INTERLEUKIN 1 MODULATES MESSENGER RNA LEVELS OF LYMPHOKINES AND OF OTHER MOLECULES ASSOCIATED WITH T CELL ACTIVATION IN THE T CELL LYMPHOMA LBRM33-1A5

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We have investigated the mechanism of action of IL 1 on T cell activation. For this purpose, we analyzed the content of specific messenger RNA for lymphokines and other genes that are associated with T cell activation in the murine IL 1-dependent T cell lymphoma LBRM33-1A5. Using cloned genes for IL 2, IL 3, TGF-β, TY5, IL 2 receptor, Ly-1, c-myc, and p53 as probes in the S1 nuclelease protection assay, we compared the amount of specific transcripts among total RNA prepared from unstimulated cells, IL 1α or PHA-stimulated cells, and PHA plus IL 1α-stimulated cells. IL 1α augmented the PHA-induced accumulation of IL 2 mRNA with a magnitude comparable to the amount of IL 2 produced, suggesting that IL 1α modulates IL 2 gene expression at the RNA level. Similar results were obtained with IL 3. We also observed that Ly-1 mRNA appears after PHA treatment and its accumulation was augmented by IL 1α addition. On the basis of the effects of IL 1α and/or PHA treatments on gene expression, we classified these genes into four groups. In all cases, IL 1α seemed to affect mRNA levels quantitatively. These observations support previously described characteristics of this cytokine as a co-stimulator of T cell activation.

The cytokine interleukin 1 (IL 1) is widely believed to be an important mediator of various immunological and inflammatory phenomena (1). It is produced by wide variety of cell types and has a broad range of target cells, mainly of mesenchymal origin (2). The role of IL 1 in T cell activation remains an important issue in immunology.

Various effects of IL 1 on T cell activation have been described. IL 1 is believed to be involved in lymphokine production by activated T cells (3, 4) and in the expression of interleukin 2 (IL 2) receptors on T cells (5). However, the mechanism of IL 1 action on T cells remains obscure. In most cases it acts as a co-stimulator of T cell activation; it does not have any detectable effects by itself, but augments the effects of other stimuli such as mitogens, or anti-T cell receptor antibodies (6).

We were interested in studying the effect of IL 1 on gene expression during T cell activation. For this purpose, we chose the mouse T cell lymphoma LBRM33-1A5 as a model system. Unlike most T cell clones and hybridomas that are fully activated to produce lymphokines by mitogens alone, this cell line requires phytohemagglutinin (PHA) and IL 1 to produce IL 2 (4, 6). Our experience in a previous study (6), in which we analyzed the stimulant requirement for the activation of this cell line, convinced us that this cell line is particularly suitable for the molecular biological analysis of IL 1 effect(s) on T cell activation. In contrast to other IL 1 assay systems that involve materials available in small amounts, which consist of heterogeneous populations such as thymocytes, this cell line is easy to grow in large quantities as a homogeneous population, which is advantageous for biochemical study.

Using the S1 nuclelease protection assay, we have analyzed the content of various messenger RNA species in these cells stimulated with PHA and/or IL 1α.

We used as probes cloned genes of IL 2, interleukin 3 (IL 3), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), Ly-1, IL 2 receptor (IL 2-R), TY5, transforming growth factor β (TGF-β), and the nuclear oncogenes c-myc and p53. Not only lymphokines but all of these molecules are believed to be associated with T cell activation; Ly-1 expression has been reported to increase on thymocytes after PHA treatment (7). TY5 was isolated by differential hybridization from an activated T cell clone cDNA library (8) and is known to be expressed in activated T cells, although the function of the gene product is still unknown. Unlike lymphokines, the induction of TY5 by mitogens occurs in the presence of dexamethasone (9), which suggests that the control of its gene expression is different from other lymphokines. TGF-β was first discovered as a factor that can alter the phenotype of fibroblast cell lines (10), but is now known to affect T cells and to be produced by activated T cells (11). The nuclear oncogene c-myc has also been described to be induced in T cells upon mitogen stimulation (12, 13). Another nuclear oncogene, p53, is known to code a cellular protein that binds to the large T antigen of SV40-transformed cells, and synergizes with the v-Ha-ras oncogene in transforming rat embryonal fibroblasts similar to mouse cells (14).

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2 Abbreviations used in this paper: IL 2-R, interleukin 2 receptor; TGF-β, transforming growth factor β; PIPES, 1,4-piperazinediethanesulfonic acid; GM-CSF, granulocyte-macrophage colony-stimulating factor.
to c-myc (14–16). This oncogene has also been reported to be associated with T cell activation (17, 18).

