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Induction of interleukin 2 receptor gene expression by p40^{x} encoded by human T-cell leukemia virus type 1

Jun-ichiro Inoue, Motoharu Seiki, Tadatsugu Taniguchi[1], Sumiaki Tsuru[2] and Mitsuaki Yoshida

Department of Viral Oncology, Cancer Institute, Kami-Ikebukuro, Toshima-ku, Tokyo 170, [1]Institute for Molecular and Cellular Biology, Osaka University, Suita, Osaka, and [2]Department of Orthopaedic Surgery and Bacteriology, National Defense Medical College, Tokorozawa, Saitama, Japan

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Human T-cell leukemia virus type 1 (HTLV-1) is an etiologic agent of adult T-cell leukemia (ATL). A viral product, p40^{x}, encoded by the *pX* sequence of HTLV-1 is a *trans*-acting transcriptional activator of the long terminal repeat (LTR) and has been suspected of involvement in leukemogenesis, activating the cellular genes. The cellular interleukin-2 (IL-2) and its receptor (IL-2R), the latter of which is expressed on ATL leukemic cells, were shown to be transiently induced by transfection of plasmid pMTPX expressing *pX* in two T-cell lines, Jurkat and HSB-2, but not in other human T- or B-cell lines. The cell type specificity of IL-2R induction by *pX* expression was the same as that by phytohaemagglutinin/phorbol ester activation, indicating the requirement for some specific cellular factors or a certain state of cellular differentiation. Induction of IL-2 and IL-2R at mRNA level was also demonstrated in transfected cells. Transfections with mutants of pMTPX in which the open reading frames for p40^{x}, p27$^{x\text{-}III}$ and p21$^{x\text{-}III}$ were inactivated indicated that p40^{x} alone was sufficient for induction of the IL-2R in inducible cells. This induction of the IL-2R by p40^{x} of HTLV-1 may contribute to preferential proliferation of HTLV-1 infected cells at an early stage of ATL development and eventually increase the number of putative target cells for malignant transformation.

Key words: pX expression of HTLV-1/mutants/activation of cellular genes/IL-2 and IL-2R/cell type specificity

Introduction

Human T-cell leukemia virus type 1 (HTLV-1) (Poiesz *et al.*, 1980; Yoshida *et al.*, 1982) is an etiologic agent of adult T-cell leukemia (ATL) (Hinuma *et al.*, 1981; Yoshida *et al.*, 1982; Wong-Staal *et al.*, 1983), a unique malignancy of helper T-cells (Uchiyama *et al.*, 1977; Hattori *et al.*, 1981). The monoclonal integration of the provirus into leukemic cells in all ATL cases examined (Yoshida *et al.*, 1984) suggested that HTLV-1 infection of a target cell is a prerequisite for leukemogenesis. Furthermore, the non-specific integration of the provirus into ATL cells in patients (Seiki *et al.*, 1984) and its immortalization of T-cells by *in vitro* infection (Miyoshi *et al.*, 1981; Yamamoto *et al.*, 1982; Popovic *et al.*, 1983) strongly suggested that a *trans*-acting viral function plays a crucial role in ATL development. In fact, HTLV-1 was demonstrated to have a *trans*-acting viral function detectable by transcriptional activation from the long terminal repeat (LTR) (Sodroski *et al.*, 1984; Fujisawa *et al.*, 1985). The *pX* sequence between the *env* gene and the 3′ LTR (Seiki *et al.*, 1983) codes for three proteins, p40^{x} (Kiyokawa *et al.*, 1984; Lee *et al.*, 1984; Miwa *et al.*, 1984; Slamon *et al.*, 1984), p27$^{x\text{-}III}$ and p21$^{x\text{-}III}$ (Kiyokawa *et al.*, 1985) from overlapping open reading frames. However, p40^{x} alone is sufficient for *trans*-activation of transcription from the LTR contained in plasmids (Sodroski *et al.*, 1985; Seiki *et al.*, 1985a, 1986). Since an enhancer sequence in the LTR is responsible for this *trans*-activation (Fujisawa *et al.*, 1986), possible activation of cellular genes through a similar enhancer was thought to be a trigger for abnormal cell growth of infected cells. On the other hand, ATL cells and T-cell lines immortalized *in vitro* by HTLV-1 infection express interleukin-2 receptors (IL-2R) on their surface (Hattori *et al.*, 1981; Depper *et al.*, 1984) and some cell lines produce IL-2. Since IL-2R are required for normal T-cell proliferation in responding to IL-2, viral function was suggested to cause overexpression of IL-2R and modify the proliferative properties of infected T-cells.

