Structural and functional characterization of the human T lymphocyte receptor for insulin-like growth factor I in vitro.

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Research Article

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Structural and Functional Characterization of the Human T Lymphocyte Receptor for Insulin-like Growth Factor I In Vitro

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Abstract

Growth factor receptors for T lymphocytes, such as interleukin 2 and insulin, are present on activated but not resting T lymphocytes. We sought to determine if insulin-like growth factor I (IGF-I) could act as a growth factor for human T cells and to characterize its receptor on resting and activated cells. Recombinant IGF-I induced two separate functions. It was chemotactic for and increased incorporation of tritiated thymidine into both unactivated (resting) and mitogen-activated T cells. High-affinity ^{125}I-IGF-I binding to human T cells was saturable with an apparent K_d of 1.2±.6 × 10^{-10} M for binding to activated T cells and 1.2±.9 × 10^{-10} for unactivated T cells. The calculated binding for activated cells was 330±90 and for resting cells 45±9 high-affinity receptor sites per cell. Affinity cross-linking of ^{125}I-IGF-I to resting or activated T cells revealed a radioligand–receptor complex of 360,000 mol wt when analyzed by SDS-PAGE without reduction and complexes of 270,000 and 135,000 mol wt upon reduction; prior incubation with excess unlabeled IGF-I prevented formation of the ^{125}I-IGF-I receptor complex. Our data suggest that both resting and activated T lymphocytes bear functional IGF-I receptors similar to those found in other tissues. These receptors may mediate T cell growth and chemotaxis.

Introduction

Insulin-like growth factor I (IGF-I)[1] is a polypeptide with insulin-like chemical structure and biologic properties (1). It circulates as a high molecular weight complex bound to a specific carrier protein. IGF-I has been shown to be identical to somatomedin C and appears to be synthesized in many mammalian tissues (2–6). Receptors for IGF-I have been identified in numerous tissues including placenta (7), fibroblasts (8), neuronal tissue (9), skeletal muscle (10), Leydig cells (11), mononuclear cells (12), and B lymphocytes (13). In vivo, IGF-I is considered to be primarily a growth hormone-dependent circulating growth factor with an important effect upon skeletal growth (1), while in high doses, IGF-I may function to regulate acute metabolic responses (14).

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1. *Abbreviations used in this paper:* hpf, high-power field; IGF-I, insulin-like growth factor I; M199, Medium 199; T, thymidine.

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Accumulation of recirculating T lymphocytes at tissue sites is thought to occur by recruitment and subsequent local proliferation. The major means by which recruitment may occur is directed migration or chemotaxis (15). In fact, several growth factors have been shown to be chemotactic for human T cells, including interleukin 1 (IL-1) (16, 17), interleukin 2 (IL-2) (18), antigen (19), and insulin (20), suggesting that such growth factors might be capable of both steps in the accumulation of T lymphocytes: chemotaxis, and subsequent proliferation.

Insulin receptors are found on activated but not unactivated (resting) T cells (21–23). Recently, we found insulin to be chemotactic for mitogen-activated human T lymphocytes in doses consistent with action on high-affinity insulin receptors (20). This effect was not seen with resting T cells unless very high doses of insulin were used, consistent with the hypothesis that insulin was acting via lower-affinity IGF-I receptors. It has been demonstrated that insulin may compete to occupy IGF-I receptors (13, 24). Because of the similarities between insulin and IGF-I and their receptors, we evaluated the chemotactic and proliferative responses of T lymphocytes to IGF-I. We determined IGF-I to be chemotactic for resting and activated T cells in doses consistent with action on high-affinity IGF-I receptors. IGF-I stimulated increased tritiated thymidine ([^{3}H]T) incorporation into resting and activated T cells in a standard proliferation assay. We also characterized the IGF-I receptor on both resting and activated T cells employing binding assays and affinity cross-linking studies.

Methods

Isolation and purification of T lymphocytes. Human peripheral blood mononuclear cells (PBMC) were isolated from the heparinized venous blood of healthy human volunteers by density centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ). Cells were recovered from the Ficoll–medium interface, washed three times in medium 199 (M199, Microbiological Associates, Bethesda, MD), supplemented with 100 U/ml penicillin and 100 μg/ml of streptomycin and 25 mM Hepes buffer (M199-HPS). Blood monocytes were removed by suspending 75 × 10^6 PBMC in 15 ml M199-HPS in sterile glass petri dishes for 1–2 h at 37°C. After incubation, the plates were washed five times with warmed (37°C) media and the monocyte-depleted supernatant recovered. Nylon wool-nonadherent human T cell-enriched populations were prepared by a modification of the method of Julius et al. (25). 3 g of prewashed sterilized nylon wool (Fenwal Laboratories, Deerfield, IL) were placed in a 30-ml syringe. The nylon wool column was soaked with M199-HPS with 0.4% bovine serum albumin (BSA) (Miles Laboratories, Inc., Elkhart, IN). Monocyte-depleted cells were then applied to the column and incubated for 1 h. After incubation, the nonadherent cells were eluted with media at a flow of 1 ml/min. 25–40% of the cells were recovered in the eluate of which > 95% were CD3+ as assessed by fluoroscein isothiocyanate-labeled (FITC) monoclonal antibody (Leu-4, Becton, Dickinson & Co., Mountain View, CA) and flow cytometric analysis with the fluorescence activated cell sorter (Becton, Dickinson & Co. FACS 440). Cell viability, checked by employing exclusion of trypan blue dye (Gibco, Grand Island, NY) was routinely > 90%.

