Interleukin-3–Induced Downregulation of the Expression of Interleukin-2 Receptor β Chain in Human T Cells

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We examined the effect of interleukin-3 (IL-3) on human CD4+ clone T cells, P607 and 1C2. By flow cytometric analysis, we found that IL-3 downregulated the surface expression of IL-2 receptor (R) β chain in a dose-dependent manner but had little effect on that of IL-2R α chain. A simultaneous [3H]-labeled IL-2 binding assay showed a decrease in the number of high-affinity, but not of low-affinity, IL-2Rs by IL-3. The downregulation of the IL-2R β chain began 3 hours after culture initiation, increased further thereafter, and was completely inhibited by anti–IL-3 antibodies. Expression of mRNA for either α or β chain was not reduced by IL-3, and this suggests that the reduction of surface β chain expression was not caused by the reduction of β chain mRNA. IL-3–accelerating internalization of IL-2R β chain appeared to be one of the mechanisms for IL-3–induced downregulation of surface IL-2R β chain expression. IL-3 alone increased the proliferation of T-cell clones but decreased the existing increment of their proliferation by IL-2. Accordingly, IL-3 may be one of the factors acting as a liaison between the hematopoietic and immune systems.

INTERLEUKIN-2 (IL-2) plays an essential role in T-lymphocyte (T-cell) activation and subsequent proliferation, exerting its effect through specific receptors expressed on the T cells. The IL-2 receptor (IL-2R) consists of at least two subunits, p55 (Tac, α chain) and p75 (β chain), and the affinity of IL-2 to its receptors is defined by each chain involved in IL-2 binding. When each IL-2R subunit is present alone, low (dissociation constant [kd] 10 nmol/L) and intermediate (kd 1 nmol/L) affinity of IL-2R is expressed, and they make a high-affinity IL-2R complex (kd 10 pmol/L) when combined. The α chain alone cannot transduce the IL-2–mediated signal, but the β chain plays a key role in transmitting an IL-2 signal.

There have been many reports about the effects of various factors on IL-2R expression. IL-2 upregulates the expression of α chain on T cells1–4 by increasing the gene transcription rate. Also producing similar effects are IL-1 (T cells5–7 and natural killer cells8), IL-5 (B cells), IL-7 (T cells9–12), adult T-cell leukemia–derived factor,13 tumor necrosis factor-α,13 and prolactin.14 However, few data are available on those factors that regulate the expression of β chain. The concept that IL-4 downregulates expression of IL-2 receptor β chain15–17 is controversial.18

IL-3 was initially defined as the factor in conditioned media from concanavalin A–stimulated splenic lymphocytes that promotes induction of 20α-hydroxysteroid dehydrogenase in nu/nu splenic lymphocytes.19 Subsequently, it has been shown to have multiple functions, to affect various cell types, and to be identical to previously reported factors20 including mast cell growth factor, P-cell–stimulating factor, histamine-producing cell-stimulating factor, burst-promoting activity factor, and multipotent hematopoietic progenitor cell growth factor acting on the early stage of development. There have been reports that IL-3 supports the continuous growth of murine helper T cells21 and promotes the IL-2–driven growth of human T cells,22 but further studies are needed to define its exact function in these cells. In the present study, we examine the effect of IL-3 on human T cells and find that IL-3 modulates the IL-2–mediated cell growth by downregulating the surface expression of the IL-2R β chain but not that of the α chain.

MATERIALS AND METHODS

Production of T-cell clones. We separated peripheral blood mononuclear cells (PBMCs) from heparinized venous blood of healthy volunteers by Ficol/Hypaque (Pharmacia LKB Biotecnology AB, Uppsala, Sweden) gradient centrifugation and cultured them for 2 to 8 weeks in medium containing 1 nmol/L recombinant IL-2 (1 U/mL = 0.5 nmol/L; kindly provided by Takeda Chemical Industries, Osaka, Japan) with repeated stimulations with 0.1% phytohemagglutinin-P (PHA-P; Difco, Detroit, MI). The cells were then cloned by limiting dilution procedures. We seeded the cells into 96-well, flat-bottom microtiter plates with 2 × 10⁵ cells/well of irradiated (40 Gy) feeder cells SUB024, an Epstein-Barr virus-transformed B-cell line derived from PBMCs of a patient with infectious mononucleosis.

