JOURNAL OF VIROLOGY, June 1996, p. 3599–3605 Vol. 70, No. 6
0022-538X/96/$04.00+0

The Human T-Cell Leukemia/Lymphotropic Virus Type 1 p12^{I} Protein Binds the Interleukin-2 Receptor β and γ_c Chains and Affects Their Expression on the Cell Surface

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Received 16 November 1995/Accepted 12 March 1996

p12^{I} is a small hydrophobic protein encoded by the human T-cell leukemia/lymphotropic virus type 1 (HTLV-1) that interacts with the 16-kDa component of the H^+ vacuolar ATPase and cooperates with bovine papillomavirus 1 E5 oncoprotein in cell transformation. Just as an important step in E5 action appears to be its binding to the platelet-derived growth factor receptor, it was found that p12^{I} binds specifically to both the β and γ_c chains of the interleukin-2 receptor (IL-2R). The IL-2R β and γ_c chains associated with p12^{I} are endoglycosidase-H sensitive, suggesting that their interaction occurs in a pre-Golgi compartment. p12^{I} stabilizes the immature forms of the IL-2R β and γ_c chains and decreases their cell surface expression. The interactions of p12^{I} with IL-2R β and γ_c may have important implications in the immunosuppressive effect of HTLV-1 in vivo as well as in the ligand-independent HTLV-1-mediated T-cell proliferation.

Human T-cell leukemia/lymphotropic virus type 1 (HTLV-1) (14, 20, 46) induces adult T-cell leukemia (ATL) as well as a chronic progressive myelopathy (15, 43, 49). HTLV-1 infects $CD4^+$ T cells and renders them ligand (interleukin-2 [IL-2]) independent (transformed) in vitro (30, 35, 47, 61). The HTLV-1 Tax protein induces expression of genes involved in T-cell activation and proliferation, including IL-2, IL-2 receptor (IL-2R) α chain, c-*fos*, and other early mitogen genes (8, 13, 21, 31, 36, 52). This autocrine/paracrine loop, mediated by Tax, is thought to be a prime event in the induction of T-cell proliferation in vitro and spontaneous T-cell proliferation in vivo (for a recent review, see reference 11). In late stages of HTLV-1 transformation in vitro, IL-2 production does not appear to be required to maintain T-cell growth (1). The IL-2R is composed of at least three chains: α chain, which increases the ligand binding affinity, and the β and γ_c chains, which upon ligand binding activate the IL-2R signaling pathway (29, 37, 38). The γ_c chain is encoded by the gene that is defective in X-linked severe combined immunodeficiency (41) and is now denoted as the common γ chain, γ_c, since it is also a functional component of the receptors for IL-4, IL-7, IL-9, and IL-15 (18, 23, 25, 26, 40, 50). Constitutive activation of the Jak/STAT pathway has been recently demonstrated in IL-2-independent, but not IL-2-dependent, HTLV-1-infected T cells (32, 59). In addition, changes in the level of expression of $p56^{lck}$ and $p59^{fyn}$ protein tyrosine kinases have been reported to occur in HTLV-1-transformed T cells in vitro (24, 33, 62), further suggesting that HTLV-1 infection might subvert the physiological mechanisms involved in T-cell activation and proliferation. The viral determinants involved in the latter steps of T-cell transformation are unknown.

p12^{I}, a membrane-associated protein (27, 28), potentiates mouse fibroblast transformation mediated by the bovine papillomavirus 1 E5 oncoprotein (12, 51) and, like E5, binds to the 16-kDa subunit of the vacuolar H^+ ATPase proton pump (10, 12, 17). The structural and perhaps functional similarity of p12^{I} to E5, which is known to bind to and activate the platelet-derived growth factor receptor (PDGF-R) (16, 39, 44, 45), prompted us to investigate whether p12^{I} might interact with the IL-2R complex (29, 34, 56) and contribute to the IL-2-independent T-cell growth induced by HTLV-1.

