Interleukin-4 (IL-4) modulates the survival, proliferation, and differentiation of hematopoietic cells. The effects are mediated through a single class of high-affinity receptors for IL-4. To understand the biologic effects of IL-4 on human T cells, we studied the regulation of IL-4 receptor (IL-4R) gene expression. We showed that IL-4R mRNA accumulation in human T cells is enhanced fourfold after activation of different secondary signaling pathways by concanavalin A (Con A), phorbol myristate acetate (PMA), the calcium ionophore A23187, and combinations of these factors. This could be ascribed to an increase in the IL-4R transcription rate and to stabilization of IL-4R mRNA resulting in a half-life of 80 to 90 minutes (v 35 to 40 minutes in resting T cells). IL-4 did enhance the IL-4R mRNA accumulation by a factor 10, which was caused by an increase in the IL-4R transcription rate and prolonging the half-life of IL-4R transcripts to 140 to 160 minutes. Finally, it was shown that A23187 induced IL-4R mRNA expression is a protein synthesis-dependent process. In contrast, Con A-, PMA-, Con A + PMA-, and Con A + A23187-induced expression of IL-4R mRNA is protein-synthesis independent. Cyclosporine A inhibited the A23187- and Con A + A23187-induced IL-4R mRNA accumulation, whereas Con A-, PMA-, and Con A + PMA-induced IL-4R mRNA expression was not affected by this drug. These data indicate that expression of IL-4 receptors on human T cells can be modulated by different intracellular signaling pathways at both transcriptional and posttranscriptional levels.

INTERLEUKIN-4 (IL-4), a product of activated T cells, is involved in the regulation of B, T, and myeloid cells. The divergent activities of IL-4 on these cell types are initiated after binding of the ligand to the IL-4 receptor (IL-4R). Receptor studies with 125I-labeled IL-4 on resting lymphocytes initially identified a trimolecular complex consisting of a 65- to 70-Kd doublet and a 120-Kd protein with an 300 high-affinity binding sites (kd 100 pmol/L). However, more recently it has been shown that the polypeptide chain with molecular weight (m.w.) of 300,000 (p70) is a breakdown product of p120,15,16 conforming the results of the cloned murine IL-4R gene. In addition, an IL-4 binding molecule with an m.w. of 40 Kd has been detected.16 The p40 seems to be the soluble, truncated form of the receptor.15,16 However, the soluble form of the IL-4R has not yet been detected in human cells.17 Although there is growing knowledge about the binding properties of the IL-4R on T and B cells, myeloid cell lines, monocytes, and acute myeloid leukemic cells, there is only limited information concerning the regulation of IL-4R gene expression in human T lymphocytes. Previous investigations have especially relied on methods using 125I-radioiodated IL-4. In the present report we investigate the regulation of IL-4R mRNA expression in human T cells and show that IL-4R mRNA expression can be upregulated by different activators of the intracellular signaling pathways and by IL-4. These processes are controlled at transcriptional and posttranscriptional levels.

MATERIALS AND METHODS

Preparation of cells. Peripheral blood cells were obtained from volunteer platelet donors, and mononuclear cell suspensions were prepared by ficoll-hypaque density-gradient centrifugation (lymphoprep; Nycomed, Oslo, Norway). T lymphocytes were isolated by panning on nylon column.2-10 The divergent activities of IL-4 on these cell types are initiated after binding of the ligand to the IL-4 receptor (IL-4R). Receptor studies with 125I-labeled IL-4 on resting lymphocytes initially identified a trimolecular complex consisting of a 65- to 70-Kd doublet and a 120-Kd protein with an 300 high-affinity binding sites (kd 100 pmol/L). However, more recently it has been shown that the polypeptide chain with molecular weight (m.w.) of 300,000 (p70) is a breakdown product of p120,15,16 conforming the results of the cloned murine IL-4R gene. In addition, an IL-4 binding molecule with an m.w. of 40 Kd has been detected.16 The p40 seems to be the soluble, truncated form of the receptor.15,16 However, the soluble form of the IL-4R has not yet been detected in human cells.17 Although there is growing knowledge about the binding properties of the IL-4R on T and B cells, myeloid cell lines, monocytes, and acute myeloid leukemic cells, there is only limited information concerning the regulation of IL-4R gene expression in human T lymphocytes. Previous investigations have especially relied on methods using 125I-radioiodated IL-4. In the present report we investigate the regulation of IL-4R mRNA expression in human T cells and show that IL-4R mRNA expression can be upregulated by different activators of the intracellular signaling pathways and by IL-4. These processes are controlled at transcriptional and posttranscriptional levels.

