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Proliferative Response of Tax1-Transduced Primary Human T Cells to Anti-CD3 Antibody Stimulation by an Interleukin-2-Independent Pathway

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The growth properties of human T-cell leukemia virus Tax1-transduced primary human T cells derived from peripheral blood lymphocytes were compared with those of the same subset of T cells transduced with a control vector. Tax1-transduced T cells exhibited slightly elevated responsiveness to externally added interleukin-2 (IL-2) and a markedly higher proliferative response to stimulation with anti-CD3 antibody. The proliferation after anti-CD3 antibody stimulation was mainly via an IL-2-independent pathway. Therefore, some other mechanism than the previously proposed IL-2 autocrine model seems to be involved in the process of deregulation of T-cell proliferation by Tax1. Moreover, Tax1-transduced T cells have continued to proliferate in medium containing IL-2 long after control T cells ceased to grow, and so they are considered to be immortalized.

Human T-cell leukemia virus type I (HTLV-I) is the causative agent of at least two kinds of disease, adult T-cell leukemia (16, 37, 57) and tropical spastic paraparesis/HTLV-I-associated myelopathy (11, 35). This virus has the potential to transform normal T cells in in vitro culture systems (32, 38, 54).

Tax1, a protein encoded in the pX region of the HTLV-I genome (41), is a transcriptional *trans* activator of this virus (6, 42, 47). Tax1 transcriptionally activates not only its own long terminal repeat but also some immediate-early genes (c-*fos*, c-*jun*, *egr-1*, etc.), cytokine genes (*IL-2*, *IL-3*, *GM-CSF*, *TGF-*β_1, etc.), and its receptor gene (*IL-2R*α) (5, 8, 9, 20, 22, 23, 27, 30, 31, 33, 45, 53). These alterations of cellular gene expression are thought to lead to T-cell transformation. Aberrant activation of the interleukin-2 (IL-2) autocrine loop by Tax1 is believed to play an especially crucial role in this process. However, most experiments demonstrating the *trans*-activating function of Tax1, which is the basis of this hypothesis, have been performed on T-cell leukemic cell lines such as Jurkat.

We examined the effect of Tax1 on the growth of normal T cells in which normal growth regulation is maintained. For this purpose, we introduced the *tax1* gene into primary T cells derived from peripheral blood lymphocytes (PBL) by using a retroviral vector and compared the growth properties of the cells with those of the same subset of T cells transduced with a control vector. We found that the Tax1-transduced T cells exhibited hyperresponsiveness to externally added IL-2 but also showed a greatly increased proliferative response to antigenic stimulation through T-cell receptor (stimulation with anti-CD3 antibody). Surprisingly, this proliferation was mediated mainly by an IL-2-independent pathway, because IL-2 mRNA was barely detectable and also because antibodies against IL-2 receptors had little inhibitory effect. Moreover, neither IL-3 nor IL-4 seemed to play a major role in this proliferative response. These results suggest that Tax1 deregulates the growth of normal T cells by some as yet undefined mechanism not involving the previously proposed IL-2 autocrine pathway.

Furthermore, Tax1-transduced T cells have continued to proliferate in medium containing IL-2 long after control T cells ceased to grow, suggesting that these cells are immortalized. Immortalization of cord blood T cells by a herpesvirus saimiri vector expressing Tax1 has been reported by another group (12).

MATERIALS AND METHODS

Cells and cell culture. Tax1-transduced primary T cells were obtained as described previously (1). Briefly, PBL stimulated with phytohemagglutinin and expanded by culture in medium containing IL-2 were infected with recombinant retrovirus DGL-Tax1 expressing Tax1 (1) by the cocultivation method. The surface phenotypes of the T-cell population prior to infection were as follows: $CD3^+$, 96.3%; $CD4^+$, 44.7%; $CD8^+$, 58.0%. T cells infected with control vector DGL (1), which lacks the entire Tax1 coding region of DGL-Tax1, were obtained in the same way. Both infectants were maintained in AIM-V medium (GIBCO) supplemented with 10% fetal calf serum (FCS), recombinant IL-2 (Takeda) at 10 ng/ml, and 0.05 mM 2-mercaptoethanol.

