Interleukin-4 Receptor Regulation in Human Monocytic Cells

By Harry de Wit, Dick W. Hendriks, M. Ruud Halie, and Edo Vellenga

The regulation of the interleukin-4 receptor (IL-4R) was studied at mRNA and protein level in monocytic cells on stimulation with activators of different intracellular signaling pathways and IL-4. Activation of protein kinase C-dependent pathways with phorbol myristate acetate (PMA) or activation of protein kinase A-dependent pathways with DBcAMP and prostaglandin E2 resulted in an augmented IL-4R expression at mRNA and protein level. Transcriptional and post-transcriptional mechanisms seemed to be involved in the promotive effect of DBcAMP because the transcription rate increased 1.8-fold, and the half-life of IL-4R mRNA was prolonged to 150 minutes compared with 120 minutes in unstimulated cells. In contrast, the effect of PMA could only be ascribed to changes at transcriptional level. However, the breakdown product of ~120-kDa was increased expression of IL4R at mRNA and protein level. In addition, recent investigations have shown that IL-4R expression is controlled by different intracellular signaling pathways. T cells stimulated with the Ca2+-dependent pathways with phorbol myristate acetate (PMA) or activation of protein kinase A-dependent pathways with DBcAMP and prostaglandin E2 resulted in an augmented IL-4R expression at mRNA and protein level. Transcriptional and post-transcriptional mechanisms seemed to be involved in the promotive effect of DBcAMP because the transcription rate increased 1.8-fold, and the half-life of IL-4R mRNA was prolonged to 150 minutes compared with 120 minutes in unstimulated cells. In contrast, the effect of PMA could only be ascribed to changes at transcriptional level.

**MATERIALS AND METHODS**

**Cell culture and stimulation.** Mono Mac 6 cells (gift of Dr. H.W.L. Ziegler-Heitbrock, Heidelberg, Germany)17 were cultured in RPMI-1640 medium (GIBCO-BRL, Gaithersburg, MD) supplemented with 15% fetal calf serum (FCS; Hyclone, Logan, Utah), nonessential amino acids (GIBCO), 1 mmol/L pyruvate (Sigma, St. Louis, MO), 1 mmol/L oxalate (Sigma), 2 mmol/L L-glutamine (GIBCO), and antibiotics. Eighteen hours before each experiment, cells were washed and resuspended in fresh medium at a cell concentration of 2 × 10⁶/mL.

Peripheral blood cells were obtained from volunteer platelet donors and mononuclear cell suspensions were prepared by Ficoll-Hypaque density-gradient centrifugation. T lymphocytes were removed by E-rosetting with 2-aminoethylisothiouronium bromide-treated sheep red blood cells. Monocytes were further enriched by plastic adherence for 1 hour at 37°C and showed a purity greater than 95%, detected by fluorescence-activated cell sorter (FACS) analysis with an anti-CD14 monoclonal antibody (MoAb; Becton Dickinson, Sunnyvale, CA) and staining. Monocytes were cultured in RPMI 1640 medium containing 10% FCS and antibiotics.

The following activators were used in the experiments: the Ca2+-ionophore A23187 (Sigma), PMA (50 ng/mL; Sigma), 2'-dibutyryl-cyclic adenosine monophosphate (DBcAMP; 10⁻⁵ mol/L; Boehringer, Mannheim, Germany), prostaglandin E₂ (PGE₂; 10⁻⁵ mol/L; Boehringer), activinomycin-D (10 μg/mL; Sigma), and rhIL-4 (gift from Dr. P. Trotta, Schering Plough, Bloomfield, NJ) with a specific activity of 10⁷ U/mg. The viability of monocytes and Mono Mac 6 cells after stimulation was greater than 95% in all experiments.

**mRNA analysis.** Total cellular RNA was isolated using the guanidium isothiocyanate/cesium chloride method. Twenty-microgram samples of total cellular RNA were size fractionated on 1.1% agarose gels with 2.2 mol/L formaldehyde and blotted onto nylon membranes (Hybond N⁺; Amersham, UK). cDNA probes were labeled with [³²P]deoxyctydine triphosphate (3,000 Ci/mmol; Amersham) using the hexanucleotide primer technique. The following cDNA probes were used: the human IL-4R (gift from Dr. M.K. Spriggs, Immunex, Seattle, WA), human macrophage colony-stimulating factor (M-CSF) (gift of Dr. S.C. H.W.L. Ziegler-Heitbrock, Heidelberg, Germany). Materials and methods.
IL-4R REGULATION IN MONOCYTIC CELLS

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Fig 1. Mono Mac 6 cells were stimulated with (A) PMA (50 ng/mL), (B) DBcAMP (10^-3 mol/L), and (C) PGE2 (10^-3 mol/L). At the indicated time points, total cellular RNA was analyzed for the IL-4R mRNA expression. Rehybridization with a 28S probe was assessed to control RNA loading in each lane.

