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Down-regulation of lck mRNA by T cell activation involves transcriptional and post-transcriptional mechanisms

Florence Paillard and Catherine Vaquero*
U 152 INSERM, ICGM, Hopital Cochin, 22 rue Mechain, 75014 Paris, France

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ABSTRACT

The p56^{lck} tyrosine kinase is most likely to be involved in signal transduction of T lymphocyte activation. After full activation through the TcR/CD3 complex lck mRNA is transiently down-modulated. This down-modulation was due to an early decrease of both transcription and stability of the lck mRNA. To study the involvment of transcriptional and post-transcriptional factors in this regulations, we have analysed the effect of cycloheximide, a protein synthesis inhibitor, on the steady-state of the lck mRNA. Cycloheximide superinduced lck mRNA by increasing its stability, although cycloheximide concomitantly decreased lck transcription. This suggests that the constitutive level of lck mRNA observed prior to activation is controlled by transcriptional activator(s) and post-transcriptional destabilizing factor(s). Second, lck mRNA down-modulation observed after full activation was inhibited by cycloheximide. It increased lck mRNA stability whereas lck transcription remained low. Therefore, full activation might increase the synthesis and/or activity of destabilizing factor(s). Cyclosporin A also inhibited the down-modulation of lck mRNA by increasing its transcription with no effect on its stability. Since, lck mRNA down-modulation was always associated with lymphokine mRNA induction, and since CsA blocks both lymphokine transcription and lck decrease of transcription, this indicates that these genes might share common regulatory pathways leading to their inverse transcriptional regulation.

INTRODUCTION

Protein kinases play a crucial role in the control of the processes leading to cell growth and differentiation. It has been suggested that various protein kinases in T lymphocytes might be involved in the initiation of the signal cascade that leads to gene activation and subsequent cellular proliferation. The tyrosine kinase p56^{lck} is physically and functionnally associated with the cytoplasmic domains of CD4 and CD8 molecules (1, 2) that compose, in association with the T cell receptor and CD3 molecules, the T cell antigen receptor complex responsible for antigenic activation (3, 4). According to this model, the intracytoplasmic p56^{lck} which is constitutively expressed almost exclusively in T lymphocytes (5, 6, 7), might thus mediate the antigenic activation signal transduction (8). Although the phosphorylation of the CD3 zeta chain on tyrosine residues after activation has been suggested to be mediated by p56^{lck} (9), the substrates of the latter remain to be identified. Moreover, p56^{lck} is itself also submitted to modifications of both its phosphorylation and activity after various activation processes (reviewed in 10). Indeed, activation mediated through the TcR/CD3 complex, CD2 molecule, or PKC leads to an increased level of phosphorylation of p56^{lck} at both serine and tyrosine residues (11, 12). However, such phosphorylations of p56^{lck} are not associated with an increased kinase activity (11). Thus, the relationship between signal transduction and p56^{lck} phosphorylation remains unclear.

In addition, the constitutively expressed mRNA of lck is also submitted to modulations after activation. Recently, Marth *et al.* demonstrated that the level of lck mRNA was transiently decreased following full activation of T lymphocytes, i.e. leading to lymphokine expression and proliferation (13). In the present report we show that the decrease of lck mRNA steady-state is due to both transcriptional and post-transcriptional regulations, i.e. to a decrease of both gene transcription and messenger stability, as we have previously demonstrated for the TcR, CD4 and CD8 genes (14). Since factors involved in transcriptional and post-transcriptional regulations of the lck gene have not been characterized, we indirectly studied these factors by analysing the effects of the protein synthesis inhibitor cycloheximide (CHX), on the constitutive level of lck mRNA and on the down-modulation of lck mRNA observed after activation.

We also focused our interest on the correlation between down-modulation of lck mRNA and lymphokine expression mediated by full activation of T lymphocytes through the CD3/TcR complex. We demonstrate that the immunosuppressive cyclosporin A (CsA) reported to block lymphokine transcription (15, 16) also inhibits the down-modulation of lck mRNA by hampering the transcriptional decrease of the lck gene. These results suggest that lymphokine and lck genes might share a common transcriptional pathway of regulation, therefore underscoring the key role of lck gene expression in the regulation of T cell activation.

