IL-4 Enhances IL-10 Gene Expression in Murine Th2 Cells in the Absence of TCR Engagement


*J Immunol* 1999; 162:238-244;
http://www.jimmunol.org/content/162/1/238

---

**References**

This article cites 43 articles, 27 of which you can access for free at:
http://www.jimmunol.org/content/162/1/238.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
IL-4 Enhances IL-10 Gene Expression in Murine Th2 Cells in the Absence of TCR Engagement

C. B. Schmidt-Weber, S. I. Alexander, L. E. Henault, L. James, and A. H. Lichtman

Both IL-4 and IL-10 are regulatory cytokines produced by Th2 cells that can down-regulate cell-mediated immune responses. The studies reported here examine the influence of various cytokines in the regulation of T cell IL-10 production. The results indicate that IL-10 gene expression by TCR transgenic Th2 cells is significantly up-regulated by IL-4 in the absence of TCR signals. IL-4 enhances both IL-10 mRNA levels and secreted protein, and this effect is not related to enhanced mRNA stability. TCR-mediated IL-10 gene expression is inhibited by cyclosporin A, but IL-4-mediated IL-10 expression is not. IL-4 also enhances IL-13 mRNA levels, to a lesser extent than IL-10, but does not significantly affect the expression of other cytokine mRNAs. Furthermore, IL-4 does not significantly enhance IL-10 expression in Th1 cells. IL-2 also enhances effector cytokine production in the absence of TCR signals, but in a subset nonspecific manner, increasing both Th2 IL-4 mRNA and Th1 IFN-γ mRNA. These data suggest that Th2 IL-4 production may contribute to the down-regulation of immune responses by directly enhancing Th2 IL-10 production. In addition, the data clearly demonstrate that exogenous cytokines can significantly influence effector cytokine production by effector T cells without the requirement for TCR signals. The Journal of Immunology, 1999, 162: 238–244.

Copyright © 1999 by The American Association of Immunologists 0022-1767/99/$02.00

Immunology Research Division, Department of Pathology, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02115

Received for publication June 3, 1998. Accepted for publication September 22, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Deutsche Forschungsgemeinschaft (to C.S.W.) and National Institutes of Health Grant 2PO1AI35297 (to A.H.L.).
2 Current address: Swiss Institute of Allergy and Asthma Research, Obere Strasse 22, CM-7270 Ilavos, Switzerland.
3 Address correspondence and reprint requests to Dr. Andrew H. Lichtman, Department of Pathology, Brigham and Women’s Hospital, 221 Longwood Ave., Boston, MA 02115-5814. E-mail address: alichtman@rics.bwh.harvard

Materials and Methods

Animals

Transgenic mice expressing the DO.11.10 TCR (DO.11) specific for chicken OVA peptide 323–339 in the context of the MHC class II molecule I-Ak (23) were bred in our pathogen-free animal facility. All animals were maintained in accordance with the guidelines of the committee on animals of the Harvard Medical School and those prepared by the committee on care and use of laboratory animals of the Institute of Laboratory Resources, National Research Council (Department of Health, Education, and Welfare.

Copyright © 1999 by The American Association of Immunologists 0022-1767/99/$02.00
The Ag used in all studies was the chicken OVA peptide 323–339 obtained from the peptide synthesis facility of the Center for Neurologic Diseases, Brigham and Women’s Hospital (Boston, MA). Supernatant containing recombinant murine IL-4 was generated from the I3L6 cell line transfected with a constitutively expressed murine IL-4 cDNA (a gift from Dr. Robert Tepper, Massachusetts General Hospital, Boston, MA). The effects of the I3L6 supernatant described in this paper could be inhibited by anti-IL-4 mAb 11B11 (see below) and reproduced with purified rIL-4 obtained from the National Biological Standards Board (Hertfordshire, U.K.). IL-2-containing supernatants were obtained from the X63-IL2 cell line, which expresses a stably transfected murine IL-2 gene (24) (obtained from Dr. Fritz Melchers, Basel Institute of Immunology, Basel, Switzerland). Recombinant murine IL-6 and IFN-γ were purchased from Endogen and Genzyme, respectively (both Cambridge, MA). Recombinant murine IL-12 was a gift from Dr. Stan Wolf, Genetics Institute (Cambridge, MA). Anti-IL-4 and anti-CD3 mAbs were generated from the 11B11 (25) and the 145-2C11 (26) hybridomas, respectively, and purified on a protein A column (Pharmacia, Piscataway, NJ).