Northern (RNA) blot analysis. Total cellular RNA was isolated by the acid-guanidium thiocyanate-phenol-chloroform method (4). The RNA (10 μg) was electrophoresed in 1.0% agarose containing formaldehyde and morpholinepropanesulfonic acid (MOPS) and blotted onto a nitrocellulose membrane. The membrane was hybridized with ^{32}P-labeled probe as described previously (15), washed with 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 65°C for 1 h, and exposed to X-ray film with intensifying screens at −70°C. The DNA fragments used as probes were as follows: human c-*fos* cDNA, a 2.1-kb *Eco*RI fragment of pSPT-fos cDNA (29); human *egr-1* cDNA, nucleotides 2530 to 3109 of ETR103 (43); human *IL-2* cDNA, a 0.6-kb *Pst*I-*Dra*I fragment of p3-16 (49); human *IL-2R*α cDNA, a 1.3-kb *Hin*dIII fragment of pKCR.Tac-2.A (34); human *IL-3* cDNA, a synthetic 60-mer oligonucleotide corresponding to the antisense strand between positions 13 and 72 of the published sequence (55); and human *IL-4* cDNA, a synthetic 60-mer oligonucleotide corresponding to the antisense strand between positions 67 and 126 of the

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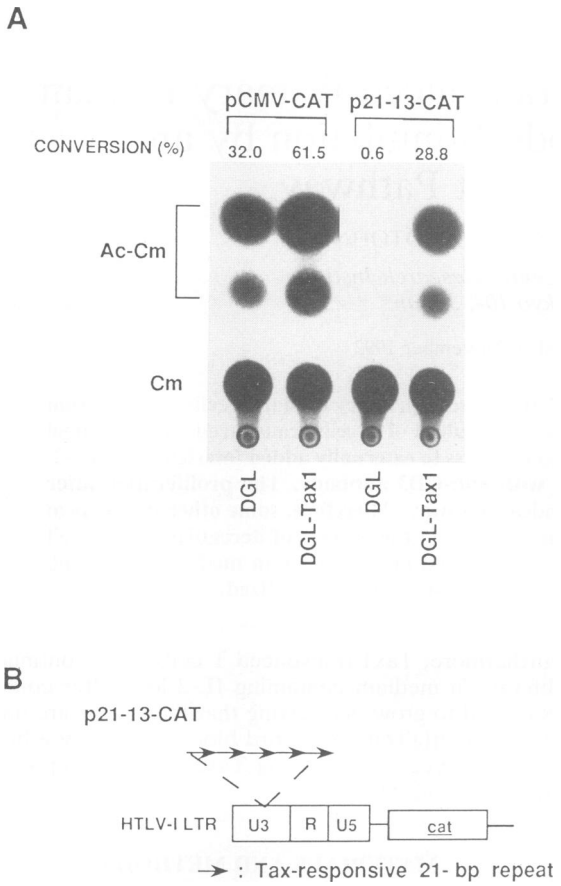

FIG. 1. *trans* activation of the Tax-responsive enhancer in Tax1-transduced PBL. (A) CAT activities in PBL infected with recombinant viruses. DGL and DGL-Tax1 infectants were transfected with pCMV-CAT and p21-13 CAT and assayed for CAT activity. Ac-CM, acetylated chloramphenicol; CM, chloramphenicol. (B) Diagram of the structure of p21-13-CAT. Arrows indicate Tax-responsive 21-bp repeats.

published sequence (56). DNA fragments of c-*fos*, *egr-1*, *IL-2*, and *IL-2Rα* were labeled with a multiprime DNA-labeling system (Amersham). The oligonucleotides for *IL-3* and *IL-4* were labeled by the 5′-end-labeling method (40).

CAT assay. Chloramphenicol acetyltransferase (CAT) reporter plasmids were transfected into T cells by electroporation by the method of Cann et al. (3) with minor modifications. T cells were resuspended at 2.5×10^7/ml in chilled culture medium, and 20 μg of plasmid DNA was added to 0.2 ml of cell suspension, mixed gently, and incubated on ice for 5 min. Electroporation was carried out in a Gene Pulser apparatus (Bio-Rad) at a voltage of 250 V and capacitance of 960 μF. CAT activity was measured as described previously (44). The reporter plasmids used were p21-13-CAT (44) and pCMV-CAT (7). The promoter of p21-13-CAT was made by inserting five repeats of the Tax-responsive 21-bp element in the U3 region of the HTLV-I long terminal repeat (Fig. 1). pCMV-CAT contains the human cytomegalovirus immediate-early region promoter.