* To whom correspondence should be addressed

MATERIALS AND METHODS

T cell culture and activation

Human peripheral blood mononuclear cells from healthy donors were obtained by Ficoll centrifugation. The T cells were then activated by PHA (1 μg/ml) and maintained in culture in RPMI with 10% foetal calf serum for 14 days in the presence of recombinant IL-2. 14 days after PHA, the cells (PHA blasts) were 100% $CD3^+$ as determined by immunofluorescence staining using anti-CD3 mAb and expressed low levels of IL-2R mRNA and no detectable lymphokine mRNA. PHA blasts were further stimulated either with anti-CD3 mAb at 1/1000 dilution of ascitis fluid, or PMA at 1 ng/ml, or both, or with anti-CD2 mAb (a combination of anti-$T11_1$ plus anti-D66) at 1/200 dilution of ascites fluid that triggers proliferation. Cells were treated at the onset of the activation with cycloheximide (CHX) at 50 μg/ml to block protein synthesis. Also, CsA (kindly provided by F.Dreyfus, Hôpital Cochin) was added at 1 μg/ml at the onset of the activation. To study the half-life of the messengers, transcription was blocked with actinomycin D at 3 μg/ml.

Northern-blot analysis

Total cellular RNA was isolated at the indicated times by the guanidinium isothiocyanate procedure (17) after various treatments of the cells. Equal amounts of total RNA (8−10 μg) were fractionated by agarose gel electrophoresis after glyoxal denaturation and transferred to a PALL membrane (PALL Industries) with a vacuum-blot apparatus (LKB). Actual amounts of RNA per lane and efficiency of transfer were assessed by ethidium bromide staining of the rRNA. After transfer, the membranes were hybridized with the labelled oligonucleotide of the 28S rRNA described by Barbu and Dautry (18). The hybridization signals of the 28S rRNA are then shown for every Northern-blot experiment to allow a good comparison of the different samples. Membranes were sequentially hybridized at high stringency with [^{32}P] labelled riboprobes obtained after *in vitro* transcription of T3T7 Bluescribe (Stratagene) vectors carrying the 1.9 kb EcoRI fragment of the human lck cDNA (R.M. Perlmutter), the 1 kb HincII fragment of the human IFN gamma cDNA (P. Sondermeyer, Transgene and ref.19) or the 1.3 kb PstI fragment of the human HLA-A3 cDNA (D. Piatier). Riboprobes were used as previously described (20) at 5.10^5 cpm/ml of hybridization medium (50% formamide, 5×SSC, 10×Dendhardt, 5 mM EDTA, 50 mM Tris pH 7.5, 0.1% pyrophosphate, 1% SDS, 500 μg/ml yeast tRNA, and 5% dextran sulphate), followed by high stringency washes, and autoradiography with intensifying screens at −80°C.

For determination of mRNA half-lives, quantitative analysis of mRNA hybridization signal was performed by scanning densitometry or by counting the radioactivity of each signal on the membrane. Lck mRNA levels were then normalized to the corresponding 28S rRNA level after hybridization with a labelled oligonucleotide (18). The mRNA half-lives were determined from 'best-fit' regression lines obtained by using the least-square method ($r > 0.9$).

Transcription in isolated nuclei ('run-on')

10^8 cells were washed in PBS and resuspended in 1 ml of lysis buffer (0.08% Triton×100, 10 mM Tris pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 7.5 mM DTT) and another 1 ml of lysis buffer was added after 3 min in ice. Nuclei were collected by centrifugation through a 3 ml cushion of 1,5 M sucrose in lysis buffer for 10 min at 2,400 rpm and resuspended in 1 ml of storage buffer (40% glycerol, 50 mM Tris pH 8.3, 5 mM $MgCl_2$, 0.1 mM EDTA, 10 mM DTT). Nuclei were then centrifuged for 4 min at 2,400 rpm, resuspended in 100 μl of storage buffer, and frozen in liquid nitrogen. Transcription reactions were performed for 1 hr at 25°C with 100 μl of the isolated nuclei in a final volume of 150 μl with 0.5 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 0.2 mM S-adenosyl methionine, 5 mM Tris pH 8, 150 mM KCl, and 2.5 mM $MgCl_2$ in presence of 200 μCi of [^{32}P]UTP . Total RNA was extracted and resuspended in 300 μl of 10 mM Tris pH 9, 0,1% SDS. The RNA was hydrolysed with 0,1 M NaOH for 10 min in ice, neutralized with 0.28 M Hepes buffer and precipitated. Equivalent amounts of radioactivity were hybridized to the PALL membrane on which 10 μg of the various linearized plasmids (including the T3T7 Bluescribe vector as a negative control) have been previously immobilized after denaturation, using a Slot-blot apparatus (Minifold II, Scleicher and Schüll). Hybridizations were performed for three days at 52°C in 50% formamide, 5×SSC, 5×Denhardt, 1 mM EDTA, 10 mM Tris-HCl pH 7.4, 0.2% SDS, 500 μg/ml salmon sperm DNA, 500 μg/ml tRNA, and 5−15 10^6cpm/ml of the labelled RNA. Non-hybridized radioactivity was removed by washing the membranes in 5×SSC, 0.1% SDS at 52°C. When background was high, the membranes were treated with proteinase K at 100 μg/ml in 2×SSC, 0,1% SDS during 30 min at 37°C.