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plasmid containing a 7.8-kb human 28S cDNA insert; (3) the 0.45-kb EcoRI/Ava II insert of human granulocyte-macrophage colony-stimulating factor (GM-CSF) cDNA (gift from Dr S. Gillis, Immunex).

Hybridization was performed at 65°C for 18 hours in 0.5 mol/L Na2HPO4, pH 7.2, 1 mmol/L EDTA, 7% sodium dodecyl sulfate (SDS). Membranes were washed once in 2× SSC, 0.1% SDS; once in 1× SSC, 0.1% SDS; and finally in 0.3× SSC, 0.1% SDS for 20 minutes at 65°C. The membranes were exposed to Kodak X-Omat XAR films (Eastman Kodak, Rochester, NY) at −80°C using an intensifying screen. Quantification of mRNA levels was performed by densitometry using a Gel Scan laser densitometer (Pharmacia LKB, Uppsala, Sweden).

**Run-on transcription assay.** For the nuclear run-on assay, 10⁶ cells were pelleted at 500g for 5 minutes, washed twice with ice-cold phosphate-buffered saline (PBS), and suspended in 4 mL of lysis-buffer (10 mmol/L Tris-HCl, pH 7.5, 3 mmol/L MgCl₂, 10 mmol/L NaCl, 0.5% NP-40). After gentle vortexing, the suspension was incubated on ice for 5 minutes. Nuclei were pelleted at 500g for 5 minutes and the lysis described above was repeated. The nuclei were resuspended in 100 μL glycerol-buffer (50 mmol/L Tris-HCl, pH 8.0, 40% glycerol, 5 mmol/L MgCl₂, 0.1 mmol/L EDTA) and incubated at 26°C for 20 minutes with 80 μL transcription buffer (12.5 mmol/L Tris-HCl, pH 8.0, 6 mmol/L MgCl₂, 125 mmol/L KCl, 2 mmol/L dithiothreitol, 1 mmol/L each of ATP, CTP, and GTP), and 12.5 μL [α-³²P]UTP (3,000 Ci/mmol; Amersham). Transcription was terminated by the addition of 40 U RNAse A, 150 μg yeast RNA, and 200 μL stop-buffer (10 mmol/L Tris-HCl, pH 7.4, 0.5 mol/L NaCl, 50 mmol/L MgCl₂, 2 mmol/L CaCl₂), and the solution was incubated at 37°C for 20 minutes. After a proteinase K digestion (750 U proteinase K/mL) in 1% SDS, the nuclear RNA was isolated by phenol-chloroform extraction and then ethanol precipitated twice in 2.5 mol/L ammonium acetate. The RNA was further purified by Sephadex G-50 column separation (Boehringer). Five micrograms of the following DNAs were immobilized on Hybond N⁺ membranes. (1) EcoRI linearized pBR322; (2) Nor I linearized Bluescript containing a 0.8-kb human IL-4R cDNA, (3) EcoRI linearized pBR322 containing a 1.3-kb rat GAPDH cDNA.²⁶ Hybridization of labeled RNA to these membranes was as described above. Washing was as described above except that an extra wash step with 2× SSC/1 μg/mL RNAse A (Boehringer) was included. Exposure and quantification of transcription rate levels were performed as described above.