Our results indicate that IL 1α augments the PHA-induced accumulation of messenger RNA for lymphokines such as IL 2, IL 3, and the surface glycoprotein Ly-1. IL 1α alone failed to induce these messages. The results of RNA analyses correspond well with bioassay observations, suggesting that IL 1α affects gene expression at the level of mRNA production. On the other hand, IL 1α alone increased messenger RNA content of IL 2-R, TGF-β, and the nuclear oncogenes c-myc and p53. These observations indicate that the effect of IL 1α varies among genes associated with T cell activation.

**MATERIALS AND METHODS**

Cells and culture media. LBRM3-1A5 cells were obtained from the American Type Culture Collection as described (8). Briefly, the culture medium was RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), essential and nonessential amino acids, 2-mercaptoethanol, and antibiotics (penicillin, streptomycin, and gentamicin). In all cases its response to IL 1α based on enhancement of IL 2 production was tested before use. After expansion to a cell density of 5 x 10^6/ml, the cells were induced as indicated. PHA (Bectin from Phaffia rorulosa) (Sigma Chemical Co., St. Louis, MO) was used at a final concentration of 2.5 µg/ml unless otherwise indicated. Recombined, purified human IL 1α was purchased from Genzyme Corp. (Boston, MA) and was added to the medium to 1 µl/Control experiments have indicated that LBRM3-1A5 exhibits the same sensitivity to human IL 1α as it does to murine IL 1α. The two reagents were added at the same time in the combined stimulation. Incubation was continued 6 hr except for those courses in which cycloheximide treatment, cells were induced with PHA and IL 1α as described, and cycloheximide (Sigma) was added at a final concentration of 10 µg/ml at the same time as PHA and/or IL 1α.

**Preparation and analysis of RNA.** At the end of the incubation, the cells were harvested and total cellular RNA was prepared from them by the standard methods of ultracentrifugation and dialysis. RNA sizes were checked by agarose gel electrophoresis (20) and their concentration was determined by measuring absorption at 260 nm.

The SI nuclelease protection assay was done as described originally by Berk and Sharp (21) with some modification (22). Briefly, 10 µg of total RNA were hybridized with 10^6 cpm/µg, 10^6 cpm/reaction) in 15 µl of solution containing 80% formamide, 0.4 M sodium chloride, 1 mM EDTA, and 50 mM PIPES (pH 6.8). The experimental design of the probes and the hybridization temperatures are summarized in Table I. After an 8-hr incubation, 150 µl of SI solution containing 1000 U/ml of SI nuclease (BRL, Bethesda, MD), 20 µg/ml denatured calf thymus DNA, 250 mM sodium chloride, 50 mM sodium acetate, and 0.1 mM zinc acetate were added to the hybridization mixture, and the incubation was continued at 37°C for 1 hr. Undigested materials were ethanol pre-
ciptitated and fractionated by 6% polyacrylamide gel electrophoresis in 7 M urea and were subjected to autoradiography performed by using an intensifying screen.

**Bioassay.** The IL 2 and IL 3 biological activities in the conditioned medium were assayed using the IL 2-dependent T cell line HT-2 and the IL 3-dependent mast cell line MC/9 as described (23, 31). Our units are defined as described elsewhere (6) (the maximal dilution of the supernatant that maintains 4000 cells/100 µl alive for 24 hr).

**RESULTS**

**Surface phenotype of LBRM3-1A5.** We began the study with the analysis of the surface phenotype of LBRM3-1A5 cells by using the fluorescence-activated cell sorter (FACS). The cell line showed the phenotype of Ly-1−, Ly-2−, L3T4−, and Thy-1+, which is similar to that of immature thymocytes. Typical results of FACS analysis are shown in Figure 1, in which the cells were stained with anti-Ly-1 antibody. In contrast to thymocytes prepared from adult thymus (panel B), this cell line appears to be completely negative for Ly-1 (panel A).

**Effect of IL 1α and PHA on the lymphokine activities produced in conditioned medium.** No IL 2 or IL 3 activities were detected in the conditioned media of unstimulated or IL 1α-stimulated cells. A small amount of IL 2 but not IL 3 bioactivity was detected in the culture medium of PHA-stimulated cells. As described previously (6), this cell line produced IL 2 maximally when stimulated with IL 1α and PHA. Cells were also induced to produce IL 3 by the combinatory stimulation. The bioassay results are summarized in Table II.

**IL 1α augments lymphokine and Ly-1 mRNA accumulation induced by PHA.** We prepared the cells as described in Materials and Methods and analyzed RNA using various cloned genes. The yield and size distribution of RNA, checked by agarose gel electrophoresis, did not show any significant change among samples. Figure 2 shows the autoradiograms of SI protection assays of various genes.

