IL-2-Dependent Expression of Genes Involved in Cytoskeleton Organization, Oncogene Regulation, and Transcriptional Control

Sabine Herblot, Patricia Chastagner, Laila Samady, Jean-Louis Moreau, Christophe Demaison, Patrick Froussard, Xinyuan Liu, Jacques Bonnet and Jacques Thèze

*J Immunol* 1999; 162:3280-3288;  
http://www.jimmunol.org/content/162/6/3280

**References**  
This article cites 41 articles, 19 of which you can access for free at: http://www.jimmunol.org/content/162/6/3280.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-2-Dependent Expression of Genes Involved in Cytoskeleton Organization, Oncogene Regulation, and Transcriptional Control

Sabine Herblot, Patricia Chastagner, Laila Samady, Jean-Louis Moreau, Christophe Demaison, Patrick Froussard, Xinyuan Liu, Jacques Bonnet, and Jacques Thèze

IL-2 induces growth, differentiation, and/or apoptosis of lymphoid cells. To study further the molecular basis of IL-2 function, we used a cDNA subtraction approach involving a cell line grown in IL-2 or IL-4. From the corresponding library, 66 nonredundant sequences were characterized; 16 of them encode identified proteins. The kinetics of in vitro expression of 8 selected sequences, the functions of which could be associated with IL-2-induced T cell activation/differentiation, was investigated using an IL-2-dependent T cell line. IL-2 increased the expression of cytoskeleton proteins (α-tubulin), oncogene-regulating proteins (CCCTC-binding factor, Jun inhibitor factor-1), and transcription factors (E2F-4, cyclic AMP-responsive element-binding protein, zfh-1). IL-2 also regulated the expression of genes coding for multifunctional proteins, e.g., β-catenin and nucleolin. These results were verified using Con A-induced T cell blasts stimulated or not by IL-2. The in vivo expression of four of these genes was also analyzed in spleen and lymph node cells of IL-2-deficient and MRL/lpr mice, which both have high numbers of activated cells, but the latter have intact IL-2 expression. The expression of β-catenin, CCCTC-binding factor, Jun inhibitor factor-1, and nucleolin was significantly higher in MRL/lpr animals. A similar analysis of thymocytes from IL-2−/− and IL-2+/− mice demonstrated the same expression patterns of the 4 sequences in these strains. The expression of the IL-2-induced genes described herein is similar to the regulatory pattern of IL-2Rα. Taken together, our data provide additional evidence for the pleiotropic action of IL-2 in the periphery and IL-2 independence of molecular processes involved in thymocyte differentiation. The Journal of Immunology, 1999, 162: 3280–3288.

Interleukin-2 is a multifunctional cytokine that regulates growth and/or differentiation of lymphocytes including T, B, and NK cells, as well as monocytes and some hemopoietic cells (1). The effects of IL-2 are mediated through its specific cell surface receptor (IL-2R), which comprises at least three subunits, IL-2Rα, IL-2Rβ, and IL-2Rγ. IL-2Rα enhances ligand affinity, and the heterodimer IL-2Rγ triggers downstream signaling events. IL-2 activates three major signaling pathways: the Ras and mitogen-activated protein kinase cascade, involved in mitogenesis; the phosphatidylinositol 3-kinase pathway, involved in cytoskeleton reorganization; and the Janus kinase-STAT pathway (2).

We and others have demonstrated that in vitro IL-2 induces the expression of its own α-chain receptor gene (3–7). Using IL-2−/− animals (8), we have verified that in vivo IL-2Rα expression is IL-2 dependent in secondary lymphoid organs. However, in IL-2−/− animals, IL-2Rα is normally expressed in thymus and bone marrow (9). IL-2-responsive elements have been localized at 1.7 and 3.7 kb upstream from the transcription start site of the mouse and human IL-2Rα genes, respectively (10–12). These responsive elements bind multiple factors, including STAT5, Elf-1, high mobility group family-(Y), and GATA family proteins; STAT5 homodimer might be the major IL-2-induced factor involved in the up-regulation of the transcription rate of the IL-2Rα gene (12, 13). More recently, we showed that the TNF-β gene is induced by IL-2, and we demonstrated that there is a STAT5-binding site with GAS sequence upstream from the transcription initiation point of this gene (14). Except for IL-2Rα and TNF-β, the target genes of the IL-2-induced signaling pathways are not well characterized. The oncogenes c-myc, c-jun, c-fos, c-myb, bcl-2, pim-1, and c-raf-1 and the tyrosine kinase gene ITK have been reported to be IL-2-inducible immediate-early genes (15). However, these genes are commonly induced by a variety of other growth factors.