**Cytofluorometric analysis of IL-4R expression.** T lymphocytes were treated for 24 hours with 25 μg/mL Con A. After washing, the cells were resuspended in RPMI 1640 medium. Aliquots of about 10⁶ cells were analyzed with respect to IL-4R expression by flow cytometry using a fluorescence-activated cell sorter (FACS) (FACSCalibur, Becton Dickinson). The percentage of IL-4R-positive cells was determined by densitometric scanning. The results shown are representative of three separate experiments.

**RESULTS**

**Regulation of IL-4R expression on activation of different intracellular signaling pathways.** The regulation of IL-4R gene expression was studied at mRNA and protein level in human T cells in response to stimulation of different intracellular signaling pathways. Exposure to Con A, PMA, A23187, and Con A + PMA resulted in a fourfold to fivefold increased expression of IL-4R mRNA as determined by Northern blotting (Fig 1). Optimal accumulation of IL-4R mRNA was observed after 2 to 4 hours of stimulation with these activators. IL-4R mRNA levels returned to baseline levels after 6 to 8 hours of exposure (data not shown). No additive effect was observed when Con A plus PMA or Con A plus A23187 was used compared with the effects of the individual agents (Fig 1). To determine whether the increased accumulation of IL-4R mRNA was caused by an increase in transcription rate, nuclear run-on assays were performed.

A representative experiment is shown in Fig 2. Unstimulated T cells demonstrated a spontaneous transcription of a concentration of 10⁶ cell/mL in a HEPES-buffered saline, containing 137 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L Na₂HPO₄, 5 mmol/L glucose, 1 mmol/L CaCl₂, 0.5 mmol/L MgCl₂, bovine serum albumin 1 g/L, HEPES 10 mmol/L pH 7.4. Cells were incubated for 10 minutes at 37°C and treated with medium (negative control), 15 ng/mL IL-4, or 25 μg/mL Con A (positive control). Cells were analyzed on FACS after 0, 10, 20, and 40 minutes of Con A or IL-4 treatment. The excitation wavelength was set to 488 nm and the emission at 525 nm was measured on a linear scale²⁸ during 3 minutes for each experiment. Temperature was maintained at 37°C throughout the experiment. Results are expressed as mean fluorescence of events.

**Fig 1.** Effect of Con A, PMA, Con A + PMA, A23187, and Con A + A23187 on the IL-4R mRNA accumulation. Northern analysis of total RNA extracted from T cells after a 3-hour treatment with the indicated activators. Hybridization with a 28S RNA probe was assessed to show equal amounts of RNA (12 μg) in each lane. Relative mRNA levels were determined by densitometric scanning. The results shown are representative of three separate experiments.
the IL-4R gene, which was enhanced with a factor 2 after activation with Con A, PMA, A23187, Con A + PMA, and Con A + A23187. This indicates that transcriptional mechanisms are involved in the increased accumulation of IL-4R mRNA on activation with these agents.

To study whether posttranscriptional mechanisms are also involved, the stability of IL-4R mRNA was examined.

The stability of IL-4R mRNA was enhanced in response to Con A, PMA, A23187, Con A + PMA, and Con A + A23187 stimulation. As depicted in Fig 3, the half-life of IL-4R mRNA in unstimulated T cells was 35 to 40 minutes as determined by densitometric scanning. The half-life was prolonged to 80 to 90 minutes after stimulation with the individual agents. The combination of Con A + PMA or
Con A + A23187 did not further increase the stability of the IL-4R transcript (Fig 3). In contrast, rehybridizing with a GM-CSF probe demonstrated a stabilizing effect of PMA on Con A-induced GM-CSF transcripts (data not shown).

