Brief Definitive Report

Chemoattraction of Human Blood T Lymphocytes by Interleukin-15

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Summary

Recombinant interleukin (IL)-15, derived from a simian kidney epithelial cell line, is a chemoattractant for human blood T lymphocytes judged by its ability to increase the proportion of cells in polarized morphology, to stimulate invasion of collagen gels containing IL-15, and to increase the proportion of locomotor cells observed by time-lapse videorecording. The ability of lymphocytes to respond was partly, but not completely, inhibited by pretreatment with anti–IL-2 receptor β-chain. The activity of IL-15 was completely abolished by preincubation with aIL-15 but unaffected by preincubation with aIL-2. No response of monocytes, neutrophils, or B lymphocytes to IL-15 was observed.

IL-15 is a newly described cytokine with an action similar to that of IL-2 as an activator of T lymphocyte growth (1). It does not share sequence homology with IL-2, but modeling suggests that the three-dimensional structure of the two cytokines is similar. Activation of growth of T cells by IL-15 is blocked by treating the cells with antibody to the β chain of the IL-2 receptor (1), suggesting that IL-15 mediates its effects through this receptor. IL-2 is a known chemoattractant for T lymphocytes (2, 3) and is more potent, as such, than IL-8 or macrophage inflammatory protein (MIP)-1α (4). We recently reported that the chemoattractant activity of IL-2 is blocked by preincubating T cells with anti–IL-2 receptor β-chain (aIL-2Rβ), but not with aIL-2Rα, suggesting a role for the IL-2Rβ chain in chemotactic activity (4). Since IL-15 binds to the IL-2 receptor, it seems plausible that IL-15, like IL-2, is a T cell attractant. Here, we report a study of the chemotactic activity of IL-15 for T lymphocytes, using several assays which measure different aspects of the response. The shape-change (polarization) assay was used to measure the initial change from spherical to polarized morphology, which accompanies, and is a prerequisite for, locomotion; a collagen gel invasion assay was used to determine the capacity of cytokines to attract cells into a matrix (the principle being similar to that of the more familiar filter assay, but using a more physiological substratum); time-lapse videorecording to study details of locomotor behavior; and a checkerboard filter assay to study the effect of IL-15 gradients on lymphocyte locomotion.

Materials and Methods

Materials. Purified recombinant IL-15 derived from a simian kidney epithelial cell line was provided by Immunex Corp., Seattle, WA, and was used at concentrations between 1 μg and 100 pg/ml. This material gave a single band at 14–15 kd on SDS-PAGE and induced proliferation of PHA-activated human T cells at concentrations between 0.1 and 10 ng/ml (1). Recombinant human (rh)IL-2

was a gift from Glaxo, Geneva, Switzerland. This material gave a single band at 15 kD on SDS-PAGE. Its specific activity was 1.6 $\times$ 10^6 U/mg, and it was used at between 200 and 0.2 U/ml (125–0.125 ng/ml). rhIL-2 from R&D Systems, Inc. (Minneapolis, MN) was also used and gave similar chemoattractant activity to the Glaxo material. rhIL-8 was from PeproTech (Rocky Hill, NJ). rhMIP-1α was provided by Dr. G. J. Graham (CRC Beatson Institute, Glasgow, United Kingdom) and originated from R&D. Monoclonal aIL-2Rβ (MIKβ1, mouse IgG2a) was provided by Dr. T. Tanaka (Tokyo Metropolitan Institute of Science, Japan). aIL-2Rα was from Dako Corp. (Glostrup, Denmark). Anti–human IL-15 (mouse IgG1) was provided by Immunex Corp. (Seattle, WA). ahIL-2 was from R&D Systems, Inc. FITC-aCD3/PE-aCD19, FITC-aCD4, and FITC-aCD8 were from Dako Corp. Hanks' solution (Gibco Ltd., Paisley, United Kingdom) was buffered to pH 7.4 with Hepes (Sigma, Poole, United Kingdom) and, when necessary, supplemented with HSA (human serum albumin) 10 mg/ml (HBSS-HSA) or 25% FCS (Gibco Ltd.) (HBSS-FCS).

Cell Preparation. PBMC were separated from blood on lymphocyte separating medium. The cells were washed thrice and either resuspended in HBSS-HSA and used immediately for polarization assays, or cultured for 24 h in HBSS-FCS + bicarbonate in 5% CO_2. Monocytes were not removed from the cell preparations before testing, because our early experience demonstrated a requirement for the presence of accessory cells for the development of locomotor activity in cultured T cells (5, 6). Locomotor monocytes were readily distinguished from lymphocytes by using phase-contrast optics or by staining for nonspecific esterase.