Therefore, in this work we examined whether IL-2 and IL-2R were induced by transient expression of the *pX* gene in various cell lines. We found that without any selection or other treat-

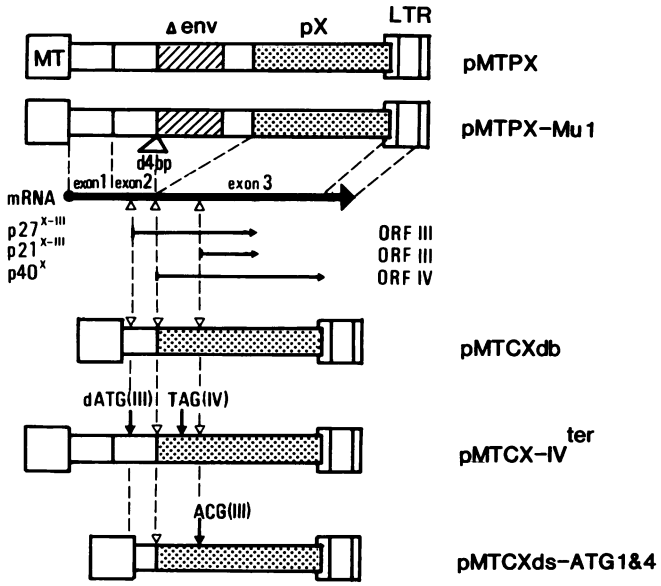

Fig. 1. Construction of expression plasmids containing the genomic or cDNA *pX* sequence. pMTPX contains the metallothionein promoter (MT), the first and second exons of *pX* cDNA, and the genomic sequences of the *env* and *pX*: the envelope gene with a deletion of the *Xho*I fragment (719 bp), the *pX* sequence and the 3′ LTR. pMTCX has the whole cDNA sequence and the 3′ LTR under the MT. pMTCXdb is a derivative of pMTCX and has a deletion in the 5′ region upstream of the *Bam*HI site that covers the first exon and a small 5′ fragment of the second exon of *pX* cDNA; thus it can code exactly the same protein as pMTCX but with higher efficiency. pMTCXds has a further deletion in the 5′ region of *pX* cDNA covering the sequence upstream of the *Sph*I site, and thus has lost the first ATG used for initiation of p27$^{x\text{-}III}$. The thick, long arrow represents the 2.1-kb *pX* mRNA and the three long, horizontal arrows represent the three proteins. △ and △ indicate an ATG codon for initiation.

Table I. Transient induction of the IL-2R on Jurkat and HSB-2 cell lines by transfection of *pX* of HTLV-1

Inducer plasmid	*pX* gene product			IL-2 receptor-positive cells (%)[a]	
	$p40^{x}$	$p27^{x\text{-}III}$	$p21^{x\text{-}III}$	Jurkat	HSB-2
None	−	−	−	0	0
pMTPX	+	+	+	1.2	0.10
pMTPX-Mu1	−	−	+	0	0
pMTCXdb	+	+	+	0.36	0.02
pMTCXds-ATG1&4	+	−	−	0.34	0.02
pMTCX-IV^{ter}	−	−	+	0	0

[a]Cells were stained by the indirect immunofluorescence method with monoclonal antibody against the IL-2R. '0' percent represents less than one, if any, positive cells among 1×10^5 transfected cells.