Fluorescence-activated cell sorter analysis. Fluorescein isothiocyanate conjugated monoclonal antibodies against CD3 (Leu4), CD4 (Leu3a), CD8 (Leu2a), CD25 (IL-2R1), CD29 (4B4), and CD45RA (2H4) were used to detect the cell surface marker. Cells were allowed to react with optimal concentrations of these antibodies and were analyzed in an argon ion laser cytofluorometer.

T-cell proliferation assay. T-cell proliferation was assayed by measuring the incorporation of [^{3}H]thymidine. T cells were washed and cultured for 24 h in AIM-V medium containing neither FCS nor IL-2 to obtain resting-state cells. Then viable cells were isolated by Ficoll-Conray gradient centrifugation, suspended in AIM-V medium containing 10% FCS and cultured in flat-bottom 96-well plates (4×10^4 cells per well) under the conditions indicated in the figure legends. After 48 h, each well was pulsed for 18 h with 1 μCi of [^{3}H]thymidine. Then the cells were harvested, and the radioactivity in the 5% trichloroacetic acid-insoluble fraction was measured in a liquid scintillation counter. Stimulation with anti-CD3 antibody was carried out by culturing the cells in OKT3 (anti-CD3 antibody)-coated plates. OKT3-coated plates were prepared by incubating 100 μl of OKT3 solution (0.1 μg/ml) in flat-bottom 96-well plates at 4°C overnight and then washing the plates with phosphate-buffered saline to remove unbound antibody (10).

RESULTS

Establishment of Tax1-transduced primary T cells derived from PBL. As described previously (1), we have developed a retroviral vector, DGL-Tax1, that expresses Tax1 and have succeeded in obtaining stable infectants of primary human T cells derived from PBL. We also prepared cells infected with DGL, a control retroviral vector of DGL-Tax1. After selection with G418, the bulk population of each infectant was used for all the following experiments without cloning the cells.

Integration and mRNA expression of the retroviral vector were confirmed by Southern and Northern blot analyses as reported previously (1). The expression of functional Tax1 protein was confirmed by a CAT assay (Fig. 1). pCMV-CAT and p21-13-CAT were transfected into DGL infectants and DGL-Tax1 infectants. pCMV-CAT contains the human cytomegalovirus immediate-early region promoter (7), which is known to have strong Tax1-independent promoter activity. p21-13-CAT has five copies of a Tax-responsive 21-bp repeat (44), and its promoter activity depends on Tax1. CAT activity was observed in both infectants transfected with pCMV-CAT, but CAT activity derived from p21-13-CAT was observed only in DGL-Tax1-infectants. These results clearly show that functional Tax1 protein was expressed in DGL-Tax1 infectants.

Under our culture conditions, using AIM-V medium supplemented with IL-2 (10 ng/ml), both DGL-Tax1 infectants and DGL infectants could be maintained in the same way for a long period (nearly 1 year). Thereafter the proliferation rate of DGL infectants gradually declined, and finally the cells ceased to grow. In contrast, DGL-Tax1 infectants have continued to grow for more than 2 years after infection. Therefore, these DGL-Tax1 infectants seem to be immortalized.

Characterization of Tax1-transduced T cells. To define the effect of Tax1, we characterized DGL-Tax1 infectants in comparison with DGL infectants 5 to 6 months after infection, several months before the proliferation rate of DGL infectants started to decrease.

First, we examined the cell surface phenotypes by fluorescence-activated cell sorter analysis (Table 1). Over 99% of both infectants were CD3 positive, and over 95% were CD4 positive; this indicates that almost all cells show the phenotype of helper T cells. The DGL-Tax1 infectants showed higher expression of CD25, corresponding to the

TABLE 1. Cell surface phenotypes of retroviral vector-infected PBL

Infectant	% of cells positive for:					
	CD3	CD4	CD8	CD25 (IL-2Rα)	CD29	CD45RA
DGL	99.7	96.5	6.7	71.8	95.0	43.0
DGL Tax1	99.4	95.3	10.3	99.3	96.5	49.2

IL-2 receptor α chain (IL-2Rα). The positivity rates of CD29 and CD45RA in the two infectants were almost the same. These results indicate that the two infectants represent almost the same subset of T cells.