Polarization Assay. This assay measures the change from a spherical shape to the shape, characterized by head-tail polarity, typical of locomotor cells (7, 8). Lymphocytes were suspended at 37°C for 30 min in the presence or absence of chemoattractants, then fixed with 2.5% glutaraldehyde. After washing, the percentage of polarized cells was scored by phase-contrast microscopy as described previously (8). A negative control value was established using HBSS-HSA alone. Lymphocyte polarization was also measured on cells that had been cultured for 24 h in HBSS-FCS. After culture, the cells were removed from culture medium, washed, and tested in 30-min shape-change assays as described above.

Collagen Gel Invasion Assay. Type I collagen was prepared from rat tail tendons (9, 10) and stored in water at pH 4.0. A gel was formed by restoring the collagen to physiological pH and osmolarity. Gels were cast in 24-well dishes and allowed to set. Cytokines were mixed with the collagen immediately before setting. Once the gel had set, it was overlaid with lymphocytes, which were allowed to settle on the top surface. The gels were incubated at 37°C for 16 h to allow the overlaid cells to invade. They were then fixed for 30 min with glutaraldehyde and washed twice very gently, taking care not to decant cells lying on top of the gel, and the proportion of cells which had remained on the upper surface and of those which had invaded the gel was determined. Duplicate gels were set up for each experiment, and cell counts were done in four fields per gel. Two measures of locomotor activity were used. The percentage of cells which had invaded the gel was determined by counting cells at 40-μm intervals of depth within the gel. The distance migrated by the leading front of cells was also determined.

Time-lapse Videorecording. Collagen gels containing HBSS were prepared as above and cast into metal filming chambers described elsewhere (10). Suspensions of PBMC direct from blood were overlaid on the upper surface of the gel in HBSS with or without IL-15 (1 μg/ml). Unactivated lymphocytes attach poorly to protein-coated plastic surfaces, which are unsuitable for analysis of their locomotion. Analysis of locomotion on the surfaces of collagen gels was preferred. Since the cytokine was present in the lymphocyte suspension, but not in the gel, few cells entered the gel and locomotion on its upper surface could be analyzed, using a videocamera and videorecorder as previously described (10). The paths of cells were traced from the videotapes thus obtained, and the proportion of locomotor cells and their speeds and turning behavior were measured.

Checkerboard Filter Assay. The influence of the absolute concentration and of the gradients of IL-15 was studied using a checkerboard filter assay (11, 12). Lymphocytes prepared directly from blood were allowed to migrate for 2 h into cellulose ester filters (8 μm poresize; Sartorius, Göttingen, Germany) with a checkerboard of IL-15 concentrations above and below the filter. After fixation and staining, the distance migrated by the leading front of the cells was measured. The distance expected of cells responding to absolute concentration alone was calculated as outlined in the appendix to the paper of Zigmond and Hirsch (11). Distances greater than expected in positive gradients and smaller than expected in negative gradients suggest a chemotactic response.

Treatment of Cells and Cytokines with Specific Antibodies. Cells were incubated with aIL-2Rβ (MIKβ1) for 2 h at room temperature. The antibody was used at 20 μg/ml, as previously determined (4, 13). As a control, normal mouse IgG2a was used at the same concentration. The cells were then washed to remove free antibodies and tested against IL-2 and IL-15 in polarization assays. IL-15 (1 μg/ml-100 ng/ml) or IL-2 (200-20 U/ml) was incubated with the anticytokine antibodies aIL-15 (1/250-1/1,000) and aIL-2 (1 μg/ml-40 ng/ml) for 1 h at room temperature. Then, the antibody-treated and untreated cytokines were tested, using lymphocytes in polarization assays.

Phenotyping of Locomotor Cells. To score the proportion of T and B cells or of CD4+ and CD8+ cells responding in polarization assays, the assay was performed as above, but the cells were fixed in paraformaldehyde (1%), which does not cause autofluorescence of cells and allows them to retain locomotor morphology. Cells were washed and stained with appropriate fluorescent antibodies. Slide and coverslip preparations were made, and the proportion of labeled cells in the polarized and the spherical lymphocyte populations was determined.

