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A Monoclonal Antibody to the T-Cell Receptor Increases IGF-I Receptor Content in Normal T-Lymphocytes: Comparison With Phytohemagglutinin

Klaus K.P. Hartmann, Thomas G. Baier, Vincenzo Papa, Michael Kronenwett, Eric J. Brown, Ira D. Goldfine, and Stephen M. Rosenthal

Division of Diabetes and Endocrine Research, Mount Zion Medical Center of the University of California, San Francisco, California 94120 (K.K.P.H., V.P., E.J.B., I.D.G., S.M.R.); Departments of Medicine and Physiology (I.D.G.) and Department of Pediatrics, University of California, San Francisco, California 94143 (K.K.P.H., E.J.B., S.M.R.); Department of Pediatric Endocrinology, Children's Hospital, Heidelberg, Federal Republic of Germany (T.G.B., M.K.)

Abstract The biological effects of the IGFs are mediated through interaction with specific cell surface receptors. It has been previously reported that mitogenic activation of T-lymphocytes by phytohemagglutinin (PHA) is associated with increased IGF-I receptor content. However, the mechanisms which regulate IGF-I receptor expression during T-lymphocyte activation are unknown. To explore further the regulation of IGF-I receptor expression in T-cells, we investigated IGF-I receptor content and mRNA abundance in T-lymphocytes after stimulation either by PHA or OKT-3, the latter being a monoclonal antibody directed against the CD-3 antigen of the T-cell receptor. IGF-I binding in T-cells demonstrated increased IGF-I receptor content after stimulation by both PHA and OKT-3. Peak binding was induced after 72 h of treatment with PHA and 48 h of treatment with OKT-3. Affinity cross-linking of ^{125}I-IGF-I to T-cell membranes demonstrated a single ~ 130 kDa band which was increased after treatment with PHA or OKT-3. This band was inhibited by the addition of α-IR3, a monoclonal antibody to the IGF-I receptor. Both PHA and OKT-3 increased IGF-I receptor mRNA abundance with peak increases at 20 h and 60 h, respectively. Parallel increases in IGF-I receptor and β-actin mRNA abundance were observed, consistent with previous studies demonstrating increased actin gene expression after T-cell activation. Thus, the increase in IGF-I receptor mRNA abundance markedly preceded the increase in IGF-I receptor content after PHA stimulation, but the increase in IGF-I receptor mRNA abundance followed the increase in IGF-I receptor content after OKT-3. These studies suggest, therefore, that IGF-I receptor content in both of these activated cells is not regulated primarily at the level of steady state mRNA.

Key words: IGF-I receptor, T-cells, OKT-3, PHA

Insulin-like growth factor (IGF) I and IGF-II exert a broad range of metabolic and mitogenic effects in many cell types through hormonal as well as autocrine/paracrine mechanisms [1]. The biological activities of these growth factors are mediated through interaction with specific cell surface receptors. The IGF-I receptor, which can mediate the mitogenic effects of both IGF-I and IGF-II in some tissues [2], is a membrane glycoprotein of Mr 300–350 kD, consisting of two extracellular α-subunits (Mr ~ 135 kDa) and two transmembrane β-subunits (Mr 90–95 kDa) [3,4]. When the ligand binds to the extracellular α-subunits, the β-subunits, which have intrinsic tyrosine kinase activity, become activated and IGF action ensues [5,6]. The IGF-I receptor is expressed in many cell types including human blood cells such as erythrocytes [7,8], platelets [9], and peripheral mononuclear lymphocytes (PBML) [10,11]. The IGF-I receptor also has been demonstrated on transformed lymphocytes including human leukemic T- and B-cell lines [12–15].

