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# Herpesvirus Saimiri Tip-484 Membrane Protein Markedly Increases p56$^{lck}$ Activity in T Cells

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**Herpesvirus saimiri (HVS) is a T-cell-specific transforming and oncogenic virus. A protein encoded by HVS known as Tip-484 (for tyrosine kinase interacting protein from HVS strain 484) is required for this transformation. Tip-484 binds specifically to the nonreceptor protein tyrosine kinase p56$^{lck}$. By transfecting Tip-484 into T cells, we now show that this interaction leads to a several hundred-fold increase in the kinase activity of p56$^{lck}$. Tip-484 is part of a protein complex which is dependent on the presence of p56$^{lck}$ and is phosphorylated. We also show that two of the complexed proteins represent two phosphorylated forms of Tip-484. Furthermore, the p56$^{lck}$ kinase activity in HVS-infected human peripheral blood T lymphocytes was at least ninefold higher than that in noninfected control cells and significantly decreased in cells infected with a Tip-484 deletion mutant virus. Finally, we report that Tip-484 is required for oncogenesis in rabbits by the survival of rabbits inoculated with Tip-484 deletion mutant HVS. The data demonstrate dramatic stimulation of the signaling pathway of p56$^{lck}$. This effect can contribute to the molecular mechanisms that lead to sustained autocrine secretion of growth factors, permanent T-cell growth, and ultimately lymphocytic tumor formation.**

Herpesvirus saimiri (HVS) naturally persists in squirrel monkeys without effect but is oncogenic when transferred to monkeys of other species. HVS is also highly oncogenic when introduced to New Zealand White rabbits. Both monkeys and rabbits die of polyclonal acute leukemias and lymphomas (20, 21). Furthermore, HVS has the ability to transform human peripheral blood T lymphocytes (PBLs) in vitro. HVS-transformed PBLs grow without the addition of interleukin-2 (IL-2) and without the need of periodic restimulation (18). An open reading frame of the HVS strain 484 genome termed orf-2 has previously been identified to be essential for transformation of human PBLs (18). orf-2 codes for a 32-kDa outer membrane protein which we have recently shown to interact with the tyrosine kinase p56$^{lck}$ (17).

p56$^{lck}$ is a lymphocyte-specific nonreceptor tyrosine kinase essential in the signaling pathway of the T-cell receptor for antigen (TCR) (for reviews, see references 3 and 7). p56$^{lck}$ may also be involved in transmitting signals from the CD4, CD8, and IL-2 β-receptor subunits (11, 23, 27). Early in TCR signaling, p56$^{lck}$ becomes activated (1, 2, 6). The mechanism of p56$^{lck}$ activation has yet to be determined. Activated p56$^{lck}$ phosphorylates tyrosine residues on the cytoplasmic portions of the CD3 and TCR-ζ subunits (14, 24, 26). Activated p56$^{lck}$ also shows increased autophosphorylation (2). The phosphorylation of CD3 and the TCR allows the recruitment of another signaling molecule, ZAP-70. ZAP-70 binds to the phosphorylated tyrosines via its SH2 domain to allow p56$^{lck}$ to phosphorylate and thereby activate ZAP-70 (8, 28, 29). Both ZAP-70 and p56$^{lck}$ are probably involved in other kinase events downstream of the TCR. The signaling pathways of phospholipase C, Ras, and protein kinase C are probably involved (reviewed in reference 3).