MATERIALS AND METHODS

Expression plasmids and antibodies. The pME18S expression vector was obtained from Atsushi Miyajima (DNAX, Palo Alto, Calif.). Transcription of pME18S DNA is regulated by a hybrid promoter between the simian virus 40 early region promoter and the R region of the HTLV-1 long terminal repeat (LTR). The p12^{I} cDNA containing the AU1 epitope; the α, β, and γ_c chains of the IL-2R; and hybrid constructs of these chains were expressed from this vector. LTR/HPR-A, encoding the human PDGF-R β chain, and LTR-2 epidermal growth factor receptor (EGF-R), which expresses the human EGF-R, were gifts of Jacalyn Pierce (National Cancer Institute, Bethesda, Md.). The expression plasmid for the human erythropoietin receptor (Epo-R) as well as the 11-88 antibody specific for this receptor were obtained from Alan D. D'Andrea (Dana Farber Cancer Institute, Boston, Mass.). The αAU1 antibody was from Babco (Richmond, Calif.). Shar4 (37) was obtained by immunization of rabbits with a peptide from the carboxyl-terminal region of human IL-2R β. TU11, Mikβ3, 561, and Mikβ1, monoclonal antibodies specific for the extracellular region of human IL-2R β, have been previously described (53–56). Anti-Tac, reactive against the extracellular region of IL-2R α, and the monoclonal antibody Mikβ1 were kind gifts from Thomas Waldmann (National Cancer Institute). Ab1 antibody, specific for the human EGF-R, was purchased from Oncogene Science (Cambridge, Mass.), and 06-131 antibody, specific for the PDGF-R β chain, was purchased from UBI (Lake Placid, N.Y.).

DNA transfection and protein binding assays. HeLa/Tat cells were plated at 10^6 cells in 100-mm-diameter dishes and the next morning were transfected by the calcium phosphate method (19). For transfection in 100-mm-diameter dishes, 10 μg of the p12^{I} expression plasmid and 5 μg of receptor expression plasmid(s) were used, and the amount of DNA transfected was normalized with the needed amount of DNA vector. Twenty-four hours after transfection, cells were metabolically labeled for 2 to 3 h with 100 μCi of EXPRE^{35}S^{35}S (ICN, Costa Mesa, Calif.) per ml in methionine- and cysteine-free medium containing 1% dialyzed serum and 2 mM L-glutamine. Cells were washed with 1× phosphate-buffered saline (PBS) and lysed with 1× radioimmunoprecipitation assay (RIPA) buffer (1% deoxycholic acid, 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 0.15 M NaCl, 50 mM Tris-Cl [pH 7.5]) containing 20 μg of aprotinin per ml, 20 μg of leupeptin per ml, and 1 mM phenylmethylsulfonyl fluoride. Cell lysates were divided in half and precleared for 2 h with normal rabbit serum and protein A-agarose beads (Boehringer Mannheim, Indianapolis,

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Ind.). The beads were pelleted, and the lysates were incubated with specific antibody overnight at 4°C with constant agitation. Immunocomplexes were bound for 1 h to protein A-agarose beads, washed four times with cold 1× RIPA buffer, and boiled for 10 min in 2× SDS–loading buffer (Novex, San Diego, Calif.). Proteins were separated on 10 and 15% polyacrylamide gels.

Endo-H and tunicamycin treatment. HeLa/Tat cells were transfected with the appropriate cDNAs and 24 h later were labeled as described above. At the end of the labeling period, cells were washed twice with PBS, and fresh medium containing 10% fetal calf serum and an excess of unlabeled methionine and cysteine (75 and 120 μg/ml, respectively [Gibco, Gaithersburg, Md.]) was added. Cells were then harvested at various times. Cell lysates were immunoprecipitated as described in the previous section, and the immunoprecipitates were resuspended in 50 μl of 0.1 M sodium citrate (pH 5.5)–2% β-mercaptoethanol–0.2% SDS–1 mM phenylmethylsulfonyl fluoride, boiled for 5 min, and quickly chilled on ice. Twenty microliters of endoglycosidase H (Endo-H) (Boehringer Mannheim) (1 U/ml) or 20 μl of 50 mM sodium phosphate (pH 6.1) for control tubes was added to each immunoprecipitate, and the mixture was incubated overnight at 37°C. At the end of the incubation period, each sample was resuspended in SDS-loading buffer and electrophoresed on an SDS-polyacrylamide (10%) gel electrophoresis (PAGE) gel. For tunicamycin treatment, transfected cells were treated with 3 μg of tunicamycin (Boehringer Mannheim) per ml 1 h before and 3 h during metabolic labeling. All subsequent manipulations were performed as described previously.

Flow cytometric analysis of cells. HeLa/Tat cells were transfected as described above, except that 1.0 μg of the IL-2R α and β and 4.0 μg of the αγγ expression plasmids were used with 20 μg of the $p12^I$ cDNA. Twenty-four hours later, cells were washed twice in PBS and collected by cell scraping. Cells were stained for 1 h at 4°C with fluorescein isothiocyanate-conjugated anti-CD25 antibody (anti-CD25-FITC) (Immunotech, Westbrook, Maine) or anti-CD122-FITC (Endogen, Cambridge, Mass.). Anti-immunoglobulin G (IgG)-FITC was used as a negative control (Immunotech). Samples were washed four times in PBS containing 10% fetal bovine serum (FBS) and 0.02% sodium azide. Cells were fixed in 2% paraformaldehyde in PBS overnight and analyzed on a Becton Dickinson FacScan (San Jose, Calif.) with Lysis II software.