Cell culture. We cultured the cells in RPMI 1640 medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratory, McLean, VA) in a humidified incubator with 5% CO₂ at 37°C. T-cell clones were cultured with 1 nmol/L IL-2 and irradiated feeder cells (SUB024) were added to the culture every 2 weeks.

Monoclonal antibodies (MoAbs). MoAbs used in this study were anti-Tac23 and 7G7/B6²⁴ (kindly provided by Dr David L. Nelson, National Cancer Institute, Bethesda, MD) as anti–IL-2R α chain, and 2R-B²⁵ and Mik-β3²⁶ (kindly provided by Dr M. Tsudo, Unitika Hospital, Kyoto, Japan) as anti–IL-2R β chain. Anti-Tac and 2R-B compete for IL-2 binding to α and β chain, respectively. MoAbs 7G7/B6 and Mik-β3 recognize the IL-2R α and β chain, respectively, but they do not inhibit IL-2 binding to the receptor.

Flow cytometry. Cells were stained and flow cytometric analysis was performed as previously described.27 In brief, we incubated 3 × 10⁵ cells for 30 minutes at 4°C with fluorescein isothiocyanate (FITC)-conjugated anti-Tac (12 μg/mL) and 2R-B (6 μg/mL) MoAbs in a final volume of 40 μL. There was a possibility that contaminated IL-2 in the culture supernatant bound to IL-2R α or β chain and blocked the binding of anti-Tac or 2R-B to the IL-2R. To address this concern, we incubated cells with 7G7/B6 or...
bionylated Mik-β3, washed them twice, and further incubated with FITC-conjugated goat antimouse IgG (Tago, Burlingame, CA) or phycoerythrin (PE)-conjugated streptavidin (Biomed, Foster, CA) in some experiments. FACScan (Becton Dickinson, San Jose, CA) analysis of stained cells yielded a mean fluorescence intensity (MFI). We determined AMFI by subtracting background MFI from the MFI of a sample examined. To determine the background fluorescence intensity, cells were stained either directly with FITC-conjugated control MoAb X39 (Becton Dickinson) or indirectly with biotinylated control MoAb X63 and PE-conjugated streptavidin.

Reagents. We used recombinant human IL-3 (1 × 10^8 U/mg) and rabbit antihuman IL-3 (both kindly provided by Dr. K. Asano, Kirin Brewery, Maebashi, Japan). In earlier experiments, anti–IL-3 antibody (Ab) was found to neutralize the activity of 10 ng (10^5 U/mL of IL-3) when used at a 1:1,000 concentration of the serum.

Radiolabeled IL-2 binding assay. The methods used have been described elsewhere. Briefly, we labeled IL-2 with Na^125I (Amersham Japan, Tokyo) by using Enzymobeads (Bio-Rad Laboratories, Richmond, CA) or chloramine T (Nacalai Tesque, Kyoto, Japan). The specific activity of this IL-2 was 14,000 to 18,000 cpm/ng. The cloned T cells, cultured without IL-2 for 24 hours, washed, and cultured in fresh medium with or without prescreened concentrations of IL-3 for another 12 hours, were then washed thoroughly to perform the ^125I-labeled IL-2 binding assay.