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A homolog of orf-2 that exists in HVS strain 488 codes for a protein referred to as Tip, for tyrosine kinase interacting protein, that has also been shown to interact with p56$^{lck}$ (4). The orf-2-expressed protein in HVS strain 484 is now referred to as Tip-484. We now show that Tip-484 dramatically increases the kinase activity of p56$^{lck}$ when transfected into Jurkat cells. An antiserum to Tip-484 brings down a complex of five proteins which are phosphorylated most likely through the action of p56$^{lck}$. Three of these proteins have been identified as p56$^{lck}$ and two different phosphorylated forms of Tip-484. We also show that the presence of Tip-484 in HVS-transformed human PBLs increases the activity of p56$^{lck}$ at least ninefold compared with control cells, and cells infected with a Tip-484-deleted virus showed a significant decrease in p56$^{lck}$ activity. Also, rabbits inoculated with Tip-484 deletion mutant HVS did not develop lymphomas, unlike those inoculated with wild-type HVS. These data provide substantial evidence that activation of p56$^{lck}$ by Tip-484 is essential in the transformation of lymphocytes by HVS strain 484.

## MATERIALS AND METHODS

**Cell lines and culture.** Jurkat T cells were maintained in RPMI 1640 (Gibco BRL, Grand Island, N.Y.) containing 10% fetal calf serum, 100 U of penicillin per ml, and 0.1 mg of streptomycin per ml. JCam cells were obtained from the American Type Culture Collection. JCam cells which have reconstituted p56$^{lck}$ expression (JCam/lck cells) were a generous gift of Arthur Weiss (University of California at San Francisco) (26) and maintained in growth medium with 250 μg of hygromycin per ml.

HVS-infected human PBLs were maintained in AIM V medium (GibcoBRL) containing 50 U of human recombinant IL-2 per ml.

**Antibodies.** Antibody to p56$^{lck}$ and the monoclonal antiphosphotyrosine antibody 4G10 were from Upstate Biotechnology, Inc. (UBI), Lake Placid, N.Y. Anti-Tip-484 is a polyclonal rabbit serum to a glutathione *S*-transferase fusion protein of Tip-484 (14). The anti-LPAP antibody was a generous gift of Burkhart Shraven (German Cancer Research Center, Heidelberg, Germany).

**Transient transfection and immunoprecipitation.** Transfection of cell lines was done by electroporation with plasmid DNA (20 μg). Parameters were 300 V, 960 μF, 3-s pulse, and $10^7$ cells in 0.5 ml of OPTIMEM (Gibco BRL). After transfection, the cells were resuspended in 5 ml of RPMI 1640. In some experiments, 10 μg of phorbol myristate acetate (PMA; Sigma) per ml and 2 μM ionomycin (Sigma) were added 24 h prior to harvest for promoter activation. At

48 h postelectroporation, $10^7$ cells were harvested and lysed in hypotonic lysis buffer (10 mM Tris, [pH 7.5] plus 1 mM $NaVO_4$, with protease inhibitors from one tablet of cOmplete [Boehringer Mannheim, Indianapolis, Ind.] per 50 ml of buffer). Cell membranes were prepared as previously described (16). Antibody to p56$^{lck}$ (UBI) or rabbit polyclonal antiserum to Tip-484 was used in immunoprecipitation using membrane extracts followed by protein A-Sepharose as described previously (16).

**In vitro kinase assay.** Immunoprecipitates were washed three times with lysis buffer, twice with 40 mM Tris (pH 7.5), and twice with kinase buffer (20 mM Tris [pH 7.5], 5 mM $MgCl_2$). Protein A beads were resuspended in 30 μl of kinase buffer, and 20 μCi of [γ-$^{32}$P]ATP was added. The kinase assay was performed for 15 min at 30°C. Beads were then washed twice and boiled in sodium dodecyl sulfate (SDS) loading buffer. Phosphorylated proteins were visualized on an SDS-polyacrylamide gel followed by autoradiography. Some autoradiographs were quantified by scanning on a densitometer (Bio-Rad 620).

Some immunoprecipitates were assayed with the Gibco BRL protein tyrosine kinase assay system as instructed by the manufacturer. Briefly, immunoprecipitation and washing were done as described above. Protein A beads were suspended in 25 μl of kinase buffer, the peptide was added at 0.5 mM along with 1 μCi of [γ-$^{32}$P]ATP, and the mixture was incubated at 30°C for 15 min. Protein A beads were pelleted, and the supernatant was spotted on phosphocellulose paper discs. The discs were incubated in 1% acetic acid twice for 5 min each time and then washed twice in water for 5 min each time. Radiolabeled discs were counted in scintillation cocktail.

