De Novo Emergence of Growth Factor Receptors in Activated Human CD4$^+$ and CD8$^+$ T Lymphocytes

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Using phytohemagglutinin (PHA)-activated human T lymphocytes, we have demonstrated de novo emergence of growth factor receptors (insulin, insulin-like growth factor-1 [IGF-1], and interleukin-2 [IL-2]) in the CD4$^+$ and CD8$^+$ subsets determined by flow cytometry. This activation was also associated with development of insulin-degrading activity (IDA) in a time-dependent fashion. These events, which are actinomycin- and cycloheximide-sensitive, occur only in activated, but not nonactivated, CD4$^+$ and CD8$^+$ lymphocytes. The emergence of these receptors, as well as IDA, which is preceded by CD69 emergence, reaches a plateau by 72 hours and is comparable in both subsets. The IDA is localized in the cytosol, and insulin binding is limited to the cell membrane. T-lymphocyte activation also initiates expression of the *IL-2* gene with the transcription of IL-2 mRNA, the level of which is further enhanced by 38% with the addition of insulin. In these activated lymphocytes, insulin binding to its receptor also caused an 83% upregulation of phosphorylated insulin receptor substrate-1 (IRS-1). In situ development of these growth factor receptors and signal transduction mechanisms in T lymphocytes upon activation, such as by proinflammatory cytokines or oxidative stress, could be an important defense mechanism in various disease states in man.

ALTHOUGH THE pathogenesis of type 2 diabetes (DM2) is not fully elucidated, insulin resistance is the hallmark of DM2 in 3 insulin-sensitive tissues: fat, muscle, and liver.[1-3] In addition, insulin resistance and DM2 are associated with increased cardiovascular events and a proinflammatory state. Recent studies have elucidated some of the mechanisms of action of insulin in insulin-sensitive tissues,[4] and the roles of insulin and insulin-like growth factor-1 (IGF-1) as growth factors in tissue differentiation have been documented.[5] Studies have shown that insulin and IGF-1 signaling occurs via the specific receptors, insulin receptor (IR) and IGF-1 receptor (IGFR), which are receptor tyrosine kinases. Insulin binding to its receptor activates the kinase domain and causes phosphorylation on tyrosine residues of insulin receptor substrate (IRS) proteins, which in turn recruit a variety of proteins such as phosphatidylinositol 3 (PI3)-kinase, Grb-2, SHP-2, etc, for subsequent activation of mitogen-activated protein kinase (MAPK) and Akt pathways. These proteins further propagate intracellular signaling, culminating in important metabolic processes such as glucose metabolism and growth-promoting functions, which have been demonstrated in the muscle and fat cells.[6]

Isolated human fibroblasts have previously been used to assess the intermediary metabolism of various substrates in response to insulin in normal and insulin-resistant states, as well as the metabolism of insulin.[7-9] However, human T lymphocytes are obtainable in greater amounts without weeks of culture, and, although removed from the donor, are reflective of the in vivo milieu. An important finding in our studies[10] and others[11-13] has been the demonstration of the ability of T lymphocytes to bind insulin upon activation. These peripheral blood cells, which usually do not bind insulin and do not respond to insulin in the normal circulating resting state, upon activation by phytohemagglutinin (PHA) or concanavalin A develop insulin binding and properties shared by insulin-sensitive tissues, which include muscle and fat. These properties include glucose utilization and metabolism in response to insulin in a dose-dependent fashion.[10-13] Furthermore, these cells are able to degrade insulin by de novo production of insulin-degrading activity (IDA).[14,15]

Another receptor that is expressed on the T-lymphocyte cell surface upon activation is the interleukin-2 (IL-2) receptor. T lymphocytes are also devoid of the cytokine IL-2; however, once activated, they release IL-2. This IL-2 can then serve as autocrine and paracrine to bind to the IL-2 receptors and promote cell cycle progression.[16]

Isolated T lymphocytes provide an easily available tissue where insulin responses of various metabolic pathways, which may be affected in normal and insulin-resistant states, could be differentiated.[10,17] The role of the CD4$^+$ helper and the CD8$^+$ suppressor T lymphocytes in the overall T lymphocyte activation, and development of insulin binding, signal transduction, insulin degradation, and other growth factor receptors is not fully elucidated.