For this assay, washed cells were cultured with various concentrations of ^125I-labeled IL-2 at 4°C for 60 minutes with or without an excess amount of unlabeled IL-2 in the binding medium (RPMI 1640 with 25 mmol/L HEPEs, 1 mg/mL of NaHCO_3, and 10 mg/mL of bovine serum albumin [BSA] at pH 7.4). We centrifuged the labeled cells over the oil cushion (20% olive oil, 80% di-n-butyl phthalate), and measured both cell-bound and free radioactivities.

The specific binding was calculated by subtracting the nonspecific binding observed in the presence of a 250-fold excess of unlabeled IL-3. The number of binding sites and Kd were calculated from Scatchard plots.

DNA probes. To detect the IL-2R α and β chain gene-specific sequences, we used the Sau3AI fragment of pKCR-Tac-2AZ8 and 2-kbp fragments encoding nucleotide positions 118 to 2,330 and 1,600 to 3,572 of the published sequence of IL-2R β chain, respectively (both kindly provided by Dr. T. Honjo, Kyoto University, Kyoto, Japan). The probes of complementary deoxyribonucleic acid (cDNA) for β-actin were purchased from Nippon Gene (Tokyo, Japan). These probes were ^32P-labeled by the multiprimed DNA labeling system (Amersham International, Buckinghamshire, England) or by a nick translation kit (Amersham).

Northern blot analysis. We isolated the total RNA from cells and performed Northern blot analysis as previously described. In brief, we washed cells in phosphate-buffered saline solution and extracted total RNA by guanidine isothiocyanate solubilization followed by centrifugation through cesium chloride. Isolated RNA (20 μg) from each sample was applied to 1% agarose gel, blotted, and hybridized with labeled probes for IL-2R α chain, β chain, and β-actin, sequentially, and exposed to an x-ray film at -70°C with an exposure time of 2 weeks.

Cytoplasmic dot hybridization. As previously described, 5 × 10^5 cells were lysed by adding 0.5% Nonidet P-40 in the presence of 10 mmol/L Vanadyl ribonucleoside complex (Bethesda Research Laboratories Inc, Bethesda, MD). After pelleting the nuclei, we incubated the supernatant with 7.4% formaldehyde and 6× standard saline citrate (0.15 mol/L NaCl, 0.015 mol/L trisodium citrate) at 60°C for 15 minutes. The samples, serially diluted with 15X standard saline citrate, were applied with suction to a 4-mm diameter spot on nylon filters starting with cytoplasmic extracts derived from 1 × 10^6 cells with a twofold serial dilution. After prehybridization, we hybridized the filters with labeled probes for IL-2R α chain, β chain, and β-actin.

Determination of the internalized IL-2R β chain. We estimated internalization of IL-2 receptor β chain by T-cell clones as internalized Mik-β3 MoAb (described elsewhere) with some modifications, using radiolabeled Mik-β3 MoAb. Mik-β3 MoAb was labeled with Na^111 using Enzymobeads with specific activity of 7,500 cpm/ng. Cells (3 × 10^5) were incubated with a half-saturating concentration (700 ng/mL) of ^111-labeled Mik-β3 MoAb with or without a 250-fold excess of unlabeled Mik-β3 MoAb for 30 minutes at 4°C in RPMI 1640 with 25 mmol/L HEPEs and 10 mg/mL of BSA at pH 7.4. Then we transferred the cells to a 37°C water bath with or without 500 U/mL of IL-3. Chloroquine (100 μmol/L; Sigma Chemical, St Louis, MO) added to each culture prevented degradation of internalized ^111-labeled Mik-β3 MoAb. After the indicated times, we added two aliquots of cells and passed one of them through an oil cushion. The other aliquot was resuspended in acidic buffer (0.2 mol/L glycine, 0.14 mol/L NaCl, and 10 mg/mL of BSA at pH 3.0) with gentle pipetting for 30 seconds at 23°C and centrifuged over an oil cushion. The radioactivity of the cell pellet was counted to determine the total cell-associated and internalized (acid-resistant) Mik-β3 MoAb, respectively. We counted all samples in duplicate, and calculated specific binding by subtracting nonspecific counts determined by the radioactivity of the cells incubated with an excess amount of unlabeled Mik-β3 MoAb. In some experiments, cells were preincubated with or without 500 U/mL of IL-3 for 3 hours at 37°C before incubation with ^111-labeled Mik-β3 MoAb at 4°C.