T lymphocyte subsets. For some chemotaxis experiments, human T lymphocytes were further separated into $CD4^+$ or $CD8^+$ subset-enriched populations by negative selection utilizing a modified "panning" technique. Magnetic beads (BioMag, Advanced Magnetics, Cambridge, MA) that were conjugated to ovine polyclonal antibody to FITC were utilized. 100 μl of commercially obtained FITC-conjugated monoclonal antibody to CD4 or CD8 (OKT4 or OKT8, Ortho Diagnostics, Raritan, NJ) was added to T lymphocytes and were incubated on ice for 20 min and then washed three times in iced M199. 5 ml of bead slurry was washed twice in M199, mixed with cells, and then incubated at 37°C for 30 min. The reaction flask was taped to a strong magnet, and after 5 min the nonadherent cells were decanted. Decanted cells were tested for adequacy of depletion by assessing staining for the depleted CD marker with the FACS; the procedure was repeated if the depleted CD marker stained > 5% of the cells.

Cell migration. A modification of the Boyden chamber technique (26) was utilized to assess T lymphocyte chemoattractant activity in response to recombinant IGF I (AmGen Biologicals, Thousand Oaks, CA). 50 μl of a T lymphocyte suspension containing 10×10^6 cells/ml of M199-HPS with 0.4% BSA was placed in the upper compartment of 48-well microchemotactic chambers (Bio-Rad Laboratories, Rockville Center, NY) separated by 8-μm pore size nitrocellulose filters (Sartorius Filters, Inc., Cherry Hill, NJ) from 25 μl of M199-HPS-BSA alone (control) or from IGF-I (10^{-11} to 10^{-7} M) in M199-HPS-BSA. Except for the absence of IGF-I the control wells were identical in every way. Gradient dependence was determined by the checkerboard technique; IGF-I was placed below the filter only (chemoattractant present with concentration gradient) as well as both above and below (chemoattractant present without concentration gradient). Increased motility over baseline in the former setting represents a chemotactic (gradient-dependent) effect, and in the latter setting a chemokinetic (gradient-independent) effect (27). Migration experiments were carried out for 3 h at 37°C in a 5% CO_2 atmosphere. The filters were fixed, stained, dehydrated, and mounted by using standard histologic methods. Lymphocyte movement was quantitated by counting the total number of cells migrating beyond a fixed distance into the filter. Five high-power fields (hpf) were counted for each of the duplicate wells. Results were calculated as mean cells per high-power field±SD. Data were also expressed as mean percentage of control migration. Student's *t* test was utilized to determine the statistical significance of migration compared with control conditions. A P value of < 0.05 was considered significant in all chemotaxis experiments.

[^{3}H]T incorporation assay. Phytohemagglutinin (PHA, 2 μg/ml; PHA-L, Sigma Chemical Co., St. Louis, MO), interleukin 2 (IL-2, 100 U/ml) (AmGen Biologicals), and IGF-I (10^{-11} to 10^{-7} M) were added separately or in combination to human T lymphocytes in HL-1 media (containing amino acids, selenium, protein, iron, transferrin, and without insulin) which were then distributed in 200-μl aliquots (10^6 cells/ml) into 96-well round-bottomed culture plates at 37°C and 5% CO_2. Controls for resting lymphocytes contained cells and media only; those for activated cells contained PHA and cells with media only. At 48 h, each well was pulsed with 1 μCi of [^{3}H]T (6.7 Ci/mmol, New England Nuclear Research Products, Boston, MA). At 72 h, the cell nuclei were harvested onto glass fiber filter paper with a Titertek Cell Harvester (Flow Laboratories, Rockville, MD) and counted (counts per minute [^{3}H]T) in a Packard Tri-Carb 300 Scintillation Counter (United Technologies Packard, Sterling, VA) for 2 min per sample. The values from quadruplicate wells were averaged and expressed as counts per minute of radioactivity incorporated by 2×10^5 cells after 72 h of incubation±SD. Values were compared with controls and underwent two-way analysis of variance. Cell cycle analysis was also performed; the cells were labeled with propidium-iodide (Sigma Chemical Co.) and analyzed with the FACS.