RESULTS

Down-modulation of lck mRNA is triggered by anti-CD3+PMA activation

The modulation of the lck mRNA level expressed constitutively in human quiescent T lymphocytes was investigated after various activation stimuli triggered either via the CD3/TcR complex or via the CD2 molecule. We found that the lck mRNA was markedly and transiently down-modulated following full activation leading to lymphokine expression mediated via the TcR/CD3 complex with anti-CD3+PMA (fig.1). Lck mRNA was decreased as early as 2 h and was fully reexpressed within 10 h post-activation (fig.1 and see also fig.4 and 7). Interestingly, the transient down-modulation of lck mRNA after anti-CD3+PMA was associated with the transient expression of lymphokine mRNA (see IFN gamma fig.1 as an example).

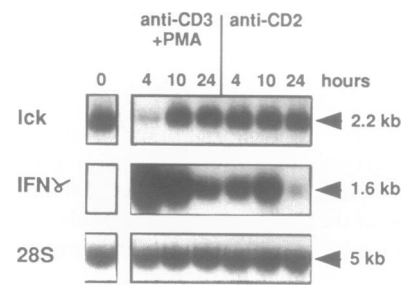

Fig. 1. Kinetics of lck mRNA expression. T cells were activated with anti-CD3+PMA or anti-CD2. Northern-blots of total RNA (8 μg) extracted at the indicated times after activation were hybridized with lck and IFN gamma riboprobes, and with the 28S rRNA labelled oligonucleotide to assess the equal loading of each RNA sample. The size of the different RNA are indicated by the arrows.

Accordingly lck mRNA was not down-modulated after activation with either anti-CD3 or PMA alone that did not induce lymphokine expression (data not shown). In addition, no modifications of the level of lck mRNA were observed in non-activated control cells (data not shown). These results were in agreement with those reported by Marth *et al.* (13).

Interestingly, and in contrast to anti-CD3+PMA, full activation via the CD2 molecule leading to lymphokine expression triggered no or only a slight down-modulation of lck mRNA (fig.1 and data not shown). This result indicated that down-modulation of lck mRNA might not be strictly associated with lymphokine mRNA expression as suggested by Marth *et al.* (13). Therefore, it seemed that strong down-modulation of lck mRNA was associated with lymphokine expression only when full activation was mediated through the TcR/CD3 complex and not through the CD2 molecule.

Down-modulation of lck mRNA is due to a decrease of both its transcription and stability

In order to study the mechanisms of regulation that could be responsible for the transient down-modulation of lck mRNA, we measured both transcription and mRNA half-life after anti-CD3+PMA activation.

First, transcription of the lck gene was analysed at 0 h, 3 h and 24 h post-anti-CD3+PMA activation, since the minimal level of the lck mRNA was reached within 3 h post-activation, and since it was fully reexpressed at 24 h. Transcription of lck gene markedly dropped to 40% of the control (average of three independent experiments, determined by scanning densitometrie) at 3 h post-activation, and recovered within 24 h post-activation the level of transcription observed prior to activation (see fig.2 for a representative experiment). As a control of activation process, we verified that the transcriptional level of IFN gamma was induced after anti-CD3+PMA activation (fig.2). In addition, HLA-A3 transcription was not significantly modified during activation (fig.2). Therefore, the specific decrease of lck transcription accounted, at least in part, for the down-modulation of lck mRNA.

Second, since the marked decline of lck mRNA steady-state could also result from an additional post-transcriptional effect, we measured the half-life of lck mRNA at 1 and 3 h post-anti-CD3+PMA activation using actinomycin D to block transcription. Before activation, the half-life of lck mRNA was 4 h ± 0.3 and declined to 2.4 h ± 0.3 at 1h post-activation (average of three experiments, fig.3). Thereafter the half-life of lck mRNA reincreased and reached almost the initial level (3.5 h ± 0.4) as soon as 3 h post-activation (fig.3). Therefore, the early decrease of lck mRNA stability seemed also to account for the early down-modulation of lck mRNA. However, since the half-life of the messenger 3 h after activation returned to a level similar to that observed prior to activation, the prolonged down-modulation of lck mRNA up to approximately 7 h (see fig.7) might be entirely due to a prolonged decline of lck transcription.