We hypothesized that upon activation both CD4$^+$ and CD8$^+$ T lymphocytes develop growth factor receptors and insulin signaling pathways for signal transduction as other insulin-sensitive tissues, and, thus, serve as easily accessible tissues for studying mechanisms of insulin action in human subjects in both normal and diseased states.

Therefore, we report our findings on the activation of both CD4$^+$ and CD8$^+$ T lymphocytes and the emergence of the insulin, IGF-1, and IL-2 receptors longitudinally, in conjunction with the CD69 (activation-inducer molecule—an early marker of cell activation), using flow cytometry, concomitant with insulin degradation studies. We also report the effect of insulin with respect

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to signal transduction, after binding to the newly emerged IR on the activated cells by determining the effect of insulin on phosphorylation of IRS-1 and on the *IL-2* gene expression by quantitating the IL-2 mRNA (mIL-2) levels in activated and nonactivated $CD4^+$ and CD 8^+ T lymphocytes.

MATERIALS AND METHODS

Blood Collection and Laboratory Processing

Fasting blood samples of the study patients were collected in EDTA from an antecubital venipuncture from 10 normal subjects who had no history of any metabolic abnormality and were on no medications. Tubes were placed on ice and processed immediately. The blood samples were centrifuged 500 $\times$ *g* for 10 minutes to separate the plasma and isolation of peripheral blood mononuclear cells (PMNs). EDTA rather than heparin was used since heparin is known to modulate IR activities.

Isolation of T Lymphocytes

After centrifugation of the blood the upper layer of plasma was removed and stored. The buffy coat was removed and placed in a tube with saline, layered over Ficoll-Hypaque, and centrifuged at 500 $\times$ *g* for 20 minutes. The PMN layer was then removed, washed with phosphate-buffered saline (PBS) pH 7.4, and incubated with Pan T Cell antibodies (Miltenyi Biotec [MACS], Auburn, CA) to selectively isolate the untouched T lymphocytes. When isolating the T4 from the T8 lymphocytes, magnetic CD4 or CD8 antibodies were also used to purify each population by negative panning techniques with MACS purification. This method consisted of multiple magnetic tagged antibodies to remove all cell populations other than the negatively selected $CD4^+$ or $CD8^+$ T lymphocytes. The purity of this method was confirmed by flow cytometry using fluorescent-labeled CD4- and CD8-specific antibodies. The $CD4^+$ and $CD8^+$ populations were then counted on a Beckman Coulter A^CT diff (Miami, FL) hematology analyzer and diluted to 10×10^6 cells per mL in RPMI 1640 with 10% fetal bovine serum (FBS). PHA [1 μg/ml] was added for the activation of the cells versus controls, which received no PHA. The cells were incubated in a 5% CO_2 incubator at 37°C for 0 , 4, 24, 48, 72, and 96 hours and then analyzed for receptors and phosphorylated IRS-1 using flow cytometry, insulin degradation, and mIL-2 transcription studies.

Isolation of RNA From T Lymphocytes

T-lymphocytes ($CD4^+$ or $CD8^+$) (1 mL of 10×10^6 cells/mL) at each of the above time points were placed in TRIZOL for extraction of RNA. Chloroform (0.2 mL) was added to the tubes containing the T lymphocytes ($CD4^+$ or $CD8^+$). The tubes were vortexed and centrifuged at 12,000 $\times$ *g*. The upper aqueous phase (containing the RNA) was then removed and placed in a DNAse/RNAse-free tube. To the RNA-containing tube, isopropanol was added; the tube was then centrifuged to precipitate the RNA. The RNA pellet was then washed with 75% ethanol, briefly dried, and resuspended in nuclease-free H_2O as previously described.[18,19]

