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STABILIZATION OF INTERLEUKIN-2 RECEPTOR α CHAIN mRNA BY HTLV-1 REX IN MOUSE L CELLS: LOWER AMOUNTS OF REX DO NOT STABILIZE THE mRNA

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SUMMARY: T cell growth factor receptor, interleukin-2 receptor α chain (IL-2Rα) is constitutively expressed on human T-cell leukemia virus type-1 (HTLV-1) infected T cells. We have established L cell lines which express both IL-2Rα and the Rex protein of HTLV-1. We found that IL-2Rα mRNA is stabilized in a cell line, Ltk/1-2a, which expresses a high amount of the Rex protein. In the presence of lower amounts of Rex, stabilization of the mRNA was not observed. These results may well explain the mechanism by which most of the lymphocytes infected with HTLV-1 escape from malignant transformation.

IL-2Rα is one of the T cell growth factor receptors (1-3). IL-2Rα is induced in helper T cells either by antigenic or mitogenic stimulation. These stimuli are supposed to activate the transcription of the IL-2Rα gene (4). IL-2Rα is also expressed on HTLV-1 infected T cells, and the constitutive expression of the receptor on these cells seems

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to have an important role in the development of adult T cell leukemia (ATL) (5, 6). The mechanisms by which IL-2Rα is induced by HTLV-1 have been investigated extensively. One of the products of HTLV-1, Tax, activates the transcription of the IL-2Rα gene (7, 8). Additionally, we have reported that another HTLV-1 product, Rex , upregulates IL-2Rα by stabilizing IL-2Rα mRNA (9). We have determined the Rex responsive element in the coding sequence of the IL-2Rα gene by employing a transient expression system using COS cells. The half life of IL-2Rα RNA, measured by using the transcription blocker actinomycin D, was apparently elongated in the presence of the Rex protein. We have also demonstrated that Rex has very little effect on the nuclear export of the IL-2Rα RNA and that the action of Rex is mainly on the cytoplasmic stabilization of the mRNA (9).

Here we report the establishment of stable transformants of mouse L cell lines in which both the IL-2Rα and HTLV-1 Rex genes are expressed. We found that the half life of IL-2Rα RNA in a cell line (Ltk/1-2a) was longer in the presence of Rex and that the stabilization of the mRNA was only observed when the expression levels of Rex were relatively high.

MATERIALS AND METHODS

Cell culture and DNA transfection: Mouse Ltk^- cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10 % fetal calf serum. Cells were transfected by electroporation (1,000 volts, 25 μFD, PBS) using a BIO-RAD GenePulser. Fourty-eight hours after transfection, cells were exposed to 500 μg/ml of G418 to obtain stable transformants.

RNA preparation and analysis of RNAs: Total cellular RNA was prepared as described (10). Northern blot analysis was carried out using IL-2Rα and ß-actin probes as described (9). Ribonuclease (RNase) protection assays were performed as described (11) using an LTR portion of the HTLV-1 RNA as probe.

Construction of plasmids: The 2.4 kb HindIII fragment from pKCR27x which contained HTLV-1 Rex DNA (9) was inserted into the HindIII site of RcCMV vector (InVitrogen, San Diego, CA). The

resultant plasmid designated RcCMV-Rex, was linearized by Bgl II and used for transfection. We obtained a fragment containing the Rex responsive element of HTLV-1 (from 8593 to 8845 (12)) by employing polymerase chain reaction using 5'- GCA GGA GTC TAT AAA AGC GT-3' and 5'-GCA GGG TCA GGC AAA GCG TG-3' oligonucleotides and the plasmid pKCR27x. Subsequently, the fragment was subcloned into pCRII plasmid (InVitrogen, San Diego, CA) to obtain a template plasmid pCRIIRxRE-HTLV for Rex probe used in the RNase protection assay.

