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# Functional Phenotype of Transformed Human αβ and γδ T Cells Determined by Different Subgroup C Strains of Herpesvirus Saimiri

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**Based on sequence divergence in the transformation-relevant region, herpesvirus saimiri strains are classified into three subgroups. Only members of subgroup C transform human T lymphocytes to continuous interleukin-2-dependent growth in culture. In this study, human cord blood T cells were immortalized by using different subgroup C strains (C488, C484, and C139). The resulting T-cell lines represented different types of T-cell clones. They were either $CD4^+$ or $CD8^+$ and expressed either the αβ or the γδ type of T-cell receptors. If transformed by the same virus strain, αβ and γδ clones were similar with respect to viral persistence, virus gene expression, proliferation, and Th1-type cytokine production. However, major differences were observed in T cells immortalized by different subgroup C strains. Strain C139 persisted at low copy number, compared to the high copy number of prototype C488. The transformation-associated genes *stpC* and *tip* of strain C488 were strongly induced after T-cell stimulation. The homologous genes of strain C139 were only weakly expressed and not induced after activation. After CD2 ligation, the C488-transformed T cells produced interleukin-2, whereas the C139-transformed cells did not. Correspondingly, the C139-transformed T cells were less sensitive to cyclosporin A. Sequence comparison from different subgroup C strains revealed a variability of the *stpC*/*tip* promoter region and of the Lck-binding viral protein Tip. Thus, closely related subgroup C strains of herpesvirus saimiri cause major differences in the functional phenotype of growth-transformed human T cells.**

Herpesvirus saimiri, a T-lymphotropic gamma 2-herpesvirus, is not pathogenic in its natural host, the squirrel monkey (Saimiri sciureus), but induces fulminant lymphoproliferation in other New World primates (25). Based on the sequence variation at one end of their nonrepetitive L-DNA, herpesvirus saimiri strains have been classified into three subgroups, A, B, and C (48, 49, 62). Attempts to transform human T lymphocytes in cell culture by using subgroup A and B strains have not been successful. We have shown that herpesvirus saimiri strain C488 transforms human $CD4^+$ and $CD8^+$ T cells to stable growth in vitro (4; reviewed in references 22 and 50). All immortalized T cells expressed the αβ type of T-cell receptors (TcR). They retain the surface phenotype and many functional properties of mature and activated T cells (4, 8, 24). While the antigen specificity of the T cells is preserved after transformation, the cytokine pattern shifts toward a Th1 profile (3, 8, 15, 65). Furthermore, the αβ T cells transformed by herpesvirus saimiri C488 are remarkably sensitive to ligation of their CD2 surface receptor. Since these T cells also express the CD2 ligand CD58/LFA3 on their surface, cell contact leads to engagement of CD2 and to autostimulation of the immortalized T cells (52). This is an important factor in the autocrine growth mechanism of the transformed T cells.

Recently, we and others have obtained permanently growing T cells expressing the γδ TcR after infection with herpesvirus saimiri strains C488 and C139 (39, 68). T cells express either αβ or γδ TcR heterodimers. The smaller γδ T-cell subpopulation plays an essential role in the defense against pathogens such as mycobacteria (reviewed in references 9 and 30). Their functional properties seem to differ greatly from those of conventional αβ T cells. For example, γ2δ2 T-cell clones did not recognize major histocompatibility complex (MHC)-bound antigenic peptides but recognized phospholipid moieties which are not presented via the classical MHC I or MHC II pathway (53, 63; reviewed in reference 9). Since it has been difficult to obtain sufficient numbers of γδ T cells for biochemical analysis from primary cultures, there is limited knowledge about signaling pathways in γδ T cells compared to αβ T cells. Whether γδ T cells are also rendered hypersensitive to CD2 ligation after transformation with herpesvirus saimiri is one of the subjects of this study.

In human T cells which are transformed to continuous growth by herpesvirus saimiri C488, virus gene expression is closely restricted. One viral bicistronic mRNA which is highly inducible in herpesvirus saimiri C488-transformed T cells in a similar way to T-cell activation genes (23) has been detected. A tyrosine kinase-interacting protein, termed Tip, is expressed at low levels from this transcript in transformed cells. Tip tightly associates with the T-cell-specific tyrosine kinase $p56^{lck}$, which plays a pivotal role in T-cell signaling (6). TipC488 strongly activated Lck both in a cell-free system and in Tip-transfected T cells as well as in insect cells (35, 66). When overexpressed in Jurkat cells, however, Tip inhibited Lck signaling (36). The same transcript encodes the saimiri transformation-associated

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protein of subgroup C virus strains (StpC), a phosphoprotein which associates with cellular Ras. *stpC* causes oncogenic transformation of rodent fibroblasts and epithelial cells as an isolated gene under heterologous regulation (31–34, 37, 56). StpC occurs abundantly in C488-transformed human αβ T cells and is highly induced after T-cell activation (23). The *stpC/tip* gene is located in a genomic region highly divergent among different strains of herpesvirus saimiri and correlating with transforming capacity (1, 5, 49). Deletion mutants of the respective region of strain A11 were nonpathogenic and did not transform T cells (18–20, 40, 57). In a subgroup C strain, both terminal reading frames were necessary for short-term T-cell transformation (46). All of these reports underscore the relevance of StpC and Tip for the transformation of human T cells. The question arose as to how minor sequence differences in the *tip* and *stpC* genes as they occur in different subgroup C strains of herpesvirus saimiri may influence the transformation efficiency of the viruses and the functional properties of the transformed cells. Systematic evaluation of a series of 18 αβ and γδ T-cell clones transformed by herpesvirus saimiri strains C488, C484, and C139 revealed different numbers of persisting viral episomes, different degrees of hyperreactivity to CD2 ligation, and different expression levels and regulation of *stpC/tip*. Surprisingly, αβ and γδ T cells showed similar features in all of these aspects, provided they were transformed with the same virus isolate. Functional differences correlated with the virus strain used for T-cell transformation.