In addition to genes implicated in growth and activation, IL-2 may induce the expression of genes negatively controlling the immune response, as suggested by the phenotype of IL-2-deficient mice, which develop a lymphoproliferative disorder involving uncontrolled T cell activation and autoimmune disease (8). Thus, immune cell activation and differentiation are induced in the absence of IL-2, but the negative control of the immune response cannot be engaged in the absence of this cytokine. The molecular mechanisms implicated in this negative regulation are still under investigation (16–18).
To identify new IL-2-inducible genes involved in these different activities, we used a cDNA subtraction hybridization method derived from genomic RDA\(^3\) (19) and a mouse T cell line grown in either IL-2- or IL-4-containing medium. Sixty-six nonredundant sequences were characterized including 16 previously identified genes. Herein, we report the influence of IL-2 on the in vitro expression of eight of these genes, which include sequences encoding cytokine receptors, oncogene regulators, and transcription factors. Their in vivo expression was analyzed in both the absence and the presence of IL-2, by comparing mRNA levels in LN and spleen cells from IL-2-deficient and MRL/lpr mice. Both strains develop a lymphoproliferative disorder with accumulation of activated lymphocytes in LN and spleen, but MRL/lpr mice have intact IL-2 gene expression (8, 20). Furthermore, the expressions of the IL-2-induced genes in the thymuses of IL-2\(^{-/-}\) and of IL-2\(^{+/+}\) mice were compared. Taken together, the data suggest that the regulation of the identified sequences differs for thymocytes and mature lymphocytes and follows the pattern of IL-2Ra expression previously described (9).

Materials and Methods

**Mouse strains**

IL-2-deficient mice were previously described (8). MRL/lpr mice were obtained from Harlan (Gannat, France). All mice were 6 to 9 wk old at the time of the experiments.

**Cell lines, cell culture, and proliferation assay**

Two cell lines were used in the course of the present work. T cell line 18.111 was used for the preparation of the cDNA subtraction library. It has been obtained after transfection by human IL-2R\(^{\alpha}\) of a murine cell line (8, 21) expressing constitutively IL-2R\(^{\beta}\) and IL-2R\(^{\gamma}\). When grown in IL-2- or IL-4-containing medium, cell line 18.111 is continuously in an activated state. It has been shown to express murine IL-2Ra only when cultured in IL-2 (21). The cytotoxic T cell line C30.1 (initially provided by Dr. F. Denizot) was used for analysis of the expression of IL-2-induced genes. In the absence of cytokines, C30.1 cells become resting and can be subsequently activated either by IL-2 or by IL-4 (21).

For standard culture in IL-2, complete medium (RPMI 1640 medium containing 5% heat-inactivated FCS, 2 mM glutamine, 10 mM HEPES, 2 mg M-2-mercaptoethanol, streptomycin, and panfungone) was supplemented with 20 ng/ml human rIL-2 (kindly donated by Roussel-Uclaf, Romainville, France). For standard culture in IL-4, mouse IL-4 was added at a final concentration of 20 U/ml. HeLa subline (H28) transfected with the plasmid pKCRIL-4 Neo was used as a source of murine IL-4. This line was provided by Pr. T. Horjoi (Kyoto University, Kyoto, Japan).

Proliferation assays were performed as previously described (22).

**Preparation and stimulation of Con A blasts**

Spleen cells from IL-2\(^{-/-}\) or IL-2\(^{+/+}\) animals on the same 129/Ola × C57BL/6 background were used. T cells were purified from spleen cell suspensions after incubation with anti-CD41 rat mAb followed by immunodepletion with magnetic beads coated with polyclonal anti-mouse Ig (Dynabeads, Dynal, Oslo, Norway). Two rounds of negative selection were performed to remove monocytes and B lymphocytes efficiently. The results are based on previous experience with the negative selection method using Dynabeads (24). Two rounds of negative selection were performed to remove monocytes and B lymphocytes efficiently. The results are based on previous experience with the negative selection method using Dynabeads (24).

**cDNA subtractive hybridization and amplification**

To identify IL-2-induced genes in mouse T cells, a cDNA subtraction library was characterized mainly from the mouse CDS\(^*\) T cell line 18.111 grown in IL-2-containing medium (18.111.2). The same cells grown in 18.111.4, were used as a negative cell population. We used a subtractive cDNA hybridization and amplification method based on RDA, which combines hybridization in solution of cDNA populations and PCR selection of target sequences. This methodology has been proved to be adequate and to be a powerful tool in the study of different genes.