Clark, Genetics Institute, Cambridge, MA), and the linearized pBr322 plasmid containing a 7.8-kb human 28S cDNA insert.

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 in 2× saline sodium citrate buffer (SSC), 0.1% SDS (65°C, 30 minutes), 1× SSC, 0.1% SDS (65°C, 30 minutes), and finally in 0.3× SSC, 0.1% SDS (65°C, 30 minutes). Membranes were then exposed to x-ray films (Kodak X-Omat XAR; 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. The transcription rate of the IL-4R gene was studied as described previously.24 In short, nuclei of 50 × 10⁶ cells were isolated and resuspended in transcription buffer containing [32P]-uridine triphosphate (3,000 Ci/mmol; Amersham) and incubated at 26°C for 20 minutes. Transcription was terminated, and nuclear RNA was isolated and hybridized to linearized pGEM and to linearized plasmids containing the cDNA inserts for the IL-4R and 28S rRNA. Hybridization, washing, exposure, and quantification of transcription rates was performed as described above.

Reverse transcription (RT) and polymerase chain reaction (PCR). A total of 0.5 μg total cellular RNA was incubated with 135 mmol/L Tris-HCl, pH 8.3; 204 mmol/L KCl; 27 mmol/L MgCl2; 0.24 ng/μL bovine serum albumin; 5.4 mmol/L of each deoxynucleotide triphosphate; 14.3% glycerol; 3 mmol/L dithiothreitol; 0.2 μg pd(N),; 5 U RNAguard (Pharmacia); and 3 U Moloney Murine Leukemia virus reverse transcriptase (Pharmacia) in a total volume of 15 μL for 1 hour at 37°C.

Specific primer pairs for the common γc and for the GAPDH gene were synthesized on a Gene Assembler Plus DNA synthesizer (Pharmacia) and purified using NAP-10 columns (Pharmacia). One microliter of 50-μmol/L 5' and 3' primers were added to each reverse transcriptase (RT) reaction sample and DEPC H2O was added to a final volume of 50 μL. The samples were then heated for 5 minutes at 95°C and placed on ice; 5 U of Taq DNA polymerase (Pharmacia) was added, and each sample was overlaid with 50 μL paraffin oil. The reaction tubes were then placed in a thermal cycler (Pharmacia), and polymerase chain reaction (PCR) was performed for 20, 25, 30, 35, and 40 cycles.
Fig 5. Experiments to determine the half-life of IL-4R mRNA. (A) Mono Mac 6 cells were stimulated with medium, PMA (50 ng/mL), and DBcAMP (10^{-8} mol/L) for 6 hours. Then actinomycin-D (10 μg/mL) was added to inhibit transcription. At various time points after actinomycin-D addition IL-4R mRNA expression was detected. (B) Graphic analysis of IL-4R mRNA half-life.

ficity of the DBcAMP effect, cells were stimulated with PGE2 (10^{-5} mol/L), which elevates intracellular cAMP levels. As depicted in Fig 1C, stimulation with PGE2 for 6 hours showed a twofold increase in IL-4R mRNA expression. In contrast, Mono Mac 6 cells stimulated with the calcium ionophore, A23187 (1 and 5 μmol/L), showed no change in IL-4R mRNA expression (Fig 2). This could not be ascribed to inadequate stimulation because rehybridization with an M-CSF probe showed a strong increase in M-CSF transcripts. Furthermore, the effects of IL-4 on the IL-4R expression were studied. Mono Mac 6 cells did not show a change in the IL-4R mRNA expression on IL-4 stimulation (Fig 3A). However, the IL-4R was functional because IL-4-stimulated cells showed an increased CD14 antigen expression of 40%, whereas the HLA-DR antigen expression decreased to 30% after IL-4 stimulation. To exclude the possibility that the unresponsiveness of the IL-4R for IL-4 was related to the used cell line, monocytes were stimulated with IL-4. As depicted in Fig 3B, no change in IL-4R mRNA expression was noticed. In three separate experiments, monocytes showed no significant increase in IL-4R mRNA expression on stimulation with IL-4 for 6 hours (1.01-fold ± 0.09-fold increase compared with unstimulated monocytes (mean ± SEM; n = 3)).