Superinduction of the constitutive expression of lck mRNA by CHX is due only to a marked stabilization of its mRNA

To further characterize the processes governing lck mRNA steady-state in non-activated T cells, we evaluated the factors positively or negatively controlling mRNA accumulation resulting from transcription and degradation by analysing the effects of cycloheximide (CHX) on lck gene expression. As shown on fig.4, CHX alone, in absence of any activation superinduced strongly lck mRNA as soon as 2 h post-treatment. As expected, CHX did not trigger the expression of the inducible IFN gamma gene (fig.4). This result suggests that CHX might block the synthesis of 'negative' regulatory factor(s) capable of controlling the level of lck mRNA constitutive expression. In order to determine whether the(se) putative 'negative' regulatory factor(s) is either transcriptional or/and post-transcriptional, we analysed the effect of CHX on both transcription and stability of lck mRNA.

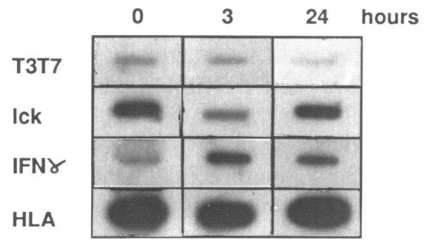

Fig. 2. Transcriptional level of lck mRNA after anti-CD3+PMA activation. Nuclear RNA run-on analyses were performed at 0, 3 and 24 h post-anti-CD3+PMA activation as indicated in Materials and Methods with the indicated linearized vectors. T3T7 vector was used as negative control of hybridization. The transcriptional level of IFN gamma and HLA-A3 were also determined as experimental controls since IFN gamma transcription is inducible during activation and HLA-A3 is constitutively expressed and remains constant during activation. This experiment is representative of three independent experiments.

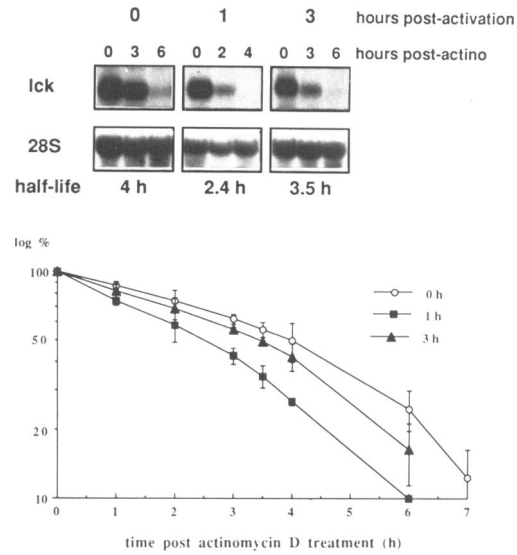

Fig. 3. Half-life of lck mRNA early after anti-CD3+PMA activation. Cells were treated with actinomycin D (actino) to block transcription before activation (0 h) and 1 h and 3 h post-activation. Total RNA was extracted at the indicated times post-actinomycin D treatment. A Northern-blot of total RNA (8 μg) was sequentially hybridized with lck riboprobe and with the 28S rRNA labelled oligonucleotide to assess the equal loading of each RNA sample. For an accurate determination of the half-lives at different times post-activation, three experiments were performed where total RNA was extracted post-actinomycin D treatment at 1) 0, 3, 6 h ; 2) 0, 1, 2, 3.5 h ; 3) 0, 2, 4, 7 h, according to the experiment. Quantitation of the lck hybridization signals, normalization to the 28S rRNA and calculation of lck mRNA half-life were performed as described in Materials and Methods. For each experiment, the normalized values were plotted as percentage of the lck mRNA level observed before actinomycin D treatment. The 'best-fit' regression lines (mean of the three experiments) are represented on the figure with standard deviation (bars). The mean values of lck mRNA half-life are indicated on the figure.

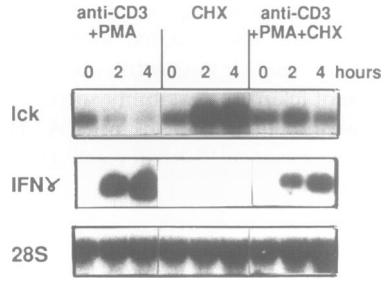

Fig. 4. Effect of CHX on lck mRNA expression. T cells were treated either with anti-CD3+PMA alone, CHX alone or the combination of both treatments. Total RNA was extracted at the indicated times after each treatment and Northern-blots of these RNA were hybridized with lck anf IFN gamma riboprobes and with the 28S rRNA labelled oligonucleotide.