Immunofluorescent detection of $p12^I$. HeLa/Tat cells were seeded onto slides (2×10^5 cells per slide) and transfected the next morning. One-fifth of the DNA-Ca_2PO_4 mix used to transfect the 100-mm-diameter plates in the flow cytometric experiments was added to the slides. Twenty-four hours later, cells were washed twice in PBS and fixed at room temperature for 10 min in 3.7% formaldehyde in PBS. The αAU1 antibody was added at 1/50 in 10% FBS–0.01% saponin in PBS and incubated in a humidified chamber at 37°C for 1 h. Slides were washed four times in PBS with 10% FBS and 0.02% sodium azide. Goat anti-mouse IgG-FITC (Cappel, West Chester, Pa.) was added at a 1/30 dilution in 10% FBS–0.01% saponin in PBS, and the mixture was incubated as described for primary antibody. After four washings, cells were counterstained with Evans blue for 10 min. Fluorescence was detected with a Nikon Optiphot microscope, and pictures were taken with a Nikon FX-35A camera (Nikon, Tokyo, Japan).

RESULTS

The HTLV-1 $p12^I$ protein binds specifically to IL-2Rβ and γ_c chains. The $p12^I$ cDNA carrying the AU1 epitope tag at the carboxyl terminus (27) was cotransfected independently with cDNAs encoding the PDGF-R β chain; the EGF-R; the IL-2R α, IL-2R β, and αγγ chains; and the Epo-R in HeLa/Tat cells (Fig. 1). The $p12^I$ protein was readily immunoprecipitated by the αAU1 monoclonal antibody when expressed in either HeLa/Tat cells (Fig. 1A, lanes 1, 2, 5, and 10) or Cos-1 cells (Fig. 1A, lane 9). When coexpressed with PDGF-R, EGF-R, Epo-R, and IL-2R α, $p12^I$ failed to bind to these proteins, as shown by the lack of coimmunoprecipitation when antibodies for the various receptors or antibodies for $p12^I$ were used (Fig. 1A, lanes 2, 3, 5, 6, 10, and 11, and B, lanes 3 and 4). In the case of PDGF-R, similar results were obtained in Cos-1 cells, which expressed PDGF-R at higher levels (Fig. 1A, lanes 8 and 9). In contrast, when $p12^I$ and the IL-2R β chain were coexpressed in HeLa/Tat cells and the cell lysates were immunoprecipitated with the Shar4 or the Mikβ1 antibodies, an association of $p12^I$ with the IL-2R β chain was clearly demonstrated (Fig. 1B, lanes 9 and 10). The difference in amount of $p12^I$ coimmunoprecipitated with the IL-2R β chain likely correlates with the different forms of the β chain recognized by the two antibodies. Shar4 was raised against a peptide from the cytoplasmic portion of the β chain and recognizes presumably equally well the immature and mature forms of the IL-2R β chain, as demonstrated by the broadness of the protein band precipitated by the Shar4 antibody (Fig. 1B, lane 6). Instead, Mikβ1, raised against the extracellular portion of the IL-2R β chain, appears to recognize a more mature form of the receptor (Fig. 1B, lane 7). $p12^I$ preferentially bound to the less mature forms of the IL-2R, as demonstrated by the smaller size of the IL-2R β chain immunoprecipitate when antibodies to the $p12^I$ tag (αAU1) were used (compare lanes 6 and 7 and lane 8 of Fig. 1B). With three additional antibodies to IL-2R β which, like Mikβ1, were raised against the extracellular portion of the β chain, a correlation is seen between the amount of $p12^I$ coprecipitated and the form of IL-2R β recognized by the antibody (Fig. 2). Those antibodies which precipitate a larger, presumably more mature form of IL-2Rβ, including Mikβ1, Mikβ3, Tull, and 561, also coprecipitate significantly less $p12^I$ than does the Shar4 antibody (Fig. 2).