Activation of T-cells through the T-cell receptor complex results in a cascade of events which lead to DNA synthesis and clonal cell proliferation [16,17]. Activated T-cells express cell surface receptors for cytokines and growth factors, including interleukin-2 and insulin [18,19]. Re-

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Address reprint requests to Dr. Klaus K.P. Hartmann, Box 0434, University of California, San Francisco, San Francisco, CA 94143.

cent studies have demonstrated that phytohemagglutinin (PHA)-induced activation of T-cells results in increased expression of receptors for both IGF-I and IGF-II [11,14]. In addition, an antiserum against IGF-I reduces the response of PBML to PHA suggesting that IGF-I may act as a growth factor for the proliferation of T-lymphocytes [20]. Furthermore, treatment of resting and activated T-cells with IGF-I results in increased thymidine incorporation and increased T-cell migration [14,20,21]. The presence of specific IGF-I receptors on T-cells and the expression of IGF peptides by activated mononuclear phagocytes [22,23] suggest that IGFs may stimulate T-cells through paracrine mechanisms. The mechanisms which regulate IGF-I receptor expression during T-lymphocyte activation, however, are unknown. To explore further the regulation of IGF-I receptor expression in T-cells, we investigated IGF-I receptor content and receptor mRNA abundance in normal T-lymphocytes after stimulation by OKT-3, a monoclonal antibody directed against the CD-3 antigen of the T-cell receptor, or after stimulation by PHA.

MATERIALS AND METHODS

Materials

Recombinant IGF-I was a gift from Ciba-Geigy, Switzerland. IGF-II was purchased from Bissendorf, FRG. ^{125}I-IGF-I was purchased from Amersham, UK; PHA from Wellcome Research Laboratories, USA; OKT-3 from Ortho-Diagnostic, FRG; and fluoresceinisothiocyanate (FITC) antihuman IgG from Behring Werke, FRG. α-IR-3 [24], a monoclonal antibody to the IGF-I receptor, was a gift from Dr. S. Jacobs. A 3.2 kb cDNA for the human IGF-I receptor was obtained as previously described [25]. β-actin cDNA was kindly provided by Cleveland et al. [26].

Cells

Human PBML were isolated from buffy coats [27], and T-lymphocytes were further isolated by the sheep erythrocyte rosette technique [28]. B-lymphocytes were isolated from the remaining cell fraction by Sephadex G10 chromatography [29]. The enriched cell fractions were tested for vitality (> 95%) using 0.25% trypan blue and for homogeneity (> 95%) by indirect immunofluorescence [28] using the T-cell specific antibody OKT-3 and by direct immunofluorescence [28] using an FITC antihuman IgG for B-cells. T-lymphocytes were maintained in RPMI 1640 plus 10% fetal calf serum (FCS) in a 5% CO_2/95% humidified air environment.

IGF-I Receptor Binding Studies

10^6 cells were incubated at 10°C for 3 h with 4.5 pM ^{125}I-IGF-I and increasing concentrations of unlabeled IGF-I in 2 ml Hepes buffer, pH 7.4 (100 mM Hepes, 120 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 10 mM Dextrose, 1 mM EDTA, 15 mM sodium acetate, and 1% BSA). Binding reactions were stopped by addition of 0.5 ml ice-cold Hepes buffer. The cells were centrifuged and the pellet was counted in a gamma counter. Binding was corrected for non-specific ^{125}I-IGF-I binding as determined in the presence of 130 nM unlabeled IGF-I. Scatchard analysis of the binding data was performed using the Ligand program [30].

Chemical Cross-linking of ^{125}I-IGF-I to Cell Membranes

^{125}I-IGF-I was chemically cross-linked to 2–3 × 10^7 intact cells with disuccinimidyl suberate (DSS) at a final concentration of 0.4 mM as previously described [9]. Cells were homogenized and centrifuged at 1,500*g* for 30 min and 100,000*g* for 90 min at 4°C. The resulting membrane pellet was resuspended in Laemmli buffer [31] with 5% v/v 2-mercaptoethanol and separated on a 6% $NaDodSO_4$ (SDS) polyacrylamide gel, and autoradiography was carried out.