Competitive binding assays. Conditions for the binding assays performed were similar to those previously utilized for investigation of the IGF-I receptor (9). ^{125}I-IGF-I (2,000 μCi/mmol, Amersham Corp., Arlington Heights, IL) was added in 20-μl aliquots (100,000 cpm/assay) to 20 μl of unlabeled ligand (10^{-11} to 10^{-7} M) or to M199-HPS-BSA alone. Human T lymphocytes in M199-HPS-BSA were added at 10^6 cells (PHA-activated cells) or 10×10^6 cells (resting cells) per 460-μl aliquot yielding a final volume of 500 μl. After incubation at 15°C for 60 min, the tubes were centrifuged for 5 min in an Eppendorf 5414 centrifuge. The supernatant was removed and the cells remaining were washed. After centrifugation, the cell pellets were obtained by cutting off the tips of the tubes. Bound ^{125}I-IGF-I was counted in a Packard Multi-Prias 1 Gamma Counter (United Technologies Packard) and background was subtracted. Binding studies were performed for both resting and 48-h PHA-activated T cells. The counts per minute bound from triplicate samples were averaged. Nonspecific binding was determined as cpm bound in the presence of 10^{-7} M unlabeled IGF-I and was subtracted from total counts per minute to get specific binding. Binding experiments were performed three times each and were reproducible; representative experiments for both resting and activated cells are shown. In addition a competition assay, comparing insulin and IGF-I binding was performed. Binding assays were each performed at least three times and were reproducible.

Affinity cross-linking of ^{125}I-IGF-I to T cells. Cross-linking was performed as described by Pilch and Czech (28, 29). Intact human resting and PHA-activated T lymphocytes (25×10^6 cells) were incubated with 10^{-9} M ^{125}I-IGF-I (10^6 cpm/assay), with or without unlabeled IGF-I (10^{-7} M), and incubated at room temperature for 30 min; unbound ligand was then removed by centrifugation at 500 *g* and discarding the supernatant. Disuccinimidyl suberate (DSS, Pierce Chemical Co., Rockford, IL) was added to yield a final concentration of 0.5 mM. After 30 min the cells were exposed to 100 μl of 1.5 M Tris (pH 8.7) while on ice for 15 min and then (total volume of 1 ml) were sonicated and centrifuged at 500 *g* for 10 min to remove nuclei. The supernatant was recovered and centrifuged (Eppendorf 5414 centrifuge) and the pellet was then suspended in 100 μl of phosphate-buffered saline, pH 7.4, plus 100 μl of Laemmli sample buffer (0.5 M Tris-HCl, pH 6.7, 2% sodium dodecyl sulfate, 10% glycerol and 0.001% bromophenol blue); reduced samples contained dithiothreitol (100 mM). Samples were boiled for 2 min and then analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a 3–10% gradient gel. Autoradiograms were developed for 48 h at −80°C, using Kodak X-Omat film (Eastman Kodak Co., Rochester, NY). The molecular weight standards used were myosin (200,000), β-galactosidase (116,250), phosphorylase B (97,400), BSA (66,200) and hen egg white ovalbumin (42,699) (Bio-Rad Laboratories, Richmond, CA). Affinity cross-linking was performed in the same manner employing HuT 78 cells, a malignant T cell line (30).

Results

Functional characteristics of IGF-I

Chemotaxis. Using a modified Boyden chamber technique to evaluate cell motility, we determined the chemoattractant effect of IGF-I on human T lymphocytes. T lymphocytes were incubated for 48 h, with or without PHA (2 μg/ml). As indicated in Fig. 1, unactivated T cells showed a statistically significant increase in motility when exposed to IGF-I with a peak migration of 24.4±2.4 cells/hpf (244±24% control migration) at 10^{-8} M IGF-I. PHA-activated cells showed a greater increase in motility and a left shift of the dose-response curve peaking at 44.2±3.2 cells/hpf (291±21% of control migration) at 10^{-9} M. There was no significant difference in baseline random motility between the activated and unactivated cells. Both unactivated and activated cells were depleted to subsets by negative selection utilizing a magnetic panning technique to deplete antibody-coated cells. The chemotactic response to IGF-I of CD4+ and CD8+ T cells were identical, and not different from the response of unseparated T lymphocytes (Fig. 2).