Inhibitors were used at a 1- to 10-μM concentration, as indicated in the figure legends, and were preincubated with the cells for 5 min at room temperature. Cyclosporin A (CsA) and ionomycin were purchased from Sigma (St. Louis, MO), rottlerin and GO 6976 were obtained from Alexis Corp. (San Diego, CA), and tyrophostin B42 (also called AG490) was purchased from Calbiochem (La Jolla, CA).

Cell preparation and culture

All cultures were conducted in RPMI 1640 supplemented with 1 mM l-glutamine, sodium pyruvate, nonessential amino acids, 5 × 10⁻³ M 2-ΜΕ, and 10% heat-inactivated FCS (all from Life Technologies, Grand Island, NY). Th1 and Th2 cells were generated as previously described (27). Briefly, CD4⁺ T cells were purified from peripheral and mesenteric lymph nodes and spleen cells of 6- to 8-wk-old BALB/c mice using CD4⁺ Dynal magnetic beads and Detach-A-Bead (both from Dynal, Lake Success, NY). The efficiency of purification was initially tested by flow cytometry and was >95% CD3⁺ CD4⁺. APCs were spleen cells purified from BALB/c mice and treated with 50 μg/ml mitomycin C (Sigma) at 37°C for 30 min as previously described (27). T cell differentiation was induced by culturing 2 × 10⁵ purified CD4⁺ TCR transgenic T cells with 2 × 10⁶ APCs, 1 μM OVA peptide 323–339, and either IL-12 (10 ng/ml) plus anti-IL-4 (1/1000 dilution of ascites) for Th1 differentiation or IL-4 (1000 U/ml) for Th2 differentiation, in 1 ml of medium. The cultures were fed with fresh medium containing 10 U/ml IL-2 after 4 days and harvested for restimulation after 6 days.

The D10.G4.1 T cell clone was obtained from the American Type Culture Collection (Manassas, VA) and maintained as previously described (28). Briefly, cells were grown in RPMI medium supplemented with 1 mM l-glutamine, sodium pyruvate, nonessential amino acids, 5 × 10⁻³ M 2-ΜΕ 10% heat-inactivated FCS (all from Life Technologies), 50 U of IL-4 (I3L6 supernatant), and 10 U of IL-2 (X63 supernatant). D10.G4.1 cells were restimulated every 7–14 days with conalbumin (Sigma) and mitomycin C-treated spleen cells from AKRJ mice. One day before the experiment, cells were rested in medium lacking IL-4.

RNome protection assay

For RNA analysis, Th1 or Th2 cells were cultured at 4 × 10⁶ cells/ml in 2-ml wells and restimulated with exogenously added cytokines or plate-bound anti-CD3 (27) as indicated in the figures. After 6 h, the cells were lysed with guanidinium thiocyanate solution. RNA was precipitated with isopropanol, washed with 70% ethanol, and subjected to RNome protection assay. Cytokine mRNA levels were analyzed by RNome protection assay using the Riboquant multiprobe set (PharMingen, San Diego, CA) following the instructions of the supplier. In brief, all RNA obtained from 4 × 10⁶ cells (~4–6 μg RNA) were hybridized overnight to the 3²P-labeled RNA probe, which had been previously synthesized from the supplied template set (mck-1 from PharMingen). Single-stranded RNA and free probe were digested by RNase A and T1. Subsequently, protected RNA was phenolized, precipitated, and analyzed on a 6% denaturing polyacrylamide gel. The quantity of protected RNAs was determined using a PhosphorImager and ImageQuant software (both from Molecular Dynamics, Sunnyvale, CA). The cytokine transcripts were identified by the length of the respective fragments. For quantitation, cytokine values were expressed as a percentage of the mean values of the housekeeping genes L32 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for each condition/gel lane.