Quantitative Reverse-Transcriptase Polymerase Chain Reaction

mRNA levels of IL-2 and GAPDH (housekeeping gene) were determined using the TaqMan one-step reverse-transcriptase polymerase chin reaction (RT-PCR) assay reagents and primers and probes (Applied Biosystems, Foster City, CA). The probes for IL-2 and GAPDH were labeled with the FAM and VIC reporter dyes, respectively. The conditions of the one-step RT-PCR were as follows: 30 minutes at 48°C (RT), 10 minutes at 95°C, then 40 cycles of amplification for 15 seconds at 95°C and 1 minute at 60°C (PCR). The fluorescence was determined during the cycles on the ABI PRISM 7700 and IL-2 and GAPDH mRNA quantitated using the PRISM software, as previously described.[18,19]

Flow Cytometry Determination of PMN Cell Surface Markers and Receptors

The EPICS Elite EPS Flow Cytometry (Beckman Coulter) was used for the 3-color studies of determination of peripheral blood lymphocyte cell types and expression of cell surface receptors. To ensure optimum instrument performance, daily quality-control procedures were performed using Beckman Coulter's Flow-Check and Flow-Set fluorospheres. Calbrite fluorospheres (Becton Dickinson, San Diego, CA) were used to set the signal overlapping or color compensation. Color compensation was verified using the same fluorochromes used in the analysis. During analysis, the lymphocytes were gated using $CD4^+$ or $CD8^+$ and the forward scatter–side scatter properties of the cells. The gated cells of interest were recorded using dual-color quadstats and single histograms, which record cell number and log fluorescence intensity.[20] The monoclonal antibodies specific for the surface epitopes were: CD4 and CD8, with PC5 labels; CD69 (activation inducing molecule) with phycoerythrin (PE) label, and IL-2 receptor (IL2R, CD25) with fluorescein isothiocyanate (FITC) label, all obtained from Beckman Coulter. Monoclonal antibodies for the receptors for insulin (IR) and IGF-1 (IGF-1R), were PE-labeled, obtained from Becton-Dickinson. The isotypic controls were IgG1-RD1, IgG1-FITC, and IgG1-PC5 (Beckman Coulter).

The cells were labeled with the cell surface probes by incubating 100 μL of l $\times$ 10^6 isolated lymphocytes per 100 μL PBS with 10 μL of the each of the fluoroprobes (CD4-PC5 or CD8-PC5, CD25-FITC, and PE-labeled antibody for IR or IGF-1). After adding antibodies, the tubes were incubated at room temperature in the dark for 30 minutes, after which 100 μL of IntraPrep Reagent I (Beckman Coulter) was added and incubated again for 20 minutes. The cells were next washed with 4 mL of PBS and centrifuged at 1,500 rpm for 10 minutes. To the cell pellet 100 μL of IntraPrep Reagent 2 was added and incubated 5 minutes at room temperature. Cells that were not to be further labeled were washed and resuspended in 0.5 mL of PBS containing 1.5% formaldehyde and analyzed on the flow cytometer.

Specific antibodies to the phosphorylated IRS-1 (2 μL of 200 μg/mL) of rabbit anti-human phosphorylated IRS-1 [pY1229]; Biosource, Camarillo, CA) or normal rabbit serum for control tubes were added to the remaining tubes, which were duplicates of the cell surface probes minus the PE fluoroprobes (IR, IGF-1, or CD69). The mixture was again incubated for 20 minutes at room temperature, washed, and centrifuged and 100 μL of PBS added to the cell pellet. A secondary antibody (donkey anti-rabbit) tagged with a PE fluorescent probe (2 μL of 500 μg/mL) was then added to each tube. After a final 20-minute incubation at room temperature in the dark, the cells were washed with 4 mL of PBS and centrifuged. The cells were resuspended in 0.5 mL of PBS containing 1.5% formaldehyde and analyzed on the flow cytometer. The results were reported as percent of lymphocytes in each sample positive for a given epitope and the absolute volume.[20]

T-Lymphocyte ^{125}I-Insulin Binding

Following incubation of ($CD4^+$ or $CD8^+$) lymphocytes for different times in the presence or absence of PHA (1 μg/mL), the cells were washed 3 times with PBS–2% FBS, resuspended in 50 mmol/L HEPES buffer, pH 7.5, counted, and checked for viability. Aliquots of cells (5×10^6) were added to tubes containing, at final volume, 1 mL buffer, 250×10^3 cpm A_{14}–^{125}I-insulin (specific activity, 360 μCi/ μg), and various concentrations (0 to 10^5 ng/mL) of unlabeled insulin for determination of total and nonspecific binding, respectively, and A_{14}–^{125}I-insulin binding specificity to the IR. Cells were then incubated 2 hours at 15°C. Triplicate aliquots of the cells were layered over dinonyl dibutyl phthalate and centrifuged at 10,000 $\times$ *g* for 2 minutes to separate free from bound hormone. The supernatant was aspirated, the

