100 µl per well. Test wells received an additional 100 µl of medium containing effectors at E:T ratios of 12.5:1 to 50:1; control wells received only medium. The plates were centrifuged 5 min at 150 × g and incubated 4 h at 37°C, 5% CO2. After 8-min centrifugation at 300 × g, 100 µl of supernatant were harvested from each well with a Gilson precision micropipet (Rainin Instrument Co., Woburn, MA) without disturbing the pellet. One set of control wells was used to determine spontaneous release of label; another set was used to determine the amount of label incorporated per well. All samples were placed in 12- x 75-mm plastic snap-cap tubes, and the amount of ^3H in each was determined by direct γ counting (Packard Autogamma 5650). Four replicate wells were prepared for each condition. The percentage of cytotoxicity was calculated as

\[
\frac{\text{Mean cpm released/test well} - \text{mean cpm spontaneously released/control well}}{\text{Mean total cpm incorporated/control well} - \text{mean cpm spontaneously released/control well}} \times 100
\]

Immunofluorescence Analysis of Lymphocyte Subsets. Lymphocytes were incubated 45 min on ice in phenol red free PBS-CS containing one of the following primary antibodies: purified monoclonal rat anti-Thy 1.2 (Becton-Dickinson, Sunnyvale, CA); monoclonal rat anti-L3T4 (purified by ammonium sulfate precipitation from supernatant of hybridoma GK 1.5, gift of Dr. F. Fitch, University of Chicago); monoclonal rat anti-Lyt-2 (purified from supernatant of hybridoma 53-6.72, from American Type Culture Collection); or polyclonal rabbit anti-asGM1 (Wako Chemicals, Dallas, TX). Normal rat IgG and rabbit IgG controls (Jackson Immunoresearch, Avondale, PA) were also run. After 3 washes in PBS-CS, the cells were incubated 30 min on ice in either FITC-conjugated mouse anti-rat immunoglobulin [Fc(β)γ, fraction] or FITC-conjugated goat anti-rabbit immunoglobulin [Fc(β)γ, fraction] (both from Jackson Immunoresearch). After a wash in PBS-CS and 2 washes in serum-free PBS, the cells were fixed 20 min in freshly prepared 1% PF, resuspended in PBS-CS, and stored overnight at 4°C. All solutions contained 0.1% sodium azide. Cells were analyzed for FITC fluorescence on a FACS 440 flow cytometer (Becton Dickinson) with a Consort 40 data analysis system. Excitation was at 488 nm, and emission was collected at 510 to 550 nm.

RESULTS

Lymphocyte Motility Increased by RIL-2 Stimulation. Murine splenic lymphocyte populations cultured in the presence or absence of RIL-2 were freed of dead cells, thoroughly washed, and allowed to locomote through collagen gel for 16 to 18 h in the absence of exogenous IL-2. The leading cell front of RIL-2-stimulated populations moved more than twice as far as that of unstimulated control populations (P < 0.05, Student’s t test) (Fig. 2). This difference in motility cannot be attributed to a difference in viability. After liberation from the collagen gel, the viability of stimulated and unstimulated populations did not differ significantly, averaging approximately 60% (not shown). RIL-2 stimulation also doubled the percentage of cells exhibiting motility, relative to unstimulated-control values (Fig. 2). The presence of exogenous RIL-2 during the assay (20 units/ml) did not significantly alter these results (Fig. 2).

Motile Subpopulations Less Tumoricidal Than Total Populations. LAK activity, measured as 4-h cytotoxicity against tumor line 410.4, varied widely among batches of RIL-2-stimulated lymphocytes. At E:T = 25:1, for example, cytotoxicity of the total population ranged from 24.1 to 92.7%. This broad range is probably attributable to the variety of RIL-2 concentrations and initial cell densities tested in a search for optimal culture conditions. Despite the range of LAK activities, a consistent pattern was observed: motile subpopulations always exhibited lower LAK activity than the total populations from which they were derived (Fig. 3). At E:T = 12.5:1, the mean LAK activity of motile subpopulations was approximately 28% lower than that of total populations; at 25:1, the difference was 44%; at 50:1, 35%. These differences were significant at all E:T ratios (paired Student’s t test, P < 0.05). In unstimulated lymphocyte populations, neither total population nor motile subpopulation exhibited significant LAK activity (not shown).