A checkerboard analysis was performed to determine if the effect of IGF-I upon motility was gradient dependent (chemo-

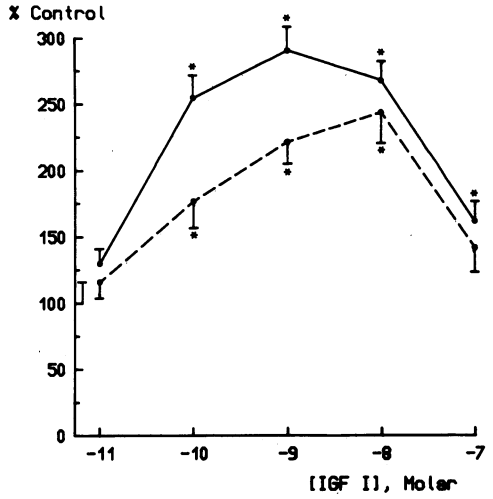

Figure 1. Chemoattractant effect of IGF-I on unactivated and PHA-activated T lymphocytes. T cell migration was assayed with a modified 48-well Boyden chamber as described in materials and methods. T cell migration was quantitated by counting the cells migrating beyond a certain depth, set to give a baseline migration under control conditions of ~ 10 cells. IGF-I concentrations in lower wells were 10^{-11} to 10^{-7} M. Lymphocyte migration is expressed as percent migration under control conditions (media without IGF-I), in which 100% of control migration was 10±1.7 (mean±SD) (unactivated) or 15.2±1.9 (PHA-activated) cells/hpf. Migration of both unactivated (*dashed line*) and PHA-activated cells (*solid line*) was markedly enhanced by IGF-I. Migration of unactivated cells peaked at 24.4±2.4 cells/hpf (244±24% of control migration) at an IGF-I concentration of 10^{-8} M. PHA-activated cells demonstrated a one-log dose-response shift with migration peaking at 44.2±3.2 cells/hpf (291±21% of control migration) at an IGF-I concentration of 10^{-9} M. In each migration assay the migratory response decreased at high concentrations of IGF-I. *Significantly different from control conditions, $P < 0.01$.

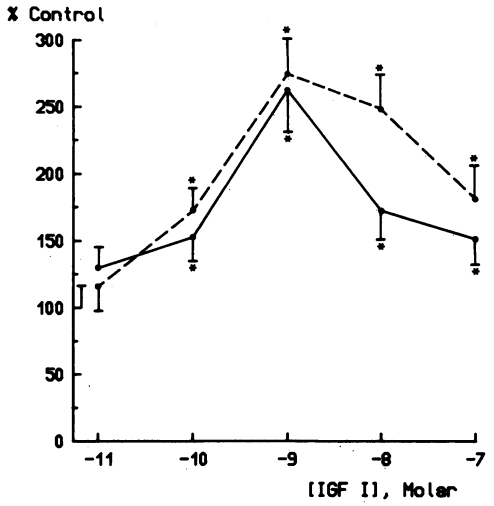

Figure 2. Chemoattractant effect of IGF-I on PHA-activated T lymphocyte subsets. IGF-I concentrations were 10^{-11} to 10^{-7} M. Lymphocyte migration is expressed as percent migration under control conditions (media without IGF-I), in which 100% of control migration was 9.1±1.5 (mean±SD) (T4 subset) or 8.3±1.2 (T8 subset) cells/hpf. The T cell subsets incubated for 48 h with PHA demonstrated similar migratory responses to IGF-I; for the T4 subset (*solid line*) migration peaked at 263±20% and for the T8 subset (*dashed line*) at 275±18% of control migration, respectively. *Significantly different from control conditions, $P < 0.01$.

tactic) or independent of the effect of a gradient (chemokinetic) (Fig. 3). The response of T cells to IGF-I was clearly greater when IGF-I was present below the filter only (i.e., gradient present). A significant chemokinetic effect was seen as well, in that cells showed significantly increased motility even when IGF-I was present in equal concentrations above and below the filter.

[^{3}H]T incorporation. IGF-I stimulated uptake of [^{3}H]T into resting T lymphocytes to a significantly greater extent ($P < .01$) than occurred in control cells, or in cells incubated with IL-2 alone (Table I). In both experiments control and IL-2 treated cells demonstrated low baseline levels of [^{3}H]T uptake, while IGF-I at 10^{-9} M induced a three- to fourfold increase in [^{3}H]T uptake. Dose-response experiments in resting cells revealed that IGF-I was active in concentrations as low as 10^{-10} M, and peaked in activity at 10^{-9} M (not shown). IL-2 FACS plots of cells stained with propidium iodide revealed an increase in the number of cells in the G_2 + M phases when resting cells were exposed to IGF-I (not shown). When the cells were activated with PHA and then incubated with IGF-I, the response was again significantly greater than with control (cells activated with PHA alone). As expected, IL-2 at 100 U/ml significantly enhanced [^{3}H]T uptake in PHA activated T cells. There was no difference in [^{3}H]T uptake between IL-2 and IGF-I treated PHA-activated cells.