**Abbreviations used in this paper:** RDA, representational difference analysis; CREB, cAMP-responsive element binding protein; CTCF, CCCTC-binding factor; JIP-1, Jun inhibitor factor-1; LN, lymph node.

---

\(^3\) Abbreviations used in this paper: RDA, representational difference analysis; CREB, cAMP-responsive element binding protein; CTCF, CCCTC-binding factor; JIP-1, Jun inhibitor factor-1; LN, lymph node.
grown in IL-4 was used as a negative cell population. This approach was designed to eliminate growth-related genes induced by either cytokines and housekeeping genes. The 122 clones randomly selected and sequenced represent 66 nonredundant sequences. Among them, 16 are highly homologous to previously characterized genes (Table I). The corresponding molecules have not been previously reported to be induced by IL-2. For our study, we retained the sequences encoding: 1) β-catenin and α-tubulin because of the cytoskeleton involvement in signal transduction and cell regulation; 2) the CTGF transcription factor and the JIF-1, because of their previously reported roles in c-myc and c-jun oncogene regulation, respectively (26, 27); 3) the E2F-4, zhx-1, and CREB transcription factors, because they could be implicated in IL-2-induced gene expression. Nucleolin was also examined because it is a multifunctional protein with transcriptional activity.

mRNA levels of selected sequences in response to in vitro IL-2 stimulation

To analyze the kinetics of induction of our selected sequences, we use C30.1 T cells, which can be easily obtained in a resting or in an activated state. The kinetics of cell size increase, as measured by flow cytometry, were similar after IL-2 or IL-4 stimulation, while IL-2 generated an amplitude threefold higher than the increment recorded with IL-4 (Fig. 1A). The kinetics of [3H]thymidine incorporation were comparable until 48 h of stimulation (Fig. 1B). As additional controls, the mRNA levels of the three IL-2R chains were examined during IL-2 and IL-4 stimulation of C30.1 cells; IL-2Rβ mRNA was transiently induced by IL-2 but not IL-4 and returned to the basal level by 18 h (data not shown). Induction of IL-2Rα mRNA by IL-2 but not IL-4 confirmed our previously reported results (Fig. 1, C and D) (21). The IL-2Rγ is used by IL-2 and IL-4 receptors, but its mRNA was induced only by IL-4 after 18 h of stimulation, reaching a maximum at 36 h. This finding is consistent with our observation that the IL-2Rγ mRNA level is higher in IL-4-dependent T cell lines than in IL-2-dependent T cell lines (data not shown).

The kinetics of induction of mRNA corresponding to the selected sequences was investigated by Northern blotting during the 48 h of IL-2 or IL-4 stimulation of C30.1 cells. Total RNA preparations were used in most cases, except for the weakly expressed transcription factors, which required poly(A)⁺ purification.

The mRNA levels corresponding to the cytoskeleton proteins β-catenin, α-tubulin, and β-actin were investigated first (Fig. 2A). Rapid increases of β-catenin and β-actin mRNA were observed in response to IL-2, with maxima being reached at 6 h. The α-tubulin mRNA level also increased rapidly after IL-2 stimulation but remained elevated for 36 h. These increases of mRNA encoding cytoskeletal proteins might reflect the increased cell size observed after IL-2-induced activation (Fig. 1A).

Table I. Comparison of the 16 selected sequences with database sequences corresponding to known genes