Then we examined the proliferative response to IL-2 and antigenic stimulation through T-cell receptors. Both infectants proliferated in a dose-dependent manner in response to externally added IL-2 (Fig. 2). The response of DGL-Tax1 infectants at any given dose of IL-2 were always higher than that of DGL infectants. Neither DGL-Tax1 infectants nor DGL infectants could proliferate in the absence of IL-2.

We examined the proliferative response to the anti-CD3 antibody OKT3, which is well known to mimic antigenic stimulation through T-cell receptors (28). Stimulation with OKT3 was performed by culturing cells in OKT3-coated plates (10). As shown in Fig. 3, the proliferative response of DGL-Tax1 infectants to OKT3 was more than 10-fold higher than that of DGL infectants. This difference in the responsiveness to OKT3 of the two infectants was much greater than the difference in their responsiveness to IL-2. This difference is not due to a quantitative difference of CD3 antigen on the cell surface of the two infectants, because both the positivity rate (Table 1) and the intensity (data not shown) of CD3 antigen expression of the two infectants are almost the same.

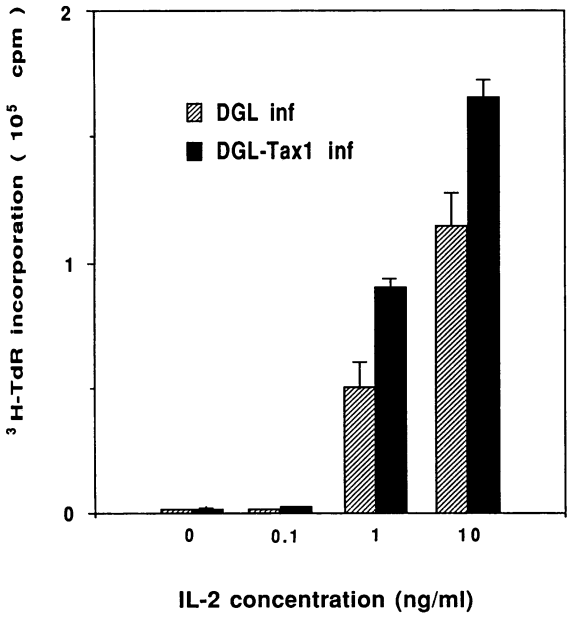

FIG. 2. Proliferative response to externally added IL-2. DGL and DGL-Tax1 infectants were cultured in AIM-V medium containing 10% FCS and the indicated concentrations of IL-2. After 48 h of culture, cells were pulsed for 18 h with 1 μCi of [^{3}H]thymidine, and the radioactivity incorporated into the fraction precipitated with 5% trichloroacetic acid was measured. Columns and bars show means and standard deviations, respectively, for triplicate samples.

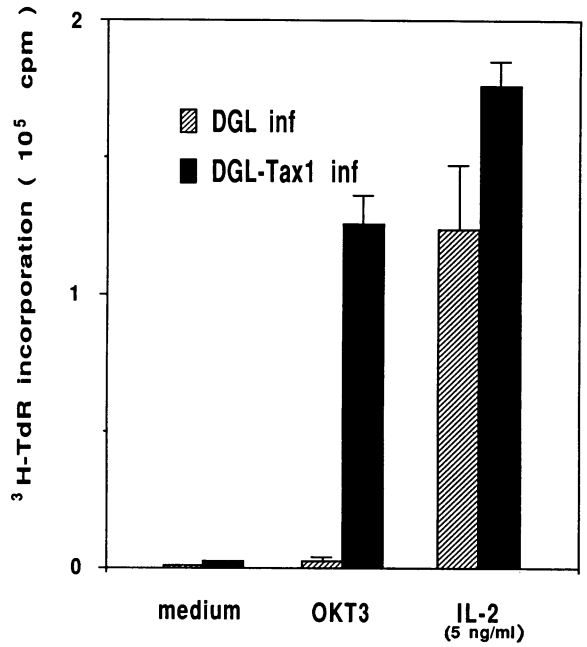

FIG. 3. Proliferative response to stimulation with the anti-CD3 antibody OKT3. DGL and DGL-Tax1 infectants were cultured for 24 h in AIM-V medium in the absence of FCS and IL-2 to obtain cells in the resting state. Then these cells were suspended in medium containing 10% FCS and cultured in medium alone in normal dishes or in dishes coated with OKT3 or in medium with IL-2 (5 ng/ml) in normal dishes. After 48 h of culture, cells were pulsed for 18 h with 1 μCi of [^{3}H]thymidine, and the radioactivity incorporated into the fraction precipitated with 5% trichloroacetic acid was measured. Columns and bars show means and standard deviations, respectively, for triplicate samples.