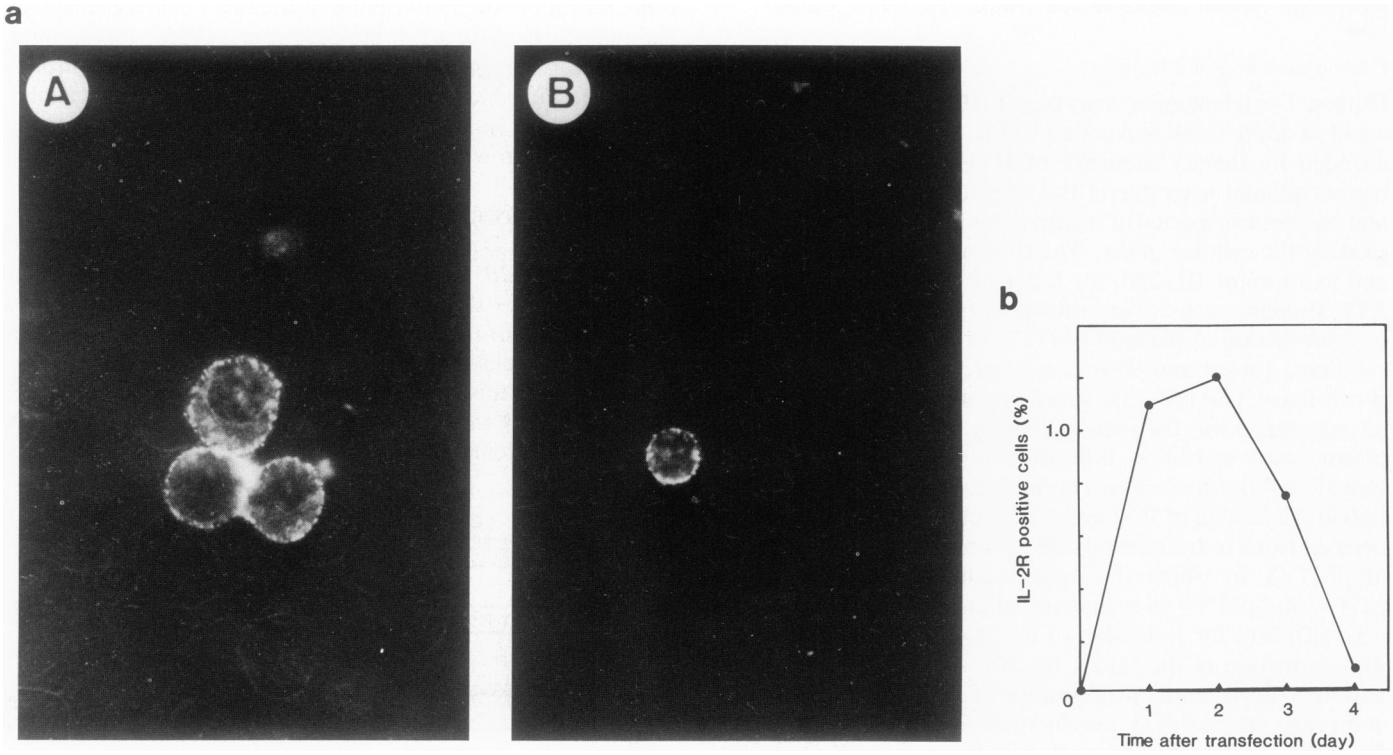

Fig. 2. Induction of the IL-2R on Jurkat and HSB-2 cells by transfection with pMTPX. (**a**) Jurkat cells (**A**) or HSB-2 cells (**B**) were transfected with pMTPX by the DEAE−dextran method as described in Materials and methods and 2 days later were stained with anti-Tac by the indirect immunofluorescence method. Less than one in 1×10^5 untransfected cells, if any, were stained. (**b**) Time-dependent change in number of fluorescence-positive Jurkat cells after their transfection with pMTPX.

ment of Jurkat and HSB-2 cell lines, expression of $p40^{x}$-induced cellular IL-2R and IL-2, but not in the other cell lines examined. Furthermore, we showed that $p40^{x}$ alone was sufficient for this induction, the expression of $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$ not being necessary. While these experiments were in progress, Greene *et al.* (1986) reported that IL-2 and IL-2R were induced in the Jurkat cell line by the *tat* gene product of HTLV-2, which is equivalent to *pX* of HTLV-1. They demonstrated these phenomena by introducing the *tat* gene of HTLV-2 into cells and then isolating several cell clones in which the *tat* gene was stably integrated. As we were examining transient expressions of $p40^{x}$ encoded by the *pX* of HTLV-1, we could analyse early events in the total cells transfected.