ELISA determination of IL-10

Cell culture supernatants were analyzed by ELISA as previously described (28). The anti-IL-10 mAb 16E3 (Endogen) was used as capture Ab, and the biotinylated anti-IL-10 mAb 2A5 (Endogen) was used for detection of plate-bound IL-10.

Electrophoretic mobility shift assays (EMSAs)

EMSAs were performed as previously described (28). Briefly, nuclear extracts of Th2 cells were prepared as follows. Cells were lysed in hypoosmotic buffer containing 10 mM KCl, 1 mM DTT, 0.5 mM EDTA, 10 mM HEPES (pH 7.9; all from Sigma), and protease inhibitors (Complete, Boehringer Mannheim, Indianapolis, IN) followed by addition of Nonidet P-40 (Sigma) to a 1% final concentration. Nuclei were pelleted by microcentrifugation for 1 min and washed once with the buffer described above. Nuclei were lysed in 50 μl of a high salt buffer containing 400 mM NaCl, 50 mM DTT, 20 mM HEPES, 0.5 mM EDTA, and protease inhibitors. The nuclear debris was removed by centrifugation at 4°C, and the supernatant was stored in a fresh tube at −80°C. Nuclear extracts were normalized for equal protein content using a colormetric assay as described by the manufacturer (Bio-Rad, Hercules, CA).

For binding reactions, nuclear extracts were incubated with an oligonucleotide (18 mer) that corresponds to a consensus STAT6 DNA-binding element of the murine IL-4 promoter (TGAATTCAGAGGAAATT) as previously described (28). The two strands of the oligonucleotides were labeled with [γ-³²P]ATP using T4 kinase (Life Technologies). Subsequently, the oligonucleotides were separated from free [γ-³²P]ATP by running the labeling mix over a Chomastpin-10 column (Clontech, Palo Alto, CA). Single-stranded oligonucleotides were eliminated by gel purification of the column eluate on a 28% polyacrylamide gel. The eluted probe was precipitated, and 5,000–10,000 cpm of the diluted probe were incubated with 15 μg of nuclear lysate in a reaction buffer containing 50 mM Tris base, 25% glycerol, 0.1 mg/ml poly(dI-dC), 5 mM DTT, and 1 ng/ml BSA. The mixture was incubated for 10 min at room temperature and loaded on a 5% nondenaturing polyacrylamide gel. Following electrophoresis, the gel was dried and subjected to autoradiography and phosphorimaging.

Statistics

The nonparametric Mann-Whitney U test was applied to analyze differences for all parameters examined. Differences were considered statistically significant at p < 0.01.

Results

IL-4 enhances IL-10 mRNA in Th2, but not in Th1, cells in the absence of TCR engagement