## RESULTS

**Tip-484 increases the kinase activity of p56$^{lck}$.** To evaluate the effects of Tip-484 on p56$^{lck}$ kinase activity in T cells, the corresponding open reading frame from HVS strain 484 was cloned into an expression vector (pZeo; Invitrogen, San Diego, Calif.) under the control of the simian virus type 2 promoter element. Constructs were then transfected into Jurkat cells by electroporation. An in vitro kinase assay was performed on p56$^{lck}$ immunoprecipitates from membrane extracts 48 h postelectroporation. To ensure promoter function, some groups were stimulated with PMA and ionomycin 24 h prior to harvest. Figure 1A shows a complex of five phosphorylated proteins brought down by anti-p56$^{lck}$ only in the presence of transfected Tip-484. This complex was seen in stimulated as well as unstimulated cells. p56$^{lck}$ could be seen clearly only in stimulated control cells (pSV2 transfected). The unstimulated control cells (pSV2 transfected) showed significantly less p56$^{lck}$ activity which was visible only after long exposure (data not shown).

Figure 1B shows results of a similar experiment in which p56$^{lck}$ kinase activity was assayed with a synthetic peptide as the substrate (Gibco BRL protein tyrosine kinase assay system). The substrate is derived from the amino acid sequence surrounding the phosphorylation site in pp60$^{src}$ and is specific for tyrosine kinases (R-R-L-I-E-D-A-E-Y-A-A-R-G). With this system, immunoprecipitates were washed as before and resuspended in kinase buffer with the addition of a peptide substrate and [γ-$^{32}$P]ATP. Figure 1B shows that the expression of Tip-484 greatly enhances the ability of p56$^{lck}$ immunoprecipitates to phosphorylate the peptide substrate. These data corroborate those obtained from the kinase assay shown in Fig. 1A.

To see if a dose-response relationship existed between the amount of pSV2-Tip DNA transfected into Jurkat cells and kinase activity of p56$^{lck}$, various amounts of plasmid were used in experiments similar to those shown in Fig. 1. Different amounts of plasmid were transfected into Jurkat cells, and the cells were assayed 48 h later for p56$^{lck}$ in vitro kinase activity. The autoradiograph was scanned with a densitometer (Bio-Rad 620), and the amount of kinase activity was quantitated. Figure 2 shows that kinase activity increased several hundredfold almost linearly with increasing amounts of pSV2-Tip DNA transfected. This increase was seen with DNA amounts up to 60 μg (data not shown). Native p56$^{lck}$ was seen in empty vector controls after longer exposure (data not shown).