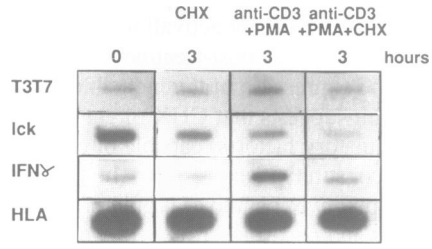

Fig. 5. Effect of CHX on the transcriptional level of lck mRNA. Nuclear RNA run-on analyses were performed in absence of any treatment (0 h), or after 3 h of treatment with either CHX alone, anti-CD3+PMA alone, or both. T3T7, lck, IFN gamma and HLA-A3 linearized vectors were used for hybridization as described in Materials and Methods. This experiment is representative of two independent experiments.

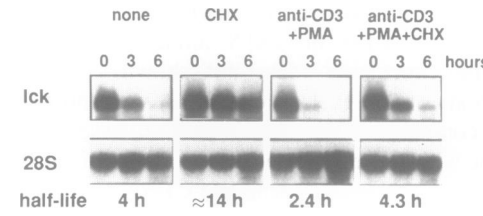

Fig. 6. Effect of CHX on the half-life of lck mRNA. T cells were left untreated or were treated at time 0 either with CHX alone, anti-CD3+PMA alone, or both. Actinomycin D was added to these cells at 0 h in the case of untreated or CHX-treated cells, and at 1 h in the case of anti-CD3+PMA and anti-CD3+PMA+CHX-treated cells. Total RNA was extracted at the indicated times post-actinomycin D treatment. A Northern-blot of total RNA was hybridized with lck riboprobe and with the 28S rRNA labelled oligonucleotide. Quantitation of the lck hybridization signals, normalization to the 28S rRNA and calculation of the lck mRNA half-life were performed as described in Materials and Methods. The values of lck mRNA half-life after each treatment are indicated on the figure.

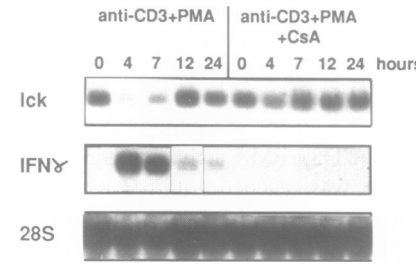

Fig. 7. Effect of CsA on lck mRNA down-modulation. T cells were treated with anti-CD3+PMA in absence or presence of 1 μg/ml of CsA. Northern-blots of total RNA (8 μg) extracted at the indicated times after treatment were hybridized with lck and IFN gamma riboprobes and with the 28S rRNA labelled oligonucleotide.

First, the increase of lck mRNA steady-state mediated through CHX (fig.4) was accompanied by a decreased level of lck mRNA transcription (60%, average of two experiments) at a time (3 h) when superinduction was fully expressed (fig.5). HLA-A3 gene transcription was not significantly altered after CHX treatment (fig.5). This suggested that CHX inhibited a positive transcriptional factor (transcriptional activator) that might control, at least in part, the constitutive expression of lck mRNA.

Second, CHX markedly increased the half-life of lck mRNA up to 14 h, i.e. more than three fold the mRNA half-life observed prior to activation (4 h) (fig.6).

Therefore, it appears that the superinduction triggered by CHX was due only to an increase of lck mRNA stability. Moreover, this stabilization of lck mRNA was strong enough to counter-balance the decrease of transcription.

Taken together, these data strongly suggested that the constitutive level of lck mRNA observed in T cells resulted from a balanced regulation between positive factor(s) acting at transcriptional level (transcriptional activator), and negative factor(s) acting at the level of lck mRNA stability (destabilizing factor).

Inhibition of anti-CD3+PMA-mediated lck mRNA down-modulation by CHX is due only to stabilization of its mRNA

As previously shown, anti-CD3+PMA activation triggered the down-modulation of lck mRNA via the decrease of both transcription and mRNA stability. To further study the regulatory factors involved in the decrease of transcription and mRNA stability, the effect of CHX was analysed on anti-CD3+PMA-mediated 1) lck mRNA down-modulation, 2) decrease of transcription and 3) decrease of mRNA half-life.