IGF-I Receptor Gene Expression

Poly(A)$^+$ RNA was isolated from 2 × 10^8 PMBL by lysing the cells in 1% SDS in a Tris buffer containing 0.2 mg/ml proteinase K [32]. Poly(A)$^+$ RNA was then directly isolated from the cell lysate by adding oligo(dT)-cellulose [32]. After being normalized by hybridization with ^{32}P-labeled oligo(dT) [32,33], 0.5 μg of poly(A)$^+$ RNA was blotted onto a nitrocellulose filter and hybridized with a human IGF-I receptor cDNA or a β-actin cDNA. Autoradiography was carried out following a final wash of 0.1 × SSC/0.1% SDS at 50°C.

RESULTS

To determine the optimal time and mitogen concentration for ^{125}I-IGF-I binding to activated T-lymphocytes, time course and dose-response studies were carried out. When isolated T-cells

were activated by OKT-3 or PHA, maximal ^{125}I-IGF-I binding was measured at concentrations of 10 ng/ml and 1 μg/ml, respectively (Fig. 1A). Using these concentrations, maximal binding of ^{125}I-IGF-I was measured 48 h after addition of OKT-3 and 72 h after addition of PHA to the culture media (Fig. 1B).

To examine the hypothesis that ^{125}I-IGF-I is principally bound to the IGF-I receptor, we cross-linked this radiolabeled ligand to T-cell membranes. The membrane fraction was then solubilized and separated by SDS gel electrophoresis under reducing conditions. In control and in T-lymphocytes activated by OKT-3 (10 ng/ml, 48 h) and PHA (1 μg/ml, 72 h), a single ~ 130 kDa band was detected (Fig. 2). The binding of ^{125}I-IGF-I to the IGF-I receptor α-subunit was completely inhibited by the addition of α-IR-3 (50 ng/ml) (Fig. 2).

To determine whether the increased ^{125}I-IGF-I binding after OKT-3 or PHA stimulation

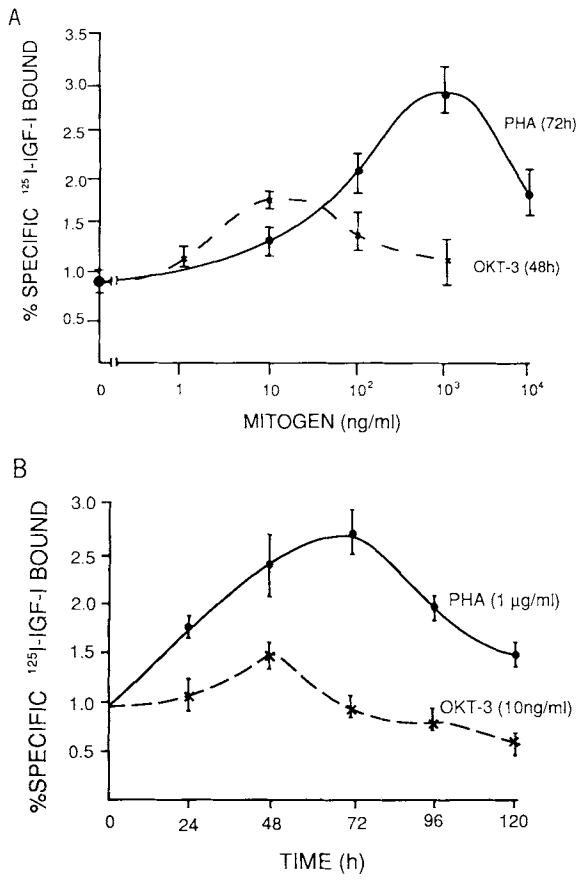

Fig. 1. Dose response (**A**) and time course (**B**) of specific ^{125}I-IGF-I binding to nontransformed T-cells following stimulation by PHA or OKT-3. Results are the mean (± SD) of four (A) and five (B) independent experiments.