Results and Discussion

Response of Lymphocytes to IL-15 and Other Cytokines. Fig. 1 shows the polarization dose-response of lymphocytes from PBMC preparations, both directly after preparation from blood and after 24 h culture in HBSS-FCS. A proportion of cells from both preparations polarized in response to both IL-15 and IL-2 and the dose responses to the two cytokines were similar (ED$_{50}$ for IL-2, 5 ng/ml; for IL-15, 7 ng/ml). Cultured cells responded in higher numbers than freshly prepared cells, largely due to an increase in background polarization. However, they also responded to lower concentrations of both cytokines (ED$_{50}$ for cultured cells to IL-2, 0.75 ng/ml; to IL-15, 0.6 ng/ml). Both IL-15 and IL-2 caused more cells to polarize (20-35% higher than control) than the chemokines IL-8 and MIP-1α, (10-15% higher than control) (Fig. 2), though the ED$_{50}$s were similar for all four cytokines. Previous work (2-4) has shown that the cells that respond to IL-2 are T cells, but, since we were using mixed cell populations, it was necessary to phenotype the responsive cells to determine which population responded to IL-15. When scoring polarization in paraformaldehyde-fixed, CD3/CD19-stained cells, almost the entire increase in polarized lymphocyte morphologies can be attributed to T cells (Table 1). Both CD4+ and CD8+ cells responded, but there was no selectivity of either subset. The CD19+ population showed no increase above control levels (not shown). Cell types other than T cells were studied separately. No response to IL-15 or IL-2
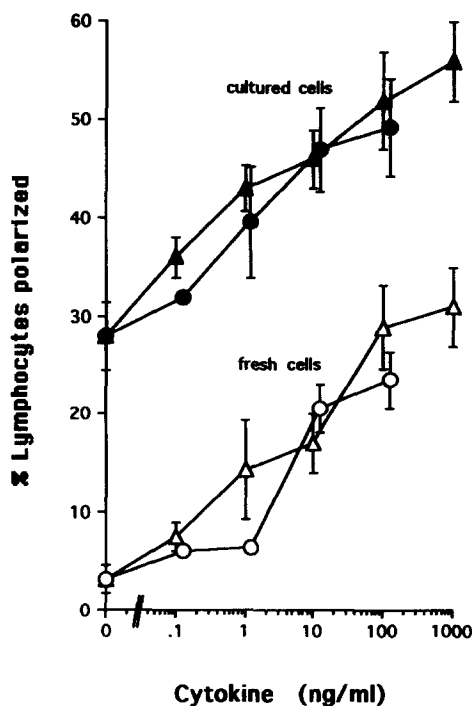

Figure 1. Dose-response of human blood lymphocytes to IL-15 (*triangles*) and IL-2 (*circles*) in polarization assays. Cells were tested directly after separation (*open symbols*) or after overnight culture in HBSS-FCS (*filled symbols*). $P < 0.01$ for both IL-15 and IL-2 at concentrations >10 ng/ml, compared with the medium control (mean ± SEM, $n = 6$).
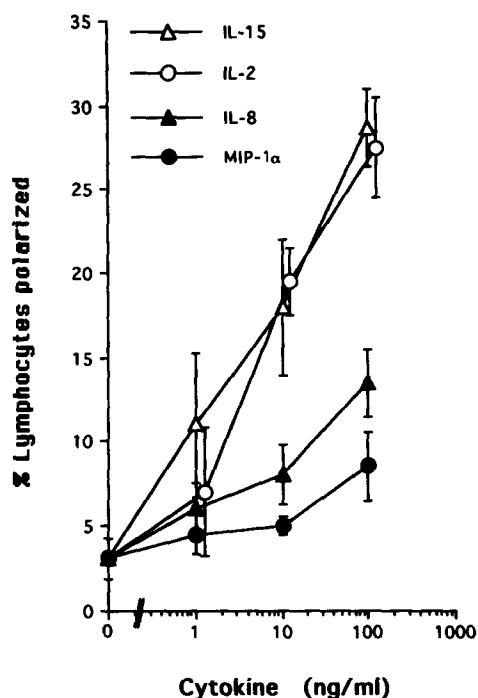
Figure 2. Comparison of dose-response of blood lymphocytes to IL-15 with that to IL-2, IL-8, and MIP-1α in polarization assays. The figure shows responses of cells tested directly after separation from blood. Similar results were obtained after overnight culture (mean ± SEM, n = 4).

was seen by using blood monocytes either from whole PBMC preparations or after purification on fibronectin-coated surfaces (14, 15). No response was seen by using neutrophils. Human tonsillar B cells were purified by removing T cells by 2-aminoethyl isothiouronium bromide (AET)-rosetting (Sigma), followed by separation on Percoll gradients (16). These B cells (>97% pure) showed no significant response to IL-15 (M. Komai-Koma, unpublished observation).