## MATERIALS AND METHODS

**Cells and cell culture.** Virus cultures were performed according to standard protocols (22). Infectious supernatants were titrated to allow efficiency comparison of various virus strains. Owl monkey kidney cells (OMK-637, ATCC CRL 1556) (13) were kept in Dulbecco modified Eagle medium with 10% fetal bovine serum and passaged once weekly by a factor of 2 to 3 relative to the flask surface area. The standard transformation procedure (22) was adapted to small cell numbers. Human cord blood mononuclear cells were isolated by density centrifugation, seeded at low density ($10^4$ cells/well) in round-bottom wells, and preincubated for 12 h at 37°C and 7.5% $CO_2$ in 50 μl of complete CG-RPMI medium (45% CG medium [Vitromex, Vilshofen, Germany], 45% RPMI 1640, 10% screened fetal bovine serum [Biochrom, Berlin, Germany]). The cultures were then infected with 50 μl of owl monkey kidney cell supernatant containing $10^6$ tissue culture infectious units of herpesvirus saimiri strain C488, C484 (17), or C139 (39) per ml. The infected cells were incubated in CG-RPMI medium with regular changes of medium but without further stimulation and in the absence of exogenous interleukin-2 (IL-2). Growth transformation in some of the wells was obvious after about 2 months, when the uninfected controls and the cells from more than 90% of the infected wells had died. The cells from up to 10% of the wells were then grown in culture with doubling times of between 2 and 7 days. After about 6 months of cultivation, the proliferation rates decreased. Therefore, IL-2 was included at a final concentration of 30 to 50 U/ml (Proleukin; Chiron, Ratingen, Germany), which restored growth rates. Two experiments, A and B, using cord blood from two newborns were performed with comparable results.

CB-15 is a $CD4^+$ αβ T-cell line derived from cord blood (4), and 3C is a $CD8^+$ polyclonal αβ T-cell line obtained from peripheral blood (24, 66) after infection with herpesvirus saimiri C488. P-1084 is a $CD8^+$ lymphoblastic αβ line transformed with strain C484 (4). The γδ T-cell line A139.1 has been analyzed before for IL-12-mediated signal transduction and was originally called 150-139-5.3 (39). L248 cells are derived from a human Hodgkin's lymphoma and express CD58/LFA3 at high density. A20 is a murine B-cell lymphoma line which expresses high levels of Fc receptors for immunoglobulin G (IgG). CTLL is an IL-2-dependent murine T-cell line. The T-leukemic cell line Jurkat (61) and the Epstein-Barr virus-free B-cell line BJA-B (38) were used for transient transfection experiments.

**Antisera and monoclonal antibodies.** The surface phenotype of transformed T cells was analyzed by either direct or indirect staining with commercially available murine monoclonal antibodies in the presence of sodium azide and excess human Ig. The antibodies were directed against TcR αβ (WT31 and BMA 031), TcR γδ (11F2), Vβ3 (8F10 and LE89), Vβ14 (CAS1.1.4), Vγ9 (IMMU 350), Vδ1 (03/1962), Vδ2 (IMMU 389), Vδ3 (P11.5B), CD2 (TS2/18.1.1), CD3 (αLeu-4, SK7), CD4 (αLeu-3a, SK3), CD8 (αLeu-2a, SK1), MHC class I (W6/32), MHC class II (HLA-DR, L243), LFA1β (TS1/18.1.2.11.4), ICAM1 (GP89-14), CD25 (2A3), CD30 (Ki-1), CD45 (αHle-1, 2D1), CD56 (αLeu-19, MY31), CD58/LFA3 (TS2/9.1.1.4.3), and CD69 (αLeu-23, L78) and were obtained from the American Type Culture Collection (Rockville, Md.), Becton Dickinson (Heidelberg, Germany), Dianova (Hamburg, Germany), Immunotech (Marseille, France), or T Cell Sciences (Cambridge, Mass.). BMA 031 was provided by R. Kurrle (Marburg, Germany), and GP89-14 was provided by J. Johnson (Munich, Germany). A fluorescein isothiocyanate-labeled rabbit anti-mouse $F(ab')_2$-specific IgG antibody (Dianova) was applied as the secondary reagent. Fluorescence analysis was performed with a FACStrak flow cytometer (Becton Dickinson). Antisera to the unique regions of Src-related kinases were donated by J. B. Bolen (DNAX, Palo Alto, Calif.) and raised by immunizing rabbits with fusion proteins containing the unique regions of murine $p56^{lck}$, $p60^{fyn}$, $p53/56^{lyn}$, and glutathione *S*-transferase (GST) (42). A rabbit antiserum against GST-StpC488 fusion protein was kindly provided by A. Tsygankov (Philadelphia, Pa.) and used for immunoblotting. Another polyclonal rabbit antiserum against an N-terminal peptide of StpC488 (CASEPNLRYPIEETG) (23) was used for immunoblotting and for intracellular staining. For that purpose, herpesvirus saimiri-transformed lymphocytes were fixed with 2% formaldehyde (10 min), permeabilized with 0.5% saponin (30 min), and incubated with the 1:2,000-diluted StpC rabbit antiserum and with a phycoerythrin-labeled goat $F(ab')_2$ anti-rabbit IgG secondary antibody (Caltag, South San Francisco, Calif.). CB219 is a murine monoclonal antibody directed against the T11.1 epitope of human CD2 (26).