Coexpression of $p12^I$ with the IL-2R α chain and immunoprecipitation with anti-Tac antibodies demonstrated clearly that the α chain does not bind to $p12^I$ (Fig. 1B, lanes 1 to 4). In contrast, a chimeric receptor (αγγ) carrying the extracellu-

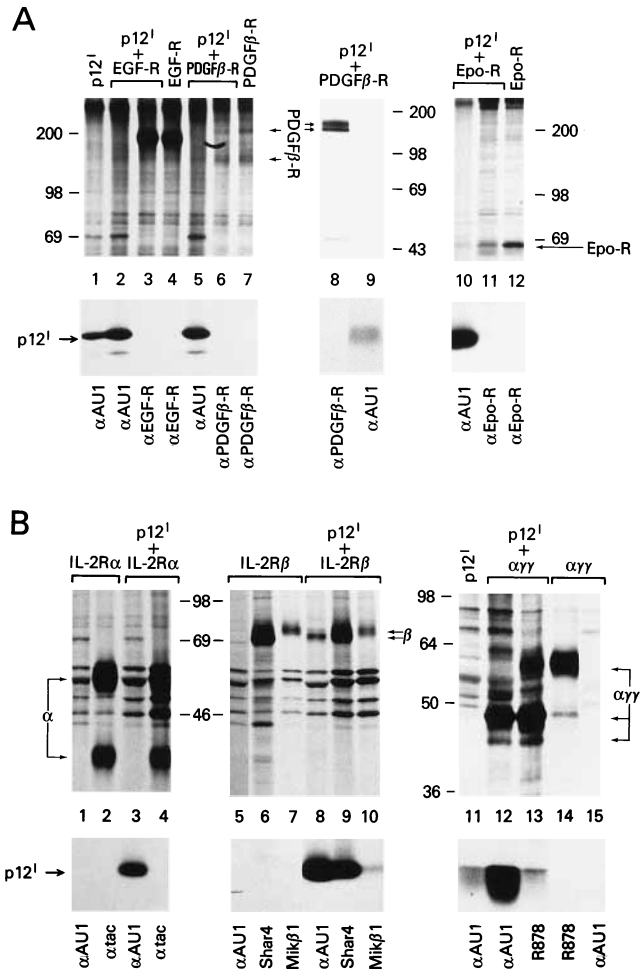

FIG. 1. Binding of $p12^I$ to growth factor receptors. (A) Binding to EGF-R, PDGF-R β chain (PDGFβ-R), and Epo-R. (B) Binding to IL-2R α, IL-2R β, and αγγ. The genes expressed in each sample are indicated above each lane, and the antibodies used are shown below each lane. (A) For lanes 8 and 9, the cells used were Cos-1; for all other lanes, the cells were HeLa/Tat. The same immunoprecipitates were electrophoresed on 10% (upper portion of each panel) and 15% (lower portion of each panel) SDS-PAGE gels. Molecular mass is given (in kilodaltons) to the side.

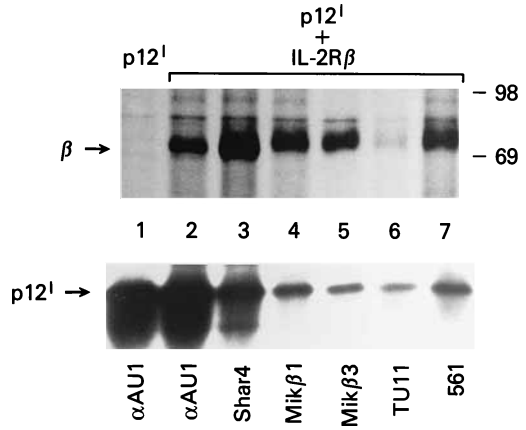

FIG. 2. Differential coprecipitation of p12^{I} by antibodies recognizing different forms of IL-2R β. HeLa/Tat cells were transfected, and the cell lysates were immunoprecipitated with the antibodies indicated at the bottom of the figure. Molecular mass is given (in kilodaltons) to the side.

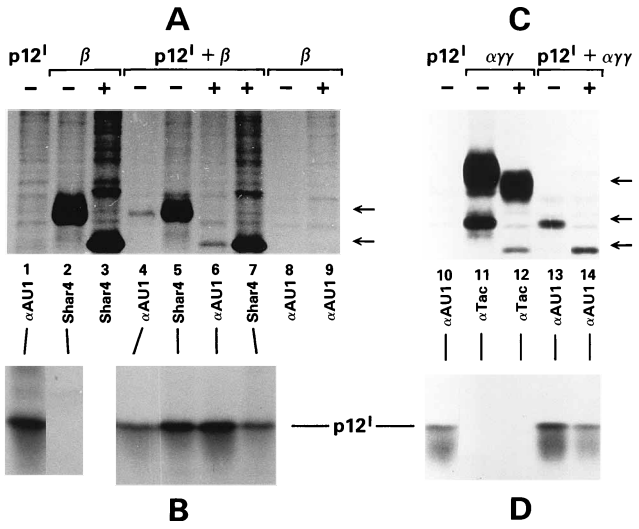

FIG. 4. Effect of tunicamycin on p12^{I} binding to the IL-2R β and γ_c chains. DNA transfection was performed as described in Materials and Methods, and the genes expressed are indicated at the top of each panel. + and −, cells were treated with or without 3 μg of tunicamycin per ml, respectively. The antibodies used for each lane are indicated. The same immunoprecipitates were electrophoresed on 8% polyacrylamide (A and C) and 15% polyacrylamide (B and D) SDS-PAGE gels.