In the cases of IL 2, IL 3, and Ly-1, RNA samples from

**TABLE I**

**Experimental design for SI protection assay**

<table>
<thead>
<tr>
<th>Gene</th>
<th>3P-Labeled Site</th>
<th>SI Protected Length</th>
<th>SI Hybridization Temperature</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL 2</td>
<td>Igκ</td>
<td>-</td>
<td>56°C</td>
<td>23, 24</td>
</tr>
<tr>
<td>IL 3</td>
<td>HindIII</td>
<td>120</td>
<td>52°C</td>
<td>25</td>
</tr>
<tr>
<td>Ly-1</td>
<td>EcoRI</td>
<td>532</td>
<td>52°C</td>
<td>26</td>
</tr>
<tr>
<td>IL 2- R</td>
<td>TaqI</td>
<td>394</td>
<td>52°C</td>
<td>27</td>
</tr>
<tr>
<td>TGF-β</td>
<td>HindIII</td>
<td>560</td>
<td>52°C</td>
<td>28</td>
</tr>
<tr>
<td>c-myc</td>
<td>HindIII exon2</td>
<td>723</td>
<td>53°C</td>
<td>29</td>
</tr>
<tr>
<td>p53</td>
<td>XhoI</td>
<td>105</td>
<td>53°C</td>
<td>30</td>
</tr>
<tr>
<td>Thy-1</td>
<td>Igκ</td>
<td>294</td>
<td>52°C</td>
<td>8</td>
</tr>
</tbody>
</table>

*Each cDNA clones or subcloned genomic DNA fragment of IL 3 or c- myc was digested by indicated restriction enzymes (a) and 5' end phosphate was replaced with 3P by using polynucleotide kinase. The size of SI nuclease undigested product (column b) is that of the complementary region between the 3P-labeled probe and mRNA, which lies between 5P- labeled site (indicated in column a) and 5' end of the cDNA insert or exon/ intron junction (in the case of c-myc). Hybridization temperatures (c) were determined empirically, which gave a good signal-to-noise ratio. The band on autoradiograms were calculated in cpm/mg of total RNA.*

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Fluorescence intensity histogram of the unstimulated LBRM3-1A5 (A) and thymocytes (B). Cells were stained by anti-Ly-1 antibody and were analyzed with a FACS. The dotted line corresponds to background staining; the x-axis indicates relative fluorescence intensity (arbitrary units).
TABLE II

<table>
<thead>
<tr>
<th></th>
<th>Unstimulated</th>
<th>IL 1α</th>
<th>PHA</th>
<th>PHA + IL 1α</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL 2</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>10</td>
</tr>
<tr>
<td>IL 3</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>640</td>
</tr>
</tbody>
</table>

* Biological activity of IL 2 and IL 3 in the conditioned medium from stimulated or unstimulated LBRM33-1A5. In control experiment, PHA or IL 1α did not support the survival of the indicator cell lines. Assay conditions and unit definition are described in Materials and Methods.

We also investigated whether GM-CSF or IFN-γ-specific mRNA were produced by LBRM33-1A5, but failed to detect any in all the samples analyzed (data not shown). IL 1α does not synergize with PHA in TY5 mRNA increase. In the case of TY5, the intensity of the bands were the same between PHA and PHA/IL 1α lanes. In contrast to other genes, IL 1α did not show an enhancing effect.

All negative lanes failed to show any detectable bands of the right size even after long exposure (data not shown). This observation correlates with the lack of lymphokine activity or Ly-1 expression observed in unstimulated or IL 1α-treated cells (6) (Fig. 1).

Unstimulated cells express IL 2-R and TGF-β transcripts that are upregulated by IL 1α alone. When the IL 2-R and TGF-β genes were used as probes, bands of the predicted size appeared in all four lanes, and their intensity increased from the left to the right of the figure. This indicates that mRNA coding these genes exist in unstimulated cells and treatment with either PHA or IL 1α increased their levels. Once again, the combination of the two reagents caused more accumulation of the specific transcripts.

Perturbation of nuclear oncogenes. Figure 2 shows the results of the S1 protection assay of c-myc and p53 using the same sample used in Figure 2. In this set of assays, the two probes were mixed in each assay and processed. Specific transcripts of c-myc and p53 were detected in unstimulated cells. Although the amount of c-myc mRNA increased with either PHA or IL 1α and further increased by the combination, p53 message increased only with IL 1α and not with PHA.