RESULTS AND DISCUSSION

Ltk/1A1 is a stably transformed cell line derived from the mouse Ltk$^-$ cell line which contains human IL-2Rα cDNA under control of SV40 early promoter (13). Ltk/1A1 cells express as many as 8.8 x 10^5 molecules of human IL-2Rα on the cell surface but do not express other IL-2R subunits (ß or γ) nor mouse IL-2Rα. In the absence of HTLV-1 Rex protein, IL-2Rα mRNA is unstable both in lymphoid cells and COS cells (9). IL-2Rα mRNA is stable, however, in the presence of the Rex protein in these cells. To see the effect of the Rex protein on IL-2Rα mRNA expression in Ltk/1A1 cells, we introduced HTLV-1 Rex expressing RcCMV-Rex plasmid (Fig. 1) into Ltk/1A1 cells and obtained 6 stable transformants (Ltk1-2a, Ltk/1-1, Ltk/2-3, Ltk/1-3, Ltk/1-2b and Ltk/2-2) resistant to G418. RcCMV-Rex contained the pX region of HTLV-1 under the cytomegalovirus (CMV) immediate early promoter as well as the

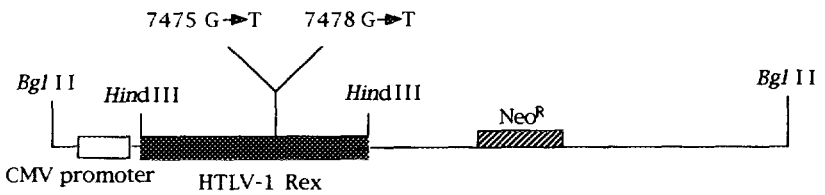

Figure 1. Construction of a HTLV-1 Rex expression plasmid. RcCMV is an expression plasmid and contains the neomycin resistant gene. The HTLV-1 Rex containing DNA fragment was inserted downstream of the CMV promoter. To avoid other HTLV-1 products than the Rex protein to be generated, point mutations were introduced at position 7475 and 7478 of the HTLV-1 genom (9, 12). The plasmid was linearized by BglII and used for transfection.

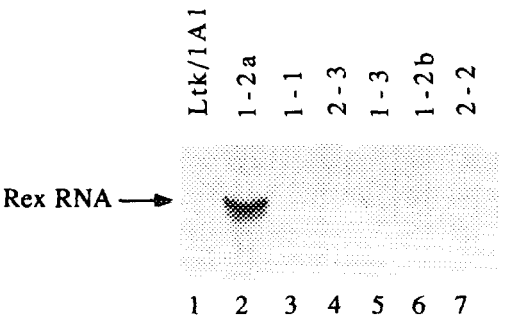

Figure 2. Expression levels of HTLV-1 Rex in mouse L-cell transformants determined by RNase protection assay. RNase protection assay was performed using 200 μg of total RNA from each cell line (lane 1: Ltk/1A1, lane 2: Ltk/1-2a, lane 3: Ltk/1-1, lane 4: Ltk/2-3, lane 5: Ltk/1-3, lane 6: Ltk/1-2b, lane 7: Ltk/2-2) and Rex probe (see materials and methods). Protected bands (253 bases) were observed on a 5 % acrylamide-7 M urea gel.

neomycin resistant gene. Although the CMV promoter is supposed to have as strong an activity as that of the SV40 early promoter, the expression levels of Rex in the transformants were so low that we could not detect the protein by using an antibody against it nor could we detect the RNA by Northern blot analysis (data not shown). We used as much as 200 μg of total RNA in the RNase protection assay to detect Rex RNA. The expression levels of Rex were variable (Fig. 2, Table 1). We could detect Rex RNA in 3 out of 6 transformants. Transformant Ltk/1-2a contained the highest amount of Rex RNA. We detected lower amounts of the RNA in Ltk/1-3 and Ltk/1-2b transformed cells. On the other hand, we could not detect the RNA in Ltk/1-1 and Ltk/2-3 and Ltk/2-2 transformants. In these cells, Rex RNA might be below the limits of detection of the assay. By comparison with the amount of Rex RNA from our previous data (14), the expression levels of Rex RNA are far less than those of lymphoid cells infected with HTLV-1. Even Ltk/1-2a cells contained several hundred folds less Rex RNA than HTLV-1 infected T cells.