**Cell proliferation and cytokine secretion.** For determination of cell proliferation, $2 \times 10^4$ cells were incubated in the presence or absence of cyclosporin A (Sandoz, Basel, Switzerland) for 48 h in 200 μl of CG-RPMI medium in round-bottom wells. [$^3$H]thymidine (0.2 μCi/well) was then added for further 16 h. Cells were harvested, and thymidine incorporation was determined by liquid scintillation counting on a beta-plate β counter (Pharmacia/LKB, Freiburg, Germany). For the measurement of cytokine production, $5 \times 10^4$ T cells were incubated in 200 μl of CG-RPMI medium in flat-bottom wells. Antibodies and $5 \times 10^4$ stimulator cells were added to the wells as indicated, and supernatants were harvested after 24 h of culture. To determine the amount of IL-2, 50 μl of culture supernatant was added to 100 μl of a suspension of the indicator cell line CTLL ($2.5 \times 10^4$ cells/ml). After 12 to 24 h, 0.2 μCi of [$^3$H]thymidine was added for further 24 h and thymidine incorporation was determined as described above. Standard error was less than 10% except for very small values. Gamma interferon (IFN-γ) was measured with an enzyme-linked immunosorbent assay (ELISA) system. Flat-bottom 96-well plates (Maxisorb; Nunc, Roskilde, Denmark) were coated with 50 μl of mouse anti-human IFN-γ monoclonal antibody GZ-4 (5 μg/ml; Boehringer, Mannheim, Germany) in carbonate buffer (pH 9.6) overnight at 4°C. Plates were then washed and blocked with 150 μl of phosphate-buffered saline containing 20% fetal bovine serum for 2 h at room temperature (RT) and then incubated with 50 μl of IFN-γ standard or samples diluted appropriately in blocking buffer for an additional 2 h at RT. After washing, the plates were incubated with 50 μl of rabbit anti-human IFN-γ serum (Genzyme, Cambridge, Mass.) diluted 1:5,000 in block buffer for 1 h at RT, washed again, and incubated with 50 μl of goat anti-rabbit Ig coupled to horseradish peroxidase (DAKO, Hamburg, Germany) diluted 1:2,000 in block buffer for 1 h. Plates were washed and incubated with 100 μl of peroxidase substrate solution (0.1 M phosphate buffer [pH 5.5] containing 10 μg of 3,5,3′,5′-tetramethylbenzidine [Sigma, Deisenhofen, Germany] per ml and 0.03% $H_2O_2$) for 20 min before the reaction was terminated by the addition of 25 μl of 2 M $H_2SO_4$. Optical densities were determined at 450 nm on a Dynatech ELISA reader. Measurements were performed in duplicate. IL-4 was also determined by ELISA, using a similar protocol. Plates were coated with 1 μg of mouse anti-human IL-4 antibody (Dianova) per ml in carbonate buffer. After blocking and washing, standards and samples were applied. Bound IL-4 was detected by incubation with 0.5 μg of mouse anti-human IL-4 conjugated to biotin (Dianova) per ml followed by incubation with streptavidin coupled to horseradish peroxidase diluted 1:10,000. The color reaction was read as described above for IFN-γ.

**Molecular biology.** DNA from virus particles was isolated as described previously (22). Molecular cloning procedures were performed according to standard techniques (2). Plasmid DNA and purified PCR products were sequenced by using the computer-assisted dye dideoxy terminator method (ABI, Weiterstadt, Germany). To demonstrate persisting virus DNA in transformed cells, synthetic primers were used to amplify viral *stpC* DNA fragments of strain C488 (N terminal, 5′-GAGTTTCCAAAATGTACTAAGCTAAC-3′; C terminal, 5′-ACTAATAAAAAGTTCCACACAACTAAC-3′) and of strain C139 (N-terminal, 5′-GAGTTTCCAAAATGTAGTAAGCTAAC-3′; C-terminal, 5′-ACTAATCAAAAGTTCCACACAACCAAC-3′). As an internal control, a 110-bp DNA fragment of cellular β-globin was amplified by using primers 5′-ACACAACTGTGTTCACTA-GC-3′ and 5′-CAACTTCATCCACGTTCACC-3′. Linear virion DNA and nonintegrated episomal DNA forms were analyzed by in situ lysis Gardella gel electrophoresis and subsequent Southern hybridization with a random-primed $^{32}$P-labeled probe representing the *stpC* open reading frame (21, 22, 27). RNA was purified from tissue culture cells (11) and subjected to Northern hybridization. The stringently washed filters were exposed and scanned on a BAS2000 imaging system, using the TINA2.0 software (Fuji, Raytest, Straubenhardt, Germany). Luciferase expression constructs were electrotransfected (Genepulser; Bio-Rad, Munich, Germany) into Jurkat and BJA-B cells (38, 61). Luciferase extracts were prepared and analyzed according to standard protocols (2).

**Immunoprecipitations and in vitro phosphotransferase assay.** Cells were incubated in the presence or absence of the phorbol ester TPA (12-*O*-tetradeca-

TABLE 1. Surface phenotype of transformed T cells

| Cell line | TcR type | Vβ or Vδ type | CD type |
|---|---|---|---|
| A488.1 | αβ | β3 | 4 |
| A488.2 | αβ | β14 | 4 |
| A488.3 | αβ | ND[a] | 4 |
| B488.1 | αβ | ND | 8 |
| B488.2 | αβ | ND | 8 L[b] |
| B488.3 | γδ | δ1 | 8 |
| B488.4 | γδ | δ1 | 8 |
| B484.1 | γδ L | δ1 L | 8 |
| A139.1 | γδ | δ1 | 8 L |
| A139.2 | γδ | δ1 | 8 L |
| A139.3 | αβ | ND | 4 |
| A139.4 | γδ | δ1 | 8 |
| B139.1 | αβ | ND | 8 L |
| B139.2 | αβ | ND | 8 L |
| B139.3 | αβ | ND | 8 |
| B139.4 | γδ | δ1 | 8 |
| B139.5 | γδ | δ1 | 8 |
| B139.6 | γδ | ND | 8 L |