Cell proliferation assay. Short-term cell proliferation was determined by measuring ™H-thymidine (™H-TdR) incorporation into cells, as previously described. That is, cells (2 × 10^5/well) were cultured in 96-well microtiter plates in the presence of the indicated concentrations of IL-2 plus IL-3 at 37°C for the required time. For the last 6 hours, we added 0.5 μCi/well of ™H-TdR to each well and then harvested the cells on a fiberglass filter. A liquid scinntillator measured incorporated radioactivity.

RESULTS

Production of T-cell clones. The established T-cell clones P607 and 1C2 were both CD4^+ cells (their clonality was shown by the analysis of rearrangement patterns of the genes encoding the β chain of the T-cell receptor; data not shown). Expression of IL-2R α and β chain on the cloned cells, determined by the reactivity with anti-Tac and 2R-B MoAbs, depended on the duration of culture from the last stimulation by SUB024 cells. We studied the time course of the IL-2R expression in the clones after the addition of irradiated SUB024. IL-2R α chain was increased by the addition of SUB024 and then was followed by a gradual reduction; β chain showed a slight change throughout the culture (data not shown). Because both α and β chain showed a stable expression from 2 weeks after the last addition of SUB024, all subsequent experiments were performed in the third week.

In six preliminary experiments, we compared the mode of IL-2R expression at different time schedule from the initiation of IL-2 starvipng. Expression of IL-2R α chain decreased gradually and that of IL-2R β chain increased rapidly during the initial 24 hours and both then reached a plateau. We confirmed that the viability of these cells cultured with medium alone could be retained for a period

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of up to 3 days. Hence, in all experiments cells were starved from IL-2 for 24 hours.

**Effect of IL-3 on the expression of IL-2R.** We used flow cytometric analysis to examine the effect of IL-3 on the expression of IL-2R α and β chain. In T-cell clones P607 and 1C2, the surface expression of IL-2R β chain determined by reactivity with 2R-B MoAb decreased with the addition of IL-3 (50% ± 6% [n = 6] and 32% ± 5% [n = 10], respectively) (Fig 1), whereas IL-3 had little effect on the expression of IL-2R α chain determined by reactivity with anti-Tac or 7G7/B6 MoAbs. IL-3 also reduced the reactivity of the T-cell clones with biotinylated Mik-β3 MoAb, which recognized the non-IL-2 binding epitope on the IL-2R β chain.

Using ^125^I-labeled IL-2 binding assay and Scatchard plot analysis, we next examined the effect of IL-3 on the expression of high- and low-affinity IL-2R on P607 and 1C2. The P607 cells cultured in the medium alone expressed 3,800 sites/cell of high-affinity IL-2R (kd 11 pmol/L) and 39,000 sites/cell of low-affinity IL-2R (kd 11 nmol/L), as shown in Fig 2. By contrast, when cultured with 100 U/mL of IL-3 for 12 hours at 37°C, the P607 cells expressed a decreased number of high-affinity IL-2R sites/cell (2,500; kd 12 pmol/L), but the number of low-affinity IL-2R sites/cell (38,000; kd 10 nmol/L) remained unchanged.

We conducted four independent ^125^I-labeled IL-2 binding assays in 1C2 cells and have summarized the results in Table 1. In cells treated with IL-3 for 12 hours, the number of high-affinity IL-2R sites decreased, but the affinity remained similar in both treated and untreated cells. However, no changes were detected either in the number or in the affinity of low-affinity IL-2R sites. The degree of reduction in binding sites of the high-affinity IL-2R calculated by Scatchard plot analysis was 28% to 44% in 1C2 cells pretreated with IL-3. The results correlated well with the reduced ΔMFI of β chain expression detected by flow cytometric analysis.