IL-2-independent proliferation of Tax1-transduced T cells after OKT3 stimulation. To clarify the mechanism of the proliferative response, we analyzed the kinetics of expression of c-*fos*, *egr-1*, *IL-2Rα*, and *IL-2* mRNA during the period of stimulation with OKT3 (Fig. 4). The induction of mRNA for c-*fos* and *egr-1* occurred 0.5 to 2 h after stimulation in both infectants. The slow kinetics of induction might be due to a time lag for the cells to interact with OKT3 antibody immobilized on the bottom of the culture dishes. DGL-Tax1 infectants showed slightly higher levels of expression at the time of maximal induction and longer periods of expression of these genes: even at 48 h after stimulation, significant levels of c-*fos* and *egr-1* mRNA were observed in DGL-Tax1 infectants. The induction of *IL-2Rα* mRNA occurred after 8 h in both infectants, and a more sustained expression was observed in DGL-Tax1 infectants.

Interestingly, no *IL-2* mRNA could be detected in DGL-Tax1 infectants before or after OKT3 stimulation, although a very faint signal was detected in DGL infectants 8 h after stimulation. The amount of IL-2 protein in the culture supernatant of these cells 24 h after stimulation was examined by using an Intertest-2 enzyme-linked immunosorbent assay (Genzyme), but its level was below the detection limit (<100 pg/ml; data not shown). These data suggested that the strong proliferative response of DGL-Tax1 infectants to OKT3 stimulation was not mediated by the IL-2 autocrine pathway. To test this possibility, we examined the inhibitory

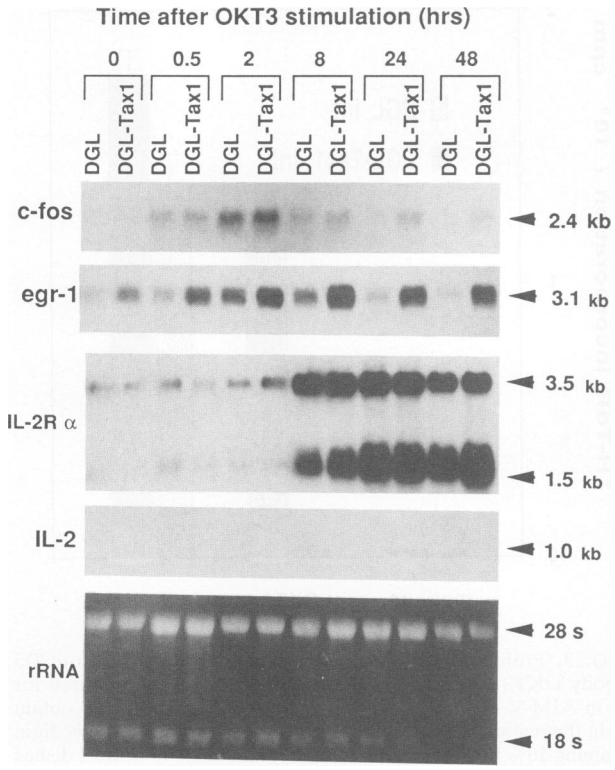

FIG. 4. Expression of c-*fos*, *egr-1*, *IL-2Rα*, and *IL-2* mRNAs after stimulation with OKT3 antibody. At various times after OKT3 stimulation (indicated above the lanes), total RNA was isolated from DGL and DGL-Tax1 infectants and analyzed by Northern blotting with specific probes for c-*fos*, *egr-1*, *IL-2Rα*, and *IL-2*. Ethidium bromide staining of rRNAs is shown at the bottom of the figure.