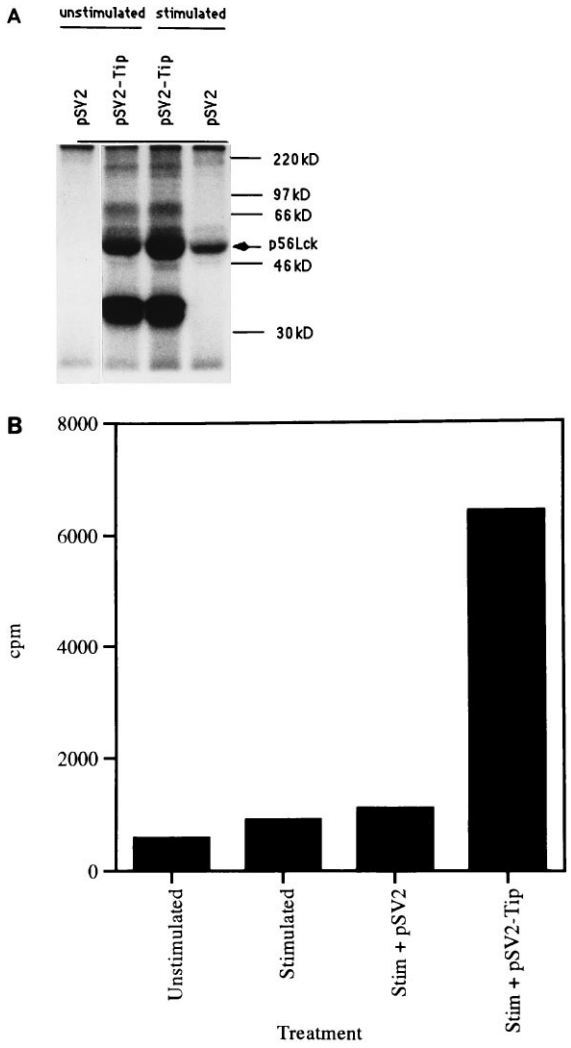


FIG. 1. Tip-484 induces protein kinase activity of p56$^{lck}$ and the phosphorylation of several proteins complexed with p56$^{lck}$ in Jurkat cells. (A) A total of $10^7$ Jurkat cells were transfected by electroporation with 20 μg of pSV2-Tip or control vector pSV2. After 24 h, cells were stimulated by 2 μM ionomycin (Sigma) and 10 μg of PMA (Sigma) per ml. At 48 h postelectroporation, cells were harvested and lysed in hypotonic lysis buffer. Membrane-bound proteins were prepared as previously described (14) and immunoprecipitated with an anti-p56$^{lck}$ antibody. Immunocomplexed proteins were used in an in vitro kinase assay with 20 μCi of [γ-$^{32}$P]ATP (see text). Labeled proteins were separated by SDS-PAGE and visualized by autoradiography. (B) p56$^{lck}$ immunoprecipitates were suspended in protein kinase buffer with 0.5 mM peptide substrate and 1 μCi of [γ-$^{32}$P]ATP for 15 min at 30°C. The supernatant was removed and spotted onto phosphocellulose, which was fixed, washed, and counted in scintillation cocktail. The graph is representative of one of several experiments. Stim, stimulated.

**p56$^{lck}$ is required for complex formation and phosphorylation.** Next, antiserum to Tip-484 was used in immunoprecipitation as was done with anti-p56$^{lck}$. Jurkat cells were transfected, and kinase was assayed as before. In this experiment, a rabbit polyclonal antiserum to Tip-484 (16) was used in some immunoprecipitations. Figure 3 shows that the same phosphorylated complex can be immunoprecipitated with either the anti-p56$^{lck}$ or anti-Tip-484 antibody. No bands were seen when preimmune serum was used as a control (data not shown). To confirm that this complex is dependent on the presence of p56$^{lck}$, we performed a similar experiment using a p56$^{lck}$-deficient T-cell line, JCam1. The JCam1 cell line is a derivative

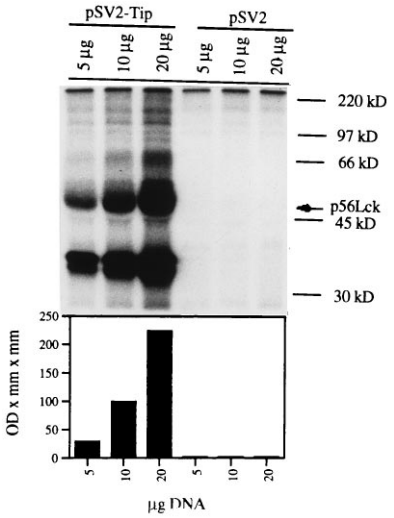


FIG. 2. Increased p56$^{lck}$ kinase activity in Jurkat cells is specific to Tip-484. A total of $10^7$ Jurkat cells were electroporated with 5, 10, or 20 μg of pSV2-Tip or control vector pSV2. After 48 h, cells were harvested and lysed in hypotonic lysis buffer. Membrane-bound proteins were prepared and immunoprecipitated with an anti-p56$^{lck}$ antibody as described in the text. Immunocomplexed proteins were used in an in vitro kinase assay with 20 μCi of [γ-$^{32}$P]ATP. Labeled proteins were visualized by SDS-PAGE. The autoradiograph was scanned with a densitometer and quantified. OD, optical density.