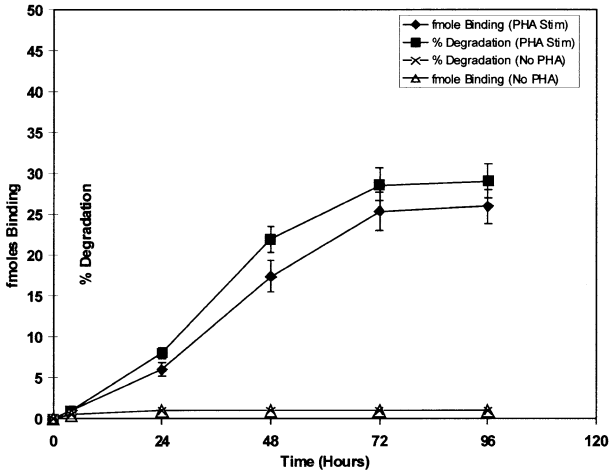

Fig 1. Emergence of ^{125}I-insulin binding and degradation in CD4$^+$ T lymphocytes at designated times of incubation in the presence or absence of PHA (1 μg/mL). Binding is reported as fmol of insulin bound per 5 × 10^6 cells at 15°C. Degradation is reported as percent of the amount of bound insulin degraded per 5 × 10^6 cells at 37°C for 30 minutes. Values are the mean ± SEM for 5 determinations.

portion of tubes containing the pellets was cut, and cell-associated radioactivity was determined. Specific binding was calculated as reported previously.[21]

Insulin Degradation

Lymphocytes were incubated as described above with ^{125}I-insulin for 2 hours at 15×C, washed 3 times with ice-cold PBS to remove unbound insulin, resuspended in HEPES buffer, and incubated at 37°C for 0 and 30 minutes. At the end of each incubation period, the reaction was stopped by adding 1 mL 10% trichloroacetic acid and the tubes were centrifuged. The percent degradation of bound insulin was calculated as follows: [(supernatant counts per min/total counts bound per min)× 100].[21]

Localization of Insulin Binding and Degradation

In order to study the mechanism of emergence of insulin binding and degradation, nonactivated and 72 hours PHA-activated T lymphocytes (CD4$^+$ or CD8$^+$), incubated without or with 35 μmol/L cycloheximide or 10 μmol/L actinomycin D, were homogenized and 100,000 × g pellets and supernatants were tested for binding and degradation of ^{125}I-insulin similar to the intact cells.

RESULTS

Figure 1 demonstrates the emergence of insulin binding and degradation in human CD4$^+$ T lymphocytes upon stimulation with PHA, using ^{125}I-insulin. Both binding and degradation reached a plateau at 72 hours, with binding of 25.4 fmol of insulin per 5 × 10^6 cells of which 28.6% of the bound insulin was degraded by 30 minutes at 37°C. A similar pattern was found with the CD8$^+$ lymphocytes (data not shown). The nonactivated cells demonstrated no binding or degradation. Table 1 depicts these data at 72 hours in the CD4$^+$ and CD8$^+$ subgroups, demonstrating 3.8% and 3.7% ^{125}I-insulin binding and 28.6% and 28% ^{125}I-insulin degradation of this bound insulin, respectively. The nonactivated T lymphocytes (ie, incubated without PHA) showed no binding or insulin degradation. Addition of cycloheximide or actinomycin D during activation completely inhibited PHA-mediated formation of IR and insulin degradation.