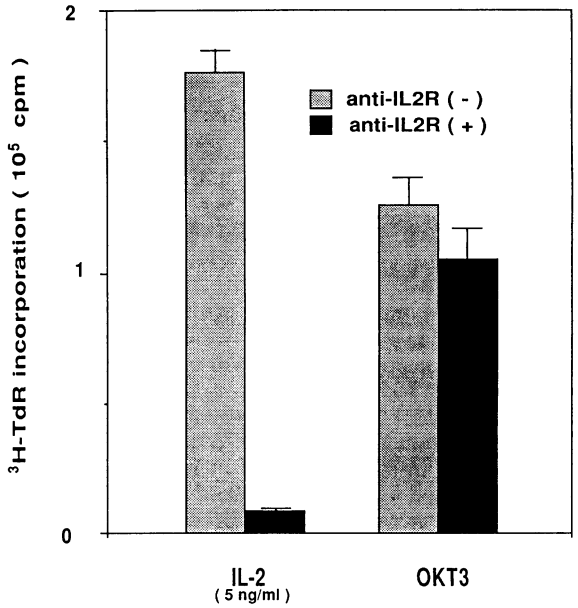

FIG. 5. Effect of anti-IL-2 receptor antibodies on the proliferation of DGL-Tax1 infectants. DGL-Tax1 infectants were stimulated with IL-2 (5 ng/ml) or OKT3 antibody (cultured in OKT3-coated dishes) in the presence or absence of anti-IL-2 receptor antibodies, and [^{3}H]thymidine incorporation was assayed as described in Materials and Methods. The anti-IL-2 receptor antibodies used were a mixture of 33B3.1 (2 μg/ml; anti-IL-2Rα chain) and Mikβ1 (25 μg/ml; anti-IL-2Rβ chain). These antibodies were added at the onset of culture. Columns and bars show means and standard deviations, respectively, for triplicate samples.

effect of anti-IL-2R antibody on cell proliferation. To do this, we stimulated DGL-Tax1 infectants with OKT3 antibody in the presence or absence of anti-IL-2R antibody and measured the incorporation of [^{3}H]thymidine (Fig. 5). The anti-IL-2R antibody used was a mixture of the anti-IL-2Rα chain (33B3.1) (21) and anti-IL-2Rβ chain (Mik-β1) (51). With this mixture, the proliferative response to externally added IL-2 was almost completely blocked. On the other hand, the proliferative responses to OKT3 stimulation were only slightly inhibited. These results indicate that the proliferative response to OKT3 stimulation observed in DGL-Tax1 infectants is mainly through an IL-2-independent pathway, not as previously proposed through an IL-2 autocrine pathway.

Expressions of IL-3 and IL-4 in DGL-Tax1 infectants. To test the possible involvement of other lymphokines in the proliferative response to OKT3 stimulation, we examined the expression of *IL-3* and *IL-4* mRNA in addition to that of *IL-2* mRNA. To do this, we used two different populations of DGL-Tax1 infectants, which were obtained in two independent infection experiments. All the infectants were cultured in medium alone or stimulated with OKT3 antibody or with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) plus ionomycin. The incorporation of [^{3}H]thymidine after 2 days of culture and the expression of mRNAs for *IL-2*, *IL-3*, and *IL-4* 8 h after stimulation were analyzed (Fig. 6), because the inductions of mRNAs for *IL-2* and *IL-4* are reported to be maximal 8 h after mitogenic stimulation (25).

Upon stimulation with OKT3, very faint bands of mRNAs for *IL-2*, *IL-3*, and *IL-4* were detected in one of the populations of DGL-Tax1 infectants but not in the other population of DGL-Tax1 infectants or in DGL infectants. When stimulated with TPA plus ionomycin, mRNAs for *IL-2* and *IL-3* were clearly detected in all three infectants, and the expression levels of these lymphokines were much higher in DGL-Tax1 infectants than in DGL infectants. *IL-4* mRNA was slightly detectable in only one of DGL-Tax1 infectants after stimulation with OKT3 or with TPA plus ionomycin.

In DGL infectants, significant proliferation occurred only after stimulation with TPA plus ionomycin when IL-2 and IL-3 were expressed. In contrast, in both DGL-Tax1 infectants, there seemed to be no such clear correlation between cell proliferation and expression of these lymphokines (Fig. 6). These results suggest that the autocrine system of these lymphokines is not the main pathway for the proliferative response of DGL-Tax1 infectants to OKT3 stimulation.