Analysis of IL-4R transcription rates. Run-on assays were performed to determine whether the effect of PMA and DBcAMP involves an increase in the transcription rate of the IL-4R gene. A total of 50 × 10⁶ Mono Mac 6 cells were stimulated with medium, PMA, and DBcAMP for 5 hours. Unstimulated Mono Mac 6 cells showed a spontaneous transcription of the IL-4R gene, which is in accordance with the constitutive mRNA expression of the IL-4R. In three separate experiments, PMA- and DBcAMP-stimulated cells showed a 1.33-fold ± 0.13-fold and 1.81-fold ± 0.32-fold
Fig 5. Experiments to determine the half-life of IL-4R mRNA. (A) Mono Mac 6 cells were stimulated with medium, PMA (50 ng/mL), and DBcAMP (10^-5 mol/L) for 6 hours. Then actinomycin-D (10 μg/mL) was added to inhibit transcription. At various time points after actinomycin-D addition IL-4R mRNA expression was detected. (B) Graphical analysis of IL-4R mRNA half-life.
increase in the transcription rate, respectively, compared with that for unstimulated cells (mean ± SEM; n = 3). A representative experiment is shown in Fig 4. These data indicate that transcriptional mechanisms are involved in the upregulation of IL-4R mRNA after stimulation of PKC- and PKA-dependent pathways.

Analysis of IL-4R mRNA stability. Mono Mac 6 cells were stimulated with PMA or DBcAMP for 6 hours, and RNA synthesis was blocked by the addition of actinomycin-D (10 μg/mL). At different time points, total cellular RNA was isolated and analyzed for IL-4R mRNA expression. Unstimulated cells showed an IL-4R mRNA half-life of approximately 120 minutes (Fig 5). On activation with PMA, IL-4R mRNA was not stabilized. However, in DBcAMP-stimulated Mono Mac 6 cells, IL-4R mRNA was slightly stabilized and showed a half-life of 150 minutes. The data indicate that the accumulation of IL-4R mRNA after stimulation of PKA-dependent pathways involves stabilization at the posttranscriptional level.

Analysis of the common γc expression. Because the common γc is an essential component of the IL-4R complex in T cells, we questioned whether the γc is also expressed in monocytic cells. Total cellular RNA from T cells and Mono Mac 6 cells was analyzed with RT-PCR. Figure 6 shows a significant expression of the common γc in T cells, whereas in Mono Mac 6 cells no transcripts could be detected even after 40 cycles of amplification. The difference could not be ascribed to an insufficient amount of RNA in view of the results obtained with the GAPDH primers.

Analysis of IL-4R protein. To determine whether the changes in IL-4R expression at mRNA level correspond with changes in IL-4R protein expression, Mono Mac 6 cells were stimulated with PMA or DBcAMP for 48 hours, and IL-4R expression was analyzed using an MoAb. Figure 7 shows that unstimulated (control) Mono Mac 6 cells express IL-4R on the cell surface. PMA stimulation results in an increased number of cells expressing IL-4R (Fig 7A) that could be further augmented by DBcAMP (Fig 7B).

Subsequently, CD23 antigen expression was studied with the focus on whether the upregulation of IL-4R in response to PMA stimulation might affect the IL-4 response. Mono Mac 6 cells cultured with IL-4 alone showed a 1.90-fold ± 0.08-fold increase in CD23 expression compared with that of unstimulated cells, whereas a 1.20-fold ± 0.03-fold increase was noticed in response to PMA. However, prestimulation with PMA followed by IL-4 during 24 hours resulted in a 3.24-fold ± 0.3-fold increase in CD23 expression (mean ± SEM; n = 3).