First, as shown on fig.4, CHX inhibited nearly completely the anti-CD3+PMA-mediated down-modulation of lck mRNA. Therefore, CHX might block the synthesis of 'negative' regulatory factor(s) acting at transcriptional and/or post-transcriptional level.

Second, we analysed the effect of CHX on the decrease of lck transcription. When T cells were treated during 3 h with both anti-CD3+PMA and CHX the drop of lck transcription was similar, if not more pronounced, to that observed after either anti-CD3+PMA or CHX (fig.5). Since the constitutive expression of lck gene appeared to be controlled by a transcriptional activator (as shown above), it is likely that the anti-CD3+PMA activation process might lead to the decrease of either the level or the activity of this positive factor. In addition, the drop in lck transcription after anti-CD3+PMA+CHX treatment could not explain the inhibition of lck mRNA down-modulation after this treatment (fig.4).

Third, we examined whether post-transcriptional regulations could be involved in the recovery of lck mRNA level. The effect of CHX on the half-life of lck mRNA was investigated at 1 h post-anti-CD3+PMA activation when lck mRNA half-life was minimal. CHX significantly increased lck mRNA half-life (4.25 h) when compared to that observed with anti-CD3+PMA

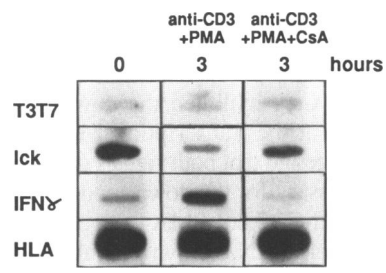

Fig. 8. Effect of CsA on the transcriptional level of lck mRNA. Nuclear RNA run-on analyses were performed at 0 and 3 h post-anti-CD3+PMA activation in absence or presence of 1 μg/ml of CsA. Transcriptional level of lck, IFN gamma and HLA-A3 was then determined as described in Materials and Methods by using the corresponding linearized vector, including T3T7 BLuescribe vector as a negative control of hybridization. This experiment is representative of two independent experiments.

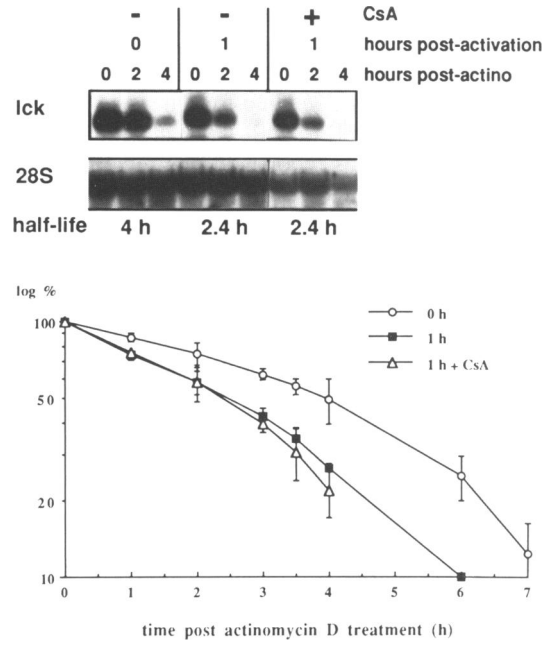

Fig. 9. Effect of CsA on the half-life of lck mRNA. Cells were left untreated or were activated with anti-CD3+PMA in absence or presence of 1 μg/ml of CsA. Actinomycin D (actino) was added at the indicated times post-activation and total RNA was extracted at the indicated times post-actinomycin D treatment. A Northern-blot of total RNA was hybridized with lck riboprobe and with the 28S rRNA labelled oligonucleotide. For accurate determination of the half-lives, three experiments were performed and the 'best-fit' regression lines plotted as described in fig.3. The mean half-lives of lck mRNA are indicated on the figure.

alone (2.4 h) (fig.6). Also at 3 h post-activation (data not shown), the half-life of lck mRNA (3.5 h) was increased in presence of CHX to 5.9 h. Since we have shown above that constitutive expression of lck gene appeared to be controlled at mRNA stability by destabilizing factor(s), it was then likely that the anti-CD3+PMA activation process might lead to the increase of either the level or the activity of this factor. Taken together, these data suggested that the recovery of the lck mRNA steady-state driven by CHX occurred essentially via the stabilization of the mRNA and was strong enough to counter-balance the marked decrease of transcription observed after treatment with anti-CD3+PMA+CHX.