When cells were pretreated with 100 U/mL of IL-3 at 4°C for 30 minutes in the binding medium containing 1 mg/mL of NaN, and an ^125^I-labeled IL-2 binding assay was performed in the presence of IL-3, the number and affinity of both high- and low-affinity IL-2R sites on 1C2 cells were not affected (data not shown).

The relationship between the concentration of IL-3 and the expression of IL-2R α or β chain measured by percentage of ΔMFI (%ΔMFI) was evaluated. Expression of IL-2R

![Fig 1. Effect of IL-3 on the surface expression of IL-2Rs.](image-url)

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Fig 2. Effect of IL-3 on the expression of high- and low-affinity IL-2R. The P897 cells were cultured with (○—○) or without (●—●) 100 U/mL of IL-3 for 12 hours at 37°C, washed, and cultured with various concentrations of 3H-labeled IL-2 for 60 minutes at 4°C. IL-2 binding assay and Scatchard plot analysis were then performed. The number of bound 3H-labeled IL-2 molecules/cell (x-axis) and the ratio of bound 3H-labeled IL-2 molecules over free concentration of 3H-labeled IL-2 (y-axis) are plotted here.

β chain gradually decreased as the concentration of IL-3 was increased, reaching a minimum level at an IL-3 concentration of 256 U/mL and followed by a plateau (Fig 3). In contrast, expression of IL-2R α chain was very slightly affected by the addition of up to 4,096 U/mL of IL-3.

Kinetics of the downregulation of IL-2R β chain expression by IL-3. We analyzed the kinetics of the IL-3-induced

Table 1. Effect of IL-3 on the Number and Dissociation Constant of IL-2R Binding Sites on 1C2 Cells

<table>
<thead>
<tr>
<th>IL-3 Addition</th>
<th>High-affinity IL-2R</th>
<th>Low-affinity IL-2R</th>
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<tr>
<td></td>
<td>No.</td>
<td>kd (pmol/L)</td>
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<tr>
<td>Assay 1</td>
<td>-</td>
<td>2,000</td>
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<tr>
<td></td>
<td>+</td>
<td>1,200</td>
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<tr>
<td>Assay 2</td>
<td>-</td>
<td>1,800</td>
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<td></td>
<td>+</td>
<td>1,300</td>
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<td>Assay 3</td>
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<tr>
<td>Assay 4</td>
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<td></td>
<td>+</td>
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1C2 cells were cultured in the presence or absence of IL-3 (100 U/mL) for 12 hours at 37°C and 3H-labeled IL-2 binding experiments were performed as described in Materials and Methods. The number and affinity of IL-2 receptors were examined by Scatchard plot analysis. Abbreviation: ND, not done.

Fig 3. Dose-response relationship of IL-3 in the expression of IL-2R in 1C2 cells. Representative results of three independent experiments are presented. Cells were cultured with various concentrations of IL-3, stained with FITC-conjugated anti-Tac MoAb (○—○) and FITC-conjugated 2R-B MoAb (■—■), and analyzed using a FACScan. The %ΔMFI [(each MFI/ΔMFI of the cells cultured without IL-3) x 100] is shown.

Fig 4. Kinetic study of the IL-3-induced downregulation of β chain expression. Kinetics of the expression of IL-2R α chain (A) and β chain (B) in 1C2 cells after coculture with (○—○) or without (●—●) 100 U/mL of IL-3 were examined using FITC-conjugated anti-Tac MoAb and FITC-conjugated 2R-B MoAb. The %ΔMFI [(each ΔMFI/ΔMFI of the cells at 0 h) x 100] was calculated and the mean of %ΔMFI in each of five independent experiments is shown.
downregulation of IL-2R β chain expression in 1C2 cells. The IL-2R β chain expression showed a marginal (11%) reduction for the first 3 hours after IL-3 was added, but this was followed by an abrupt and progressive decrease at 3 hours, reaching a minimum plateau by 8 hours (Fig 4). The kinetics contrasted with those of IL-2R α chain in which no essential difference was disclosed in cultures with or without IL-3. Similar results, including an abrupt decrease at 3 hours of incubation, also were obtained in P607 cells (data not shown).