Results

Induction of IL-2R by pX gene expression

Transient expression of the *pX* gene can activate transcription from the LTR, eventually enhancing gene expression controlled by the LTR (Sodroski *et al.*, 1985; Chen *et al.*, 1985; Felber *et al.*, 1985; Seiki *et al.*, 1985a, 1986). To examine whether the *pX* gene products can activate expression of the cellular IL-2R gene, a plasmid pMTPX that can express all three *pX* gene products (Kiyokawa *et al.*, 1985; Seiki *et al.*, 1985a, 1986) (Figure 1) was transfected into Jurkat cells, a human T-cell leukemia line. After transfection, the cells were analysed by indirect immunofluorescence staining with monoclonal antibody against IL-2R, 2A3 (Urdal *et al.*, 1984), which blocks the binding of anti-Tac to IL-2R. Strong staining of the membrane was seen on ~1−1.2% of the transfected cells, as shown in Figure 2aA, while no staining was seen on non-transfected Jurkat cells (Table I). The number of stained cells was maximal on day 2 after transfection and then gradually decreased (Figure 2b). This time dependency was similar to those of transient expression of other transfected plasmids, suggesting that the membrane fluorescence resulted from the presence of plasmid pMTPX. Membrane stain-

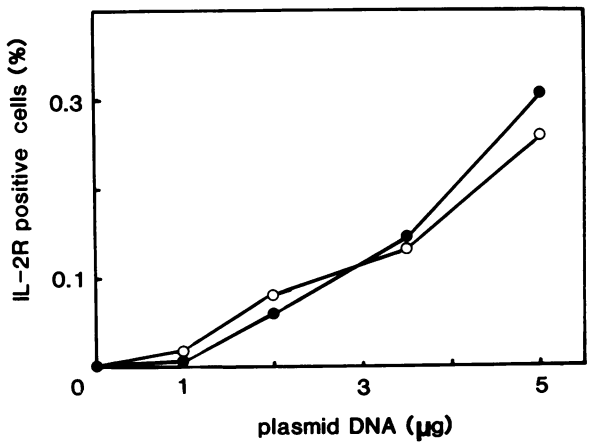

Fig. 3. DNA-dependent expression of cellular IL-2R on Jurkat cells. Jurkat cells were transfected with increasing amounts of DNA of pMTCXdb (—●—) or pMTCXds-ATG1&4 (—○—) and 2 days later cells expressing IL-2R on their surface were stained with anti-Tac.

Table II. Cell type specificity of the IL-2R induction by pMTPX or PHA and TPA

Cell line	IL-2R-positive cells (%)[a]		SV40 T-Ag-positive cells (%)[b]
	pMTPX	PHA/TPA	pMT-1
T-cells			
Jurkat	1.2	100	3.3
HSB-2	0.1	100	0.96
CEM	0	0.1	0.27
MOLT-3	0	1.0	0.76
RPMI8402	0	20	0.02
B-cells			
Ball-1	0	0	3.4
Raji	0	0.04	4.5

[a]Expression of the IL-2R was detected by anti-IL-2R 2 days after transfection with pMTPX or 1 day after treatment with PHA and TPA.
[b]Expression of SV40 T-antigen was assayed by the indirect immunofluorescence method with anti-SV40 T-antigen serum for estimating the efficiency of transfection.

ing of a similar number of transfected cells was also observed with anti-Tac antibody (Uchiyama *et al.*, 1981) (data not shown). The number of cells stained with anti-IL-2R antibody increased in a DNA-dependent fashion (Figure 3), clearly indicating that expression of IL-2R on the cell surface resulted from transfection with pMTPX. Transfection of a mutant pMTPX/Mu1 (Seiki *et al.*, 1986), which has a 4-bp deletion in the coding region, did not induce a significant number of stainable cells. Therefore it was concluded that expression of the *pX* gene was required for induction of IL-2R on the cell surface.

Cell type specificity of IL-2R induction

The *trans*-activation of pLTR-CAT with $p40^x$ expression is not restricted to T-cells, but observed in many types of cells (Sodroski *et al.*, 1985; Seiki *et al.*, 1985; Fujisawa *et al.*, 1986). So we examined the cell type specificity of the induction of IL-2R. Transfection of pMTPX into another human T cell line, HSB-2, also induced IL-2R, although at an efficiency of 0.1%, which was one-tenth of that with Jurkat cells. This lower expression of IL-2R on HSB-2 cells was explained simply by a low efficiency of transfection into this cell line, because expression of large T antigen in HSB-2 cells after transfections of pMT-1, an expression plasmid of SV40 T antigen, was less than one third of those in Jurkat cells (Table II). Stained HSB-2 cells were as brilliant as stained Jurkat cells (Figure 2aB), indicating efficient IL-2R expression in the successfully transfected cells.