<table>
<thead>
<tr>
<th>Putative Identification</th>
<th>% Total Sequences</th>
<th>Length (bp)</th>
<th>Homology (%)</th>
<th>Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoskeleton proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tubulin</td>
<td>6.4</td>
<td>104–225</td>
<td>99</td>
<td>AF027905</td>
</tr>
<tr>
<td>β-Catenin</td>
<td>1.1</td>
<td>115</td>
<td>98</td>
<td>AF027945</td>
</tr>
<tr>
<td>Transcription factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CREB</td>
<td>5.3</td>
<td>132–205b</td>
<td>89</td>
<td>AF027911</td>
</tr>
<tr>
<td>E2F-4</td>
<td>1.1</td>
<td>93</td>
<td>97</td>
<td>AF027946</td>
</tr>
<tr>
<td>zhx-1</td>
<td>2.1</td>
<td>180–219a</td>
<td>93.5–97</td>
<td>AF027908</td>
</tr>
<tr>
<td>Nucleolin</td>
<td>1.1</td>
<td>197</td>
<td>98</td>
<td>AF027892</td>
</tr>
<tr>
<td>Oncogene regulator</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTGF</td>
<td>1.1</td>
<td>171</td>
<td>100</td>
<td>AF027943</td>
</tr>
<tr>
<td>JIF-1 (QM)</td>
<td>2.1</td>
<td>101–180b</td>
<td>98</td>
<td>AF027941</td>
</tr>
<tr>
<td>Nuclear proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mMIS-5</td>
<td>1.1</td>
<td>160</td>
<td>98</td>
<td>AF027907</td>
</tr>
<tr>
<td>hnRNP</td>
<td>1.1</td>
<td>65</td>
<td>90</td>
<td>AF027898</td>
</tr>
<tr>
<td>U2-srRNP</td>
<td>2.1</td>
<td>179–190b</td>
<td>90</td>
<td>AF027942</td>
</tr>
<tr>
<td>Ribosomal proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBP L35</td>
<td>1.1</td>
<td>141</td>
<td>86.5</td>
<td>AF027944</td>
</tr>
<tr>
<td>RBP L37a</td>
<td>3.2</td>
<td>108–170b</td>
<td>98</td>
<td>AF027887</td>
</tr>
<tr>
<td>RBP S12</td>
<td>2.1</td>
<td>122–188b</td>
<td>93</td>
<td>AF027910</td>
</tr>
<tr>
<td>RBP S15 (RIG)</td>
<td>1.1</td>
<td>188</td>
<td>92.5</td>
<td>AF027895</td>
</tr>
<tr>
<td>Transporter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺/K⁺ ATPase</td>
<td>2.1</td>
<td>184–224b</td>
<td>92</td>
<td>AF027896</td>
</tr>
</tbody>
</table>

* Accession number to EMBL database.

** Isoalted two or more times.

The mRNA levels of the nuclear factors CREB, E2F-4, zhx-1, and nucleolin were also investigated, (Fig. 2C). E2F-4 mRNA increased after IL-2 stimulation with two peaks at 6 and 36 h. Because E2F-4 is involved in cell cycle regulation, these two peaks might correspond to two cell division cycles during the 48 h of the experiment. CREB and zhx-1 were induced by IL-2, but not IL-4, attaining their maximum levels after 6 h of cytokine stimulation. The basal nucleolin mRNA level at t = 0 was high and then declined sharply over 3 h in the presence of IL-2 before rising again to peak at 24 h. A more precipitous and prolonged decrease of the nucleolin mRNA level was observed during IL-4 stimulation, and this level, despite a slight rise, never returned to baseline.

Taken together, these different induction kinetics for the selected sequences after cytokine stimulation of murine T cells suggest that the responses might be attributable to specific functions acquired after IL-2 stimulation.

In vitro expression of selected sequences by T cell blasts

Purified T cells from IL-2⁺/⁻ animals were analyzed for in vitro expression of IL-2Rα, β-catenin, nucleolin, and JIF-1 sequences. We found that culturing the cells during 18 h at low density (10⁵ well) limits their proliferation. Under those experimental conditions, the kinetics of induction of c-jun and JIF-1 mRNA were similar and do not allow any conclusion to be drawn concerning the involvement of JIF-1 in c-jun expression.

The kinetics of induction of mRNA corresponding to the selected sequences was investigated by Northern blotting during the 48 h of IL-2 or IL-4 stimulation of C30.1 cells. Total RNA preparations were used in most cases, except for the weakly expressed transcription factors, which required poly(A)⁺ purification.

The mRNA levels corresponding to the cytoskeleton proteins β-catenin, α-tubulin, and β-actin were investigated first (Fig. 2A). Rapid increases of β-catenin and β-actin mRNA were observed in response to IL-2, with maxima being reached at 6 h. The α-tubulin mRNA level also increased rapidly after IL-2 stimulation but remained elevated for 36 h. These increases of mRNA encoding cytoskeletal proteins might reflect the increased cell size observed after IL-2-induced activation (Fig. 1A).

CTCF transcription factor and JIF-1 have been previously reported to be, respectively, a transcriptional repressor of the c-myc gene and an inhibitor of c-Jun (26, 27). Therefore, we investigated CTCF, c-myc, JIF-1, and c-jun mRNAs levels after IL-2 or IL-4 stimulation of C30.1 cells (Fig. 2B). c-myc mRNA peaked 6 h after IL-2 stimulation and then declined regularly until 48 h. During this second period, the CTCF mRNA level continued to rise, reaching its maximum at 36 h. These kinetics suggest a relationship between the expression of the oncogene with respect to the expression of its regulator. In contrast, under our experimental conditions, the kinetics of induction of c-jun and JIF-1 mRNA were similar and do not allow any conclusion to be drawn concerning the involvement of JIF-1 in c-jun expression.