DISCUSSION

In this work, we characterized Tax1-transduced primary human T cells in comparison with the same subset of T cells transduced with a control vector that does not express Tax1. Tax1-transduced T cells have continued to proliferate in medium containing IL-2 long after T cells transduced with the control vector ceased to grow. Thus the primary T cells derived from PBL seem to have been immortalized by introduction of Tax1. Grassmann et al. reported that a herpesvirus saimiri vector containing the *tax1* gene could immortalize primary human CD4$^+$ cord blood lymphocytes in culture but that their vector could not immortalize T cells derived from PBL (12, 13). Considering that authentic HTLV-I, as well as our vector, immortalized CD4$^+$ PBL, it is likely that the immortalizing action of Tax1 is not limited

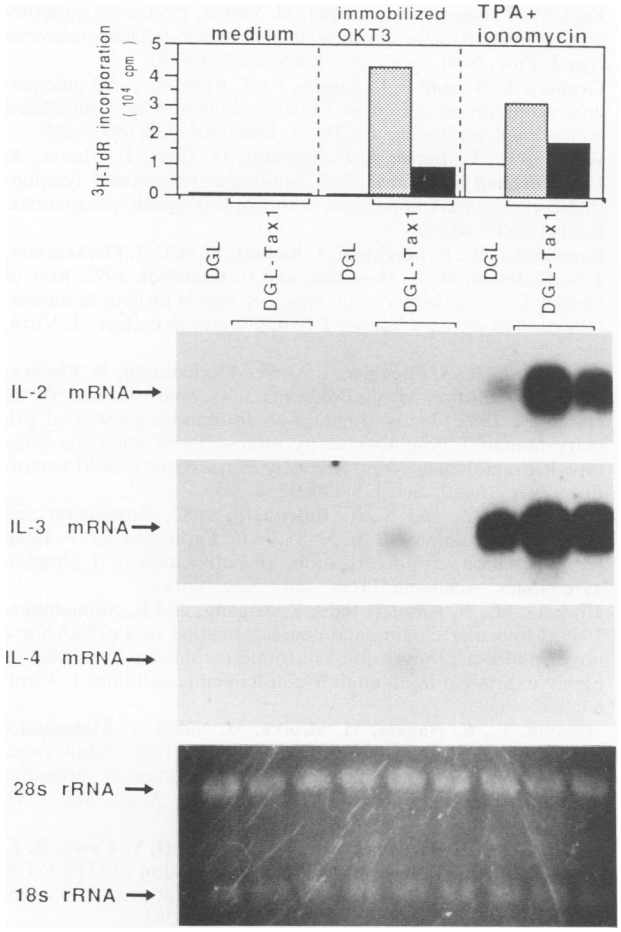

FIG. 6. Levels of proliferation and *IL-2*, *IL-3*, and *IL-4* mRNA expression. DGL infectants and two independently obtained DGL-Tax1 infectants were cultured in medium alone or with OKT3 antibody (in OKT3-coated dishes) or with TPA (1 ng/ml) plus ionomycin (0.5 μM). [^{3}H]thymidine incorporation was measured after 2 days of culture (upper panel), and the RNA isolated 8 h after stimulation was analyzed by Northern blotting (lower panel). On stimulation with TPA plus ionomycin, the medium was changed after 8 h to avoid the toxic effects of these reagents.

to cord blood T cells. The life span of the control T cells in our system (about 1 year) seems very long compared with that reported for primary T cells (14). Primary T cells are well known to undergo a so-called crisis period after 30 to 60 days in culture (14). However, Perillo et al. demonstrated in a systematic study that this crisis period could be avoided by improving the culture conditions (for example, using AIM-V medium) and that normal human T cells have a proliferative life span, with the cumulative population doublings corresponding to that of human fibroblasts (50 ± 10 doublings) (37). Because we did not measure the number of population doublings of the control T cells (DGL infectants), we do not know whether the culture period was longer than the time supposed to be required for senescence. Our results suggest, however, that under optimal culture conditions the in vitro life span of normal T cells is longer than previously thought.

To define the effect of Tax1 on the growth of normal T cells, we compared the growth characteristics of Tax1-transduced T cells and the same subset of T cells transduced with a control vector before they became senescent. We found that Tax1-transduced T cells showed a higher proliferative response to externally added IL-2. This hyperresponsiveness probably reflects a higher expression level of CD25 (IL-2Rα chain), as shown in Table 1. Tax1-transduced T cells still remain IL-2 dependent, unlike most HTLV-I-infected T-cell lines, which grow in the absence of IL-2. In contrast to HTLV-I-infected T-cell lines, which usually harbor multiple copies of the *tax1* gene (18), DGL-Tax1 infectants have a single copy (data not shown). Therefore, the gene dosage of *tax1* in DGL-Tax1 infectants may be insufficient to achieve an IL-2-independent state. However, T cells infected with a herpesvirus saimiri vector containing the pX region have multiple copies of the *tax1* gene but still proliferate in an IL-2-dependent manner (13), suggesting that in addition to *tax*, another viral gene, such as *env*, may be required to establish a fully IL-2-independent growth state.