In three experiments, sufficient lymphocytes were obtained to permit assay not only of LAK activity but also of NK activity, although only at a single E:T ratio (25:1) (Fig. 4). The mean NK activity of motile subpopulations was approximately 65% lower than that of the total populations from which they were derived (P < 0.05, paired Student’s t test).

In one experiment, sufficient cells were obtained to permit assay of LAK activity at two time points: immediately after the locomotion period and after a 24-h rest period (suspension culture in the presence of RIL-2, 700 units/ml). At both time points, the motile subpopulation exhibited less LAK activity than did the total population (Table 1).

In type I collagen gel, cells locomote through a meshwork of...
Lymphocyte Motility and Tumoricidal Activity

Fig. 3. LAK activity of RIL-2-stimulated lymphocytes, measured as 4-h cytotoxicity versus mammary adenocarcinoma line 410.4. T, total populations obtained after 16- to 18-h locomotion through collagen gel; M, corresponding motile subpopulations. Points, mean of 6 independent experiments; bars, SD. *, total population values significantly greater than corresponding motile-subpopulation values ($P < 0.05$, paired Student's $t$ test).

Fig. 4. NK activity of RIL-2-stimulated lymphocytes, measured as 4-h cytotoxicity versus YAC-1 lymphoma. E:T = 25. O, total populations; □, motile subpopulations. Columns, mean determined from 3 independent experiments; bars, SD.

Table 1 LAK activity of RIL-2-stimulated lymphocytes, assayed both immediately after separation of total populations and motile subpopulations, and after 24-h additional culture at $5 \times 10^6$ cells/ml of DME and 700 units/ml of RIL-2

<table>
<thead>
<tr>
<th>Time of assay (h post locomotion)</th>
<th>% of cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E:T</td>
</tr>
<tr>
<td>Zero</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>24 h</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

* Mean ± SD of 4 replicate wells.

** NT, not tested.

collagen fibers. This situation can potentially produce a sorting of cells according to size. In the present experiments, only a slight size-sorting effect was observed. The forward scatter profiles of total populations and bulk populations were determined by flow cytometry and found to be nearly indistinguishable (Fig. 5). The mode of the forward scatter profile of motile subpopulations was located only 3 to 5% to the left of that of total populations, indicating a very slight bias towards smaller cells.

Difference in Subset Distribution of Total Populations and Motile Subpopulations. Subset distributions are displayed in Table 2. The percentage of asGM1+ cells in total populations was approximately twice as great as that in motile subpopulations. Total populations and motile subpopulations did not differ significantly with respect to the percentage of Thy-1$^+$ cells (T-cells). Of the T-cells in total populations, however, 29.6% were "T-null," bearing neither L3T4 nor Ly-2. Motile subpopulations were poorer in these T-null cells, containing a mean of only 11.4%. Motile subpopulations contained a significantly higher percentage of L3T4$^+$ cells than did total populations. In summary, total populations were richer in asGM1+ and T-null cells and poorer in L3T4$^+$ cells than motile subpopulations. As dual immunofluorescence was not performed, percentages of doubly positive cells are not available.

Interferon-stimulated Motility. The preceding experiments involved spontaneous lymphocyte motility, i.e., motility in the absence of exogenous chemotactic or chemokinetic factors. In some experiments, exogenous RIL-2 was present, but did not measurably modify motility (Fig. 1). Lymphocyte motility was increased, however, by the continuous presence of rIFN-αA/D ($10^3$ to $10^4$ units/ml) in the culture medium during the locomotion periods. The stimulation appeared to be chemokinetic; downward motility (into the bases, away from the culture medium) and upward motility were both increased by 30 to 35% (Fig. 6). The relationship of LAK activity to rIFN-αA/D-stimulated motility was much the same as that observed for spontaneous motility; the motile subpopulation exhibited only 35 to 75% as much activity as the total population from which it was derived (Fig. 7).