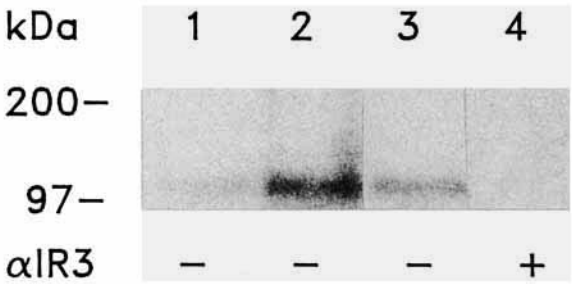

Fig. 2. Cross-linking of ^{125}I-IGF-I to isolated control and activated T-cells. In the absence (**lanes 1–3**) or presence (**lane 4**) of α-IR-3 (50 ng/ml), a monoclonal antibody to the IGF-I receptor, 2–3 × 10^7 cells [control, lane 1; PHA (1 μg/ml, 72 h), lanes 2,4; OKT-3 (10 ng/ml, 48 h), lane 3] were incubated with ^{125}I-IGF-I. Samples were cross-linked with DSS and subjected to electrophoresis and autoradiography as described in Materials and Methods. The positions of the Mr standards are indicated. A representative experiment is shown.

reflected changes in receptor content or affinity, Scatchard analyses were carried out. OKT-3 treatment resulted in a 50–60% increase in receptor content in comparison to control cells (Table 1). No change in IGF-I receptor affinity was seen after OKT-3 treatment. Similar effects on IGF-I receptor content and affinity were seen after PHA treatment. In contrast, B-lymphocytes did not show any specific binding of ^{125}I-IGF-I (Table 1).

To examine IGF-I receptor gene expression in mitogen-activated T-cells, poly(A)$^+$ RNA was isolated and probed with an IGF-I receptor cDNA. Time course analysis of IGF-I receptor mRNA accumulation following OKT-3 (10 ng/ml) treatment demonstrated a 60–70% increase in IGF-I receptor mRNA abundance at 60 h (Fig. 3A). Following PHA (1 μg/ml) treatment, a transient increase in IGF-I receptor mRNA abundance was seen at 20 h. However, after 60 h, a 50% decrease in IGF-I receptor mRNA abundance in comparison to control cells was observed. Analysis of β-actin mRNA abundance was also carried out during these time course studies. Following incubation of cells with OKT-3, β-actin mRNA abundance increased with highest levels noted after 60 h, the time at which maximal IGF-I receptor mRNA abundance was detected (Fig. 3B). After PHA treatment there was a transient increase in β-actin mRNA at 20 h, paralleling the increase in IGF-I receptor mRNA abundance (Fig. 3B). Subsequently a decline in β-actin mRNA was observed.

TABLE I. Scatchard Analysis of ^{125}I-IGF-I Binding in T-Lymphocytes*

	Control	PHA	OKT-3	B-lymphocytes
Kd (nM)	0.16 ± 0.03	0.14 ± 0.02	0.20 ± 0.03	n.d.[a]
IGF-I receptor sites per cell	390 ± 30	770 ± 45[b]	610 ± 40	n.d.

*Normal T-lymphocytes were stimulated for 48 h with OKT-3 (10 ng/ml) (n = 4) or for 72 h with PHA (1 μg/ml) (n = 4). Binding in B-lymphocytes (n = 4) is also indicated. Values are expressed as mean ±SD.
[a]n.d. = not detectable.
[b]$P < 0.05$ vs. control T-cells, unpaired t-test.