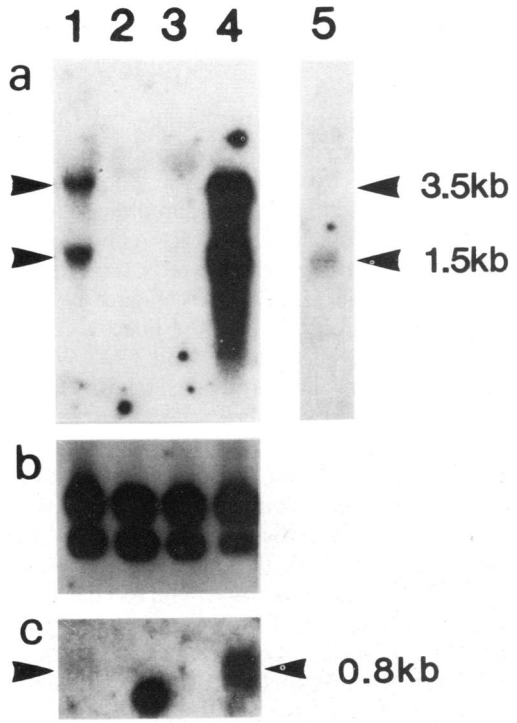

Fig. 4. Detection of IL-2R and IL-2 mRNA in Jurkat cells transfected with pMTPX. Jurkat cells (1×10^7) were transfected with DNA or treated with PHA (1%) and TPA (50 ng/ml). Cytoplasmic RNA was extracted by the vanadyl complex method 40 h after transfection or 12 h after PHA/TPA activation. RNA containing poly(A) equivalent to 4×10^6 of the original Jurkat cells or equivalent to 1×10^4 of the original HUT102 cells was denatured with 2.2 M formaldehyde at 65°C for 10 min, and examined by blotting analysis. The filter was probed with nick-translated [^{32}P]DNA of IL-2R cDNA (**a**), of tubulin cDNA (**b**), or of IL-2 cDNA (**c**). RNAs were from Jurkat cells transfected with pMTPX (**lane 1**) or pMTPX-Mu1 (**lane 2**), without DNA (**lane 3**), or activated with PHA/TPA (**lane 4**) and from HUT102 cells (**lane 5**). In panel (**c**), **lane 4** represents RNA from Jurkat cells treated with concanavalin A for 6 h.

On the other hand, transfection into other human T-cell lines, such as CEM, Molt3 and RPMI8402, or the human B-cell lines Raji and Ball-1 did not induce a significant amount (<0.001% if any) of the IL-2R on the cell surface (Table II). The absence of significant induction of IL-2R on these cell lines could not be explained by a low efficiency of transfection, because all these cell lines except RPMI8402 (see later) expressed SV40 T antigen after transfection with pMT-1, although it was variable. These results clearly indicate that induction of IL-2R by *pX* expression shows cell type specificity, suggesting that it requires some cellular factor or cellular potentiality. To test these possibilities, we treated various cell lines with phytohaemagglutinin (PHA) and 12-O-tetradecanoylphorbol-13-acetate (TPA), which are used to induce IL-2 and IL-2R on normal T lymphocytes. On this treatment, almost all Jurkat and HSB-2 cells expressed IL-2R, but none of the other cell lines listed above (i.e. CEM, Molt3, Raji and Ball-1 cells) expressed significant IL-2R. Therefore, receptor inducibilities by the *pX* gene products seems to be correlated with PHA and TPA treatment. We found that ~20% of the RPMI8402 cells expressed IL-2R on PHA and TPA treatment, but that IL-2R was not induced by pMTPX transfection. This exception is explained by the extremely low efficiency of DNA

transfection into RPMI8402 cells, as demonstrated by the expressions of T antigen after transfection of pMT-1 (Table II).