DISCUSSION

The regulation of lymphocytic traffic through tumors is poorly understood. The endothelium of tumor vasculature may have selective lymphocyte-binding properties. Soluble chemotactic factors, locomotion inhibitors, and substrate-bound adhesion gradients probably all coexist and interact in the tumor microenvironment (26). We are currently developing a collagen-gel-based system for the study of lymphocyte migration in and out of masses of cultured tumor and tumor-infiltrating cells. This system should aid in the identification of the most important tumor-derived modulators of lymphocyte motility. In the absence of such information we have focused, in this initial study, mainly on random motility, a fundamental lymphocyte property. Both random and chemotactic motility have been found to correlate with the ability of lymphocytes to extravasate...
LYMPHOCYTE MOTILITY AND TUMORICIDAL ACTIVITY

Table 2. Subset distribution of total populations and motile subpopulations

<table>
<thead>
<tr>
<th>Subset distribution</th>
<th>Thy-1* (%)</th>
<th>L3T4* (%)</th>
<th>Ly-2* (%)</th>
<th>T-null* (%)</th>
<th>asGM1* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total population</td>
<td>84.6 ± 10.6</td>
<td>30.0 ± 1.5</td>
<td>39.2 ± 5.9</td>
<td>29.6 ± 7.6</td>
<td>28.0 ± 5.2</td>
</tr>
<tr>
<td>Motile subpopulation</td>
<td>89.6 ± 6.6</td>
<td>47.0 ± 1.0</td>
<td>41.6 ± 2.3</td>
<td>11.4 ± 2.7</td>
<td>14.6 ± 2.3</td>
</tr>
</tbody>
</table>

* Expressed as percentage of Thy-1* cells.
* Thy-1* cells exhibiting neither L3T4 nor Ly-2; calculated for each experiment as 100 - (% of L3T4* + % of Ly-2*).
* Mean ± SD of 3 experiments.
* Significantly different from total population, P < 0.05.

Fig. 6. Chemokinetic effect of interferon (rIFN-aA/D) on RIL-2-stimulated lymphocytes. Locomotion was for 18 h in the absence of exogenous RIL-2. Leading-front distances were measured upward (into the overlays) and downward (into the bases). Columns, mean of 6 replicate assays in a single experiment; bars, SD.

Fig. 7. LAK activity of RIL-2-stimulated lymphocytes, measured as 4-h cytotoxicity versus mammary adenocarcinoma line 410.4.7; total population obtained after 18-h locomotion through collagen-gel in the presence of rIFN-aA/D, 10^9 units/ml; M, corresponding motile subpopulation. Points, mean of 4 replicate assays in a single experiment; bars, SD.

into tumors and other inflammatory lesions (11–13).

We found that the overall random motility of murine splenic lymphocyte populations was increased significantly by 72 to 80 h of nonspecific stimulation by RIL-2. The distance traveled by the leading cell front in 16 to 18 h was more than doubled over control values. Proliferation of the RIL-2-stimulated lymphocytes may have contributed slightly to this result; at the end of the locomotion period, the stimulated populations usually contained approximately 10% more viable cells than did unstimulated populations (results not shown). The leading-front-distance method of motility measurement, however, is relatively insensitive to the size of the population tested. Leading-front distance varies approximately as the square root of population size (27), so a 10% increase in population would have contributed no more than 4% to the observed 240% increase in leading-front distance. Furthermore, a measure which is completely insensitive to population size—the percentage of viable cells exhibiting motility—was approximately doubled by RIL-2 stimulation.

Overall motility was assayed in both the absence of exogenous interleukin 2 and the presence of 20 units of RIL-2 per ml. This is a concentration which is achievable in serum after i.v. RIL-2 infusion (28). It was not surprising that the presence of this concentration of RIL-2 had no chemokinetic effect upon unstimulated lymphocytes, as these cells generally lack IL-2 receptors (29). The lack of chemokinetic effect upon RIL-2-stimulated lymphocytes was unexpected and may indicate that the motility of these lymphocytes is already maximally stimulated by endogenously produced lymphokines.

The observation that overall lymphocyte motility is increased by RIL-2 stimulation is not a novel one. A variety of activation protocols are known to increase both the random motility and chemotactic response of lymphocytes (20, 30–35). Few attempts have been made, however, to compare the relative motilities of phenotypically and functionally diverse subsets which comprise heterogeneous lymphocyte populations. Such comparisons are easily made in the collagen-gel system, in which total populations and motile subpopulations can be liberated from the locomotion matrix.