The most remarkable feature of Tax1-transduced T cells is their hyperresponsiveness to antigenic stimulation through T-cell receptors. The proliferative response of Tax1-transduced T cells to stimulation with OKT3 anti-CD3 antibody is about 10-fold higher than that of control T cells. Surprisingly, this proliferative response seems to be independent of the IL-2 autocrine pathway, because IL-2 mRNA was barely detectable by Northern blot analysis during this proliferation and also because addition of anti-IL-2R antibodies to culture medium had little inhibitory effect on this proliferative response. Moreover, neither IL-3 nor IL-4 plays a major role in this proliferation. Tax1 *trans* activates some immediate-early genes and cytokine genes (5, 8, 9, 20, 22, 23, 27, 30, 31, 33, 45, 53). It is widely supposed that aberrant activation of the IL-2 autocrine loop by Tax1 plays a crucial role in T-cell transformation by HTLV-I. However, this IL-2 autocrine mechanism has been demonstrated only rarely in HTLV-I-infected cell lines (2). Several lines of evidence suggest the existence of a pathway other than the IL-2 autocrine mechanism. For example, HTLV-I-infected CD4$^+$ T-cell clones derived from HTLV-I-associated myelopathy patients proliferate spontaneously without production of IL-2 (17). In the course of transformation of normal T cells by cocultivation with HTLV-I-producing cells, IL-2 expression was seen only very transiently at the beginning of coculture (24). Moreover, when human PBL were induced to proliferate by addition of Tax protein to the culture medium, no production of IL-2 could be detected (26). Considering the ability of Tax1 to transform murine fibroblasts (39, 48), it seems likely that Tax1 promotes cell growth by some mechanism other than the IL-2 autocrine pathway. From this point of view, *trans* activation of immediate-early genes, such as c-*fos* and *egr-1*, by Tax1 seems to be very important (8). This possibility is supported by a recent report that the ability of Tax1 to transform rat fibroblasts is correlated with the CREB/ATF pathway, which is involved in the activation of c-*fos* as well as the HTLV-I long terminal repeat, but not with the NF-κb pathway, which is involved in the activation of IL-2 and IL-2Rα (46). In this regard, it seems necessary to analyze the contributions of these immediate-early genes (c-*fos* and *egr-1*) to the proliferative response to OKT3 stimulation without using the IL-2 autocrine pathway in Tax1-transduced T cells.

It would be interesting to know whether proliferation independent of the IL-2 autocrine pathway could be observed in HTLV-I-infected cells on stimulation through T-cell receptors. Expression of the CD3–T-cell receptor complex and responsiveness to antigenic stimulation are known to be markedly decreased in HTLV-I-infected T cells (19, 50, 58). However, experiments with T-cell clones have shown that HTLV-I-infected cells express the CD3–T-cell receptor complex at the same level as do uninfected control

cells at an early stage of infection (19, 58). Volkman et al. reported that an HTLV-I-infected CD4$^+$ T-cell clone, which shows a normal level of CD3 expression, proliferates in response to antigenic stimulation without production of IL-2 (52). From these findings, it seems likely that proliferation by an IL-2-independent pathway actually occurs in HTLV-I-infected T cells in response to stimulation through T-cell receptors at an early stage after infection.

The possibility that the growth properties of Tax1-transduced T cells described here are those of some peculiar clone, such as one in which some c-*onc* is activated by the integration of retroviral vector, can be excluded for the following reasons: (i) T cells transduced with control vector, which were infected at the same time and maintained for the same period under the same culture conditions, never showed outgrowth of clones with the above growth characters; and (ii) another population of DGL-Tax1 infectants with different proviral integration patterns, which we obtained in an independent infection experiment, showed a similar proliferative response to that of the infectants described in this paper (data not shown).

The existence of another pathway than the IL-2 autocrine pathway involved in the deregulation of T-cell proliferation by Tax1, as demonstrated in this work, provides a new insight into the process of T-cell transformation by HTLV-I.

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