Th1 or Th2 populations that had been differentiated from purified DO.11 CD4⁺ T cells were stimulated for 6 h with medium, IL-4, or plate-bound anti-CD3 as a positive control. To all cultures lacking exogenously added IL-4, anti-IL-4 Abs were added to neutralize any endogenously produced IL-4. Without stimulation, Th1 cells expressed only traces of IL-10, IL-5, IL-10, or IL-13 mRNAs. When Th2 cells were incubated with IL-4, anti-IL-4 Abs were added to neutralize any endogenously produced IL-4. Without stimulation, Th1 cells expressed only traces of IL-10, IL-13, and IL-2 and low levels of IFN-γ mRNA (Fig. 1A, lane 2). In contrast, Th2 cells expressed a detectable, but low, amount of IL-10 mRNA, and only trace or undetectable amounts of message for other cytokines (Fig. 1A, lane 6). To confirm the success of the subset differentiation, T cells were restimulated with plate-bound anti-CD3 Abs. As expected, anti-CD3-stimulated Th2 cells showed marked induction of mRNAs encoding IL-4, IL-13, IL-10, and, to a lesser extent, IL-5. In contrast, IL-2 and IFN-γ mRNAs were expressed in only very limited amounts (Fig. 1A, lane 8). Anti-CD3-stimulated Th1 cells expressed marked levels of IFN-γ message and very little IL-4, IL-5, IL-10, or IL-13 mRNAs. When Th2 cells were incubated with IL-4 in the absence of anti-CD3, there was a significant and consistent enhancement of IL-10 mRNA (Fig. 1A and B). In 13 independent experiments, IL-4 increased the expression of

4 Abbreviations used in this paper: CsA, cyclosporin A; EMSA, electrophoretic mobility shift assay; PKC, protein kinase C.
IL-10 mRNA 7-fold ($p < 0.01$). In addition, IL-4 caused a 4-fold enhancement of IL-13 mRNA. IL-13 mRNA levels were about one-half as high as IL-10 mRNA levels if compared with the level of housekeeping gene expression. In contrast, IL-4 did not enhance expression of IL-2, IL-5, IL-6, IL-9, or IFN-γ. Similar observations were made using a well-established Th2 cell clone, D10.G4.1 (data not shown). Although the experiments in the present study were performed at saturating levels of IL-4 (1000 U), titration experiments demonstrated increased IL-10 mRNA levels in DO.11 Th2 cells stimulated with as little as 50 U of IL-4 (data not shown). When Th1 cells were incubated with IL-4, no IL-10 induction could be observed. Interestingly, an approximately 6-fold increase in the level of IFN-γ was observed (Fig. 1A); however, this level was small in relation to levels of housekeeping gene expression.

**Specificity of cytokine-induced up-regulation of IL-10 mRNA**

Additional cultures were set up to determine whether other cytokines besides IL-4 could up-regulate T cell IL-10 production in the absence of TCR engagement. In Th1 cells, no substantial effect of IFN-γ, IL-12, IL-2, or IL-6 on IL-10 mRNA expression could be detected. IL-2, however, enhanced IFN-γ production 7-fold, whereas IL-4 or IL-12 enhanced IFN-γ expression only 1.7- or 1.6-fold, respectively (Fig. 2, lane 3). IFN-γ or IL-6 had no effect on IFN-γ expression. None of the added cytokines enhanced Th1 expression of IL-4, IL-5, IL-10, IL-13, IL-15, IL-9, IL-2, or IL-6 mRNA.

In Th2 cells, a 7-fold increase in IL-10 mRNA was observed following incubation with IL-4, similar to the results described above, and a 4.7-fold increase in IL-10 mRNA was observed following incubation with IL-2 (Fig. 2, lanes 9 and 12). IFN-γ, IL-12, or IL-6 had no effect on IL-10 mRNA expression. Other Th2 cytokines were not significantly induced with IL-4, IFN-γ, IL-12, IL-2, or IL-6.

**IL-4 and IL-2 enhance IL-10 protein expression in Th2 cultures in the absence of TCR engagement**

To determine whether the effects of IL-4 on IL-10 mRNA levels correlated with enhanced IL-10 protein expression, cytokine ELISAs were performed on supernatants taken 24 h following addition of the cytokines. Protein levels were too low to be measured.
at earlier time points. The IL-10 production of Th2 cells in the absence of any stimulation was either under the detection limit of the ELISA (10 pg/ml) or slightly above it. The addition of IL-4 increased the level of IL-10 to 80 pg/ml after 24 h (Fig. 3). Incubation with IL-2 increased the IL-10 level to 40 pg/ml. These results correlate very well with the mRNA levels reported above.