DISCUSSION

In the present study, we analyzed the involvement of IL-4 and different intracellular signaling pathways in the regulation of IL-4Rs on human monocytic cells. The results show an upregulation of the IL-4R on activation of the PKC- and c-AMP-dependent pathways, whereas stimulation with IL-4 or the calcium ionophore A23187 did not affect the receptor expression. These findings suggest that the control of IL-4R expression on monocytic cells is more restricted than that on T cells because T cells show an augmented IL-4R mRNA expression on stimulation with PMA. A23187, anti-CD3, concanavalin A, and IL-4.17,18 A remarkable observation was the fact that PMA did augment the receptor expression, whereas no effect of IL-4 was observed. The unresponsiveness to IL-4 was not caused by a lack of functional receptors because IL-4 modulated the CD14 and CD23 antigen expression. Arruda and Ho26 recently observed that IL-4-exposed monocytes showed a significant redistribution of PKC activity from cytosol to the nuclear fraction, indicating that PKC-dependent signaling is triggered on IL-4 stimulation. The apparent discrepancy between the IL-4-mediated signaling and PMA may be connected to activation of different PKC isoforms on stimulation with one of the activators. Alternatively, the composition of the IL-4R may be different between monocytic cells and T cells because a strong increment in IL-4R expression is noticed in both lymphoid cells in response to IL-4 stimulation.18,27 Recently performed studies have shown that binding of IL-4 to the TF-1 cell line can be competed for by IL-13, suggesting that an IL-4R complex may be composed of different subunits28 as has been shown for IL-3/IL-5 and IL-7 receptor families.29,30 In fact, the IL-4R shares the common γc with the IL-2R and IL-7R.30 However, in the Mono Mac 6 cell line, γc transcripts were not detected with RT-PCR, whereas in T cells a significant expression was noticed. This is in accordance with findings of Takeshita et al31 who could only detect γc mRNA in lymphoid cells, whereas in the monocytic cell line THP-1, γc mRNA was absent. The absence of the γc might affect the IL-4 response in T cells and monocytic cells. This is supported by findings of Russell et al12 who showed tyro-
sine phosphorylation of the insulin receptor substrate-1 (IRS-1) in response to IL-4 in murine L cells, which constitutively express the IL-4R and were transfected with the \( \gamma_c \). L cells that were not transfected with the \( \gamma_c \) did not phosphorylate IRS-1 in response to IL-4 stimulation, indicating that \( \gamma_c \) expression is essential for at least some IL-4-mediated signaling events.

Previously, we showed that monocytes incubated with PMA for 1 hour showed a reduced binding of \(^{125}\)I-IL-4,\(^{34}\) whereas in the present study, an augmented IL-4R expression was noticed after a longer period of stimulation. The findings may reflect different cellular processes in a sequence of events. The primary response to PKC activation is a downregulation of the IL-4R as result of shedding or receptor internalization, which is followed by a protein-dependent upregulation of the receptor. Comparable observations have been reported for the granulocyte-macrophage CSF (GM-CSF) receptor with regard to stimulation with GM-CSF and PMA.\(^{35-37}\)

Little has been reported regarding the regulation of IL-4R expression on myeloid/monocytic cells. Feldman et al\(^{38}\) showed an increment in IL-4R expression on the murine myeloid leukemia M1 cells in response to IL-6 stimulation, whereas interferon-\( \gamma \) did not modulate the receptor expression. In contrast, interferon-\( \gamma \)-stimulated murine macrophage-like cells J774.16 showed an increased IL-4R expression.\(^{39}\) Studies with additional physiological stimuli will further reveal the exact regulation of IL-4Rs on monocytic cells.

A profound effect on IL-4R mRNA accumulation was observed by DBcAMP and PGE\(_2\), which was caused by changes at transcriptional and posttranscriptional level in parallel with an increased IL-4R protein expression. The cAMP-dependent pathway seems to be of importance for the signal transduction in monocytic cells, because it affects the expression of different cytokines and receptors. An increased expression of tumor necrosis factor receptors has been observed in monocytic cells in response to stimulation with DBcAMP.\(^{40}\) In addition, cAMP augments the expression of IL-1 and IL-6 at mRNA and protein level,\(^{41,43}\) whereas M-CSF is downregulated in activated monocytic cells.\(^{42}\) The modulating effects of cAMP on the IL-4R expression might be an important feedback mechanism during an inflammatory response. High concentrations of PGE\(_2\) can be produced locally by monocytic cells in response to endotoxin stimulation\(^{44}\) and result in the induction of different cytokines, such as IL-1, IL-6, and IL-8, by direct and paracrine pathways. Because of the interactive process between T cells and monococytes, the secreted IL-4 will downregulate the production of the different cytokines.\(^{44,45}\) In addition, the controlling effects of IL-4 on monocyctic cells are sustained during the inflammatory response as result of an upregulation of IL-4R on activation of cAMP-dependent pathways.

In summary, the data show a PKA- and PKC-dependent
regulation of IL-4R in monocyctic cells, whereas stimulation of Ca2+-dependent pathways and stimulation with IL-4 had no effect. IL-4R regulation in monocyctic cells seems to be different from the regulatory mechanisms in human T lymphocytes that might relate to the expression of different IL-4R components.

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