The mRNA levels of the nuclear factors CREB, E2F-4, zhx-1, and nucleolin were also investigated, (Fig. 2C). E2F-4 mRNA increased after IL-2 stimulation with two peaks at 6 and 36 h. Because E2F-4 is involved in cell cycle regulation, these two peaks might correspond to two cell division cycles during the 48 h of the experiment. CREB and zhx-1 were induced by IL-2, but not IL-4, attaining their maximum levels after 6 h of cytokine stimulation. The basal nucleolin mRNA level at t = 0 was high and then declined sharply over 3 h in the presence of IL-2 before rising again to peak at 24 h. A more precipitous and prolonged decrease of the nucleolin mRNA level was observed during IL-4 stimulation, and this level, despite a slight rise, never returned to baseline.

Taken together, these different induction kinetics for the selected sequences after cytokine stimulation of murine T cells suggest that the responses might be attributable to specific functions acquired after IL-2 stimulation.
Analysis of mRNA for β-catenin, nucleolin, and JIF-1 was also performed with cells from IL-2+/- and IL-2-/- animals before culture (Fig. 3). It is striking to see that cells from IL-2+/- animals produce more mRNA before culture than after 18 h in vitro without stimulation. With cells from IL-2-/- animals, this phenomenon is less striking (as shown with β-catenin, Fig. 3B), therefore suggesting that IL-2 is involved in this in vivo stimulation.

IL-2-dependent expression of the selected sequences in spleens and LN of IL-2-/- and MRL/lpr mice

IL-2-deficient mice generated by gene targeting (8) provide an additional experimental model to investigate the influence of IL-2 on the expression of the selected genes. In these animals, spleen and LN cells are activated, as assessed by the expression of the CD69 and CD71 markers on the CD4+ and CD8+ subpopulations (9). Because activation was observed in the absence of IL-2, expression of the genes under consideration might be negatively modulated. In agreement with this hypothesis, IL-2Rα was not expressed at the surface of mature CD4+ T lymphocytes from IL-2-/- animals. To compare cell populations with similar activation status, we chose MRL/lpr mice, the lymphocytes of which are spontaneously activated (20). The presence of IL-2, mRNA levels in MRL/lpr spleen, and LN were evaluated by RT-PCR (data not shown).

The IL-2Rα mRNA was barely detectable in spleen cells from IL-2-/- mice, in contrast to their strong expression in MRL/lpr spleen cells (Fig. 4). These results confirm our previous observations made by FACS analysis (9). Similarly, the expression of β-catenin, CTCF, JIF-1, and nucleolin mRNA was higher in MRL/lpr than IL-2-/- spleen cells. Although variations among different animals were found, the mean hybridization ratios were 4.5- to 8-fold higher in MRL/lpr than in IL-2-/- spleen (0.001 < p < 0.01).

The results obtained from the mRNA analysis of IL-2-/- and MRL/lpr LN cells confirmed and extended these data (Fig. 5). As expected, IL-2Rα was not expressed in IL-2-/- animals, and β-catenin, JIF-1, CTCF, and nucleolin levels were low in these mice. The differences between expression levels of the five genes analyzed, assessed by their hybridization ratios in MRL/lpr and IL-2-/- LN, were statistically significant (0.001 < p < 0.008).

IL-2-independent expression of the selected sequences in thymuses from IL-2-/- and IL-2+/- mice

Because FACS analysis showed that 30% of the CD4+CD8- thymocytes from IL-2-/- mice, like the IL-2+/- strain used as controls, express IL-2Rα (9), expression of this chain at the surface of IL-2-/- thymocytes seems normal. These data were confirmed by Northern blotting. Fig. 6A shows that IL-2Rα mRNA is normally expressed in the thymuses of IL-2-/- animals; when compared
with the IL-2Rα mRNA levels in the thymuses of IL-2−/− animals, the ratios obtained were not statistically different (p = 0.29).

The same experiment was performed with spleen and LN cells from IL-2−/− and IL-2−/− animals. In the secondary lymphoid organs of IL-2−/− mice, the frequency of activated cells was low as compared with IL-2−/− and MRL/lpr animals (9). However, as expected from the results shown in Figs. 4 and 5, we found significant differences between the mRNA levels of IL-2Rα in IL-2−/− and IL-2−/− animals; IL-2−/− mice express significantly more IL-2Rα than IL-2−/− animals (0.0006 < p < 0.01) (Fig. 6B).