Induction of IL-2R mRNA

Since the $p40^x$ encoded by the *pX* sequence of HTLV-1 is a transcriptional *trans*-activator, it was expected that induction of the IL-2 receptor was activated at a transcriptional level. For examination of this prediction, we measured the level of mRNA of the IL-2R in transfected cells. RNA from Jurkat cells transfected with pMTPX gave two mRNA bands of 3.5 and 1.5 kb when probed with IL-2R cDNA (Figure 4a). These sizes were the same as those found in HTLV-1 infected cells, HUT102 and Jurkat cells stimulated with PHA and TPA. When the cells stained with monoclonal antibody against the IL-2R were sorted out with a fluorescence-activated cell sorter, the fraction of stained cells showed increased densitites of these two bands of mRNA, while the unstained cell fraction did not give any significant bands of mRNA (data not shown). Untransfected Jurkat cells and cells transfected with an inactive mutant, pMTPX/Mu1, also did not give any significant bands of the mRNA (Figure 4a). As control, hybridization of the same filter with tubulin cDNA probe confirmed the equal amounts of RNA in each lane (Figure 4b). Thus, expression of the IL-2R on the cell surface was associated with induction of the mRNA by *pX* gene expression.

Requirement of $p40^x$ for induction of the IL-2R

We demonstrated previously that three proteins, $p40^x$, $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$, are coded by a single species of spliced 2.1-kb mRNA (Nagashima *et al.*, 1986). In a transient assay the $p40^x$ was responsible for *trans*-activation of the LTR in plasmids, neither $p27^{x\text{-}III}$ nor $p21^{x\text{-}III}$ being required (Seiki *et al.*, 1985a, 1986). However, $p27^{x\text{-}III}$ has been suspected of being involved in the regulation of integrated provirus expression or cellular gene expression because of its nuclear localization (Kiyokawa *et al.*, 1985). To identify the product responsible for the induction of the cellular IL-2R gene, we used mutants of pMTCX containing the cDNA of 2.1-kb mRNA under the metallothionein promoter, because the mutagenesis on pMTCX derivatives was easier than on pMTPX with genomic construction. A wild-type derivative in *pX* expression, pMTCXdb, was active in activating the cellular IL-2R gene, although its activity was a quarter of that with pMTPX. This difference between pMTPX and pMTCXdb was explained by the fact that introns in pre-mRNA resulted in higher activity than RNA without introns (Breathnach and Chambon, 1981), and thus do not reflect any preferential expression of one of the pX proteins. Exactly the same difference between pMTPX and pMTCX in the transient activation of pLTR-CAT was observed previously (Seiki *et al.*, 1986).

The mutant pMTCXds-ATG1&4 (Nagashima *et al.*, 1986) had defects in the initiation codons ATGs for both $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$. Thus it cannot code for any fragments of these proteins, but it can code for the intact $p40^x$. Transfection of the mutant pMTCXds-ATG1&4 induced IL-2R on the Jurkat cell line. Furthermore, the dose-dependency of the activity with this mutant was the same as that with the wild-type pMTCXdb (Figure 3). On the other hand, the mutant pMTCX-IV^{ter}, which carries a termination codon in open reading frame IV coding for $p40^x$, was completely inactive, as described in the previous section. The pMTCX-IV^{ter} DNA did not inhibit the activity of pMTPX in a co-transfection assay, excluding the possibility of an inhibitory activity of the mutant. These observations indicated that $p40^x$ is required for induction of the cellular gene of the IL-2R, and also suggest that $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$ are not necessary for this induction.

Effect on IL-2 and HLA expression

Since some cell lines infected with HTLV-1 are known to produce consecutively IL-2 and express HLA (Popovic *et al.*, 1983), effects of *pX* proteins on IL-2 and HLA expression were also examined. When Jurkat cells were transfected with *pX* expression plasmid, pMTPX, the IL-2 mRNA was detected in the transfected cells, but not in untransfected cells or cells transfected with an inactive mutant pMTPX/Mu1. The mRNA signal was the same as that induced by concanavalin A treatment of Jurkat cells, however, the signals were rather weak (Figure 4c). These results clearly indicated that the level of the IL-2 mRNA was elevated by expression of the pX proteins, thus suggesting production of IL-2 in the transfected cells, although this was not directly demonstrated.