lar portion of the IL-2R α chain and the transmembrane and cytoplasmic portion of the IL-2R γ_c bound to p12^{I}, as demonstrated by the detection of p12^{I} with the R878 antibodies (40, 50) specific for the γ_c chain (Fig. 1B, lanes 11 to 15). The p12^{I} protein also binds to the wild-type γ_c chain (data not shown), but because of the limited availability of γ_c antibodies, the αγγ construct was used in these studies. As in the case of the IL-2R β chain, p12^{I} bound to the immature, not fully glycosylated form of the αγγ receptor, since only the low-molecular-weight form of the receptor coimmunoprecipitated with p12^{I} (Fig. 1B, lane 12).

Thus, p12^{I} associated specifically with the IL-2R β and γ_c chains and not with the other receptors tested. This association is not cell specific, since the same results were obtained in Cos-1 cells (data not shown). The identity of the IL-2R β protein coimmunoprecipitated with p12^{I} was also verified by Western blotting (immunoblotting) (data not shown).

In an attempt to demonstrate p12^{I} binding to both IL-2R β and γ_c simultaneously, we performed triple transfections and subsequently immunoprecipitated each of the expressed proteins. As shown in Fig. 3, however, an association between these receptor molecules was detected even in the absence of p12^{I}. The IL-2R β chain was coprecipitated with antibodies to γ_c as well as to the αγγ chimera (Fig. 3, lanes 4 and 10), and the immature form of αγγ was readily coprecipitated with antibodies to IL-2R β (Fig. 3, lane 5). This association did not seem to be a simple matter of receptor overexpression, because

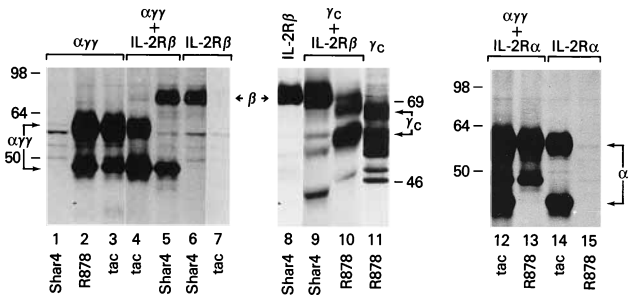

FIG. 3. Interaction of IL-2R β with γ_c and αγγ in HeLa/Tat cells. Transfections were performed as previously described with the DNA indicated above each lane. The antibodies used in the immunoprecipitation are shown below each lane. Molecular mass is given (in kilodaltons) to the side.

the IL-2R α chain was not coprecipitated with antibodies to the γ chain (Fig. 3, lane 13). This interaction does not appear to be a cell-specific event, because similar results were obtained in Cos-1 cells (data not shown). The biological relevance of this receptor interaction remains to be determined. In addition, to establish whether or not p12^{I} indeed affects IL-2R heterodimerization awaits the development of other experimental systems.

The p12^{I} protein binds to the immature form of the IL-2R β and αγγ chains. To better define the nature of the IL-2R β and γ_c forms that interact with p12^{I}, experiments were performed in the presence of tunicamycin, an inhibitor of N-linked glycosylation. HeLa/Tat cells expressing the IL-2R β chain alone or in combination with p12^{I} were cultured in the presence or absence of tunicamycin. Tunicamycin affected the glycosylation of the IL-2R β chain, which migrated faster on the SDS-PAGE gel (Fig. 4A, lanes 2 and 3). Association of the IL-2R β chain with p12^{I} was still detected in tunicamycin-treated cells (Fig. 4A and B, lanes 6 and 7). Similarly, in treated cells coexpressing p12^{I} and αγγ, p12^{I} bound to the faster-migrating form of the chimeric αγγ receptor (Fig. 4C and D, lanes 13 and 14). Thus, p12^{I} is able to bind to the immature, unglycosylated forms of IL-2R β and αγγ.