In addition, experiments were performed with CHX to test whether expression of the IL-4R gene was dependent on ongoing protein synthesis. No difference in the accumulation of IL-4R mRNA was observed on stimulation with Con A, PMA, Con A + PMA, or Con A + A23187 in the presence or absence of CHX (Fig 4). In contrast, A23187-induced IL-4R mRNA expression was inhibited by CHX, indicating that only the calcium-dependent upregulation of IL-4R expression is a protein synthesis-dependent process. To demonstrate the effectiveness of CHX, the filter was exposed to staurosporine (10 ng/mL). As shown in Fig 9, the effect of Con A on Con A-induced GM-CSF transcripts (data not shown). The role of Ca²⁺ in regulating IL-4R mRNA levels was analyzed by means of flow cytometric measurement of cytoplasmic free calcium with fluo-3 in IL-4–treated cells. Fluorescence was measured for 3 minutes directly after addition of IL-4 and after 10, 20, 40, and 60 minutes of IL-4 addition. In two independent experiments, no increase in Ca²⁺ concentration was observed in cells that were treated with IL-4 compared with untreated cells (mean fluorescence events control: 41 ± 1 [mean ± SD]; IL-4: 41 ± 1 [mean ± SD]). As a positive control, Con A-treated cells demonstrated a strong transient mobilization of Ca²⁺ (mean fluorescence events: 132 ± 4 [mean ± SD]) that peaked after 140 seconds.

**Regulation of IL-4R protein.** To determine whether the changes at mRNA level correspond with changes at protein level, human T cells were incubated with an MoAb against the IL-4R. After 24-hour stimulation with Con A, an enhanced expression of the IL-4R was noticed compared with unstimulated T cells. The mean fluorescence intensity as determined by FACS analysis increased from 4.7 ± 3.0 (mean ± SD, n = 5) for unstimulated cells to 9.4 ± 6.3 (mean ± SD, n = 5) for Con A–treated cells. A representative experiment is shown in Fig 9. The effects of Con A were also tested in the presence of anti-IL-4 to exclude the possibility that the Con A–mediated upregulation was caused by secreted IL-4 in response to Con A stimulation. However, no difference in expression was noticed in the absence or presence of anti-IL-4.

**DISCUSSION**

Since the cloning of the IL-4R cDNA, it has become possible to study the expression of the IL-4R gene in detail. In the present report, we examine the control of IL-4R expression in human T cells and show that different...
pathways regulate IL-4R gene expression. Firstly, constitutively expressed IL-4R mRNA in resting T cells could be upregulated by activators of different secondary signaling pathways such as Con A, PMA, and A23187. Upon treatment, an approximate fourfold increase in IL-4R mRNA accumulation was shown that could be ascribed to a moderate increase in transcription rate of the IL-4R gene and to stabilization of the IL-4R transcripts. The combinations of factors did not further augment the IL-4R expression. Secondly, IL-4 upregulated the IL-4R mRNA accumulation, which was accomplished at both transcriptional and posttranscriptional levels. However, the increase in IL-4R transcription rate as well as the stabilization at posttranscriptional level were more pronounced by IL-4 than by Con A, PMA, A23187 or combinations of these agents. These data suggest that upregulation of the IL-4R by IL-4 depends on additional signaling pathways, besides the PKC and Ca2+ route. Alternatively, IL-4 exhibits its effect independent of the PKC- and Ca2+-dependent pathway. This is also supported by the findings that binding of IL-4 to the receptor on murine B cells did not stimulate Ca2+ mobilization or PKC translocation. Furthermore, inactivation of PKC by staurosporin treatment had no effect on the IL-4-induced IL-4R mRNA accumulation in human T cells, nor did IL-4 stimulate Ca2+ mobilization in these cells. In contrast, Finney et al\textsuperscript{33} showed that IL-4 stimulation of human B lymphocytes causes a transient increase in Ca2+. This apparent discrepancy could reflect a difference in cell type. For human T cells, tyrosine kinase complexes may be involved for transducing the signal as shown for different cytokine receptors.\textsuperscript{34} However, examination of the predicted cytoplasmic domain sequence of the human IL-4R showed no phosphorylation acceptor sites for protein tyrosine kinases.\textsuperscript{16,17}