Effect of IL-4 on IL-10 mRNA stability

One way in which IL-10 could act to increase steady state IL-10 mRNA levels is to enhance mRNA stability. To address this possibility, IL-10 mRNA levels were examined in IL-4-activated Th2 cells after the addition of actinomycin D, which blocks de novo transcription. Fig. 4 shows that IL-10 message decayed at the same rate with or without IL-4 present. Interestingly, the level of IL-10 mRNA dropped dramatically within 30 min (>50% of the amount at 0 min; Fig. 4, A and B), whereas housekeeping genes remained unaffected for up to 5 h. The rapid loss of IL-10 message was also observed in D10.G4.1 cells. When D10.G4.1 cells were stimulated with anti-CD3 instead of IL-4, IL-10 mRNA disappeared faster than the other Th2 cell cytokines, IL-4, IL-5, and IL-13 (data not shown).

IL-4 enhancement of IL-10 mRNA is not blocked by CsA or JAK kinase inhibitors

The effects of specific inhibitors of protein kinases and phosphatases on IL-4 induction of IL-10 mRNA were examined to determine which signaling pathways may be involved in the effect (Fig. 5). The effect of the drugs were tested after a 6-h incubation period. The cell viability in all conditions shown in Fig. 5 was >95% as assessed by trypan blue dye exclusion. CsA was used to block potential residual NF-AT-dependent TCR signaling in the differentiated cells. Interestingly, while CsA effectively blocked anti-CD3-induced IL-10 mRNA expression in Th2 cells (data not shown) as well as ionomycin-induced IL-4 expression (Fig. 5A, lane 6), this drug did not block IL-4-mediated enhancement of IL-10 mRNA expression (Fig. 5A, lane 9). Furthermore, IL-13 enhancement by IL-4 was also resistant to CsA, while IL-13 enhancement by ionomycin was not. We also found that the JAK kinase inhibitor tyrophostin B42 (30, 31) could block STAT6 activation in DO.11 cells in a dose-dependent manner (Fig. 5C), but this drug had no effect on IL-4 enhancement of IL-10 mRNA expression (Fig. 5B). Only protein kinase C (PKC) inhibitors, in particular rottlerin, which preferentially blocks the PKCδ isoform, interfered with IL-4 enhancement of IL-10 expression (Fig. 5A, lanes 4 and 5). Maximal inhibition was achieved all across a titrated rottlerin concentration range from 0.5–300 μM. At concentrations >200 μM, 40% trypan blue-positive cells were observed. IL-10 mRNA could never be 100% inhibited, suggesting that a basic level is constitutively expressed in Th2 cells and therefore

![FIGURE 2. Specificity of IL-4-enhanced IL-10 mRNA expression. A phosphorimage of an RNase protection gel is shown. Both Th1 (lanes 2–7) and Th2 (lanes 8–13) cells were incubated with IL-4 (1000 U/ml; lanes 3 and 9), IFN-γ (1 ng/ml; lanes 4 and 10), IL-12 (1 ng/ml; lanes 5 and 11), IL-2 (1000 U/ml; lanes 6 and 12), and IL-6 (1 ng/ml; lanes 7 and 13) for 6 h before lysis and RNA isolation. Lane 1 shows the undigested 32P-labeled RNA probe. The gel shown is representative for three separate and independent experiments.](image)

![FIGURE 3. IL-10 protein levels of Th2 cells after a 24-h incubation. Th2 cells were incubated with IL-4 or IL-2 (both 1000 U/ml) for 24 h. In two cases IL-10 levels of the medium group were below the detection limit and were considered as 0 ng/ml. Data shown represent the mean of three independent experiments; error bars show the SD of the mean.](image)
regulated by transcriptional elements independent of PKC activation. IL-4 enhancement of IL-13 was also sensitive to PKC inhibitors. In contrast to IL-10, IL-13 induction was most sensitive to GO 6976, a selective blocker of PKCα, -β, and -γ isoforms (Fig. 5A, lane 3). These results suggest that PKC might play an important role in IL-10 expression. Fig. 5D demonstrates that, in fact, PMA, a potent inducer of PKC activation, was sufficient to induce IL-10 and IL-13 mRNA expression and, to a lesser degree also IL-5. In contrast, PMA did not induce IL-4 mRNA expression.