p12^{I} binds to and stabilizes the IL-2R β and chimeric αγγ chains in a pre-Golgi compartment. Endo-H hydrolyzes N-linked high mannose oligosaccharides which are added co-translationally in the endoplasmic reticulum. Glycoproteins such as the IL-2R β and γ_c chains traverse the Golgi apparatus, and additional processing of the oligosaccharides renders the glycoprotein resistant to cleavage by Endo-H. To investigate in which cellular compartment(s) the binding of p12^{I} to the IL-2R β and γ_c chains occurred, we tested the Endo-H resistance of the receptor molecules coimmunoprecipitated by p12^{I}. HeLa/Tat cells, cotransfected with p12^{I} and the β chain or p12^{I} and the αγγ chain, were metabolically labeled, and a cold amino acid chase was performed for 45, 90, or 180 min. As demonstrated in Fig. 5A, most of the β chain detected by the Mikβ1 antibody was resistant to Endo-H after a 3-h chase (Fig.

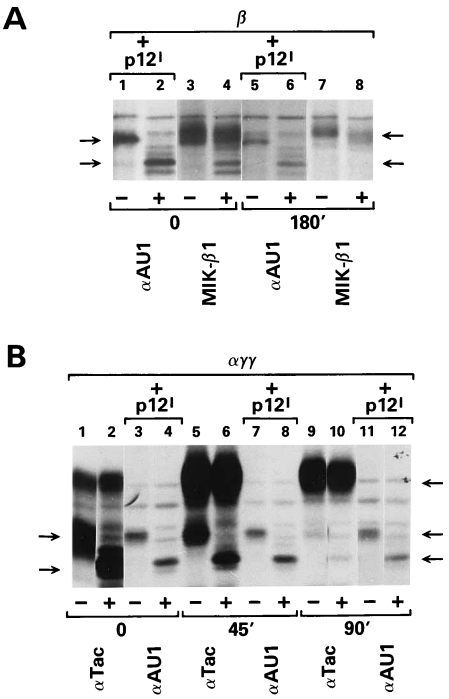

FIG. 5. Analysis of the Endo-H sensitivity of the IL-2R β (A) and αγγ (B) chains coprecipitated with $p12^I$. + and −, treatment with or without Endo-H, respectively. The genes expressed in each lysate are indicated at the top of each panel, and the antibodies used in each immunoprecipitation are indicated below each panel. The numbers shown at the bottom of the panels represent the nonradioactive chase period in minutes.

5A, compare lanes 4 and 8). However, the β chain coimmunoprecipitated with $p12^I$ was clearly sensitive to Endo-H cleavage, even after a 3-h chase (compare lanes 6 and 8). In the case of the αγγ chain, within 90 min, most of the αγγ molecules were resistant to Endo-H, as shown in Fig. 5B (compare lanes 1, 2, 5, 6, 9, and 10). In contrast, in the presence of $p12^I$, equivalent amounts of the Endo-H-sensitive αγγ chain were immunoprecipitated by the αAU1 antibody at each time point (Fig. 5B, lanes 3, 4, 7, 8, 11, and 12). These data indicate that both the IL-2R β and αγγ chains likely bind the $p12^I$ protein before reaching the Golgi apparatus and are retained in a pre-Golgi compartment. Furthermore, both IL-2R chains appear to be stabilized by this interaction with $p12^I$.

$p12^I$ disrupts the IL-2R β and αγγ trafficking and decreases their expression on the cell surface. The stabilization observed in the $p12^I$-IL-2R protein complex in a pre-Golgi compartment suggested that $p12^I$ might interfere with the normal trafficking of the receptor molecules to the cell surface. To investigate this hypothesis, each of the IL-2R chains was expressed with $p12^I$, and cell surface staining for the specific receptor was compared with staining in cells transfected with receptor alone. Flow cytometric analyses with the αCD25 antibodies for the α and αγγ chains and αCD122 for the β chain were performed with the transfected HeLa/Tat cells. As demonstrated in Fig. 6, surface expression of the α chain was not substantially affected by $p12^I$ expression in HeLa/Tat cells. In contrast, a statistically significant loss of surface expression of the β chain was observed (Fig. 6 and Table 1). In addition, surface expression of αγγ was noticeably affected by coexpression of $p12^I$ and, although the difference did not reach statistical significance (Table 1), it was suggestive of a downregulation trend of surface expression of the IL-2R αγγ chain. The

TABLE 1. Effect of $p12^I$ on surface IL-2R expression[a]

Surface receptor	Mean % loss ± SD[b]	95% confidence interval[c]
IL-2R α	10.3 ± 7.2	1.2–21.8
IL-2R β	47.0 ± 10.1	31.1–62.9
IL-2R αγγ	29.3 ± 9.5	13.7–40.3

[a] Values were derived from four independent experiments.
[b] Mean (± standard deviation [SD]) percent loss of surface receptor expression due to coexpression of the $p12^I$ protein.
[c] Confidence interval for each population mean.

numerical values reflecting these differences are shown in Table 1. The percentages of cells expressing $p12^I$ in the different samples were comparable (approximately 25%), and the amounts of p12 expressed per cell were also comparable, as shown in Fig. 6. Thus, the $p12^I$ protein binds the IL-2R β and γ_c chains in a pre-Golgi compartment, and presumably this interaction interferes with the trafficking of the receptor molecules to the cell surface.