To examine the expression of HLA, Jurkat cells transfected with pMTPX were stained with monoclonal antibody against HLA-DR, L243, however, no positive cells were detected. The positive cells should be less than one, if any, in 1×10^5 transfected cells. The same cell population was confirmed to express IL-2R, thus it was clearly concluded that the HLA-DR was not induced at least in a similar efficiency to that of the IL-2R induction. To examine the effect of *pX* expression on the mRNA level, a filter containing the same set of RNA preparations as in Figure 4 was hybridized with cDNA clones of HLA-DRβ, HLA-DPβ and HLA-DQβ separately, however, we could not clearly detect the HLA mRNA in cells transfected with pMTPX (data not shown). These results indicate that the *pX* proteins could not induce HLA gene expression efficiently. The induction, if it occurs, might be at too low a level to detect in analysis of total cell population or the induction may be a delayed event after expression of *pX* proteins.

Discussion

In this report, we have demonstrated that one of the *pX* gene products of HTLV-1, $p40^x$, can induce expression of the cellular IL-2 and IL-2R. Transfection of Jurkat cells with plasmids expressing $p40^x$ induced the mRNA of IL-2R within 20 h and the time dependency was similar to that of transient expression of general plasmids. Therefore, the cellular IL-2R induction seems to be an early event after $p40^x$ expression. Only $1-1.2\%$ of the transfected cells expressed the IL-2R, but this low efficiency of expression simply reflected a low efficiency of the DNA transfection as evidenced by a similar level of expression of T antigen by transfection of pMT-1, an expression plasmid of SV40 T antigen (Table II). Therefore, rather efficient induction of the cellular IL-2R seemed to occur in successfully transfected cells. For this induction, $p40^x$ only was sufficient; the other two *pX* proteins $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$ were not required, because defects in the initiation codons for the translations of $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$ did not affect the activity of the pMTCX. This requirement for $p40^x$, but not for $p27^{x\text{-}III}$ or $p21^{x\text{-}III}$, was the same as that for transient activation of the transcriptional activity from the LTR (Seiki *et al.*, 1985a, 1986). These similarities suggest that the cellular IL-2 and IL-2R genes were also activated at a transcriptional level. This conclusion is supported by preliminary observations that the CAT gene inserted under the promoter of the IL-2R gene was transiently activated by $p40^x$ in Jurkat cells (T.Taniguchi, in preparation). In fact, induction of the mRNA of the IL-2R was shown in cells transfected with pMTPX, but not in cells transfected with mutants, which cannot express $p40^x$. Although only 1% of the transfected cells expressed the IL-2R, strong bands of mRNA of the IL-2R were observed. A rough estimation of

the mRNA level based on the cell numbers from which RNA was extracted showed that the mRNA level in each positively stained cell was almost equal to that of HUT102, assuming that only stained cells expressed the mRNA.

This induction of the cellular IL-2R gene was observed in certain human T-cell lines such as Jurkat and HSB-2, but not in the other T-cell lines tested, CEM, Molt3 and RPMI8402, or in the B-cell lines Raji and Ball-1. This apparent cell specificity for induction of the IL-2R clearly indicates that the induction by p40^x requires a certain stage of T-cell differentiation, that is some cellular potential or specific factor, because p40^x was functionally expressed in all cell lines, as evidenced by *trans*-activation of pLTR-CAT. In fact, Jurkat and HSB-2 cells, but not other cell lines, could be activated to express the IL-2R by treatment with PHA and TPA (Greene *et al.*, 1984). Therefore, it is conceivable that only infection with HTLV-1 of specific T-cells that have differentiated to a certain stage can induce expression of IL-2R. B-cell lines infected with HTLV-1 were also reported to express IL-2R on their surface, although at lower levels. However, on transfection of pMTPX into B-cell lines we could not detect any induction of IL-2R. This inability was probably a characteristic of B-cell lines, because infection of these cell lines with HTLV-1 by thier co-cultivation with HTLV-1 producing cell lines MT2 did not induce IL-2R expression.