[a] ND, not determined.
[b] L, low intensity.

noylphorbol-13-acetate; 2 ng/ml; Sigma) at 37°C for the time indicated. They were then washed once in serum-free medium and lysed in TNE buffer (50 mM Tris [pH 8.0], 150 mM NaCl, 2 mM EDTA, and 1% Nonidet P-40) supplemented with 1 mM sodium orthovanadate, 5 mM NaF, and 10 μg each of aprotinin and leupeptin (Sigma) per ml at a density of $1 \times 10^7$ to $2 \times 10^7$ cells/ml for 20 min on ice. Lysates were cleared at 14,000 × *g* for 5 min, and the protein concentration in the supernatants was determined. Five microliters of antiserum/mg of protein was added for at least 1 h at 4°C to precipitate Src-related kinases, followed by incubation with 50 μl of a 10% (vol/vol) suspension of *Staphylococcus aureus* particles (Pansorbin; Calbiochem, Bad Soden, Germany). The immunoprecipitates were washed five times in TNE buffer and once in kinase buffer (20 mM morpholinepropanesulfonic acid [MOPS; pH 7.0], 5 mM $MgCl_2$). The pellets were then incubated for 5 min at RT in 25 μl of kinase assay mix containing 1 μM ATP (Boehringer) and 10 μCi of [γ-$^{32}$P]ATP (Amersham, Braunschweig, Germany) in kinase buffer. The phosphotransferase reaction was stopped with sample buffer (62.5 mM Tris [pH 6.8], 2% sodium dodecyl sulfate [SDS], 10% glycerol, 5% β-mercaptoethanol). Samples were then incubated for 30 min at RT and centrifuged. Supernatants were denatured at 95°C for 5 min before separation by SDS-polyacrylamide gel electrophoresis (PAGE) on 8% polyacrylamide gels. Gels were dried and exposed to Kodak XAR5 film or to Fuji imaging screens.

**Anti-StpC immunoblots.** For immunoblotting, cell lysates (50 μg/lane) were separated by SDS-PAGE (12% polyacrylamide gels) and transferred to nitrocellulose membranes. Blots were incubated for 1 h at RT in blocking buffer (phosphate-buffered saline [pH 7.4], 5% bovine serum albumin, 0.5% Tween 20) followed by incubation with anti-StpC rabbit serum diluted 1:1,000 in blocking buffer. After thorough washing in phosphate-buffered saline containing 0.5% Tween 20, blots were incubated with 1 μCi of $^{125}$I-protein A (Amersham) per ml in blocking buffer for at least 1 h, washed again, dried, and exposed to Kodak XAR5 film. Quantitative analysis was performed on a PhosphorImager (Molecular Dynamics, Krefeld, Germany).

**Nucleotide sequences.** The transformation-associated terminal L-DNA sequences of herpesvirus saimiri strains C139 and C484 are available from the EMBL data library under accession numbers X99518 and X99519.

## RESULTS

**Immortalization of human T cells by different subgroup C strains of herpesvirus saimiri.** In contrast to uninfected controls, initial growth stimulation was regularly observed after infection of low numbers ($10^4$) of cord blood mononuclear cells with herpesvirus saimiri strains C488, C484, and C139 at the same titer ($10^6$/ml). However, stable transformation by herpesvirus saimiri C488 and C139 was achieved only in about 5 to 10% of these cultures, corresponding to a transformation efficiency of 0.5 to 1 in $10^5$ cells. Infection with virus strains C484 was even less efficient and resulted in only one permanently growing culture (B484.1) out of 120 infected samples.