Characterization of the IGF-I receptor

Competitive binding studies. Specific binding of IGF-I to both resting and PHA-activated cells was demonstrated. The resulting curvilinear Scatchard plot for PHA-activated cells is shown in Fig. 4 and for resting cells in Fig. 5. A representative competition assay comparing IGF-I binding to that of insulin is

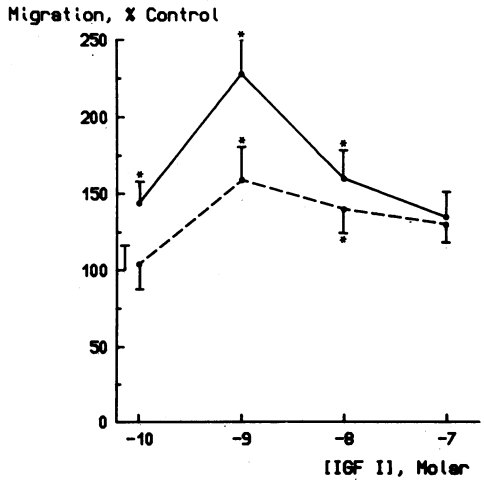

Figure 3. Chemoattractant effect of IGF-I on activated T cells: effect of a gradient. IGF-I concentrations were 10^{-10} to 10^{-7} M in the lower chamber (concentration gradient present: *solid line*) or in both chambers (gradient absent: *dashed line*). Lymphocyte migration is expressed as percentage of control migration in media without IGF-I. Migration is clearly enhanced in the presence of a concentration gradient, peaking at 228±25% with 10^{-9} M IGF-I; in the absence of a gradient migration peaked at 159±26%. *Significantly different from control conditions, $P < 0.01$.

Table I. Effect of IGF-I on [^{3}H]T Incorporation by Resting or PHA-activated T Cells

Sample	[^{3}H]T	
	Experiment 1	Experiment 2
	cpm	
Control (cells/media)	161±15	368±22
IGF-I (10^{-9} M)	721±59*	1,139±74*
IL-2 (100 U/ml)	165±30	412±48
Control (PHA 2 μg/ml)	40,624±7,651	163,096±9,839
PHA + IGF-I (10^{-9} M)	85,298±2,886‡	203,784±4,018‡
PHA + IL-2 (100 U/ml)	123,923±16,616‡	197,479±20,946‡

IGF-I or IL-2 were added to human T cells in HL-1 media (containing amino acids, selenium, protein, iron, transferrin, but without insulin), and then distributed in 200-μl aliquots (10^6 cells/ml) into 96-well round-bottomed culture plates and incubated at 37°C and 5% CO_2. Controls for resting lymphocytes contained cells and media only; those for activated cells contained PHA (2 μg/ml), cells and media. Cells were pulsed with 1 μCi of [^{3}H]T. At 72 h, cell nuclei were harvested and counted. Mean values from quadruplicate wells are presented. Results are expressed as counts per minute of radioactivity incorporated by 2×10^5 cells after 72 h of incubation. Resting cells exposed to IGF-I incorporated [^{3}H]T to a significantly greater degree than control cells or cells exposed to IL-2 (*$P < 0.01$). PHA-activated cells exposed to IGF-I also incorporated [^{3}H]T to a significantly greater degree than control PHA-activated cells (‡$P < 0.01$) but not to a greater degree than IL-2 treated PHA-activated cells.

also shown in Fig. 5 (*inset*). Scatchard analysis (31) of competitive binding assays revealed an apparent K_d of $1.2 \pm 0.6 \times 10^{-10}$ M determined for the steep portion of the curve for activated cells and $1.2 \pm 0.9 \times 10^{-10}$ for resting cells. For activated cells,

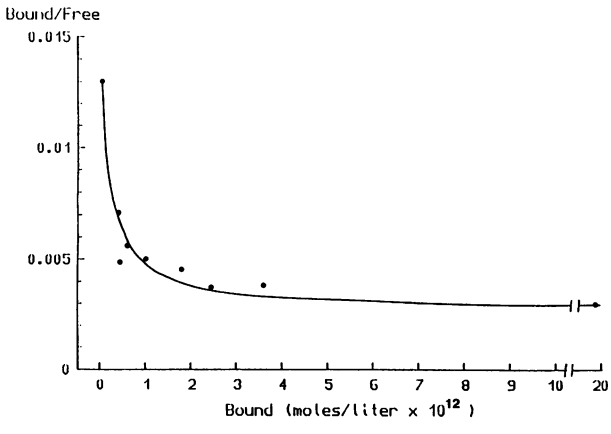

Figure 4. Representative Scatchard plot of ^{125}I-IGF-I binding to PHA-activated human T lymphocytes. ^{125}I-IGF-I (100,000 cpm per assay) was added in 20-μl aliquots to 20 μl of unlabeled ligand (10^{-11} to 10^{-7} M) or to media alone. Human T lymphocytes were added at 10^6 cells per 460-μl aliquot yielding a final volume of 500 μl. After incubation at 15°C for 60 min, the tubes were centrifuged and the cell pellet was resuspended and washed. Gamma counting of bound ^{125}I-IGF-I was performed. Each point represents the mean of triplicate assays. Plots were reproducibly curvilinear. The apparent K_d for the receptor was 1.3×10^{-10} M and the calculated binding for activated cells was 390 high-affinity receptor sites per cell.