CsA inhibits the anti-CD3+PMA-mediated down-modulation of lck mRNA by hampering the decrease of its transcription

Since lck mRNA down-modulation was always associated with lymphokine mRNA expression when T cells were stimulated with anti-CD3+PMA, the effect of the immunosuppressor CsA that blocks lymphokine expression (15, 21, 16) was investigated on lck mRNA down-modulation. CsA at 1 μg/ml almost completely inhibited the anti-CD3+PMA-mediated down-modulation of lck mRNA as well as the IFN gamma mRNA induction (fig.7). In addition, CsA alone did not alter the constitutive expression of lck mRNA (data not shown) showing that this was not due to a non-specific effect of CsA. We investigated if the inhibition of lck mRNA down-modulation by CsA resulted from transcriptional or/and post-transcriptional regulation mechanisms.

The transcriptional level of lck gene was measured at 3 h post-anti-CD3+PMA activation in presence or absence of CsA (1 μg/ml). As shown on fig.8, the decrease of lck transcription mediated by anti-CD3+PMA activation was clearly inhibited by CsA addition. We confirmed that in these conditions of activation, CsA was able to inhibit the transcription of IFN gamma gene (fig.8) as described by Krönke *et al.* (15). Moreover, with CsA addition we did not observe any modification of the transcriptional level of HLA (15). This strongly suggests that regulation of transcription plays a key role in the CSA-mediated inhibition of lck mRNA down-modulation.

Since we could not exclude an additional post-transcriptional effect of CsA, we analysed the effect of CsA on the half-life of lck mRNA at 1 h post-anti-CD3+PMA-activation, when lck mRNA half-life was minimal. As shown on fig.9, almost no change in the lck mRNA half-life was observed after CsA treatment. This result shows that the inhibition of lck mRNA down-modulation by CsA was not due to post-transcriptional regulations, but mainly to transcriptional regulations.

DISCUSSION

Full activation of T lymphocytes mediated through the TcR/CD3 complex and leading to lymphokine expression and cellular proliferation induced a down-modulation of lck mRNA. This observation is in agreement with that of Marth *et al.* who have previously shown that stimulation of T lymphocytes with either of Con A, anti-CD3, PHA, or ionophore, in combination with PMA induces a down-modulation of lck mRNA (13). However, in the present report we show that full activation mediated through the CD2 molecule did not induce a marked down-modulation of lck mRNA. This difference between the TcR/CD3 and CD2 activation pathways is intriguing since both types of activation share common features such as a rise of intracellular calcium, activation of PKC, and modifications of $p56^{lck}$. This suggests that modifications of the $p56^{lck}$ might not be the only condition, if any, of the subsequent down-modulation of the mRNA. In favour of this observation was also that the down-modulation of lck mRNA by anti-CD3+PMA was accompanied by phosphorylation of $p56^{lck}$ protein (11, 22, 13 and our unpublished data), and that neither PMA nor anti-CD3 alone down-modulated lck mRNA, whereas PMA triggered the phosphorylation of $p56^{lck}$ protein (22 and our unpublished data) and anti-CD3 did not (data not shown).

The early down-modulation of lck mRNA observed within the first hours of T cell activation with anti-CD3+PMA was due

to transcriptional and post-transcriptional regulations, i.e. to a transient decrease of both lck gene transcription and mRNA stability. We have previously described a striking correlation in the regulation of the genes coding for the molecules composing the T cell receptor complex (TcR, CD4 and CD8) (14) and also the lck gene (13 and this report). In this regard, it is tempting to speculate that coordinate regulation of these genes is linked to their physical and functionnal relationship. Such coordinate regulation might occur through the presence of common regulatory sequences within either their promoter or/and other additional sequences located in non-coding and/or coding regions. It has recently been shown that transcription of the lck gene is driven by two structurally distinct promoter elements separated by 35 kb in the human gene (23, 24). The upstream promoter is responsible for specific expression of lck gene in mature T lymphocytes (25). A search for sequence homologies within the various promoters showed that the decanucleotide sequence described in 14 murine V beta promoters (26) was also present within the lck promoter. This decanucleotide was also found in other human TcR promoters (27, 28), as well as CD4 (D. Littman unpublished data) and CD8 promoters (29). However, this decanucleotide is also present in constitutive, CD2, and inducible genes, IL2-R alpha, IFN gamma and IL-2 genes that do not share the same transcriptional regulation. Therefore, it is likely that additional elements not yet identified might be responsible for the coordinated regulation of the transcriptional rate of TcR, CD4, CD8 and lck gene. Also, it is interesting to note that the transient decrease of lck gene transcription does not seem to be due to a differential usage of either the upstream or the downstream promoter during the activation process (25). In addition, the lck transcripts initiated from three start sites of the upstream promoter in non-activated T lymphocytes coordinately declined after 4 to 7 h post-activation and coordinately reincreased at 24 h (25).