We tested the stability of IL-2Rα RNA in the transformed cells by using actinomycin D (Fig. 3). Zero and six hours after the

TABLE I

Summary of mRNA Expression and Decay of IL-2Rα in Tranformed Cells

	Expression of § IL-2Rα mRNA	Expression of § Rex mRNA	Decay of ** IL-2Rα mRNA
Ltk/1A1	+++	-	R
Ltk/1-2a	++++	++++	S
Ltk/1-1	+++	-	VR
Ltk/2-3	+++++	-	R
Ltk/1-3	+	+	R
Ltk/1-2b	++	+	R
Ltk/2-2	+++	-	R

§Amounts of mRNA are shown by semiquantitative estimation as described in the text.
** R: rapid decay, S: stable, VR: very rapid decay.

administration of the transcription blocker, cells were harvested, total cellular RNA was prepared and RNA levels were compared using Northern blot analysis. There seemed to be various expression levels of IL-2Rα RNA among the cell lines we analyzed. Ltk/2-3 cells contained much more IL-2Rα RNA than original Ltk/1A1 cells, while Ltk/1-3 cells contained less. We can observe the decay profiles of IL-

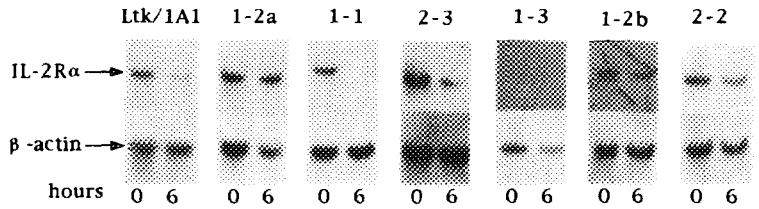

Figure 3. Decay profiles of IL-2Rα mRNA in L-cell transformants. Cells were treated with 5 μg/ml of actinomycin D. Northern blot analysis using 10 μg of total RNA at time 0 and 6 hours after treatment with actinomycin D showing the decay profiles of IL-2Rα RNA in each cell line. After hybridization with IL-2Rα probe, filters were hybridized with ß-actin probe to monitor the amounts of RNA loaded in each lane.

2Rα RNAs by comparing the amounts of the RNA at 0 and 6 hours after the administration of actinomycin D. Decay profiles of IL-2Rα RNA could be grouped into three categories. Very rapid decay (VR), rapid decay (R) and stable groups (S) (Table I). In the original Ltk/1A1 cells which only expressed IL-2Rα RNA, IL-2Rα RNA decayed rapidly with a half life approximately 2.5 hours, which is similar to that observed in lymphoid cell lines or PHA-stimulated peripheral blood lymphocytes not expressing HTLV-1 Rex. Ltk/2-3, Ltk/1-3, Ltk/1-2b and Ltk/2-2 cells showed similar decay patterns of IL-2Rα RNA compared to the original Ltk/1A1. And Ltk/1-1 cells showed very rapid decay pattern of IL-2Rα RNA (half life < 1 hour). On the other hand, in Ltk/1-2a cells which expressed high amount of Rex RNA, IL-2Rα RNA was stable. The levels of IL-2Rα RNAs in Ltk/1-2a seemed unchanged during actinomycin D treatment. We used endogenous ß-actin RNA as an internal standards to verify the amounts of RNA loaded on the same filter. ß-actin RNAs seemed rather stable. In Ltk/1-2a cells, IL-2Rα RNA appeared more stable than ß-actin RNA.

Ltk/1-2a cells contained more than a hundred times as much Rex compared to the other cell lines we analyzed and the stabilization of IL-2Rα RNA was only observed in this cell line. This result is consistent with the stabilizing effect of HTLV-1 Rex on IL-2Rα RNA demonstrated in our previous study. Ltk/1-3 and Ltk/1-2b cells may have expressed too little Rex to stabilize IL-2Rα RNA.

Development of ATL seems to be related to the constitutive expression of IL-2Rα (15). Our results strongly suggest that HTLV-1 Rex has an important role on the levels of IL-2Rα by a posttranscriptional mechanism. In addition, present data indicate that Rex does not stabilize IL-2Rα RNA unless it reaches a certain level during the course of development of ATL, which may explain the fact that most of the cells infected with HTLV-1 escape from the

malignant transformation. It is assumed that Ltk/1-2a cells which express Rex protein contain IL-2Rα RNA stabilizing factor related to HTLV-1 Rex. This kind of factor may also be the potential physiological regulatory factor which has a significant role in the T cell immune activation system. Establishment of Ltk/1-2a cells may enable us to identify the factor that is directly associated with the Rex responsive region of the IL-2Rα RNA.

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