DISCUSSION

A direct role for hematopoietic growth factor receptors in leukemogenesis has been demonstrated. For example, the spleen focus forming virus gp55 protein interacts with Epo-R, resulting in receptor activation and erythroleukemia even in the absence of the natural ligand (Epo) (64). This event appears to correlate with the constitutive activation of STAT-1 and STAT-3 observed in cells infected by spleen focus forming virus and grown in the absence of exogenous Epo (42). The gp55 interacts with the Epo-R in the endoplasmic reticulum (63, 64), and dimerization of the Epo-R by gp55 has been proposed as the mechanism for ligand independence induced by spleen focus forming virus (64). This hypothesis is further supported by the finding of natural Epo-R mutants which form homodimers and transform cells (57). In another retroviral model of oncogenesis, the B-cell leukemia induced by the Abelson murine leukemia virus, binding of v-*abl* and constitutive activation of the Jak1 and Jak3 kinases have been demonstrated (9). Similarly, in some HTLV-1-infected and transformed T-cell lines, ligand (IL-2) independence correlated with constitutive activation of STAT-3, STAT-5, and Jak3 (32, 59). Thus, interference with receptor signaling pathways by viral proteins appears to be a frequent event in retrovirus-transformed cells. Some DNA viruses also appear to use this mechanism. The bovine papillomavirus 1 E5 oncoprotein is thought to function in an analogous fashion by binding and activating the PDGF-R; this binding has been correlated with the ability of E5 to transform murine fibroblasts (7, 16, 39, 44, 45).

The data presented here suggest that the HTLV-1-encoded $p12^I$ protein might interfere with the normal IL-2-dependent proliferation by binding to and perhaps inducing heterodimer formation of IL-2R β and γ_c chains. In this regard, heterodimerization of the β and γ_c cytoplasmic domains has been shown to be necessary and sufficient for IL-2R signaling (37, 38). At present, we are unable to address directly this issue because of the interaction of IL-2R β and γ_c in the absence of p12 in the experimental system used. The development of additional reagents, such as antibodies specific for the wild-type, non-tagged $p12^I$, and retroviral vectors expressing high levels of $p12^I$ will help in addressing the interaction of p12 with