All results were subjected to densitometric tracing and were compared quantitatively. The summary of the perturbation of mRNA is shown in Table III.

Effect of cycloheximide on IL 2 gene expression. For further characterization, we chose IL 2 as the representative of our "Type I" genes (Table III). Figure 4A shows a time course of IL 2 mRNA accumulation. The kinetics of IL 2 mRNA accumulation did not show any significant change among the three conditions tested. We also investigated the effect of cycloheximide, a protein synthesis inhibitor, on IL 1α action (Fig. 4B). Unlike previous ob...
**TABLE III**

| mRNA MODULATION BY IL 1 | 2517 |

<table>
<thead>
<tr>
<th>Effect of IL 1a or PHA on the relative amount of specific transcripts.</th>
<th>Type</th>
<th>Gene</th>
<th>Unstimulated</th>
<th>IL 1a</th>
<th>PHA</th>
<th>PHA + IL 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type I</strong></td>
<td>IL 2</td>
<td>—</td>
<td>—</td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL 3</td>
<td>—</td>
<td>—</td>
<td>41</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ly 1</td>
<td>—</td>
<td>—</td>
<td>22</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Type II</strong></td>
<td>TYP</td>
<td>—</td>
<td>—</td>
<td>95</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Type III</strong></td>
<td>IL 2-R</td>
<td>8</td>
<td>16</td>
<td>29</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TGF-β</td>
<td>10</td>
<td>28</td>
<td>59</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c-my</td>
<td>27</td>
<td>51</td>
<td>64</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Type IV</strong></td>
<td>p53</td>
<td>74</td>
<td>116</td>
<td>64</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

* Results of RNA analysis were quantified and summarized. The intensity of specific bands in autoradiograms were measured by using a densitometer, and each value was then converted into the percentage of the level of IL 1a plus PHA-stimulated samples for each probe. These numbers correspond to the relative concentration of mRNA. Dash represents "undetectable limit." Genes were classified into four types according to the pattern of perturbation by the stimulation.

1. Not detected in "unstimulated" or "IL 1a." "PHA" < "PHA + IL 1a." "Not detected in "unstimulated" or "IL 1a." "PHA" < "PHA + IL 1a." "Unstimulated" = "PHA" < "IL 1a." "PHA + IL 1a." "Unstimulated" = "PHA" < "IL 1a." "PHA + IL 1a." "PHA" < "IL 1a." "PHA + IL 1a."

Figure 4. A. RNA samples of 3 hr and 6 hr incubated cells treated with PHA, PHA plus IL 1a, or high concentration of PHA (10 μg/ml) were used for IL 2 mRNA detection. IL 2 cells were treated with PHA, cycloheximide, IL 1a, or their combination as indicated and were subjected to IL 2 message analysis. IL 2 specific bands are indicated with arrows. Bands appearing in the "RNA" lane are nonspecific background. Film was exposed for 12 hr.

The data presented here indicate that IL 1a regulates the expression of the genes studied at the level of mRNA. In the case of IL 2, the difference in messenger RNA amount induced by IL 1a corresponds roughly with the IL 2 activity observed in the conditioned medium, suggesting that a major effect of IL 1a in gene expression is the control of mRNA content.

The S1 nucleosome protection assay, used for these studies, is a very sensitive and specific method to analyze RNA. However, since this assay only reveals relative concentrations of a given mRNA species, we cannot determine conclusively whether IL 1a actually increases the transcriptional rate or the RNA processing rate, or stabilizes the message, all of which could lead to the observed increase of the relative concentration of the specific mRNA species. It is also possible that IL 1a acts on the translation, processing, or secretion of the protein product. In experiments currently underway we will aim to investigate these possibilities.

We also found that LBRM33-1A5 produces IL 3 mRNA by PHA stimulation. We confirmed the observation by assaying the IL 3 biological activity in the conditioned medium. IL 1a synergistically increased PHA-induced IL 3 message accumulation like that of IL 2, although the amount of IL 3 production was considerably lower compared with IL 2. We failed to detect any GM-CSF mRNA in this cell line. LBRM33-5AA, a related clone derived from the same parental line from which LBRM33-1A5 was derived, does not require IL 1a to produce IL 2 (4, 6). PHA-activated 5AA cells produce large amounts of IL 2, IL 5, and GM-CSF (6). In view of the surface phenotype (5AA cells are Ly-1, Ly-2, L3T4+, Thy-1−) and IL 1a responsiveness, these two cell lines may represent two different developmental stages of T cell ontogeny. As mature T cells do not seem to require IL 1a when activated by mitogens (34), 1A5 cells seem to be more "immature" compared with 5AA. It is tempting to speculate that the difference of lymphokine production spectrum may reflect developmental changes.