Time-lapse filming confirmed that cells responding by shape-change to IL-15 were also active locomotor cells: 45% of lymphocytes in a uniform concentration of IL-15 (1 μg/ml), but

Table 1. *T Cell Polarization in Response to IL-15*

Marker population	Percent of all cells that are positive for marker	Percent of marker positive cells that are in polarized morphology	
		Medium control	IL-15 (1 μg/ml)
Cells direct from blood			
CD3	76.4	<5	44.6
Cells after 24 h culture			
CD3	87.4	24.5	53.5
CD4	59	20.7	47.2
CD8	28.5	24.3	48

only 15.1% of cells in the medium control, showed active locomotion on collagen gels. The median speed of the cells in IL-15 was 5.9 μm/min, and the cells moved by a persistent random walk (median displacement; 5.5 μm/min).

Effect of Anti-IL-15 and Anti-IL-2. The effect of these antibodies is shown in Table 2. Each antibody strongly inhibited lymphocyte shape change induced by its own specific cytokine but had little inhibitory effect on the activity of the others.

Effect of Anti-IL-2Rβ. Fig. 3 shows that incubation of lymphocytes fresh from blood with aIL-2Rβ (MIKβ1) significantly reduced their capacity to respond to both IL-2 and IL-15. Similar results were obtained using lymphocytes cultured overnight before incubation with aIL-2Rβ. Anti-IL-2Rα had no inhibitory effect on the response to either cytokine (data not shown).

Collagen Gel Invasion. The invasion by blood lymphocytes of collagen gels containing IL-2 and IL-15 is shown in Table 3. Overnight, more cells invaded gels containing the cytokines than the control gels (these proportions were very similar to those for overnight polarization shown in Fig. 1). Also, the leading front of cells migrated further in the presence than in the absence of cytokines. Anti-IL-15 antibody did not completely inhibit the invasion of IL-15–containing gels (not shown). Interpretation was difficult, because this antibody (mouse IgG1), itself, attracted lymphocytes into gels, and, in unpublished experiments in this laboratory (M. Komai-Koma), it was observed that mouse IgG1 may stimulate the locomotion of B cells.

Checkerboard Filter Assay. Table 4 shows the distances migrated by lymphocytes in a checkerboard assay. Cell migration increased as the absolute IL-15 concentration was increased (along the diagonal from upper left to lower right). In positive gradients (above the diagonal), migration was greater than calculated on the basis of a response to absolute concentration alone; in negative gradients (below the diagonal), it was lower. These results suggest that IL-15 induces both a chemokinetic and a chemotactic response.

The results presented here show that IL-15 stimulates locomotion of human T lymphocytes, judged by polarization, time-lapse filming, and invasion of collagen gels. A filter checkerboard assay suggested that locomotion was enhanced, both by increasing the absolute concentration of IL-15 and by exposing the cells to positive gradients, implying that IL-15 causes

Table 2. *Inhibitory Effect of aIL-15 and aIL-2 on Lymphocyte Polarization Induced by IL-15 and IL-2*

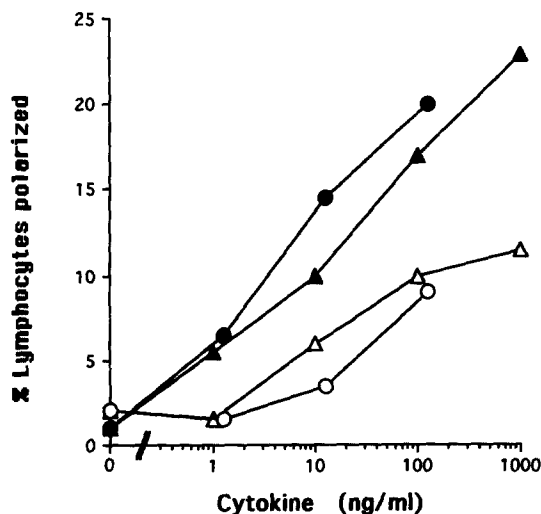| Antibody	Percent inhibition of response to		
	IL-15 (100 ng/ml)	IL-2 (200 U/ml)	IL-2 (20 U/ml)
aIL-15: 1/500	93.8*	0	5
1/1,000	74.3*		
aIL-2: 200 ng/ml	16.1	82.7*	85*

* P <0.01 compare non–antibody-treated control.