IL-4-mediated upregulation of the IL-4R was reported earlier by others.\textsuperscript{35-37} Ohara and Paul\textsuperscript{35} demonstrated by means of flow cytometric analysis using biotin–IL-4 that virtually all subsets of murine T cells stimulated by IL-4 express higher amounts of the receptor, resulting in an overall increase by approximately fivefold. In agreement with our results, Renz et al\textsuperscript{36} noticed an upregulation of IL-4R mRNA levels in response to IL-4 stimulation in murine B and T cells. Armitage et al\textsuperscript{37} described the accumulation of IL-4-induced IL-4R mRNA in human T cells after 45 hours of stimulation. They did not detect an IL-4 effect after 16 and 24 hours of stimulation. In contrast, we noticed a 10-fold IL-4R mRNA increase after 3 hours of IL-4 treatment. This could indicate that this first peak at 3 hours is followed by a second peak of IL-4R mRNA expression at 45 hours. This is underscored by our observation that Con A-treated T cells expressed a second peak of IL-4R mRNA accumulation after 24 hours of stimulation (unpublished results, December 1991).

Furthermore, evidence was obtained that IL-4R expression is less stringent controlled than IL-4 expression in human T cells as was described for IL-2 and its receptor.\textsuperscript{38} This is supported by the finding that Con A-, PMA-, Con A + PMA-, and Con A + A23187-induced IL-4R mRNA expression is independent on the synthesis of new protein. Moreover, CsA did not affect the Con A-, PMA-, and Con...
Fig 7. Effect of IL-4 on the transcription rate of the IL-4R gene. Cells were treated with medium (control) or with 15 ng/mL IL-4 for 5 hours. The run-on assay was performed as described in Fig 2.

Fig 8. Effect of IL-4 on the stability of IL-4R mRNA. T cells were treated with 15 ng/mL IL-4 for 3 hours. The IL-4R mRNA half-life study was performed as described in Fig 3.

Fig 9. Effect of Con A on IL-4R expression of human T cells. Cells were treated with or without 25 μg/mL Con A after 24 hours. IL-4R expression was then analyzed by flow cytometric analysis. Number of cells are on the Y-axis. Control cells were incubated with FITC-conjugated rabbit-antimouse IgG antibody (- - -) or with FITC-conjugated rabbit-antimouse IgG antibody plus mouse-antihuman IL-4R antibody (---); Con A-treated cells were incubated with FITC-conjugated rabbit-antimouse IgG antibody (-----) or with FITC-conjugated rabbit-antimouse IgG antibody plus mouse-antihuman IL-4R antibody (----). Depicted is one representative experiment out of five.

A + PMA-induced IL-4R mRNA accumulation. In contrast, the induction of IL-4 mRNA in human T cells, which can only be induced by Con A and Con A + PMA, is protein-synthesis dependent and can be inhibited by CsA.30 These findings suggest that different underlying mechanisms are involved with regard to the expression of IL-4 and IL-4R genes in activated T lymphocytes. However, CHX lowered the A23187 induced IL-4R mRNA expression, indicating that the calcium-mediated IL-4R mRNA expression partly depends on protein synthesis. CsA inhibited the A23187- and Con A + A23187-induced IL-4R mRNA accumulation. These results are in agreement with studies showing that CsA blocks a calcium-dependent pathway.39

The interactive process between IL-4 and its receptor may be an important immunoregulatory process. The binding of IL-4 to the receptor provides T lymphocytes the possibility to respond continuously to exogenous IL-4 by upregulating the receptor expression and subsequently to be controlled by the modulatory effects of this cytokine.

In conclusion, we demonstrate that regulation of IL-4R gene expression is controlled at both transcriptional and posttranscriptional levels by IL-4 and in response to different activation signals.

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Interleukin-4 (IL-4) receptor expression on human T cells is affected by different intracellular signaling pathways and by IL-4 at transcriptional and posttranscriptional level

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