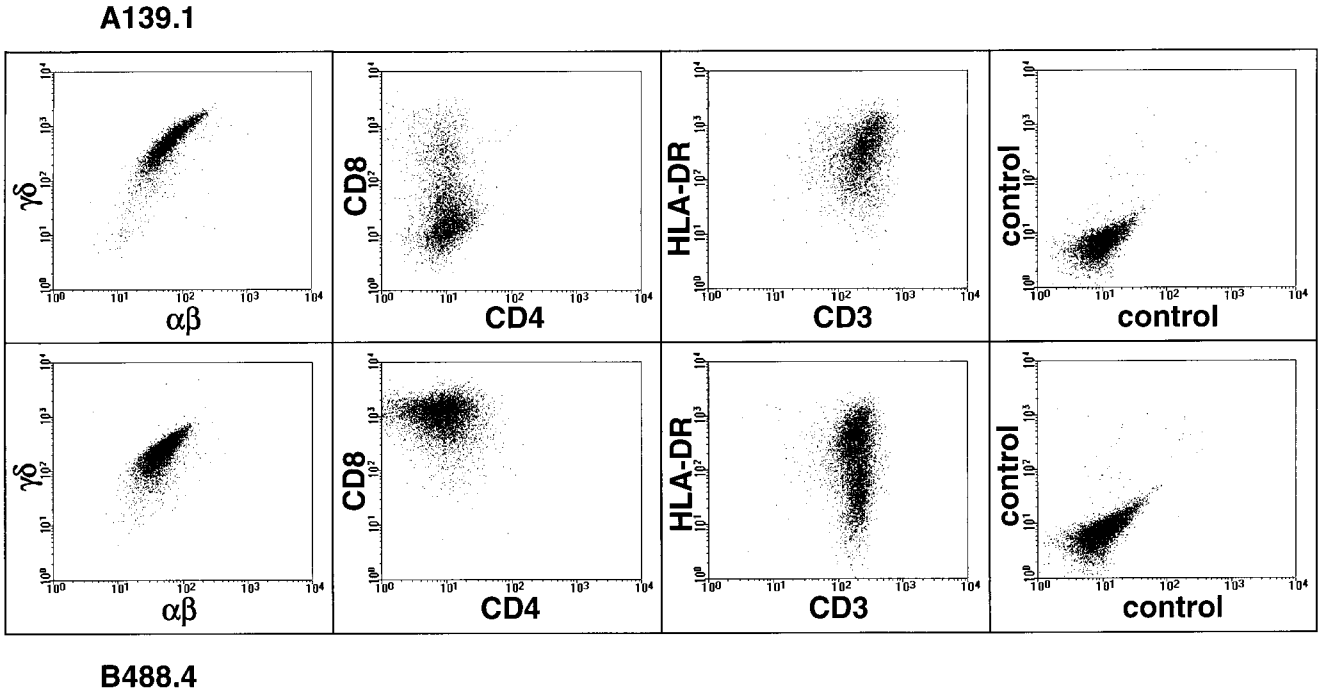


FIG. 1. Examples of surface phenotypes of two transformed γδ T-cell lines. The γδ cell lines A139.1 and B488.4 were subjected to direct two-color flow cytometry. The *x* and *y* axes show stainings with fluorescein isothiocyanate-labeled antibodies and phycoerythrin-labeled antibodies, respectively, both at logarithmic scale. There is clear staining for γδ TcR. The weak staining with the anti-αβ TcR monoclonal antibody WT31 is due to cross-reaction with CD3 in activated αβ-negative T cells (59). Another monoclonal antibody directed against αβ TcR (BMA 031) did not stain these T-cell clones (data not shown). The transformed γδ T cells carry either high or intermediate to low amounts of CD8 molecules. All transformed T cells were positive for CD3 and HLA-DR. Isotype-matched directly labeled antibodies were used as controls.

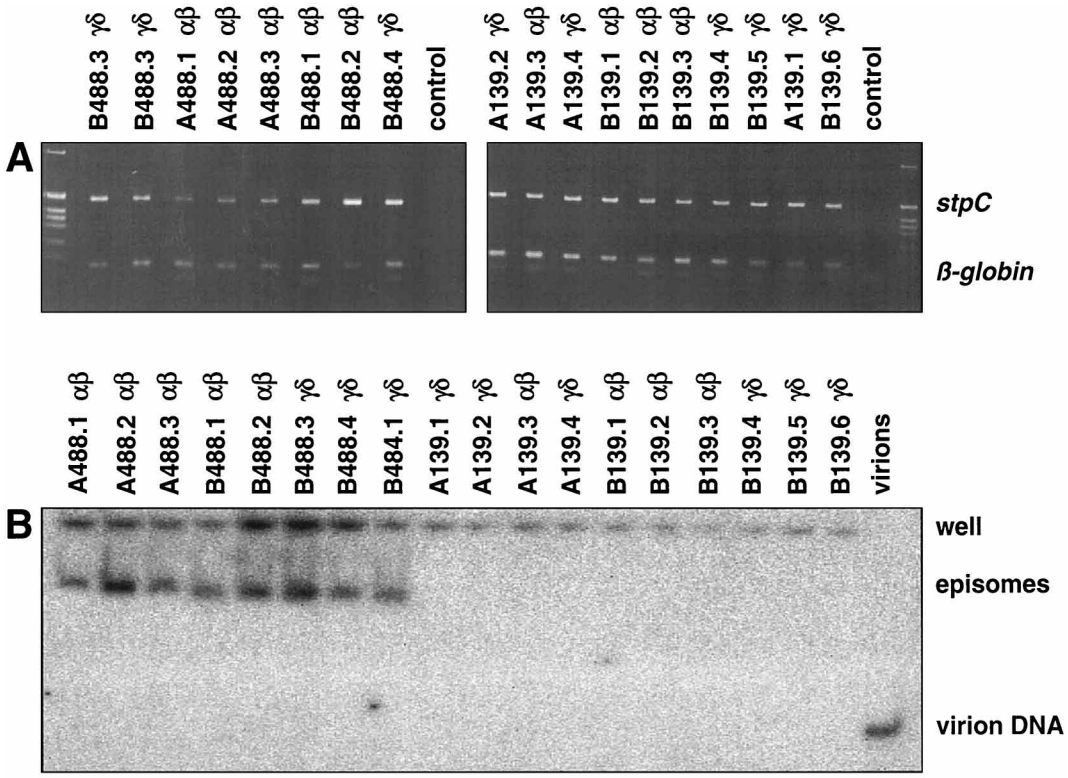


FIG. 2. Persistence of virus DNA in transformed T cells. (A) Presence of viral DNA from the *stpC* region in transformed T cells. Due to sequence variation, separate pairs of primers were used to amplify *stpC* DNA fragments of strains C488 (490 bp) and C139 (482 bp). C488 primers did not allow efficient amplification of C139 DNA, and vice versa. As an internal control, a 110-bp cellular β-globin DNA fragment was amplified. To analyze the state of virus persistence, Gardella in situ agarose gel electrophoresis was performed, followed by Southern hybridization with an *stpC*-specific $^{32}$P-labeled probe (B). T-cell lines transformed by strains C488 and C484 harbor exclusively nonintegrated episomes at high copy number. In contrast, only faint episomal bands appeared after long exposure in C139-transformed T cells.

Since growth was observed in only 5 to 10% of C488- and C139-infected wells, most likely the resulting T-cell lines were of clonal origin. This was confirmed by the mutually exclusive expression of αβ or γδ TcR in the different T-cell lines (Table 1). Moreover, in two cases the Vβ families could be identified by monoclonal antibodies. The T-cell line A488.1 was stained homogeneously for Vβ3, and A488.2 was stained for Vβ14. All γδ T-cell lines expressed Vδ1 (but neither Vδ2 nor Vδ3) except for cell line B139.6, which was negative for Vδ1, Vδ2, and Vδ3. No T-cell line expressed Vγ9. Cell line B484.1, which was immortalized by herpesvirus saimiri C484, had an unusual phenotype: CD3 expression was very low and the cells carried low amounts of Vδ1-positive and Vγ9-negative TcRs (data not shown). The characteristics of all stably growing T-cell lines investigated in this study are summarized in Table 1. All T-cell lines expressed either CD4 or CD8. Surface density of CD8 was either uniformly high or variable to low, as depicted for two representative γδ T-cell lines in Fig. 1. In addition, MHC class I, MHC class II, CD2, CD3, LFA1β, ICAM1, CD25, CD30, CD45, CD56, CD58/LFA3, and CD69 were regularly expressed on the cell surface.