It has been suggested that the lymphocyte surface antigen Ly-1 may be functionally related to IL 1. Anti-Ly-1 antibodies have been reported to be able to substitute IL 1 in the thymocyte proliferation assay, and Ly-1 has been postulated to be related to the IL 1 receptor (7). PHA upregulates Ly-1 mRNA in this cell line, which strongly suggests that PHA induces surface Ly-1 expression as observed in thymocytes (7). Because preincubation with IL 1 fails to augment PHA action (4), IL 1 apparently requires PHA-priming to cause augmentation of lymphokine production. As discussed above, PHA priming causes the induction of Ly-1, which is consistent with the postulated relationship between the IL 1 receptor and Ly-1. However, we think that Ly-1 is not likely to be the IL 1 receptor itself because IL 1a by itself, without PHA priming, causes some perturbation of gene expression in LBRM33-1A5, which appears to be completely negative for Ly-1 surface staining as well as for mRNA. Although we do not think that Ly-1 is the IL 1 receptor, this surface molecule is probably a receptor for a ligand(s) that has not been identified yet. The predicted Ly-1 sequence shows an extracellular cystein-rich region, which is a common feature among many growth factor receptors (26, 35). Ly-1 also has a long cytoplasmic domain with a potential phosphorylation site that may be involved in signal transduction (26).

As shown in Figures 2 and 3, IL 1a alone upregulated the levels of mRNA specific for the IL 2-R, TGF-β, and the nuclear oncogenes c-myc and p53. This observation is consistent with the interpretation that LBRM33-1A5 expresses IL 1 receptors without PHA stimulation, as suggested by Dower et al. (36). Although IL 1 by itself has various direct effects in other systems, this cytokine has been mostly described as a co-stimulator in T cell activation. Our observations suggest that IL 1 has direct actions on T cells. However, because mRNA for all four genes are detectable in the
absence of any stimulation, IL 1α only induced a quantitative change in the expression of these genes.

The characteristics of the mode of action of IL 1 also seem to apply in the case of the induction of IL 2 mRNA expression. When the PHA-induced IL 2 gene activation was blocked by cycloheximide, IL 1α could not restore IL 2 expression, which suggests that IL 1α fails to trigger further transcription of the IL 2 gene. In contrast, cycloheximide caused superinduction of c-fos mRNA (data not shown), indicating that this is a specific effect on IL 2 mRNA expression.

The gene named TY5, whose physiological function is still unknown, is strongly induced in mitogen-activated helper T cell clones (8). In contrast to other genes, IL 1α failed to augment PHA-induced message accumulation of this gene. This is probably due to an apparent full induction by PHA alone.

On the basis of the pattern of the effect of PHA and/or IL 1α on gene expression, we tried to classify the genes under investigation into four groups as shown in Table III. Both type I and type II genes are induced by PHA but not by IL 1α alone. However, although IL 1α increases the PHA-induced mRNA accumulation of type I genes, type II gene (TY5) is minimally affected by IL 1α. The transcripts of type III and type IV genes are detectable in unstimulated LBRM337-A15 cells. In both types, IL 1α causes mRNA accumulation to some extent; however, the effect of PHA is different among the two: type III genes are induced by PHA and optimally induced by PHA plus IL 1α, but PHA fails to increase mRNA of type IV gene (p53) with or without IL 1α. Thus, as Table III indicates, the regulation of gene expression is different among the four types (see Table III footnotes).

In all the cases described above, IL 1α action was predetermined by the state of gene expression at the time of IL 1α addition. This observation suggests that IL 1α acts as an amplifier of the signals that regulate the amount of mRNA.

The intracellular event caused by IL 1 stimulation is still unknown. However, because the IL 1 signal can be phenotypically substituted by phorbol ester and not by calcium ionophore (6, 34), IL 1 and phorbol ester may result in similar molecular events(s) in the activation of T cells. A recent report (37) shows that phorbol myristate acetate (PMA) caused the persistent accumulation of GM-CSF mRNA in T lymphoblastoid cells, suggesting that PMA alters the stability of the messenger. The mechanism that involves mRNA half life control in addition to the transcriptional activation may account for the effect of IL 1 described above.

Because fetal calf serum was present during the induction phase in our experiments, we cannot exclude the effect of the xenosensor. Current experiments are aimed at the analysis of a serum-free system.

In conclusion, IL 1α acts as a modifier of gene expression in LBRM 33-1A5. We believe that the use of this cell line will provide further understanding of the molecular mechanism of action of IL 1 in T cell activation.

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