With the *tat* gene of HTLV-2, induction of IL-2R was recently reported by Greene *et al.* (1986). Their strategy was selection of cell clones that contained the *pX* sequence (*tat* gene) of HTLV-2 in a sense or anti-sense orientation in a retroviral vector, and examination of expression of IL-2 and IL-2R in the cloned cells. In contrast, we used the *pX* sequence of HTLV-1, which is an etiologic agent of T-cell leukemia, and analysed total cells with the transient expression. Thus we could examine the effects of mutants of the *pX*, the dose−response, the time dependence, and the cell type specificity of the activation.

Since IL-2 and IL-2R are required for T-cell proliferation, induction of IL-2 and its receptor by the *pX* gene product would induce selective proliferation of infected T-cells without specific antigen stimulation, if this activation occurs in primary T-cells. Thus, HTLV-1 infection resulted in an unregulatable growth of infected T-cells, but not other cell types, and eventually may increase the number of putative target cells for malignant transformation. Primary leukemic cells of ATL patients express the IL-2R on their cell surface, but usually do not express any HTLV-1 antigens including p40^x. Therefore, the viral function of p40^x in activating the IL-2R gene may account for an early event in ATL development, but may not be required for the constitutive expression of the IL-2R on ATL cells.

Materials and methods

Cell lines, antibodies and plasmids

Jurkat, HSB-2, CEM, Molt3 and RPMI8402 are T-cell leukemia cell lines and Raji and Ball-1 are B-cell lines. Jurkat cells were a gift from Dr J.Hamuro, Ajinomoto Co., and HSB-2 cells were a gift from Dr T.Uchiyama, Kyoto University. All lymphoid cell lines were maintained in RPMI1640 medium supplemented with 10% FCS. Monoclonal antibodies against IL-2R, 2A3 (Urdal *et al.*, 1984) and HLA-DR, L243, were products of Becton Dickinson Co. and monoclonal antibody anti-Tac was kindly provided by Dr T.Uchiyama, Kyoto University. Plasmids for expression of the *pX* gene, pMTPX and pMTCX (Figure 1), in which the genomic or cDNA sequence of the *pX* is under control of the metallothionein promoter, were described previously together with those of mutants (Seiki *et al.*, 1985a, 1985b, 1986; Nagashima *et al.*, 1986). cDNA clones of HLA β-chains, pDRβ102, pDQβ101 and pDAβ5, were generously supplied by Drs H.Inoko and K.Tsuji, Tokai University. pMT-1 (Sugano and Yamaguchi, 1984), an expression plasmid of SV40 T antigen, was a gift from Dr N.Yamaguchi, The University of Tokyo.

Transfection into lymphoid cells and indirect immunofluorescence assay

The plasmids were transfected into lymphoid cells as described previously (Fujisawa *et al.*, 1985). Briefly, 2 × 10^6 cells were washed once with Tris-buffered saline (TBS; 25 mM Tris−HCl, 137 mM NaCl, 5 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$ and 0.6 mM Na_2HPO_4, pH 7.5) and suspended in 0.4 ml of transfection cocktail,, consisting of 5 μg of DNA and 200 μg of DEAE dextran in TBS at room temperature. After 30 min, the cocktail was replaced by 4 ml of culture medium and the suspension was cultured at 37°C in a 5% CO_2 incubator. The cells were washed once with TBS 40 h after transfection and mixed with monoclonal antibody against IL-2R. After incubation for 30 min at 4°C, they were washed twice with TBS containing 5% calf serum, and then mixed with FITC-conjugated anti-mouse immunogloblins and incubated for 30 min at 4°C. Finally the cells were washed twice with PBS containing 5% calf serum and then observed by fluorescence microscopy.

RNA analysis

Total RNA was extracted from cells with vanadyl complexes by the procedure of Berger and Birkenmeier (1979) and RNA containing poly(A) was isolated by an oligo(dT)-cellulose column. The RNA was denatured with 2.2 M formaldehyde and applied to agarose gel in the presence of formaldehyde. After electrophoresis, the RNA was transferred to a nitrocellulose filter and hybridized with ^{32}P-labeled cDNA of IL-2 or IL-2R at 42°C for 18 h in a mixture containing 50% formamide, 4 × SSC (1 × SSC: 0.15 M NaCl and 0.015 M sodium citrate), 50 μg sonicated and denatured *Escherichia coli* DNA and 20 μg of poly(A). The filter was finally washed with 0.1 × SSC, 0.1% SDS at 65°C for 15 min.

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