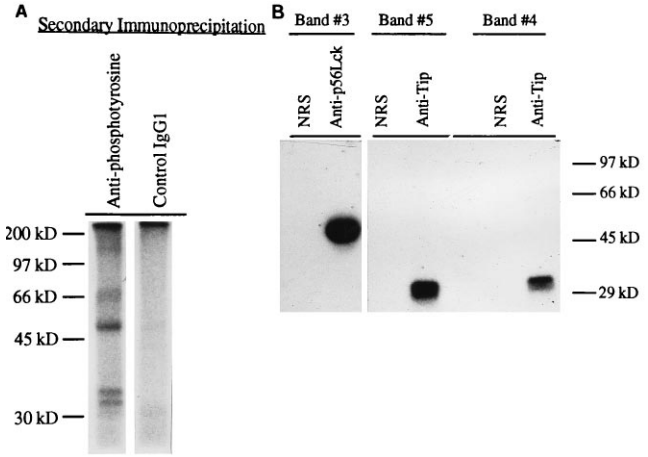


FIG. 4. Identification of p56$^{lck}$-complexed proteins phosphorylated on tyrosine residues. (A) In vitro protein kinase-radiolabeled Tip-484 immunoprecipitates were boiled in loading buffer, and the supernatant was diluted to 0.5 ml in lysis buffer with 0.5% Nonidet P-40. The antiphosphotyrosine antibody 4G10 was used in a second round of immunoprecipitation followed by proteinA-Sepharose. Labeled proteins were visualized by SDS-PAGE and autoradiography. IgG1, immunoglobulin G1. (B) Proteins were immunoprecipitated with p56$^{lck}$ and labeled with [γ-$^{32}$P]ATP protein kinase (see Fig. 3). After electrophoresis, bands were excised and electroeluted. Eluted proteins were then subjected to immunoprecipitation and resolved by SDS-PAGE and autoradiography. NRS, normal rabbit serum.

of the Jurkat cell line in which p56$^{lck}$ is not expressed due to a splicing defect (26). Figure 3 shows that in the absence of p56$^{lck}$, the anti-Tip-484 antiserum did not precipitate a complex and no phosphorylation took place. As expected, p56$^{lck}$ immunoprecipitates were also negative in JCam1 cells (data not shown). To check if Tip-484 was being expressed in the transfected JCam cells, a p56$^{lck}$-reconstituted JCam cell line (JCam/lck) was used in the same type of experiment. A similar level of Tip-484 was expressed as in the Jurkat cells, but p56$^{lck}$ was expressed at a much higher level in untransfected JCam/lck cells and it did show enhanced activity in the presence of

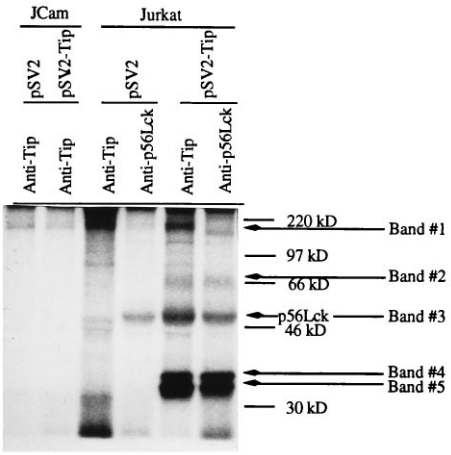


FIG. 3. Tip-484-induced p56$^{lck}$ activity requires the presence of p56$^{lck}$. A total of $10^7$ Jurkat or JCam1 (p56$^{lck}$-deficient) cells were electroporated with 20 μg of pSV2-Tip or control vector pSV2. At 48 h postelectroporation, cells were harvested and lysed in hypotonic lysis buffer. Membrane-bound proteins were prepared and immunoprecipitated with an anti-p56$^{lck}$ antibody or polyclonal anti-Tip-484 antibody as described in the text. An in vitro kinase assay was performed as described in the text. Labeled proteins were visualized by SDS-PAGE and autoradiography.