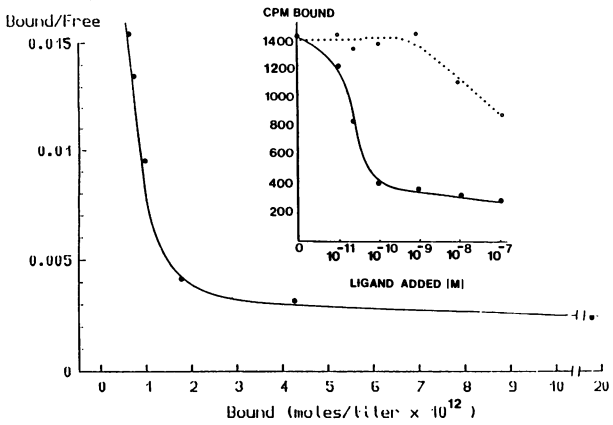

Figure 5. Representative Scatchard plot of ^{125}I-IGF-I binding to resting (unactivated) human T lymphocytes. The assays with resting cells were performed in the same manner as those with activated cells except that more cells (10×10^6 cells per point) were required to demonstrate binding. Each point represents the mean of triplicate assays. Plots were reproducibly curvilinear. The apparent K_d for the receptor was 1.2×10^{-10} M and the calculated binding for resting cells was 51 high-affinity receptor sites per cell. (*Inset*) A competition assay comparing displacement of ^{125}I-IGF-I by increasing concentrations of unlabeled insulin (*dashed line*) or IGF-I (*solid line*). There was no significant displacement of ^{125}I-IGF-I until high concentrations of insulin (10^{-7} M) were reached.

330±90 receptor sites were calculated per cell. There was less specific binding of IGF-I to resting T cells (45±9 binding sites per cell) but binding could be clearly demonstrated when cell concentration was increased 10-fold (10×10^6 cells/aliquot) (Fig. 5). Displacement of ^{125}I-IGF-I by insulin from resting T cells could be demonstrated only with very high doses of insulin.

Affinity cross-linking. Utilizing DSS, ^{125}I-IGF-I was effectively cross-linked to 48-h PHA-activated as well as to resting T cells (Fig. 6). Under nonreducing conditions, the major labeled species was a 360,000-mol wt moiety compatible in size with the IGF-I receptor (32). Polypeptides corresponding to 270,000 and 135,000 mol wt (after subtraction of the 7,500 mol wt contributed by the cross-linked ^{125}I-IGF-I) were identified under reducing conditions. Excess unlabeled IGF-I effectively prevented the cross-linking of radiolabeled ligand in each case. Although the 270,000-mol wt band corresponds roughly in size with the insulin-like growth factor II (IGF-II) receptor under reducing conditions, there was no band compatible with the IGF-II receptor under nonreducing conditions. We therefore believe that the 270,000 mol wt species most likely represents α chain–α chain cross-linking, as previously described (33). The 135,000-mol wt band corresponds in size to the α chain of the IGF-I receptor. We obtained labeling of a band of identical size by cross-linking ^{125}I-IGF-I to HuT 78 cells (data not shown).

Discussion

We investigated the effect of recombinant human IGF-I on cellular growth and motility of purified human blood T lymphocytes. We found IGF-I to be chemotactic and chemokinetic for both resting and activated human T lymphocytes. Chemotactic responsiveness was identical in CD4$^+$ and CD8$^+$