In all cell lines, the presence of viral DNA was demonstrated by PCR with primers from the *stpC* region (Fig. 2A). Due to sequence variations, two separate pairs of primers were used to detect virus DNA. In cell lines transformed by herpesvirus saimiri C488 and C484, viral episomes were observed at high copy number as usual for herpesvirus saimiri-immortalized human T cells. In contrast, episomal signals were hardly detectable in T cells transformed by herpesvirus saimiri C139 (Fig. 2B), although virus DNA had been demonstrated by PCR with C139-specific primers (Fig. 2A). There was no indication of virus production from the transformed T cells. Linear virion DNA was not detectable in Gardella analysis (Fig. 2B), and repeated attempts to isolate virus from the transformed human T-cell cultures by cocultivation with permissive owl monkey kidney cells were not successful.

**T cells transformed by herpesvirus saimiri C488 and C139 differ in their responses to CD2 ligation.** For maximal stimulation, T cells were incubated with phytohemagglutinin (PHA) in the presence of L428 accessory cells. Under these conditions, most T-cell lines produced large amounts of IL-2 (5 to 200 U/ml) and IFN-γ (50 to 900 ng/ml), with no significant differences between αβ or γδ T cells or between cells transformed by herpesvirus saimiri C488 and C139 (Fig. 3, upper panels). Cell line A139.4 did not produce IL-2, and cell lines A139.3, B484.1, and B139.1 did not secrete IFN-γ. Only cell line B139.5 was able to produce low amounts of IL-4. This means that in both αβ and γδ T cells, the Th1-like cytokines IL-2 and IFN-γ predominated after transformation by herpesvirus saimiri. $CD4^+$ and $CD8^+$ cells secreted comparable amounts of these cytokines. Next, we examined the response of the transformed T cells to ligation of CD2. This was achieved by the CD2 ligand CD58/LFA3, which is expressed on L428 cells, or by cross-linking the anti-CD2.1 monoclonal antibody CB219 on Fc receptors expressed on A20 cells. αβ and γδ T cells which were immortalized by herpesvirus saimiri C488 responded to these stimuli with the secretion of large amounts of IL-2. The IL-2 production induced by L428 cells was completely abolished by blockade of CD2/CD58 interaction with anti-CD2 antibodies, underscoring the essential role of CD2