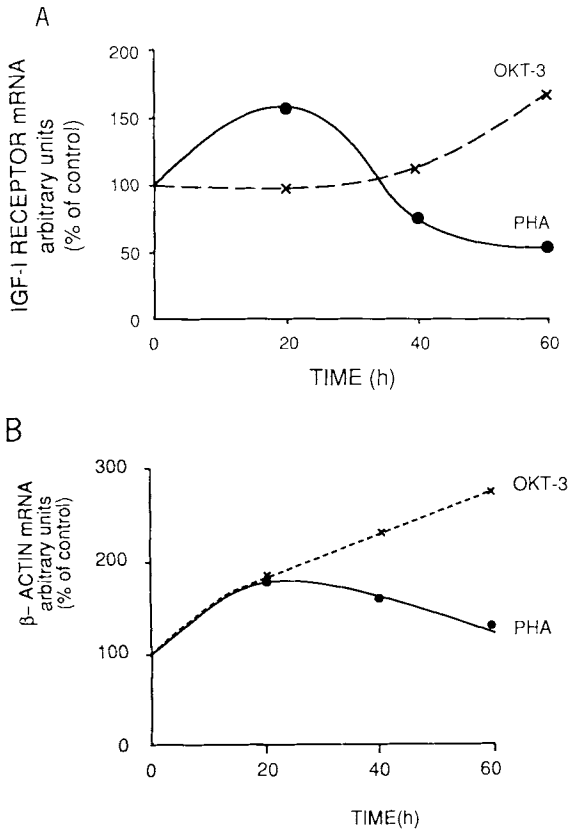

Fig. 3. Time course of IGF-I receptor and β-actin mRNA abundance in control and mitogen stimulated normal T-lymphocytes. Poly(A)$^+$ RNA was isolated from control and PHA (1 μg/ml) or OKT-3 (10 ng/ml) stimulated cells, and slot blotted onto a nitrocellulose filter and hybridized with an IGF-I receptor (**A**) or β-actin (**B**) cDNA. A representative experiment is shown.

DISCUSSION

IGF-I receptor expression in human non-transformed T-lymphocytes can be increased by mitogenic activation of these cells. Stimulation of T-lymphocytes by either PHA, in agreement with earlier studies [11,14], or by OKT-3 was followed by an increase in IGF-I receptor content. The present studies demonstrate that the increased IGF-I receptor content following treatment with PHA and OKT-3 was associated with increases in IGF-I receptor mRNA abundance. The parallel changes in IGF-I receptor and β-actin mRNA abundance were consistent with previous reports demonstrating increased actin gene expression after T-lymphocyte activation [34,35]. However, a discordance between IGF-I receptor content and gene expression both in PHA- and OKT-3–stimulated cells was indicated by the observation that peak IGF-I receptor mRNA abundance followed peak IGF-I binding in OKT-3–stimulated cells and preceeded peak IGF-I binding in PHA-treated cells.

Discordance between IGF-I receptor content and gene expression is not unique to T-lymphocytes. Such discordance has been observed during differentiation of mouse 3T3-L1 pre-adipocytes to adipocytes [36]. In these cells, both insulin and IGF-I binding increased with differentiation [36]. However, while there was a parallel increase in insulin receptor mRNA abundance in the mature adipocytes, IGF-I receptor mRNA abundance decreased [4].

Discordance between gene expression and protein content has also been described in other systems. Mitogen activation of human mononuclear macrophages results in increased expression of IGF-I peptide [22,23]. However, while IGF-I transcription and nuclear IGF-I mRNA abundance increase in these cells, cytoplasmic IGF-I mRNA abundance decreases due to decreased mRNA stability [23]. In addition, it has been recently reported that adult rats and rabbits express high levels of lactase mRNA at a time when lactase content as determined by bioassay is very low [37]. A similar discrepancy between lactase mRNA abundance and enzyme activity has been observed in some lactose-intolerant humans [37].

One possible explanation for the discordance between IGF-I receptor mRNA abundance and receptor content in T-lymphocytes could be an increased efficiency of translation of specific mRNAs. White et al. [34] described that mitogen activation of bovine lymphocytes by Concanavalin A (ConA) induced a fivefold discrepancy

of the mRNA and enzymatic activity of ornithine decarboxylase (ODC). This discrepancy was consistent with a change in efficiency of translation of ODC mRNA as ODC mRNA in activated cells was enriched in polysomes [34]. Thus, a decrease in the amount of untranslated ODC mRNA but an increase in the amount associated with polysomes could result in increased biosynthesis of the enzyme.

In summary, IGF-I receptor expression in T-lymphocytes is increased either through stimulation of the CD-3 antigen of the T-cell receptor or through activation by PHA. However, for both mitogens, stimulation of IGF-I receptor binding was not mediated primarily via an increase in IGF-I receptor RNA abundance.

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