Table 1 also shows the localization of insulin binding and degradation results. The cytosol and pellet fractions from the PHA-stimulated CD4$^+$ lymphocytes showed 64% and 0% insulin degradation, respectively, which was inhibited with actinomycin or cycloheximide added during the cell activation period. In contrast, the nonactivated activated cells demonstrated no insulin degradation in either fraction. Only the pellet fractions demonstrated insulin binding, and this was also inhibited with actinomycin or cycloheximide. Both CD4$^+$ and CD8$^+$ T lymphocytes showed similar results. This demonstrates that (1) the insulin-degrading enzyme is localized only in the cytosol fraction; (2) emergence of the IR and degradation events occurs during activation of the lymphocytes; and (3) these events are cycloheximide- and actinomycin-sensitive.

Using flow cytometry to measure the cell surface receptors of insulin (IR), IGF-I (IGFR), and IL-2 (IL2R) during various times of incubation, we detected no appearance of these receptors on the surface of nonactivated T lymphocytes, until after activation with PHA. Figure 2a depicts the flow cytometry profile of these cells after 72 hours of activation using the forward scatter–side scatter and the CD4 or CD8 antibodies to gate on the lymphocytes. Figure 2b and c show the PE-labeled IR antibody binding to the CD4$^+$ T-lymphocyte IRs before and after activation with PHA, respectively. Quadrant 1 of 2b shows only CD4-PC5 antibody bound to the CD4$^+$ cells and quadrant 2 of 2c shows that the majority of the CD4$^+$ cells are labeled with the PE-labeled IR antibody, as well as with the

Table 1. Localization of Insulin Receptors and Insulin-Degrading Activity in Activated and Nonactivated T Lymphocytes

Cell Treatment	% Insulin Binding		% Insulin Degradation	
	CD4$^+$	CD8$^+$	CD4$^+$	CD8$^+$
Nonactivated cells (intact)*	0	0	0	0
Supernatant†	0	0	0	0
Pellet†	0	0	0	0
Activated cells (intact)*	3.8 ± .4‡	3.7 ± .5‡	28.6 ± 2.9‡	28.0 ± 3.1‡
Supernatant†	0	0	64.1 ± 4.1‡	59.3 ± 3.8‡
Pellet†	5.1 ± 1.2‡	4.7 ± 1.3*	0.4 ± 0.2	0.2 ± 0.1

*The results are from 10 subjects and expressed as mean ± SE.

†The results are from 5 subjects and expressed as mean ± SE.

‡Significantly different from nonactivated cells (P = .001).