Under the same experimental conditions, we investigated the expression of some of our selected sequences: β-catenin, CTCF, JIF-1, and nucleolin. As for IL-2Rα, we found that these sequences were comparably well expressed in the thymuses from IL-2−/− and IL-2−/− animals (0.1 < p < 0.4) (Fig. 6A). We also compared the expression of these sequences in spleen and LN of IL-2−/− and IL-2−/− mice. Spleen and LN cells from IL-2−/− animals express more β-catenin, JIF-1, and nucleolin mRNA than the corresponding cells from IL-2−/− animals (p < 0.05). As expected from the frequency of activated cells, the ratios calculated for the expression...
of theses genes in IL-2^{-/-} animals were lower than those determined for MLR/lpr (Figs. 4 and 5). Unlike IL-2R α, β-catenin, JIF-1, and nucleolin, the amounts of CTCF mRNA were comparable in IL-2^{-/-} and IL-2^{-/-} animals.

**Discussion**

Regulation of IL-2R α gene expression has been the subject of intense research efforts. IL-2 is the main biological signal influencing IL-2R α expression. In this study, we characterized families of genes with regulation patterns paralleling that of IL-2R α. They correspond to different types of molecules (cytoskeleton structural proteins, oncogene regulators, and transcription factors) and depend on IL-2 for their expression in secondary lymphoid organs. These data highlight the pleiotropic molecular activity of IL-2. In contrast, the expression of these genes in the thymus is IL-2 independent, a result that emphasizes the absence of any major influence of IL-2 on thymic differentiation.

Using a cDNA subtraction procedure with mRNA from a T cell line grown in medium containing either IL-2 or IL-4, we characterized 66 nonredundant sequences preferentially induced by IL-2 (Table I and data not shown). Our simplified cDNA RDA method gives rise to few false positives, unlike many other subtractive procedures or differential display PCR. Our methodology, based on cDNA amplification, can be applied to very small amounts of starting material and can be used in a variety of systems to isolate mRNA specifically modulated throughout cell activation or cell differentiation processes. Analysis of the mRNA levels of eight selected sequences, which encode cytoskeleton proteins, oncogene regulators, and transcription factors, was conducted both in vitro and in vivo. In vitro, T cells were stimulated by either IL-2 or IL-4, as a control. Fig. 1 shows that both cytokines stimulate cell proliferation, although the increased cell size induced by IL-2 suggests a major contribution of the cytoskeleton after IL-2 stimulation (see below). All sequences studied were induced to various degrees by IL-2 (Fig. 2). In most cases, maximum induction occurred at 6 h, although α-tubulin and nucleolin exhibited different kinetic profiles. In vitro experiments performed with T cells blasts from IL-2^{-/-} or IL-2^{-/-} animals confirm that IL-2 is required for the induction of IL-2R α, β-catenin, nucleolin, and JIF-1. In vivo studies were conducted to compare the expression of four sequences (β-catenin, JIF-1, CTCF, and nucleolin) in spleen and LN
cells from IL-2\(^{-/-}\) and MRL/lpr mice. In both strains, lymphocytes were activated (9), but only MRL/lpr animals expressed intact IL-2. \(\beta\)-Catenin, JIF-1, CTCF, and nucleolin mRNA levels were significantly higher in MRL/lpr than IL-2\(^{-/-}\) spleen and LN cells (Figs. 4 and 5). Since MRL/lpr and IL-2\(^{-/-}\) spleen and LN cells are activated by different mechanisms, they may contain subsets of lymphocytes of distinct phenotypes and express different sets of genes independently of IL-2 expression. However, the role of IL-2 in the control of the selected sequences was verified by comparing the results obtained with spleen and LN cells from IL-2\(^{-/-}\) and IL-2\(^{+/+}\) animals (Fig. 6). We also show that thymuses from IL-2\(^{-/-}\) animals express normally \(\beta\)-catenin, JIF-1, CTCF, and nucleolin mRNA as they express IL-2R \((\text{Fig. 6}).\)

In this report, we have shown that IL-2 induced the expression of \(\beta\)-catenin, \(\alpha\)-tubulin, and \(\beta\)-actin mRNAs, corresponding to molecules comprising part of the cytoskeleton network. The enhanced synthesis of these structural proteins may reflect the increased cell size and intense membrane movements observed after IL-2 stimulation but not after exposure to IL-4 (Trautmann et al., unpublished observations). \(\alpha\)- and \(\beta\)-catenin provide a link between cell surface-expressed cadherins and the actin cytoskeleton filaments and represent key molecules connecting cellular adhesion to signal transduction pathways. Cadherin molecules expressed on T lymphocytes (28) form a complex with \(\beta\)-catenin (29) and might be involved in the interactions between activated T cells and their cellular targets or the extracellular matrix. In addition, \(\beta\)-catenin could participate in IL-2-induced activation, acting as a cofactor for members of the high mobility group family of transcription factors such as lymphocyte enhancer factor-1 or T cell factor-1 (30, 31).