Discussion

The data reported here demonstrate that IL-4 enhances IL-10 mRNA and protein expression by differentiated Th2 cells in a TCR-independent manner. Thus, IL-4 secreted by Th2 cells may contribute to the regulation of immune responses by enhancing IL-10 production in nearby T cells. The ability of IL-4 to enhance IL-10 synthesis represents a potentially important mechanism by which Th2-cells could down-regulate macrophage activation. Furthermore, this effect of IL-4 may promote the differentiation of IL-10-producing regulatory T cells.

The data indicate that IL-4 does not enhance IL-10 in Th1 cells despite the capability of these T cells to respond to IL-4 (27, 32, 33). This is in agreement with previous studies showing that IL-10 is an exclusive Th2 cytokine in the mouse; in contrast, human Th1 cells are reported to produce IL-10 (15, 34). The finding that IL-4 also enhanced IFN-γ gene expression to some degree was unexpected; however, the induced levels were low compared with those of housekeeping genes. Although the biological significance of this finding is not clear, it should be noted that IFN-γ expression is impaired in IL-4 knockout mice (35).

In addition to the IL-4 effects, the data reported here indicate that IL-2 enhances T cell cytokine gene expression in the absence of TCR signals. For example, IL-2 increases IL-10 mRNA and protein expression in Th2 cells. Similarly, IL-2 enhances TCR-induced IL-10 production in human T cells (36). IL-2 induction of IL-10 by human Th1 cells apparently leads to down-regulation of Ag-presenting functions of macrophages and therefore reduced IL-2 production and proliferation of Th1 cells. This has been invoked as a mechanism of negative feedback regulation of Th1 responses (15, 16, 36). An IL-10-mediated negative feedback regulation has also recently been reported in an in vivo model of allergic encephalomyelitis (37). In the experiments reported here, IL-2 also increased IFN-γ expression 7-fold in Th1 cells. These results confirm previous data showing that IL-2 can induce IFN-γ expression in murine T cell lines (38). It is perhaps useful to compare the effects of IL-2 on Th1 and Th2 cells. In the former case, IL-2 synergizes with IL-12 to induce IFN-γ, and this would favor macrophage activation. In the latter case, IL-2 appears to work with IL-4 to induce IL-10, favoring down-regulation of macrophage function.

The data reported in this paper also show that IL-4 significantly enhances IL-13 mRNA production by Th2 cells, albeit to lower levels than that induced by IL-10. This finding contradicts a report that IL-4 does not influence IL-13 mRNA expression (39). This discrepancy may reflect the sensitivity of the methods used; the quantitative RNase protection assays employed here are probably more sensitive than semiquantitative RT-PCR, which does not allow reliable detection of less than 5-fold differences.

The present study shows that IL-4 enhanced IL-10 mRNA expression is presumably a result of transcriptional activation of the IL-10 gene and not a result of mRNA stabilization, as it has been reported for several cytokines (reviewed in Ref. 40). Interestingly, the IL-10 mRNA rapidly drops after inhibition of RNA de novo synthesis by actinomycin D. We also have preliminary evidence that TCR induced IL-10 mRNA drops much faster than other Th2