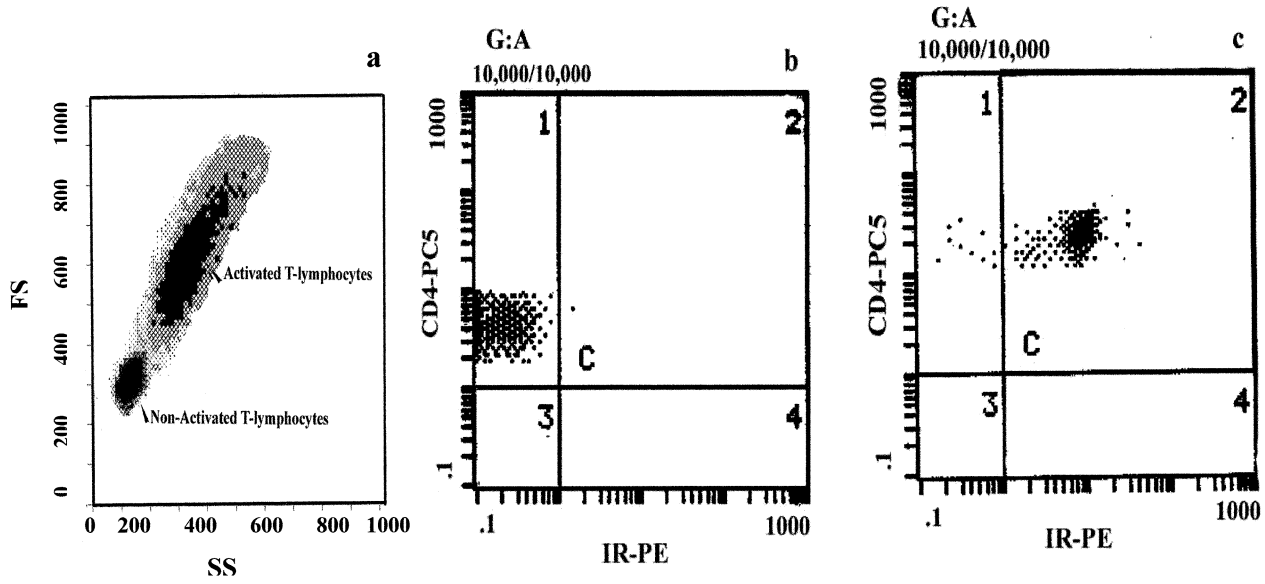

Fig 2. Flow cytometry forward scatter–side scatter presentation of nonactivated and 72-hour–activated lymphocytes (a). (b and c) Dual-color quadstat of nonactivated CD4$^+$ lymphocytes and activated CD4$^+$ lymphocytes, respectively, with the cells labeled with the CD4-PC5 and PE-labeled IR antibodies (IR-PE). Experiments were performed on a total of 10 subjects.

CD4-PC5 labeled antibody. This shows that IRs are not expressed on the lymphocyte cell surface until after activation. The same pattern was also seen in the CD8$^+$ lymphocytes using the CD8-PC5 antibody (data not shown). As state earlier, our method of separation removes all other leukocytes except CD4$^+$ or CD8$^+$ T lymphocytes.

The appearance of IL2R was noted first at 24 hours, followed by the appearance of IR and IGFR, with maximum response of all 3 receptors by 72 hours (Fig 3). The figure also demonstrates the emergence of the CD69, an activation inducer molecule involved in the early events of lymphocyte activation for both CD4$^+$ and CD8$^+$ lymphocytes. As can be seen from Figs 1 and 3, there is a strong relationship between specific ^{125}I-insulin binding and percent receptor expression determined by flow cytometry using fluorescent monoclonal antibodies.

To study the effect of increased insulin levels on the expression of IR, IGFR, and IL2R, 10 ng/mL or 100 ng/mLl of insulin was added to the cell cultures 26 hours prior to binding with the receptor antibody. The IR antibody binding was downregulated by 63% ± 5% and 91% ± 6% with 10 and 100 ng/mL insulin at 72 hours stimulation, respectively. IGFR antibody binding was downregulated by 12% ± 2% and 23% ± 4%, respectively, whereas IL2R antibody binding was increased by 6% ± 1% and 11% ± 2%, respectively. The addition of 10 ng/mL or 100 ng/mL of IGF-1, instead of insulin to the T cells under the same conditions, caused no change in the expression of IR at 10 ng/ml and only showed a 6% ± 2% decrease with 100 ng/mL. However, IGF-1 did downregulate the IGFR antibody binding by 32% ± 3% and 58% ± 5% with 10 ng/mL and 100 ng/mL IGF-1, respectively, whereas no changes were observed in IL2R antibody binding. This shows that insulin binds with the IR, and at higher insulin concentrations interacts with IGFR;