**Direct effect of IL-3 on the reduction of IL-2R β chain.** Adding rabbit antihuman IL-3 antisera but not normal rabbit serum to the culture resulted in almost complete inhibition of IL-2R β chain downregulation (Fig 5), and this indicates that the inhibition is the effect of IL-3 itself. Furthermore, we tested IL-2 activity in the culture supernatant by the proliferation of the IL-2-dependent T-cell line Kit 225 in which growth was not affected by the addition of IL-3. The analysis disclosed no detectable amount of IL-2 (<2 pmol/L; data not shown).

**Effect of IL-3 on the expression of IL-2R β chain mRNA.** To study the mechanism by which IL-3 downregulates the surface expression of IL-2R β chain, we first examined the expression of IL-2R β chain mRNA by Northern blot analysis. Specific bands for IL-2R α chain, β chain, or β-actin were detected. Compared with mRNA for β-actin, the expression of mRNA for IL-2R α or β chain was not decreased by adding IL-3 (Fig 6). In a further analysis with cytoplasmic dot hybridization technique, addition of IL-3 induced a twofold increase in the mRNA for IL-2R β chain at 3 hours (Fig 7A, lane h). We detected an increase in the expression of mRNA for β-actin by IL-3, but not to the degree observed for IL-2R β chain (Fig 7B, lane h). No difference in the expression of mRNA for the IL-2R β chain between cells cultured with medium alone and with IL-3 was disclosed at 6, 9, and 12 hours. IL-3 did not affect the mRNA concentration for IL-2R α chain (data not shown).

**Effect of IL-3 on the internalization of IL-2R β chain.** Because the decrease in the surface expression of IL-2R β chain did not correlate with the decrease in mRNA expression, we next examined the internalization of IL-2R β chain. After 12 hours of incubation, a twofold increase in the mRNA for IL-2R β chain was observed (Fig 7B, lane h). To study the mechanism by which IL-3 downregulates the surface expression of IL-2R β chain, we used a monoclonal antibody against Tac (IL-2R β chain) and examined the cell surface expression of IL-2R β chain after 12 hours of incubation. The results showed that the antibody was able to bind to the surface of 1C2 cells, indicating that the decrease in the surface expression of IL-2R β chain was due to internalization of the receptor instead of degradation.
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Effect of IL-3 on the IL-2-mediated proliferation of T-cell clones. When we added only IL-3, we saw dose-dependent stimulation of the proliferation of both P607 and 1C2 cells, evaluated by the incorporation of 3H-TdR. At an IL-3 concentration of 100 U/mL, the count increased from 5,948 ± 888 to 12,152 ± 636 in P607 and from 2,577 ± 487 to 7,029 ± 672 in 1C2 (Fig 9). The addition of an excess amount of anti-Tac MoAb (100 μg/mL) inhibited the IL-2-mediated but not IL-3-mediated cell proliferation, suggesting that the cell proliferation induced by IL-3 was not due to the IL-2 secreted from T-cell clones on stimulation with IL-3. However, a significant decrease in the magnitude of IL-2–induced cell proliferation was seen after 100 U/mL of IL-3 was added (Fig 9). This effect was dose-dependent; we saw IL-2–induced cell proliferation less inhibited at a concentration of 10 U/mL IL-3, and this became marginal at 1 U/mL.