Figure 3. Effect of pretreatment of uncultured blood lymphocytes with aIL-2Rβ on their response to IL-15 and IL-2. The figure shows results of a single experiment. Cells pretreated with medium alone, *filled symbols*; cells pretreated with aIL-2Rβ, *open symbols*. IL-15, *triangles*; IL-2, *circles*. $P < 0.01$ in five experiments comparing antibody-treated with untreated cells for both cytokines at concentrations >10 ng/ml.

T cells both to accelerate (chemokinesis) and to move directionally (chemotaxis). These results are closely similar to those observed with the sister cytokine IL-2, and, weight-for-weight, these cytokines are more powerful attractants than IL-8 or MIP-1α (Fig. 2). There is little homology between the two cytokines and there was no cross-inhibition by their respective antibodies. As in the first report of T cell activation by IL-15 (1), the T cell locomotor activity of this cytokine was reduced by pretreatment of the cells with aIL-2Rβ, though inhibition was only partial, suggesting that other cell surface molecules may contribute to activation by IL-15. Table 1 shows that 40–50% of the T cell population polarized in response to IL-15. Our unpublished observations suggest that this was also the proportion which was positive for IL-2Rβ, judged by FACS® analysis. Moreover, variations in IL-2Rβ

expression in different blood samples were reflected in the response of lymphocytes from those samples to IL-15.

The role in the migrations of T lymphocytes in vivo played by the various cytokines that have been reported to attract T lymphocytes in vitro is unknown. The effective concentration of IL-2, a cytokine released chiefly by T cells, as a stimulant of T cell locomotion is higher than that required to stimulate T cell proliferation, as observed here. The concentrations of IL-2 found in inflammatory sites, such as rheumatoid synovial fluids and tissues (17), or following stimulation of PBMC from normal subjects with PHA (18) or aCD3 (unpublished observations of the authors) would, based on the dose-response curves shown here, be suboptimal for attracting T lymphocytes. Moreover, IL-2-gene knockout mice are still capable of mounting immune responses that classically require IL-2 (19). Possibly, IL-15, which is made by non-lymphocytic cells, including mononuclear phagocytes, can substitute for IL-2 as a T cell activator under conditions where little IL-2 is present, though IL-15 levels in various sites of antigen deposition or response to tissue injury are, as yet, unknown.

Other than recruitment into inflammatory sites, T cell locomotion is probably also important in T cell–accessory cell

Table 3. *Invasion by Lymphocytes of Collagen Gels Containing Cytokines*

Attractant in gel	Percent cells invading gel (mean ± SEM)	Leading front value μm/16 h (mean ± SEM)
Medium alone	24.4 ± 5.0	154.9 ± 21.4
IL-2 (50 U/ml)	44.6 ± 2.4*	261.3 ± 16.1*
IL-15 (1 μg/ml)	44.8 ± 3.7*	242.8 ± 22.7*
IL-15 (50 ng/ml)	49.4 ± 3.0*	223.1 ± 16.2‡

* $P < 0.01$ compare medium control ($n = 8$).
‡ $P < 0.05 > 0.01$ compare medium control ($n = 8$).

Table 4. *Checkerboard Filter Assay*

		Distance migrated (μm in 2 h) by leading front of cells (mean ± SEM)			
		IL-15 (ng/ml) below filter			
		0	10	100	1,000
IL-15 (ng/ml) above filter	0	25 ± 3	51 ± 3 (27)*	65 ± 5 (34)	68 ± 4 (49)
	10	45 ± 5 (34)	35 ± 5	52 ± 6 (37)	44 ± 4 (48)
	100	43 ± 4 (46)	32 ± 5 (45)	49 ± 3	59 ± 5 (50)
	1,000	46 ± 4 (53)	46 ± 4 (53)	49 ± 5 (53)	54 ± 5

* Figures in parentheses show the calculated distance that would be migrated if cells responded to the absolute concentration of IL-15, but not to the gradient (reference 11). They are for comparison with the experimentally observed distances migrated in each chamber.

interactions. In time-lapse studies of immune cell clustering, we observed that clustering occurred chiefly after random contacts between lymphocytes and accessory cells, but, at very close range (within a cell diameter), lymphocytes showed orientation towards nearby monocytes, suggesting that the latter cells might be releasing an attractant detectable only at close range (20). A subsequent study showed that monocytes in clusters with lymphocytes released large quantities of IL-8, sufficient to stimulate lymphocyte locomotion under conditions where IL-2 levels were too low to exert an effect (6). It will be important to determine whether accessory cells make IL-15 in sites of immune reactions and whether this contributes to the recruitment of T cells into such sites.

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