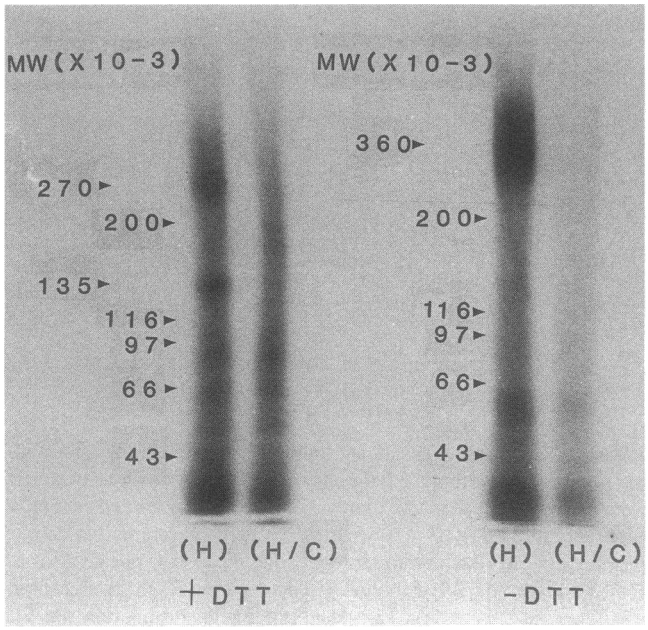

Figure 6. Affinity cross-linking of ^{125}I-IGF-I to PHA-activated T lymphocyte membranes. ^{125}I-IGF-I was bound to T cell membranes (25×10^6 cells per assay) under high-affinity conditions using disuccinimidyl suberate (DSS) as a cross-linking agent. SDS-polyacrylamide gel electrophoresis (3–10% gradient gel) was performed followed by autoradiography. Samples were analyzed in the absence (*H*) or presence (*H/C*) of excess unlabeled IGF-I (10^{-7} M). Reduction of disulfide bonds with dithiothreitol (+*DTT*) (*left*) revealed complexes of 270,000 and 135,000 mol wt; the former represents α–α cross-linking and the latter complex the α (ligand-binding) subunit of the IGF-I receptor. Under nonreducing conditions (*right*), the labeled species appears at 360,000 mol wt and represents the intact IGF-I receptor. MW, molecular weight.

cells. IGF-I also stimulated increased uptake of [^{3}H]T into T lymphocytes cultured in a defined medium containing no growth factors such as insulin. This effect was demonstrated in both resting and PHA-activated T cells. The lack of increased thymidine incorporation seen in resting cells treated with IL-2 (100 U/ml) suggests that these cells were indeed in a resting state, and that entry of cells into the cell cycle was induced by IGF-I alone. The effect of IGF-I on PHA-activated cells was equivalent to that seen with IL-2. Flow cytometric analysis was utilized to confirm the effects of IGF-I on [^{3}H]T incorporation. Dose response analysis suggested that IGF-I induced cellular motility and entry into the cell cycle at doses consistent with action on a high affinity IGF-I receptor.

We then investigated whether the effects of IGF-I on purified T cells might be mediated by the receptor for IGF-I. We have shown that IGF-I binds specifically to both resting and activated T lymphocytes. Scatchard analysis for IGF-I binding revealed a K_d similar to that found in other tissues (1.2×10^{-10} M). Finally, we have demonstrated the presence of the receptor on the surfaces of both resting and activated purified T cells and on a malignant T cell line by affinity cross-linking.

The molecular mechanisms of leukocyte chemotaxis are complex and are reviewed elsewhere (34). Cell locomotion is dependent upon complex cytoskeletal changes. IGF-I has been shown to effect reorganization of the cytoskeleton in a human squamous cell line (35), suggesting that it might be a growth factor capable of inducing cell motility. The effect of IGF-I upon lymphocyte motility has not been previously demonstrated, although other growth factors including IL-1 (16, 17), IL-2 (18), and insulin (20) have recently been shown to be chemotactic for either activated (IL-2) or both resting and activated (IL-1, insulin) T cells. We believe that the effect of IGF-I upon cell motility is receptor-mediated. It is possible that a nonspecific growth factor effect not mediated through receptor binding is responsible for the results we obtained. We have demonstrated that IGF-I is chemotactic for resting and activated human T lymphocytes with a half-maximal concentration consistent with the K_d of the receptor, providing evidence that the effect shown is mediated via high-affinity IGF-I receptors. In addition, the gradient-enhanced motility (chemotaxis) and high-dose inhibition demonstrated resemble that seen with insulin (20), IL-2 (18), and with other receptor-mediated chemotaxis models such as *N*-formylated-methionyl peptides for neutrophils (36, 37) and platelet-derived growth factor for neutrophils and monocytes (38, 39). However, the precise mechanism through which IGF-I enhances T cell motility is unclear.

Insulin receptors are not present on resting T cells, but are inducible by mitogen activation (21–23, 40). Krug et al. (21) demonstrated about 350 insulin molecules bound per transformed (activated) human lymphocyte with virtually no specific receptors for insulin on resting peripheral lymphocytes. Helderman et al. (23) found the lymphocyte insulin receptor to be a universal marker for lymphocyte activation. We have previously shown that insulin is chemotactic for resting as well as activated T cells, but that there is a two-log leftward dose response shift after PHA activation. Because of the structural, functional and antigenic similarities of the insulin and IGF-I receptors, we suggested that IGF-I receptors might mediate the chemotactic effect of insulin on resting cells (20). Evidence for the presence of IGF-I receptors on the surface of resting T cells now further supports this hypothesis. Others have determined that insulin at high doses may compete to occupy IGF-I receptors (13, 24) and it has been proposed that the major mitogenic actions of insulin result from its binding to the IGF-I receptor rather than to the insulin receptor itself (41). IGF-I is chemotactic for both CD4$^+$ and CD8$^+$ T cells, similar to the identical response of these subsets to insulin (20). This is in contradistinction to IL-2, which is chemotactic only for CD4$^+$ T cells (18).