DISCUSSION

Immunologic events are controlled by a network of various cytokines, but the exact role of IL-3 in human T cells has remained unclear. The reported distribution of IL-3R analyzed by radiolabeled IL-3 binding assay has been restricted to the myeloid and pre-B cells. However, researchers have reported that IL-3 affects the growth of T cells; hence, there may be a trace amount of IL-3R on T cells.

In this study, a flow cytometric analysis using anti–IL-2R β chain MoAbs 2R-B and Mik-β3 showed that IL-3 downregulates in a dose-dependent manner the expression of IL-2R β chain in cloned T cells P607 and 1C2. The addition of 100 U/mL of IL-3 induced a 32% and 50% reduction in the expression of β chain in 1C2 and P607 cells, respectively. This result is consistent with the observation that IL-3 downregulates IL-2R β chain expression in cloned T cells.

fig 7. Time-course analysis of IL-2R mRNA expression after the addition of IL-3 examined by cytoplasmic dot hybridization. The 1C2 cells were cultured with (100 U/mL, lanes h through k) or without (lanes c through g) IL-3 and harvested at 0 (c), 3 (d and h), 6 (e and i), 9 (f and j), and 12 hours (g and k). The cytoplasmic extract of each sample was spotted onto each filter with a twofold serial dilution starting with the extract derived from 1 x 10⁶ cells. Expression of mRNA for IL-2R β chain (A) and β-actin (B) was analyzed. The expression of mRNA in ED515-D (a) as a positive and HL60 (b) as a negative control for IL-2R β chain mRNA expression are also shown.

fig 8. Internalization of ¹²⁵I-labeled Mik-β3 MoAb bound to IL-2R β chain on 1C2 cells. The cells were labeled with 700 ng/mL of ¹²⁵I-labeled Mik-β3 MoAb at 4°C for 30 minutes with or without an excess amount of unlabeled Mik-β3 MoAb, washed, and warmed to 37°C in the presence (O—O) or absence (●—●) of 500 U/mL of IL-3, which was added to the cells immediately before transfer to a 37°C water bath. Chloroquine (100 μmol/L) was added to each culture. A time-course analysis of the acid treatment-resistant radioactivity at 37°C, which represented the internalized ¹²⁵I-labeled Mik-β3 MoAb, is shown (A). The 1C2 cells were precultured with (O—O) or without (●—●) IL-3 at 37°C for 3 hours before incubation with ¹²⁵I-labeled Mik-β3 MoAb at 4°C (B). These are the representative results of two independent experiments.

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follows: (A) 5,948 were cultured without IL-2 for 24 hours, and were plated at 2 x 10^6/well in 96-well microtiter plates in the presence of various concentrations of IL-2 with (C--C) or without (B--B) 100 U/mL of IL-3 for 24 hours at 37°C. 'H-TdR incorporation for the last 6 hours was measured and the mean counts per minute from triplicate wells were calculated. The concentrations of added IL-2 (x-axis) and the fold increase of the 'H-TdR uptake ('H-TdR uptake with IL-2/ 'H-TdR uptake without IL-2: y-axis) are plotted. The count without IL-2 is as follows: (A) 5,948 ± 588 (without IL-3); 12,152 ± 638 (with IL-3); (B) 2,577 ± 487 (without IL-3); 7,020 ± 672 (with IL-3).

respectively. The binding assay with 125I-labeled IL-2 disclosed that IL-3 induced a decrease in the number of high-affinity IL-2 receptors. The results are compatible with the reduction of β chain surface expression proved by flow cytometric analysis. However, on the contrary, IL-3 showed no effect on the expression of IL-2R α chain in the simultaneous analysis using these methods. In murine, IL-3 has been reported to enhance the expression of α chain in the hematopoietic cell lines FDC-P1 and DX-1, but it has no obvious effect on T cells.36