Tip-484 (data not shown). This overexpression of p56$^{lck}$ was probably due to the fact that the p56$^{lck}$ gene was encoded in an episomal vector driven by the long terminal repeat promoter and not its native promoter.

**Identification of complexed proteins.** A double immunoprecipitation was done to verify that the complexed proteins were phosphorylated on tyrosine residues as assumed if only p56$^{lck}$ is involved. After a radiolabeled in vitro kinase assay, Tip-484 immunoprecipitated complexes were released from antibody and protein A beads by boiling in SDS loading buffer for 5 min. The supernatant was removed and diluted in 0.5 ml of extraction buffer (hypotonic lysis buffer with 0.5% Nonidet P-40). Immunoprecipitation was then repeated with a second antibody, the antiphosphotyrosine antibody 4G10 (UBI) or a control antibody. Figure 4A shows that all five protein species could be precipitated with 4G10 and are therefore phosphorylated on tyrosine residues. This assay would not exclude phosphorylation on other amino acids such as serine or threonine.

To identify the complexed proteins, radiolabeled p56$^{lck}$ immunocomplexes were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and the specific bands were excised from the gel and electroeluted. Eluted proteins were analyzed by SDS-PAGE to ensure that they were quantitatively eluted from the gel and intact (data not shown). Immunoprecipitation experiments were done to identify the eluted proteins. Band 3 (Fig. 3) was specifically immunoprecipitated with antibody to p56$^{lck}$ as was expected (Fig. 4B). Initially, it was suspected that one of the bands (4 or 5) may be a protein known as LPAP, a 32-kDa protein found to form a complex with p56$^{lck}$ and CD45 in T cells (25). Therefore, immunoprecipitation was performed on bands 4 and 5 with an antiserum to Tip-484 and anti-LPAP. Only the Tip-484 antiserum was able to bring down both bands 4 and 5 independently (Fig. 4B). These lower bands probably represent two distinct phosphorylation states of Tip-484. This experiment shows that Tip-484 forms a complex with p56$^{lck}$ in vivo and that Tip-484 is probably a substrate for p56$^{lck}$. Bands

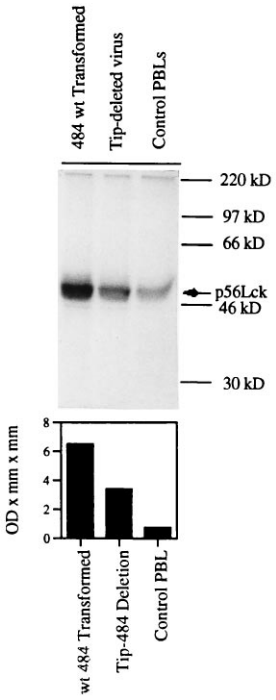


FIG. 5. Tip-484 increases p56$^{lck}$ activity in HVS-transformed human PBLs. Human PBLs were infected with either wild-type HVS strain 484 (484 wt) or a Tip-484 deletion mutant or remained uninfected (control). Four weeks after infection, $5 \times 10^7$ cells were harvested and lysed in hypotonic lysis buffer. Plasma membranes were prepared by differential centrifugation. Membrane-bound proteins were extracted in extraction buffer, protein concentration was measured, and equal amounts of protein were subjected to immunoprecipitation with anti-p56$^{lck}$. An in vitro kinase was performed on the immunocomplexed p56$^{lck}$ with 20 μCi of [γ-$^{32}$P]ATP at 30°C for 15 min. Labeled proteins were then loaded on an SDS–10% polyacrylamide gel and autoradiographed. The resulting autoradiograph was scanned with a densitometer, and the p56$^{lck}$ labeling was quantitated. OD, optical density.