FIGURE 4. Effect of IL-4 on IL-10 mRNA stability. A, mRNA was isolated from Th2 cells after the indicated treatments and was analyzed by RNase protection as described previously. Lane 1 shows the undigested 32P-labeled RNA probe. Lane 2 represents RNA from cells incubated for 6 h in medium alone, and lane 3 represents RNA from cells stimulated with 1000 U/ml IL-4 for 6 h. All other RNAs (lanes 4–11) were isolated from cells that were treated for 6 h with IL-4, washed, and recultured with actinomycin D (5 μg/ml) with or without IL-4 for the indicated times. The gel shown is representative of two independent experiments. B, Protected mRNAs shown in A were quantitated using the PhosphorImager software. Zero on the x-axis indicates the time point when actinomycin D was added (after the initial 6-h IL-4 treatment). The closed circles represent RNA from cells that were recultured with IL-4 plus actinomycin D; the open circles represent RNA from cells that were recultured in the presence of actinomycin D alone.
cytokines, such as IL-4, IL-5, and IL-13. The short half-life of IL-10 mRNA might be important to limit the duration of IL-10-mediated down-regulation of bystander T cells and macrophages.

The fact that one T cell cytokine enhances expression other T cell cytokine genes. Cytokine induction in the absence of TCR receptor engagement has been reported previously for a mouse Th2 cell clone (D10.G4.1). These cells express IL-5, IL-6, and IL-10 following stimulation in vitro with IL-1 (41). Some T cell cytokines, such as granulocyte-macrophage CSF and IL-2, are characterized by a strict requirement of activation of both the PKC- and calcium-dependent pathways. IFN-γ, IL-3, and IL-4 are all partially induced by calcium ionophore alone, and IL-5, IL-6, and IL-10 are partially induced by either PMA or calcium ionophore alone (41, 42). The data presented here indicate that IL-10 can be induced by PMA alone or ionomycin alone, and it is of interest to know whether the IL-4 pathway of IL-10 enhancement involves either PKC activation or calcium/NF-AT signals. IL-4-enhanced IL-10 expression was not blocked by PKA or mitogen-activated protein kinase inhibitors (data not shown) or by CsA (Fig. 5). In contrast, CsA did prevent calcium ionophore-induced IL-10 expression. These results indicate that IL-10 gene expression can be enhanced by at least two independent signaling pathways. Surprisingly, the JAK kinase inhibitor (tyrophostin B42) did not show any effect on IL-4-induced IL-10 expression. However, the PKC inhibitor rottlerin did inhibit the IL-4-induced IL-10 expression. These results suggest that IL-4 engages a signaling pathway...
that is distinct from the JAK/STAT pathway and involves PKC.

However, pharmacological inhibitors are never completely sub-
strate specific, and therefore further biochemical analysis of this
putative pathway will be required. Nonetheless, the association
of IL-4R signaling and PKC activation has been demonstrated in
macrophages (43). Preliminary results from our laboratory indicate
that IL-7 also induces IL-10 mRNA in DO.11 and D10.G4.1 T
cells. We speculate that the common γ-chain that is shared by
the receptors for IL-2, IL-4, and IL-7 is involved in a PKC-depen-
dent, STAT6-independent signaling pathway that indicates IL-10
expression.

In conclusion, IL-4 markedly enhances IL-10 gene expression in
primary Th2 cells but not in Th1 cells. This subset-specific effect
of IL-4 is independent of TCR signaling and may have important
consequences on the paracrine regulation of the immune re-
sponses. In addition, IL-4 enhancement of IL-10 may be involved
in T cell differentiation toward the recently described T repressor
phenotype (18). We hypothesize that IL-4-enhanced IL-10 expres-
sion represents a negative feedback regulation of immune
responses.

References

1. Abbas, A. K., K. M. Murphy, and A. Sher. 1996. Functional diversity of helper

2. Davies, J. D., G. Martin, J. Phillips, S. E. Marshall, S. P. Cobbold,
and H. Waldmann. 1996. T cell regulation in adult transplantation tolerance. J. Im-
umunol. 157:529.