With the use of 125I--IL-2 binding assay, we showed that IL-3 induces a decrease in the number of high-affinity IL-2 receptors in human T cells. This effect was not observed when 100 U/mL of IL-3 was added to the culture at 4°C in the presence of NaN3, 30 minutes before the assay was performed, thereby refuting the suggestion that the receptor decrease was due to the competitive inhibition by IL-3 of 125I-labeled IL-2 binding to its receptor, as in the case of IL-3 and granulocyte-macrophage colony-stimulating factor.37,39

In the T-cell clones used here, IL-2 also reduced the surface expression of IL-2R β chain within 1 hour (data not shown). However, the IL-3-mediated β chain downregulation does not seem to be an indirect effect of IL-2 produced by the cells stimulated with IL-3, as no detectable IL-2 was secreted in the culture medium. IL-3 also reduced the reactivity of the T-cell clones with biotinylated Mik-β3 MoAb, which recognized the non--IL-2 binding epitope on the IL-2R β chain. This indicates that the reduction of the IL-2R β chain determined by 2R-B MoAb was not the consequence of underestimation of the number of β chain due to the blockage of binding of 2R-B MoAb to the IL-2R β chain by receptor-bound IL-2. Furthermore, IL-2 induced the reduction of IL-2R α chain (60% decrease) but IL-3 had no effect on the surface expression of IL-2R α chain at 12 hours.

Previously, we reported that IL-4 downregulates the expression of β chain in T cells.37 However, in the T-cell clones P607 and 1C2, the addition of IL-4 resulted in a marginal reduction of β chain level (data not shown), suggesting that IL-4 is not involved, at least in P607 and 1C2 cells, in the mechanism of β chain expression downregulation by IL-3.

It has been reported that protein kinase C is involved in the signal transmission of IL-3.40 However, at present, the contribution of tyrosine phosphorylation reaction to this transmission is considered more important because: (1) insertion of the oncogene belonging to the tyrosine kinase family into IL-3--dependent cells resulted in the loss of IL-3 dependency,42,43 and (2) a 140- to 150-Kd membrane-bound glycoprotein undergoes IL-3--specific tyrosine phosphorylation.44 In the T-cell clones P607 and 1C2 the addition of phorbol esters, a potent activator of protein kinase C, induced the reduction of IL-2R β chain surface expression within 1 hour and increased α chain expression (200% increase) at 12 hours (data not shown). By contrast, IL-3 did not affect IL-2R α chain surface expression. The results suggest that the mechanism, except the activation of protein kinase C, is related to the IL-3--induced reduction of IL-2R β chain, although it is clearly possible that the activation of protein kinase C is related to the IL-3--induced downregulation of β chain.

IL-3 did not induce a decrease of the mRNA expression for β chain and the downregulation of its surface expression by IL-3 was not disturbed by the presence of brefeldin A (data not shown) that blocks the transportation process of substances between the Golgi body and endoplasmic reticulum.44,45 Based on these observations, IL-3 does not seem to downregulate the surface expression of IL-2R β chain by inhibiting the expression of β chain mRNA or transportation of its precursor protein to the cell surface membrane. As the accelerated internalization of β chain occurring at 150 minutes preceded the decrease of β chain surface expression that occurred at 180 minutes of incubation, it is likely that the IL-3--induced acceleration of β chain internal-
IL-3 DOWNREGULATES IL-2 RECEPTOR β CHAIN

The precise mechanism of IL-3–induced downregulation of β chain expression has not yet been fully addressed and deserves further study. In the past several years, successful cloning of the receptors of various cytokines led to the notion that many of them share structural similarities (eg, IL-2 [β chain], IL-3, IL-4, IL-6, IL-7, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, erythropoietin, prolactin, and growth hormone). They subsequently have been categorized as a “new cytokine receptor family.” An IL-3–dependent cell line was reported to become IL-2–responsive when transfected with the IL-2R β chain. It is possible that IL-3 downregulates the surface expression of IL-2R β chain through an intracytoplasmic signal transduction pathway common to IL-2 and IL-3, or by their cross-communication or interaction.

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