Mitogenic effects of IGF-I have been demonstrated upon IM-9 lymphocytes, a lymphocytoblastoid B cell line (42), and other cell types as well. We have found IGF-I to initiate entry of resting cells into the cell cycle, as well as increasing the [^{3}H]T incorporation of mitogen-activated cells. The growth promoting activity of IGF-I for mitogen-activated cells was similar to that seen with IL-2, suggesting a possible mechanism for IGF-I action in these cells, i.e., stimulation of production of IL-2. This possibility is under study in our laboratory. That this mechanism is not responsible for the cell cycle entry seen in resting cells is suggested by the fact that IL-2 alone did not enhance [^{3}H]T incorporation into resting cells in our assay (Table I). A recent study (43) reported no effect of IGF-I (at doses similar to the ones which we employed) on IL-2 production, nor on antigen-driven proliferation as measured by [^{3}H]T incorporation. However, since our experiments and experimental conditions were different (i.e., different media, no antigen, different assay), we cannot speculate on the reasons for

their negative results. Similarly, suppressive effects of IGF-I upon IL-2–induced lymphocyte proliferation have been demonstrated (44); we examined the effect of IGF-I and IL-2 separately in PHA-activated cells.

Evidence for presence of the IGF-I receptor on resting T cells by competitive binding and affinity cross-linking provides a basis for its behavior as a competence factor. These data suggest that IGF-I produced locally in tissues such as thymus (6) or inflammatory sites might affect the growth and function of IGF-I receptor-bearing T lymphocytes. IGF-I is vital to normal growth. A defect in IGF-I secretion during puberty prohibits growth acceleration; this defect is exemplified in African pygmies (45). Low IGF-I levels also occur in Laron dwarfism (46) and in malnutrition (47, 48). However, the extent of the biologic importance of IGF-I may not yet be realized. In situ hybridization histochemistry studies suggest ubiquitous IGF synthesis with fibroblasts and mesenchymal cells within connective tissues as predominant sites (6). The IGF-I receptor is found in numerous tissues as well and the mitogenic actions of IGF-I upon multiple tissues (1, 3) support a more extensive role for this ligand–receptor system. In addition, fibroblasts both synthesize IGF-I (4, 49, 50) and bear IGF-I receptors on their surfaces (51, 52); the cellular production of "somatomedin-like peptides" has been suggested as an example of the autocrine model of growth regulation (53). Because T lymphocytes are frequently involved with fibrotic tissue reactions the link between the chemotactic and growth factor activity of IGF-I may be important in the recruitment to and activation of effector lymphocytes at sites of fibroblast activation.

The chemotactic and mitogenic phenomena, which we have determined, prompted us to pursue more definitive proof of the IGF-I receptor on the surface of T cells. We demonstrated competitive binding of IGF-I on the surfaces of both resting and activated T cells and analyzed the results by the method of Scatchard (31). The resultant curvilinear plot could be interpreted as representative of a heterogenous population of receptors, i.e., high and low affinity receptors. Other possibilities such as negative cooperativity (54) or tighter binding of labeled IGF-I than of the unlabeled ligand (55) might be considered. Both linear (9, 56) and curvilinear (57, 58) plots have been reported with IGF-I binding in other human tissue types. Our results demonstrate that IGF-I binds specifically to the surface of T lymphocytes. In addition, a cold competition assay was performed comparing the relative abilities of unlabeled insulin and IGF-I to displace ^{125}I-IGF-I from resting T cells; insulin binding was demonstrated only with very high doses of insulin. We believe that this provides a compelling argument for IGF-I binding to resting T cells. It is difficult to be absolutely certain that our results represent a small amount of high-affinity IGF-I receptors on all of the cells; possibly a small subpopulation of cells has a larger number of receptors. Use of the fluorescent activated cell sorter enabled us to be certain that the cells were at least 95% CD3+. The concentration of ^{125}I-IGF-I used in our cross-linking assays was low enough to exclude the possibility of IGF-I binding to insulin receptors which are present on activated T cells. Affinity cross-linking has been utilized in characterizing the quaternary structure of protein oligomers (59, 60) including the insulin (28, 29) and IGF-I (26, 61) receptors. We found the size of the IGF-I receptor complex and its binding subunit to be compatible with that found in other tissues (26, 62–64).

We conclude that IGF-I is chemotactic and mitogenic for both resting and activated human T cells in vitro. The IGF-I receptor that we have demonstrated to be present on the surface of T cells likely mediates these phenomena.

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