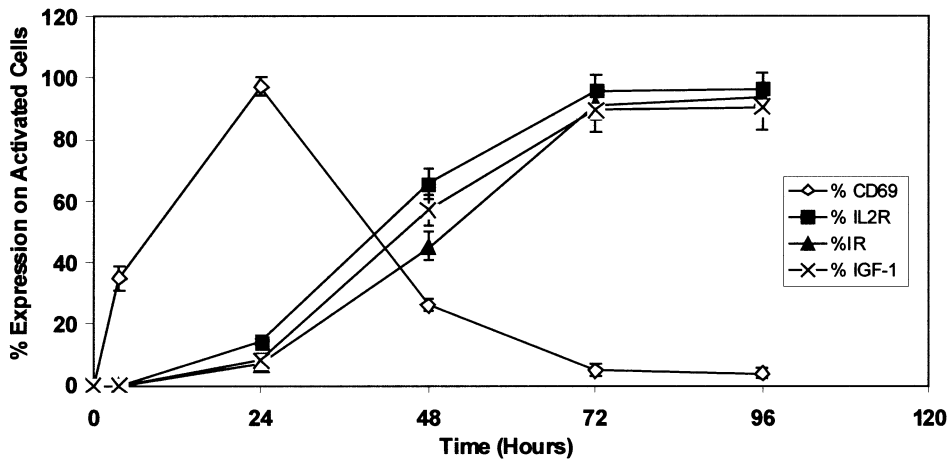

Fig 3. Flow cytometry demonstration of growth receptors and CD69 emergence on CD4$^+$ lymphocytes during various times of incubation after addition of PHA. Values are mean ± SEM of the percent of fluorescent intensity within each channel of the gated cells obtained from 5 determinations. The nonactivated (no PHA added) CD4$^+$ lymphocytes showed 0% fluorescence in the PE channel for the growth receptors antibodies binding over 96 hours (data not shown).

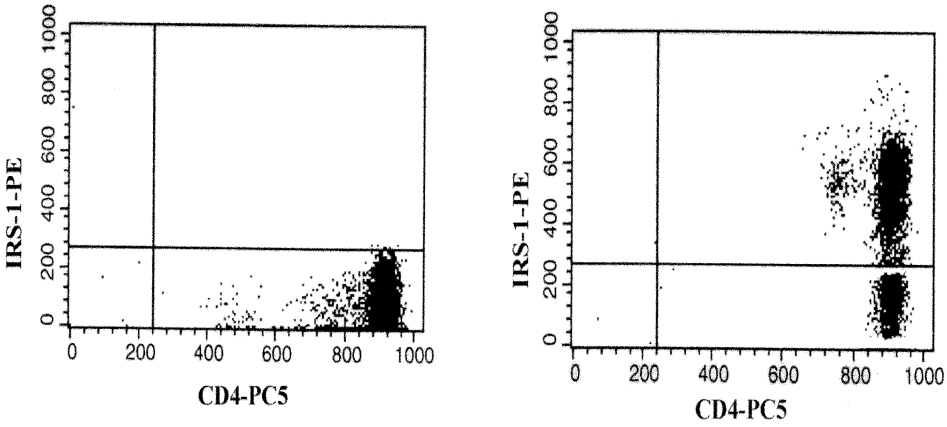

Fig 4. Flow cytometry dual-color quadstat of activated CD4$^+$ lymphocytes labeled with the CD4-PC5 and phosphorylated IRS-1-PE antibodies. (A) No phosphorylated IRS-1 antibody (IRS-1-PE) binding before insulin was added to the PHA-stimulated CD4$^+$ cells (b) IRS-1-PE antibody binding after 1 hour of incubation with 100 μ U/mL human insulin. Experiments were performed on a total of 10 subjects.

however, IGF-1 does not appear to interact with the IR to any appreciable extent.

Figure 4 shows the dual-color quadstat of the flow cytometry analysis of the intracellular binding of the PE-labeled antibody to phosphorylated IRS-1 in the CD4$^+$ T lymphocytes. The binding increased from 0% (in both the nonactivated and PHA-activated CD4$^+$ T lymphocytes) to 83% $\pm$ 7% fluorescent intensity in the 72-hour–activated lymphocytes upon the addition of 100 μU/mL of insulin and incubation for 1 hour (n = 10). However, the addition of insulin resulted in no binding of the phosphorylated IRS-1 antibody to the nonactivated cells. The results were similar in the CD8$^+$ lymphocytes, with an increase in fluorescent intensity of 78% $\pm$ 6% with the addition of insulin to the activated cells.

To assess the effect of insulin on the enhancement of the immune system, we performed quantitative RT-PCR using the RNA extracted from the T lymphocytes, and the results are provided in Table 2. These studies demonstrate that PHA stimulated the transcription of IL-2 mRNA from 26 to 1,540 amol per million cells. This was further enhanced by about 38% with the addition of insulin; however, these stimulatory effects were inhibited by actinomycin. Nonactivated T-lymphocytes demonstrated no changes in IL-2 mRNA levels.