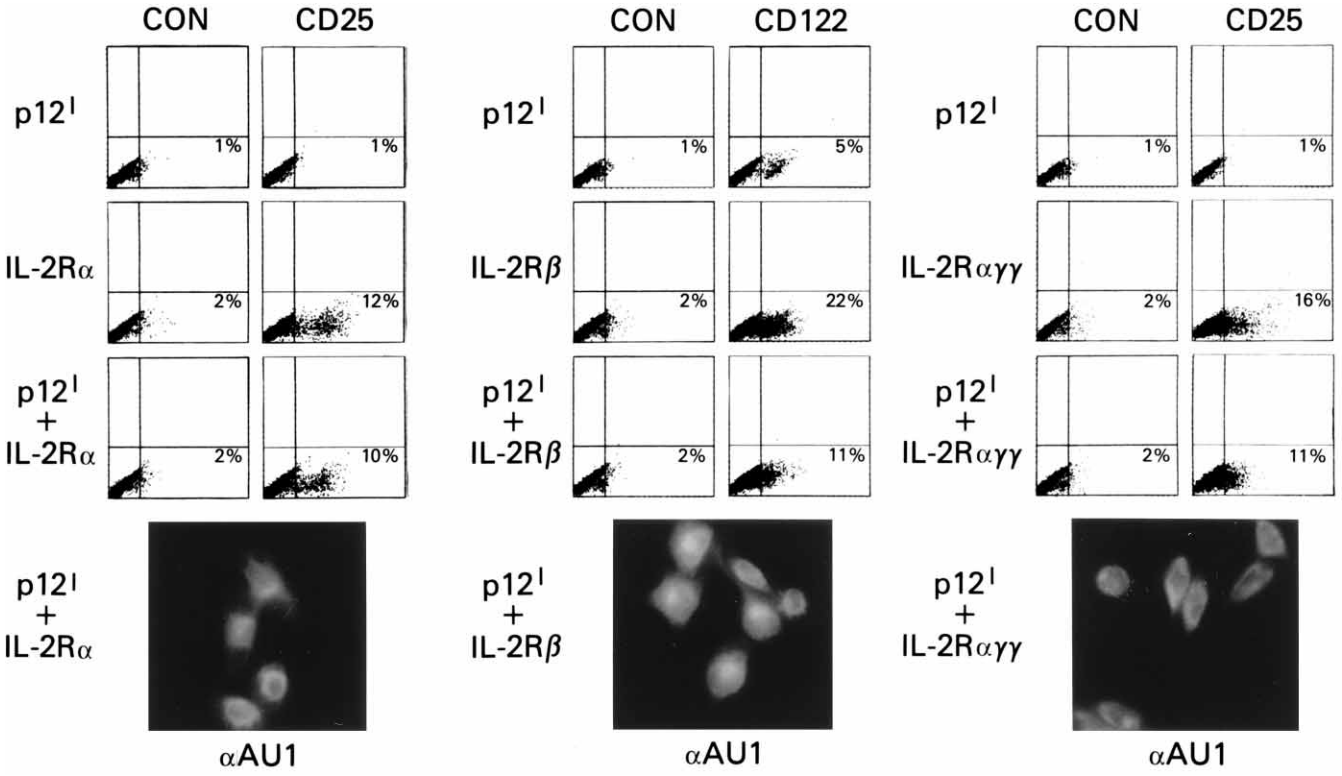

FIG. 6. Effect of p12^{I} on the surface expression of IL-2R chains. The proteins expressed in each sample are indicated for each dot plot. Fluorescence intensity is expressed linearly along the *x* axis (lower right panel). CON indicates staining of cells with an FITC-conjugated IgG antibody. For each cotransfection, the expression of p12^{I}, as detected by immunofluorescence with the αAU1 antibody, is shown below the panel.

IL-2R and its biological relevance in T cells, the natural target of HTLV-1 infection.

The downregulation of the IL-2R β and γ_c chains observed in vitro could correlate with a decrease in the responsiveness of HTLV-1-infected cells to normal antigen stimulation (4). If indeed HTLV-1 infection of T cells in vivo also induces downregulation of the IL-2R β and γ_c chains, this event could underlie the mild immunosuppression observed in HTLV-1-infected patients (3, 60). In addition, downregulation of the IL-2R β and γ_c chains would allow for an escape from the physiological regulation by exogenous ligand(s), including IL-2, IL-4, and IL-7. These events, in conjunction with alteration of cell cycle genes known to occur in HTLV-1-infected cells (5), may be crucial in determining T-cell transformation in vitro and perhaps in vivo as well.

As noted above, γ_c is also a functional component of the IL-4, IL-7, and IL-9 receptors (23, 25, 26, 40, 50), and both IL-2R β and γ_c are involved in the IL-15 receptor (18). What role, if any, the p12^{I}-γ_c receptor interactions may play in these other receptor systems is not known but warrants further study.

It has previously been demonstrated that two HTLV-1-encoded proteins, Tax and Rex, relate to the IL-2R system. Tax induces T-cell proliferation, in part by inducing IL-2 and IL-2R α expression (8, 21, 31, 52), whereas Rex increases IL-2R α by prolonging the IL-2R α mRNA half-life (22, 58). Here, we demonstrate that a third HTLV-1-encoded protein, p12^{I}, directly interacts with IL-2R β and γ_c. Since neither Tax and Rex nor p12^{I} mRNAs are detected consistently in the leukemic cells of ATL patients (2, 28), these HTLV-1 proteins might be important in the early stages of HTLV-1 leukemogenesis. Possibly, multiple oncogenic events are needed to determine the full leukemic phenotype observed in ATL patients. Recently, the stabilization of wild-type p53 (65) in HTLV-1-transformed T-cell lines (48) and p53 mutations in the leukemic cell DNAs of one-fourth of ATL patients (6) have been demonstrated, consistent with the occurrence of multistep oncogenic events. The enhanced proliferative capacity of HTLV-1-infected T cells in vivo could predispose to the accumulation of a number of genetic defects that can lead to the neoplastic phenotype.

ACKNOWLEDGMENTS

We are very grateful to R. C. Gallo for support; T. Waldmann, M. Tsudo, A. D. D'Andrea, and R. Schlegel for generosity in providing reagents; A. LoMonico, E. Boeri, and D. Goldstein for help with some of the experiments; A. Mazzuca and L. Anderson for editorial assistance; and Y. Nakamura and T.-S. Migone for valuable discussions.

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