1 and 2 have not yet been identified because these bands electroeluted poorly from the gel.

**Tip-484 increases p56$^{lck}$ activity in HVS-infected human PBLs.** To analyze the effect of Tip-484 on p56$^{lck}$ in HVS-transformed cells, an in vitro kinase assay was performed with p56$^{lck}$ immunoprecipitates from either wild-type-transformed human PBLs, human PBLs infected with a Tip-484 deletion mutant virus (18), or uninfected PBLs (control). Viral infection was confirmed by the presence of circular DNA by the method of Gardella et al. (10) (data not shown). p56$^{lck}$ activity was quantitated by scanning densitometry. Figure 5 shows that HVS strain 484-transformed cells have approximately ninefold-greater p56$^{lck}$ kinase activity than uninfected PBLs. In Tip-484 deletion mutant-infected cells, p56$^{lck}$ activity was significantly decreased but still higher than that of control cells. This result indicates that Tip-484 is involved but another element in the HVS strain 484 genome can also increase the activity of p56$^{lck}$.

**Tip-484 is required for oncogenic transformation in rabbits.** To evaluate the role of Tip-484 in oncogenesis, two 6-week-old New Zealand White rabbits were inoculated with $10^7$ PFU of Tip-484 deletion mutant virus (18) as described previously (19). These rabbits survived over the 3-year observation period and appear healthy and normal, while all three control animals infected with wild-type virus died of typical lymphomas within 30 days. These experiments show that Tip-484 is required for lymphocytic oncogenesis in the rabbit model.

## DISCUSSION

Our previous work showed that Tip-484 interacts with p56$^{lck}$ (17). We now show that this interaction leads to an increase in p56$^{lck}$ kinase activity as well as complex formation. Figures 1 and 2 show that when Tip-484 is expressed in Jurkat cells, p56$^{lck}$ kinase activity is enhanced dramatically. We rule out the possibility of the increase in activity being due solely to the expression of a foreign protein by the fact that both the empty vector and pSV2-Tip express a foreign protein for resistance to Zeocin, an antibiotic which can be used for selection purposes. Such a significant increase in kinase activity due to a viral protein interaction has not been shown before. The results from the tyrosine kinase assay using a peptide substrate agree with the increase seen in p56$^{lck}$ kinase activity. This assay does not rule the possibility of an additional tyrosine kinase immunoprecipitated with the complex.

Tip-484 induces a complex of at least five proteins which are phosphorylated. Formation of this complex requires the presence of p56$^{lck}$, as shown by the experiments done with the JCam cells. One of the complexed proteins was identified as p56$^{lck}$. The two lower-molecular-weight proteins were precipitated with anti-Tip-484. We believe that these two proteins represent two specific phosphorylated forms of Tip-484. It is unlikely that this observation is the result of degradation due to the presence of protease inhibitors in our lysis buffer and the distinctness of the bands. Also, these bands have been reproduced in more than 15 experiments. There is the possibility that additional proteins of similar sizes form complexes with Tip-484 which may coprecipitate.

The previous data along with the double immunoprecipitation with the antiphosphotyrosine antibody strongly suggest that the proteins in the complex mediated by p56$^{lck}$, a tyrosine kinase, and Tip-484 are phosphorylated on tyrosine residues by p56$^{lck}$. However, we recognize a number of alternative scenarios. First, another kinase could be present in the complex and may phosphorylate proteins on tyrosine residues. Second, the antiphosphotyrosine immunoprecipitation does not exclude the possibility that some of the proteins are phosphorylated on serine or threonine. If this is the case, it may also be possible that only one protein in the complex is tyrosine phosphorylated and that this protein is responsible for immunoprecipitation with the antiphosphotyrosine antibody should the complex reform after release from the first immunoprecipitation.