The precise regulation of c-myc and c-jun gene expression in response to IL-2 is essential for controlling cell growth. Although the downstream IL-2R-signaling pathways leading to c-myc and c-jun oncogene induction have been extensively studied (32–34), little is known about the molecules involved in the regulation of these oncogenes. We have identified two oncogene regulators with mRNA levels that are enhanced after IL-2 stimulation of mouse T cells. First, the CTCF transcription factor, initially described as a c-myc transcriptional repressor, is characterized by a DNA-binding domain consisting of 11 zinc fingers and 2 repression domains (26). Its singular property of combining different sets of zinc fingers to bind different nucleotide sequences suggests that it could have several target genes. CTCF is therefore a multivalent transcription factor the activity of which might depend on the activation status of the cell. We described herein a relationship between the decrease of c-myc mRNA and the increase of CTCF mRNA in IL-2-stimulated C30.1 cells, suggesting the possible CTCF repression of c-myc. The second regulator, JIF-1, was initially described as a jun inhibitory factor and is a highly conserved protein with tumor suppressor properties (27). This factor binds c-Jun in a

![Figure 6](http://www.jimmunol.org/)

**Figure 6.** In vivo expression of IL-2R \(\alpha\), \(\beta\)-catenin, CTCF, JIF-1, and nucleolin mRNA in lymphoid organs of IL-2\(^{-/-}\) and IL-2\(^{+/+}\) mice. A, Total RNA were extracted from the thymuses of IL-2\(^{-/-}\) and IL-2\(^{+/+}\) animals and subjected to Northern blot analysis. Mean hybridization ratios \pm SD are shown. B, mRNA were prepared from the spleen and LN cells of these strains and assayed as indicated in the legend of Fig. 3.
Zn²⁺-dependent manner and inhibits the AP1 complex formation. The role of IL-2 in the control of API formation is therefore supported by our observation.

Four sequences encoding nuclear factors were isolated in our cDNA-subtraction library. 1) E2F-4 transcription factor belongs to the E2F family, which comprises five E2F-related proteins and three DP proteins crucial for cell cycle progression and DNA replication (35, 36). It has been reported that in quiescent primary T cells and CD34⁺ hematopoietic progenitors, the major E2F transcription factor is composed of E2F-4 complexed with DP-1 and p130 (37). 2) CREB plays a crucial role in T cell activation and proliferation, as indicated by the markedly impaired thymocyte proliferation and IL-2 production in transgenic mice expressing a dominant-negative CREB (38). CREB triggers the expression of growth-related genes, including the peripheral cell nuclear Ag, c-Jun A, as well as c-fos, c-jun, fra-2, and fosB involved in API complex formation, which are essential for IL-2-induced proliferation (38, 39). 3) Zfh-1 is a newly described transcription factor characterized by two zinc finger motifs and five tandem homeodomain A, as well as c-fos, c-jun, fra-2, and fosB involved in API complex formation, which are essential for IL-2-induced proliferation (38, 39). 3) Zfh-1 is a newly described transcription factor characterized by two zinc finger motifs and five tandem homeobox domains involved by screening a mouse cDNA library of bone marrow stroma cell lines (40). 4) Nucleolin is a ubiquitously expressed multifunctional protein involved in ribosomal biogenesis, DNA replication, and transcription. Herein we showed that IL-2 induced E2F-4, CREB, zfh-1, and nucleolin mRNA, but their different kinetics of induction (Fig. 2) suggest the sequential involvement of these transcription factors after IL-2 stimulation.

The similarities between the expression patterns of the genes isolated with our subtractive cDNA hybridization procedure and the IL-2Ra gene, both in vitro and in vivo, suggest that some common factors could regulate their transcription. Three proximal regulatory regions have been characterized in the IL-2Ra gene promoter (11, 12). Transcription factor STAT5 may account for most of the IL-2-induced expression of the IL-2Ra gene. Among the previously described promoters of β-catenin, CREB, and nucleolin gene sequences (41–43), we have not found either GAS or regulatory sequences analogous to the S¹-region of the IL-2Ra gene. The three S¹-flanking regions are characterized by the presence of CpG islands and numerous Sp1 transcription factor sites, both of which are features of regulated housekeeping genes. Therefore, these genes could be constitutively expressed at low levels and up-regulated in secondary lymphoid organs by transcription factors modulated directly or indirectly by the IL-2-IL-2R system. The phosphatidylinositol 3-kinase pathway or the Ras/mitogen-activated protein kinase cascade could be implicated. The expression of these genes in the thymus would be enhanced by yet unknown signals. More genomic sequences corresponding to the genes characterized as inducible by IL-2 are needed to draw more definitive conclusions concerning the mechanism(s) involved in the activation of this cytokine.