Through the use of the collagen-gel system, we found that motile subpopulations of RIL-2-stimulated lymphocytes were significantly poorer in LAK and NK activity than were total populations. The most likely explanation for this difference is that tumoricidal cells were less motile than nontumoricidal cells. It is not likely that the depletion of tumoricidal activity from motile subpopulations reflected transitory phenomena such as the exhaustion of metabolic energy during locomotion or the cell-cycle position of the motile cells. Even after a 24-h postlocomotion rest period, the depletion was still evident. Furthermore, total populations and motile subpopulations differed significantly in their content of stable, serologically defined phenotypes. The most notable difference was in the asGM1* subset, which has been associated with both NK and, in some cases, LAK activity (14–17, 36). The relatively low asGM1* content of the motile subpopulation might thus account for its relatively low tumoricidal activity. The significance of other differences between total populations and motile subpopulations is less clear. The motile subpopulation was relatively enriched in L3T4* cells. These cells have not been implicated as effectors of LAK or NK activity, and they may have acted as bystanders, passively diluting the cytotoxic activity of the motile subpopulation or perhaps even competing with cytotoxic cells for IL-2. The role of the "T-null" subset, which was relatively depleted from the motile subpopulation, is also unclear. These cells have previously been reported in RIL-2-stimulated lymphocyte populations, but no definite function
LYMPHOCYTE MOTILITY AND TUMORICIDAL ACTIVITY

has yet been assigned to them (14). Long-term cultures and more extensive characterization of total populations and motile subpopulations are planned.

The physiological basis for the relatively low motility of the most tumoricidal cells is another subject for further study. Possible determining factors include adhesiveness, membrane fluidity, metabolism, cytoskeletal organization, enzyme secretion, and chemotactic or haptotactic response to degraded matrix products (26, 35, 37). It is not likely that the tumoricidal cells locomoted poorly simply because they were larger. The forward scatter profiles of total populations and motile subpopulations were nearly identical. The small effect of cell size upon motility in collagen gel can probably be attributed to the nature of this matrix. Locomoting cells do not encounter rigid channels, as in a controlled-pore filter, but rather a deformable, degradable lattice work of collagen fibers.

Tumoricidal lymphocytes proved relatively low not only in spontaneous motility but also in chemokinetic response to rIFN-αA/D (Fig. 7). It will be important to examine their locomotory responses to other lymphokines and to tumorderived factors. The design of the collagen-gel system should permit the detection of chemotactic effects. Motility up and down a chemotactic gradient can be measured simultaneously. In practice though, chemotaxis has not yet been observed in the collagen-gel system. This is likely attributable to the short life of concentration gradients in the gel, which is hydrated and extremely permeable. When the high-molecular-weight dye trypan blue is added to the medium, it diffuses evenly throughout the base and overlay slabs within an hour. This problem may be overcome by gradual release of factors from inert polymers or from viable tumor-cell cultures placed above the overlays.

The finding that tumoricidal activity and motility are independently and perhaps inversely regulated is surprising, but not without precedent. Hoffmann et al. (31) found that allosensitized murine lymphocytes which locomote completely through nitrocellulose membranes are poorer in cytotoxic activity than the total population, with the fastest-moving cells expressing the least cytotoxicity. It will be interesting to test a variety of cytotoxic T-lymphocytes, tumor-infiltrating lymphocytes, and activated NK cells in the collagen-gel system in order to determine whether an inverse relationship between cytotoxicity and lymphocyte motility can be taken as a general principle.

The in vivo localization patterns of RIL-2-stimulated lymphocyte populations of known motility and tumoricidal capability are currently under examination. In one set of experiments, total populations and motile subpopulations of RIL-2-stimulated lymphocytes were labeled with 111In and injected i.v. into mice bearing s.c. 410.4 tumors. After 18 h, motile subpopulations exhibited tumor localization indices 1.5 to 2 times higher than those of the total populations from which they were derived. In light of the present finding that motile subpopulations are poor in tumoricidal activity, it would appear that adoptive transfer is not a maximally efficient method of deliver ing tumoricidal lymphocytes to tumor masses. This inefficiency might be overcome by increasing the motility of lymphocytes before adoptive transfer (methods are currently under investigation), or by delivering chemotaxis to the tumor site. Conversely, therapeutic strategies in which lymphocytes are activated in situ might be especially efficient, since the most tumoricidal cells would tend to remain at the tumor site.

ACKNOWLEDGMENTS

The authors thank Diane Jackson and Craig Mueller for expert technical help, Mark Kukuruga of the Ben Kasele Flow Cytometry Laboratory for flow cytometric analyses, and Margaret Peterson for the preparation of this paper.

REFERENCES


Motility and Tumoricidal Activity of Interleukin-2-stimulated Lymphocytes

Stuart Ratner and Gloria H. Heppner


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/12/3374

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.