Using a Tip-484 deletion mutant virus, we showed that Tip-484 increases p56$^{lck}$ phosphorylation in HVS-transformed human PBLs. Unfortunately, we could not detect any complex in the human cells. This can be attributed to the very low levels of Tip-484 message expressed in the human cells (18). Although Tip-484 is expressed at very low levels in HVS-transformed human cells, a modest effect on p56$^{lck}$ activity was seen. This is expected given the tremendous increase seen when Tip-484 is overexpressed (Fig. 1 and 2). The wild-type virus-infected cells showed at least a ninefold increase in p56$^{lck}$ activity compared to uninfected cells. Deletion of Tip-484 correlated with significantly lower p56$^{lck}$ activity, but this activity still remained above that of uninfected cells. This increased activity over uninfected cells indicates that HVS probably has one or more other elements which could alter the p56$^{lck}$ signaling pathway. One such element may be STP (saimiri transforming protein). STP from HVS strain 488 has been shown to activate the *ras* pathway (12). A homologous protein, STP-484, also exists in strain 484. STP-484 is required for the transformation and the production of IL-2 in human PBLs infected with HVS strain 484 (9, 18). This cellular activation by STP may also directly activate p56$^{lck}$ or possibly allow indirect activation of p56$^{lck}$ by

the autocrine action of secreted IL-2 by HVS-infected lymphocytes.

Another herpesvirus, Epstein-Barr virus (EBV), also expresses a protein, LMP2a, which binds to a Src family kinase. LMP2a has been shown to bind stably to the Lyn kinase in B cells (5). p56$^{lck}$ did bind LMP2a in some EBV cell lines, but only weakly (5). Like Tip-484, LMP2a is constitutively phosphorylated, but in contrast, LMP2a downregulates the activity of the Lyn kinase (22). It is thought that this downregulation serves to maintain the latency of EBV in the B cell and prevent reactivation. Another major difference between Tip-484 and LMP2a is that LMP2a is not required for transformation (15), while previous data (18) and the data presented in this report show that Tip-484 is required for transformation by HVS.

There are two conflicting reports regarding the effect of Tip on p56$^{lck}$. Jung et al. report a downregulation of tyrosine phosphorylation in cells expressing HVS strain 488 Tip (Tip-488) (13), while Wiese et al. report a marginal activation of p56$^{lck}$ by Tip-488 (30). Our data agree with the general conclusions of the work of Wiese et al., who claimed an increase in p56$^{lck}$ activity in a cell-free system. Our data present evidence that this activation takes place in HVS-transformed cells in vivo. Moreover, our in vitro assays show that Tip-484 is a much more potent activator of p56$^{lck}$ than Tip-488. Sequence comparison of Tip-488 and Tip-484 shows duplication of a C-terminal sequence in Tip-488. This additional sequence predicts a larger protein, and this additional duplicated protein domain could interfere with protein kinase activity of p56$^{lck}$. Although Jung et al. report a decrease in tyrosine phosphorylation in cells expressing Tip-488 and also a decrease in ZAP-70 phosphorylation after CD3 stimulation, this does not exclude the possibility that other proteins may interact with Tip-488 and exert different effects on various signaling pathways.

In conclusion, we have shown that Tip-484 can substantially increase the kinase activity of p56$^{lck}$. Tip-484 also induces the formation of a complex of several proteins, including two phosphorylated forms of Tip-484. HVS strain 484-infected human PBLs display significantly higher p56$^{lck}$ activity than uninfected control cells. When Tip-484 is deleted from the virus, p56$^{lck}$ activity decreases. Also, rabbits inoculated with a Tip-484-deleted form of HVS do not succumb to lymphomas to which those inoculated with wild-type HVS succumb. These data provide substantial evidence for an essential role of Tip-484 in the transformation of T lymphocytes by HVS. To maintain a permanent state of transformation, Tip-484 must be present to induce the p56$^{lck}$ signaling pathway, which then allows infected T cells to reside in a continuous state of activation and thereby produce the factors necessary for long-term survival.

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