The selective regulation by IL-2 of cytoskeleton proteins, oncogene regulators, and transcription factors provides new elements contributing to a better understanding of the role of IL-2 at the level of mature lymphocytes and thymocytes. The observation that the corresponding mRNAs were expressed normally in the thymuses of IL-2−/− mice is in agreement with the normal thymic differentiation observed in these animal and suggests that at the thymic level, a cell surface molecule or another cytokine might be involved in the up-regulation of the genes under consideration. The possible involvement of IL-15 was explored, but no up-regulation in the IL-2−/− thymus was detected (9). The underexpression of these genes in the peripheral lymphocytes of IL-2−/− animals might contribute to the phenotype of these animals. Abnormal proliferation may be facilitated by the uncontrolled expression of oncogenes and transcription factors. Similarly faulty adhesion due to defective cadherin-β-catenin complexes may result in abnormal lymphocyte circulation and homing and may also allow lymphocytes to escape normal regulatory signals. Taken together, underexpression of these sets of genes may contribute, at least in part, to the lymphoproliferation and autoimmune disorders observed in IL-2−/− animals.

Acknowledgments

We thank B. Dujon and his associates for their valuable advice and assistance in sequence analysis. We also thank Drs. J. Bertoglio and M. Yaniv for critical reading of the manuscript and C. Baran and C. Corel for expert secretarial assistance.

References

24. Hultman, T., S. Stahl, E. Hornes, and M. Uhlen. 1989. Direct solid phase se-
quencing of genomic and plasmid DNA using magnetic beads as solid support.
Nucleic Acids Res. 17:4937.
25. Ansorge, W., T. Kristensen, H. Voss, S. Wiemann, C. Schwager, and
J. Zimmermann. 1994. DNA sequencing, advance approaches, automated meth-
ods and analysis. EMBO Practical Course: EMBL.
26. Filippova, G. N., S. Fagerlie, E. M. Klenova, C. Myers, Y. Dehner, G. Goodwin,
P. Neiman, S. J. Collins, and V. V. Lobanenkov. 1996. An exceptionally con-
served transcriptional repressor, CTCF, employs different combinations of zinc
fingers to bind diverged promoter sequences of avian and mammalian c-myc
61:514.
30. Behrens, J., J. von Kries, M. Kuhl, L. Brahn, D. Wendhich, R. Gosschedl, and
W. Birchmeier. 1996. Functional interaction of β-catenin with the transcription
31. Molenaar, M., M. van de Weretering, M. Oosterweling, J. Perterson-Maduro,
S. Godsave, V. Kornek, J. Roose, O. Destreé, and H. Clevers. 1996. XTcf-3
transcription factor mediates β-catenin-induced axis formation in Xenopus em-
32. Taniguchi, T. 1995. Cytokine signaling through nonreceptor protein tyrosine ki-
33. Miyazaki, T., Z. Liu, A. Kawahara, Y. Minami, K. Yamada, Y. Tsujimoto,
signaling pathways mediated by bcl2, c-myc, and Ick cooperate in hematopoietic
34. Takeshita, T., T. Arita, M. Higuchi, H. Asao, K. Endo, H. Kuroda, N. Tanaka,
K. Murata, N. Ishii, and K. Sugamura. 1997. STAM, signal transducing adaptor
molecule, is associated with Janus kinases and involved in signaling for cell
Genet. 11:173.
lation of E2F family members by cyclin-dependent kinases. Mol. Cell. Biol. 17:
3667.
cycle status and E2F complexes in mobilized CD34+ cells before and after cy-
38. Barton, K., N. Muthusamy, M. Chanyangam, C. Fisher, C. Clendenin, and
J. Leiden. 1996. Defective thymocyte proliferation and IL-2 production in trans-
genic mice expressing a dominant-negative form of CREB. Nature 379:81.
activates cyclic AMP-responsive element-binding protein in T lymphocytes.
Zbt-1: a novel mouse homeodomain protein containing two zinc fingers and five
41. Nollet, F., G. Berx, F. Molemans, and V. F. Roy. 1996. Genomic organization of
the human β-catenin gene (CTNNB1). Genomics 32:413.
The mouse CREB (cAMP responsive element binding protein) gene structure,
43. Bourbon, H.-M., M. Pudhomme, and F. Amaldi. 1988. Sequence and structure of
the nucleolin promoter in rodents: characterization of stringently conserved