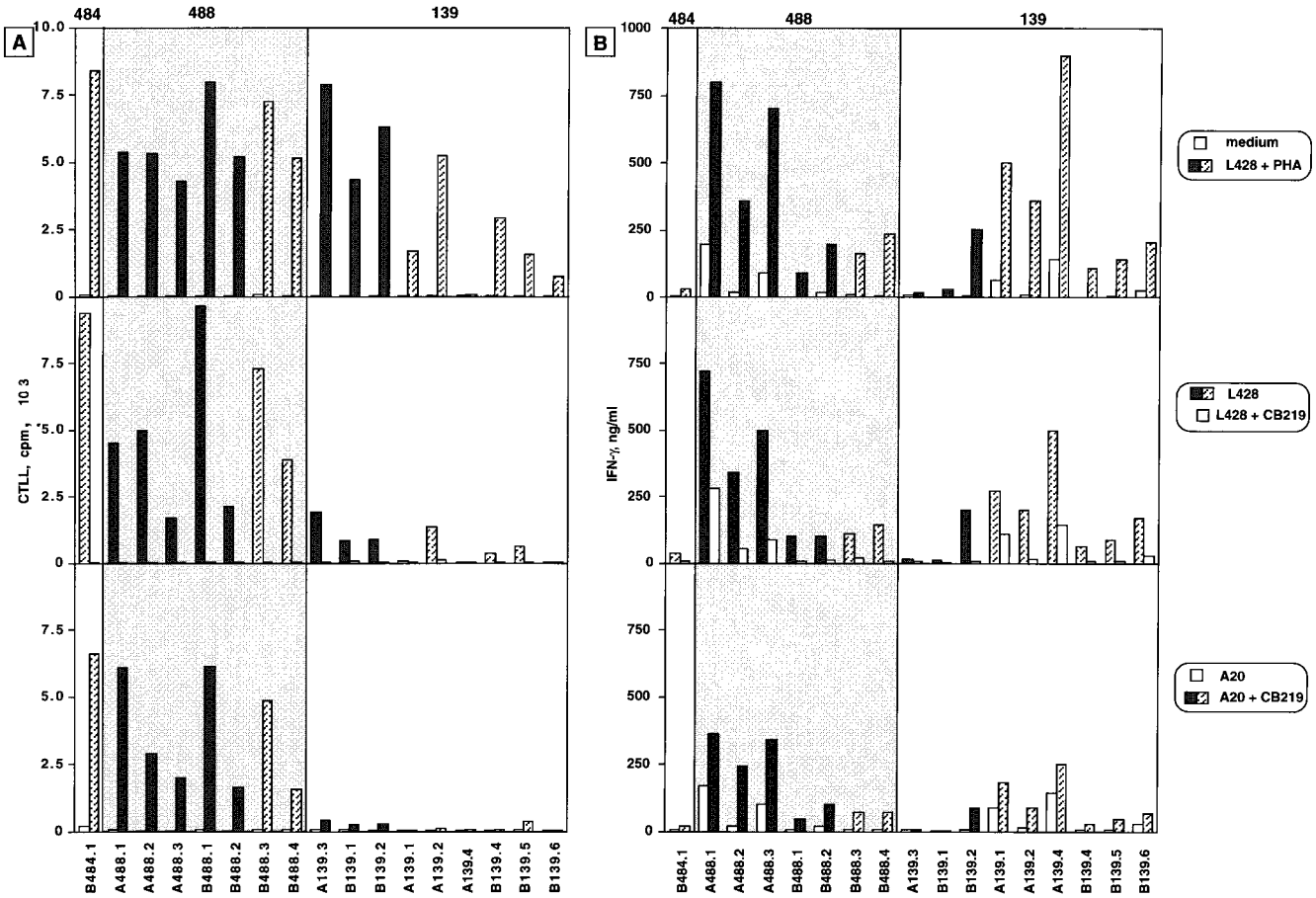


FIG. 3. Production of IL-2 and IFN-γ after CD2 stimulation or after treatment with accessory cells and PHA. T-cell clones transformed by herpesvirus saimiri C484, C488, and C139 were assayed for production of IL-2 (A) and IFN-γ (B). Grey bars, induced values for αβ T cells; hatched bars, induced values for γδ T cells. The stimuli are given on the right. Basal activities are shown by white bars. The upper panels show the medium control and the maximal stimulation with PHA and L428 stimulator cells. The middle panel shows activation by L428 cells alone, which express high levels of the CD2 ligand CD58/LFA3. This stimulation was abrogated by blockade of the CD2-CD58 interaction with the anti-CD2 monoclonal antibody CB219. The lower panels depict the effect of CD2 ligation with CB219 which is cross-linked via Fc receptors expressed on A20 cells. Induction of IL-2 via CD2 engagement was significantly lower in T-cell clones transformed by herpesvirus saimiri C139.

stimulation. In contrast, αβ and γδ T cells, which were transformed by herpesvirus saimiri C139, did not produce significant amounts of IL-2 after ligation of the CD2.1 epitope (Fig. 3, middle and lower panels). However, as demonstrated above, they were principally able to elaborate comparable levels of IL-2 after stimulation with PHA in the presence of L428 accessory cells. Inducibility of IFN-γ secretion after CD2.1 stimulation did not differ between T cells transformed by the two virus strains (Fig. 3, middle and lower panels). T-cell line B484.1, which was immortalized by herpesvirus saimiri C484 and which expressed only small amounts of TcR and CD3, behaved like its C488-transformed counterparts (Fig. 3). The transformed T cells expressed both CD2 and its ligand CD58/LFA3; therefore, cell contact leads to CD2 ligation. One might expect that this will elicit the secretion of significant amounts of IL-2 under basal conditions only in T-cell lines transformed by herpesvirus saimiri C488, not in those transformed by herpesvirus saimiri C139. Interestingly, T cells immortalized by herpesvirus saimiri C139 were less sensitive to growth inhibition by cyclosporin A than their C488-transformed counterparts (Fig. 4). This result suggests that the cells that were less able to elaborate IL-2 were also less dependent on it for growth.

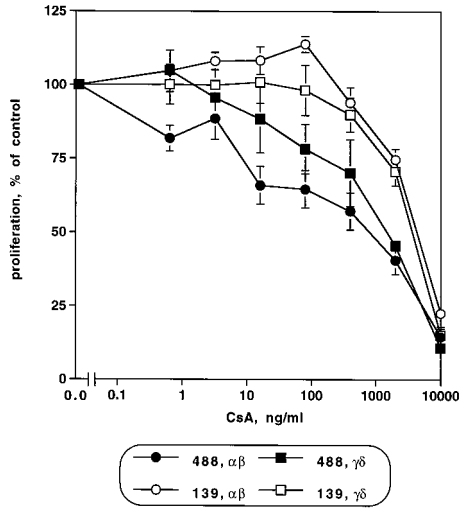


FIG. 4. Sensitivity to cyclosporin A of T cells transformed by different virus strains. The proliferation of different types of transformed T cells was measured under increasing concentrations of cyclosporin A (CsA; 0.01 to 10,000 ng/ml). The values are averages of multiple experiments using two to six cell lines for each T-cell type. Standard error values are indicated. T cells transformed by C139 are less sensitive to cyclosporin A than C488-immortalized cells.