3. Donckier, V., M. Wissing, C. Bruyns, D. Abramowicz, M. Lybin,
M. L. Vanderhaeghen, and M. Miki. 1998. Administration of IL-4 prevents
autoimmune diabetes but enhances pancreatic insulitis in NOD mice. Clin. Im-
umunol. Immunopathol. 86:209.

imune diabetes by adjuvant therapy in the non-obese diabetic mouse: the role of

derlying counterregulation of autoimmune diabetes by IL-4. Immunity 7:411.

Interleukin 10 secretion and impaired effector function of major histocompati-

1997. Adenosine-mediated gene transfer of viral interleukin-10 inhibits the
immune response to both alloantigen and adenviral antigen. Hum. Gene Ther.
8:1365.

intrathymic apoptosis of CD4⁺ CD8⁺ TCR⁺ thymocytes in vivo. Science 250:
1720.

constitutively secrete large quantities of IL-2, 3, 4 or 5, using modified

10. Finkelmann, P. D., I. M. Kalscheuer, J. F. Urban, Jr., C. M. Snapper, J. Obara,
and W. E. Paul. 1986. Suppression of in vivo polyclonal IgE responses by monoclonal
USA 83:9675.

of transformed T cell growth in vitro by monoclonal antibodies directed against

and A. H. Lichtman. 1996. Cytokine transcriptional events during helper T cell subset

152:48.

1997. Human endothelial cell costimulation of T cell IFN-γ production. J.
Immunol. 159:3247.


K. Mitani, Y. Yasaki, and H. Hira. 1998. c-EB1 is tyrosine-phosphorylated by
interleukin-4 and enhances mitogenic and survival signals of interleukin-4 recep-
tor by linking with the phosphatidylinositol 3’-kinase pathway. Blood 91:46.

Th1 and Th2 populations. Int. Immunol. 7:869.

1995. Developmental commitment to the Th2 lineage by extinction of IL-12 signaling.

1
and -13 inhibit tumor necrosis factor-alpha mRNA translational activation in
macrophage colony-stimulating factor receptor in murine macrophages. J. Leu-

and -13 inhibit tumor necrosis factor-alpha mRNA translational activation in

21. Hart, P. H., G. M. Giudizi, D. R. Burgess, G. A. Whitty, D. S. Piccoli, and
M. G. Giudizi. 1997. Protection from autoimmune diabetes in mice by recom-

22. Rahimivarch, A., W. L. Suarez-Paniz, O. Sorensen, R. C. Bleackley,
R. F. Power, and R. V. Rajotte. 1995. Combined therapy with interleukin-4 and
interleukin-10 inhibits autoimmune diabetes recurrence in syngeneic islet-trans-
planted nonobese diabetic mice: analysis of cytokine mRNA expression in the
graft. Transplantation 60:568.

23. Rapoport, M. J., A. Jaramillo, D. Zopris, A. H. Lazarus, D. V. Serreze,
reverses T cell proliferative unresponsiveness and prevents the onset of diabetes

24. Dello Sbarba, P., E. Rovida, B. Ciacci, L. Nencioni, D. Labardi, A. Paccagnini,
L. Savini, and M. G. Coppolissi. 1996. Interleukin-4 rapidly down-modulates the
macrophage colony-stimulating factor receptor in murine macrophages. J. Leu-
kocyte Biol. 60:644.


27. Kuhn, R., J. Lohler, D. Rennick, K. Rajewsky, and W. Muller. 1993. Interleukin-
10-deficient mice develop chronic enterocolitis. Cell 75:263.

28. de Waal Malefyt, R., J. Haanen, H. Spits, M. G. Roncarolo, A. te Velde,
C. Klein, K. Johnson, R. Kastelein, H. Yssel, and J. E. de Vries. 1991. Inter-
leukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell
proliferation by diminishing the antigen-presenting capacity of monocytes via
dowregulation of class II major histocompatibility complex expression. J. Exp.
Med. 174:915.