In addition to their target sequences in the lck gene, the factors controlling the level of transcription and stability of the lck messenger have to be identified. We indirectly approached this issue by using CHX to block the synthesis of regulatory factors and determined whether such factors act positively or negatively. We observed that the CHX-mediated superinduction of lck mRNA was accompanied by a decrease of transcription and an increase of mRNA stability. These data suggest that prior to activation the constitutive expression of lck gene results from the balance between a positive transcriptional factor (transcriptional activator) and a negative post-transcriptional factor (destabilizing factor) that maintains the lck mRNA steady-state. Second, CHX did not alter significantly the decrease of transcription observed early after anti-CD3+PMA activation. In addition, the decline of mRNA half-life observed after anti-CD3+PMA was abolished by CHX. Taken together our data suggest that anti-CD3+PMA activation most likely induces 1) the decline of either the synthesis or the biological activity of transcriptional activator(s), and 2) the increase of either the synthesis or the biological activity of mRNA destabilizing factor(s). Since anti-CD3+PMA activation induces an increase of intracellular calcium and PKC activation, both pathways might be responsible within minutes after activation for post-translational modifications of the factors. Indeed, it has been demonstrated that the calcium rise (reviewed in 30) and the PKC activation could induce the phosphorylation of certain transcriptional regulatory factors. Thus, phosphorylation and dephosphorylation mechanisms might also be responsible for the modifications of activity of the factors involved in the regulation of lck mRNA during activation of T lymphocytes. These rapid post-transcriptional modifications of the factors might explain the early features of the down-modulation kinetics.

We showed that CsA inhibited the down-modulation of lck mRNA exclusively at the transcriptional level. We and others have previously suggested that both signals (calcium rise and PKC activation) are necessary for the down-modulation of lck mRNA (13) since neither anti-CD3 nor PMA (triggering only one of these signals (4) induce lck mRNA down-modulation. CsA has been shown to block exclusively the calcium-dependent pathway by inhibiting the calmodulin (31, 26), without altering the PKC activation pathway (8). Thus, the calcium pathway appears to be necessary but not sufficient for the decrease of transcription presumably via the modifications of the transcriptional activator. However, CsA did not modify the decrease of lck mRNA stability mediated through anti-CD3+PMA activation. Thus, the decrease of lck mRNA stability would not be mediated by the calcium-dependent pathway, but rather by PKC or/and another unidentified pathway.

Finally, the transient down-modulation of lck mRNA was always associated with transient induction of lymphokine mRNA (13) triggered through activation via the TcR/CD3 complex. The link between these two phenomenons was reinforced by the fact that CsA, which inhibits activation-induced transcription of lymphokine genes, also blocked the activation-mediated decrease of lck mRNA. Similar results were independently obtained by Perlmutter *et al.* (personal communication). However, Takadera *et al.* (25) found that the down-modulation of lck mRNA induced by PHA+TPA was not inhibited by CsA at 1 μg/ml. Similarly, in some of our experiments where lck mRNA showed a very prolonged down-modulation, 1 μg/ml of CsA only partially inhibited the lck mRNA down-modulation whereas 5 μg/ml was fully efficient (data not shown). Thus, it might be interesting to evaluate the effects of similar higher concentrations of CsA in PHA+TPA-activated T cells. In addition, since CsA exerted its inhibitory effect on both lymphokine expression and lck down-modulation at the transcriptional level only, lymphokine and lck genes might share common regulatory pathways leading to their inverse transcriptional regulation. Therefore, it is tempting to speculate that the same transcriptional regulatory pathway could alternatively and inversely modulate lymphokine and lck gene expression. In agreement with this hypothesis was that the magnitude of induction of lymphokine mRNA was related to the magnitude of the lck mRNA disappearance (data not shown). Prior to activation, the lck gene is constitutively expressed. The activation signal delivered via TcR/CD3 complex could induce the modification of a factor(s) that would lead, on the one hand to the inhibition of lck transcription and on the other hand to the activation of lymphokine transcription. The activation signal delivered via CD2 does not lead to a marked lck down-modulation. Although lck mRNA down-modulation appeared to be associated to lymphokine induction after activation via TcR/CD3 complex, the role of this phenomenon in T cell activation physiology is still unresolved.

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