DISCUSSION

Insulin is an immunomodulating hormone in that after binding to its receptor, it can enhance cytotoxic T-cell function, permit mature cell differentiation, and maintain the activated state of lymphocytes.[10-13,22] Although resting human peripheral T lymphocytes are devoid of IR, as determined by ^{125}I-insulin binding to the lymphocytes, these receptors emerge upon activation of cells by specific antigens or mitogens. Insulin has been shown to exert its classical effects on carbohydrate metabolism in the stimulated T lymphocytes, thereby validating the use of activated T lymphocytes for studying the pathogenesis of metabolic and immune disorders and the mechanism(s) by which insulin exerts its effects. In activated T lymphocytes, insulin stimulates glucose uptake, glucose oxidation,[11-13] pyruvate flux and pyruvate dehydrogenase activity,[9,10] amino acid transport, and protein synthesis.[12] Through its ability to enhance nutrient uptake and raise the levels of intermediary cellular metabolism, insulin is believed to maintain the alloactivated state of lymphocytes, enhance cytotoxic responsiveness, and support or possibly promote the actions of immune-derived regulatory growth and differentiation factors.[5,22]

Table 2. Effect of Insulin and Actinomycin on IL-2 mRNA Levels in Activated and Nonactivated T Lymphocytes

Cell Treatment	amol mRNA per 10^6 cells	
	CD4$^+$	CD8$^+$
Cells	26 $\pm$ 2	27 $\pm$ 3
+ INS	28 $\pm$ 3	27 $\pm$ 4
+ PHA	1,540 $\pm$ 65*	1,585 $\pm$ 71*
+PHA + INS	2,125 $\pm$ 83*†	2,143 $\pm$ 92*†
+PHA + INS + Act	25 $\pm$ 3	26 $\pm$ 4

NOTE. The results are from 10 subjects and expressed as mean $\pm$ SE.

Abbreviations: INS, insulin; Act, actinomycin D.

*Significantly different from nonactivated cells (P = .001).

†Significantly different from PHA alone–activated cells (P = .001).

In previous reports, we have found that insulin binding, processing, and responsiveness in PHA-activated T lymphocytes are reflective of the donor's glycemic status and ambient insulin levels,[17,23-25] which has also been described in monocytes[26] and other tissues.[27] Thus, in the insulin-resistant state with hyperinsulinemia, activated T lymphocytes respond to insulin less than in normal individuals.[17,25]

In this study using 3-color flow cytometry has provided distinct advantages over our prior method using radioactive labeled ligands because with the flow cytometer the quantity of CD4$^+$ or CD8$^+$ T lymphocytes can be determined, and of those cells, the quantity that are activated and the quantity of the receptors, such as insulin, IGF-1, IL-2, on each activated cell type. In addition, intracellular signaling mechanisms can be determined on these same cells by the flow cytometer. In contrast, the method for quantitation of receptors using radioactive labeled ligand (such as ^{125}I- insulin) only yields a percent of the ligand bound to all the cells relative to the total counts.

As to the reason and mechanism for development of these growth factor receptors (IR, IGFR, and IL2R) in activated T lymphocytes, it is tempting to hypothesize that during a proinflammatory states, including those of uncontrolled diabetes,[28-30] production of cytokines, such as tumor nrcrosis factor-α and IL-2, can activate T lymphocytes with emergence of these growth factor receptors. The IDA, which is detected

upon T-lymphocyte activation, is probably due to insulin-degrading enzyme (IDE); although our studies did not confirm by purification or specific IDE antibodies, studies by us and others previously have determined IDE to be the major enzyme responsible for insulin degradation in most tissues.[7,21,27] The appearance of signal transduction mechanisms, such as we observed with the phosphorylation of IRS-1, in conjunction with IR, could plausibly increase the ability of the immune system, ie, T lymphocytes, to combat any unfavorable effects of stress, insulin depletion, or cell death.[31] Further studies will be needed to investigate activation responses of lymphocytes in situ in various states of oxidative stress and diabetic complications[32] to verify this hypothesis.

In summary, we have demonstrated the emergence of 3 important growth factor receptors in activated human $CD4^+$ and $CD8^+$ T lymphocytes preceded by the emergence of CD69. These cells then become responsive to insulin, as determined by phosphorylated IRS-1 levels and enhancement of transcription of IL-2 mRNA. The importance of these cells as markers of in situ inflammatory state and their metabolic responses to insulin might be an important means by which these cells can overcome oxidative stress.

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