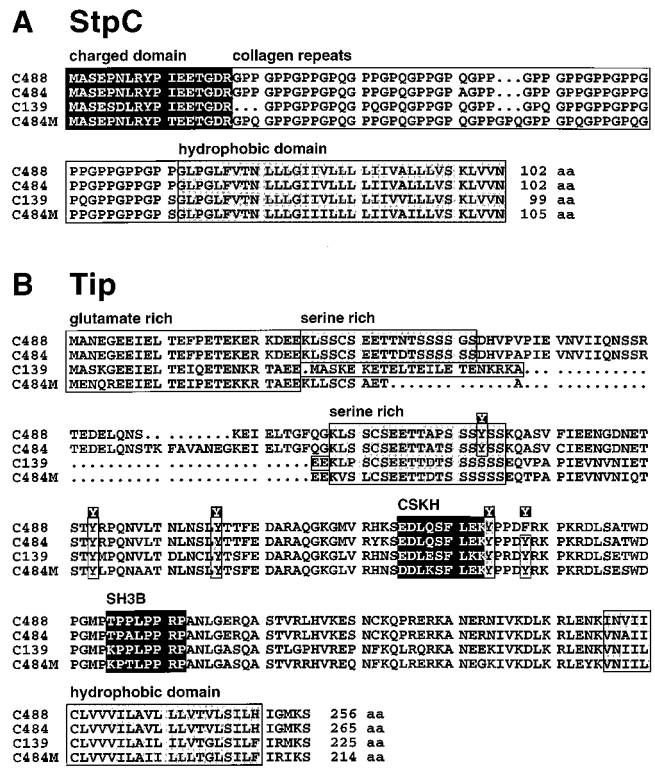


FIG. 5. Sequence alignments of transformation-associated proteins from different subgroup C strains of herpesvirus saimiri. The sequences of the proteins StpC (A) and Tip (B) of strains C488, C484, C139, and C484M are aligned. StpC sequences vary in the number of collagen repeats. In the N-terminal charged domain, there are few amino acid changes in C139 and C484M. Tip sequences exhibit more pronounced heterogeneity. Within the N-terminal portion, there are duplications of glutamate-rich or serine-rich regions. In the central part, there are five tyrosine residues, three of which are conserved among the four subgroup C strains. In the CSKH and SH3B domains, some amino acids are changed.

**Primary structure and regulated transcription of the transformation-relevant gene *stpC/tip* in different virus strains.** To understand the functional differences between T-cell lines transformed by different virus strains, we cloned and sequenced the transformation-relevant region of the strains C484 and C139 from purified virion DNA. We obtained a *Pst*I-*Xba*I clone (3,734 bp), harboring the whole terminal L-DNA region of strain C484, and a shorter *Pst*I-*Xba*I clone for strain C139. Both clones comprise the *stpC* open reading frame and the promoter upstream regulatory region including two U-RNA genes, *HSUR1* and *HSUR2*, up to the conserved *Xba*I site. The cloned C139 fragment stops between *stpC* and *tip* at an additional *Pst*I cleavage site. The sequences of C139 downstream of the *Pst*I cleavage site including the spacer between the reading frames, the *tip* open reading frame, and the H-DNA transition region were determined from cloned PCR fragments or by direct sequencing of primary PCR products. In total, 3,168 bp of terminal L-DNA of strain C139 were obtained. The sequences were compared with the corresponding published sequences of strains C488 (accession number M55264 [5]) and C484 (accession number M31964 [28, 29]). Surprisingly, the sequence obtained from strain C484 was not identical to the published C484 sequence (28, 29); the latter is therefore termed C484M throughout this report. We confirmed our C484 sequence by direct PCR sequencing from a reference virus stock frozen early after isolation and provided by J. Jung and R. Desrosiers (17) and proved the identity of both samples. We further confirmed the published sequence of strain C484M by PCR from reference virion DNA donated by P. Medveczky (28, 29). By PCR, we were unable to amplify TipC484 sequences from C484M virion DNA. Correspondingly, the DNA sequence of the Tip counterpart of C484M was not recovered by PCR from the C484 reference stock. We presume that two distinct subgroup C virus strains, C484M and C484, had been isolated from the same squirrel monkey, 484/77.

In summary, the sequences of all subgroup C strains showed a high nucleotide sequence homology of 82.2 to 99.3%. C488 and C484 (99%) as well as C139 and C484M (92%) formed two pairs with higher homology. The sequence variations of highest functional relevance are shown as sequence alignments for StpC (Fig. 5A), for Tip (Fig. 5B), and for the promoter upstream region (Fig. 6A). The StpC protein sequences varied mainly in the number of collagen triplet repeats and in few amino acid changes in the N-terminal charged domain. The Tip proteins also had a high degree of homology. The most pronounced variation was found in the N-terminal third of Tip. TipC484 carries a 9-amino acid insertion in this region. TipC488 harbors a single copy of a glutamate-rich stretch and two copies of a serine-rich domain. In contrast, TipC139 has a duplication of the glutamate-rich region but only a single serine-rich stretch. There are five tyrosine residues in the Tip sequence, three of which are conserved in all virus strains analyzed. There are two motifs of special interest, a C-terminal Src kinase homology (CSKH) sequence and an SH3 binding (SH3B) motif. They are highly conserved between herpesvirus saimiri C488 and C484. In C139, there are two amino acid changes in the CSKH domain, Q to E and E to K. Also, there is a T-to-K variation in the SH3B sequences of C139 and C484M.

StpC and Tip are translated from a bicistronic mRNA of 1.7 kb. The *stpC/tip* transcription unit is preceded by a complex regulation unit (Fig. 6). In strain C488-transformed T cells, the *stpC/tip* transcription was found to be highly inducible upon T-cell stimulation via mitogen (PHA) or phorbol ester (TPA), similar to what was found for T-cell activation genes. In contrast, transcript levels were hardly detectable and not inducible in either αβ or γδ T cells transformed by strain C139 (Fig. 7). The promoter upstream sequences of the different virus strains were also highly homologous (Fig. 6A). The major differences between C488 and C139 were located within a *Hin*dIII/*Nsp*I promoter upstream fragment. In strain C139, several potential functional promoter elements are deleted. This fragment was tested at enhancer position in front of the herpes simplex virus thymidine kinase (TK) promoter and the luciferase gene after transient expression in Jurkat and BJA-B cells. Whereas the longer C488 sequence strongly activated luciferase expression, the shorter C139 sequence only weakly induced luciferase expression (Fig. 6B and C).

**Expression of StpC and Tip proteins.** The amount of StpC present in the transformed cells was determined on immunoblots with or without treatment of the cells with TPA (Fig. 8A). For this purpose we used two rabbit sera, one directed against a GST-StpC488 fusion protein and the other recognizing an N-terminal peptide of StpC488. The results for the two sera were identical. Basal expression levels of StpC were about sixfold lower in the cells transformed by herpesvirus saimiri C139, with no significant differences between αβ and γδ T cells. In the cells transformed by herpesvirus saimiri C488, TPA treatment led to a strong increase (8- to 15-fold) in the abundance of StpC, which was not observed in the cells immortalized by herpesvirus saimiri C139 (increase of 0.5- to

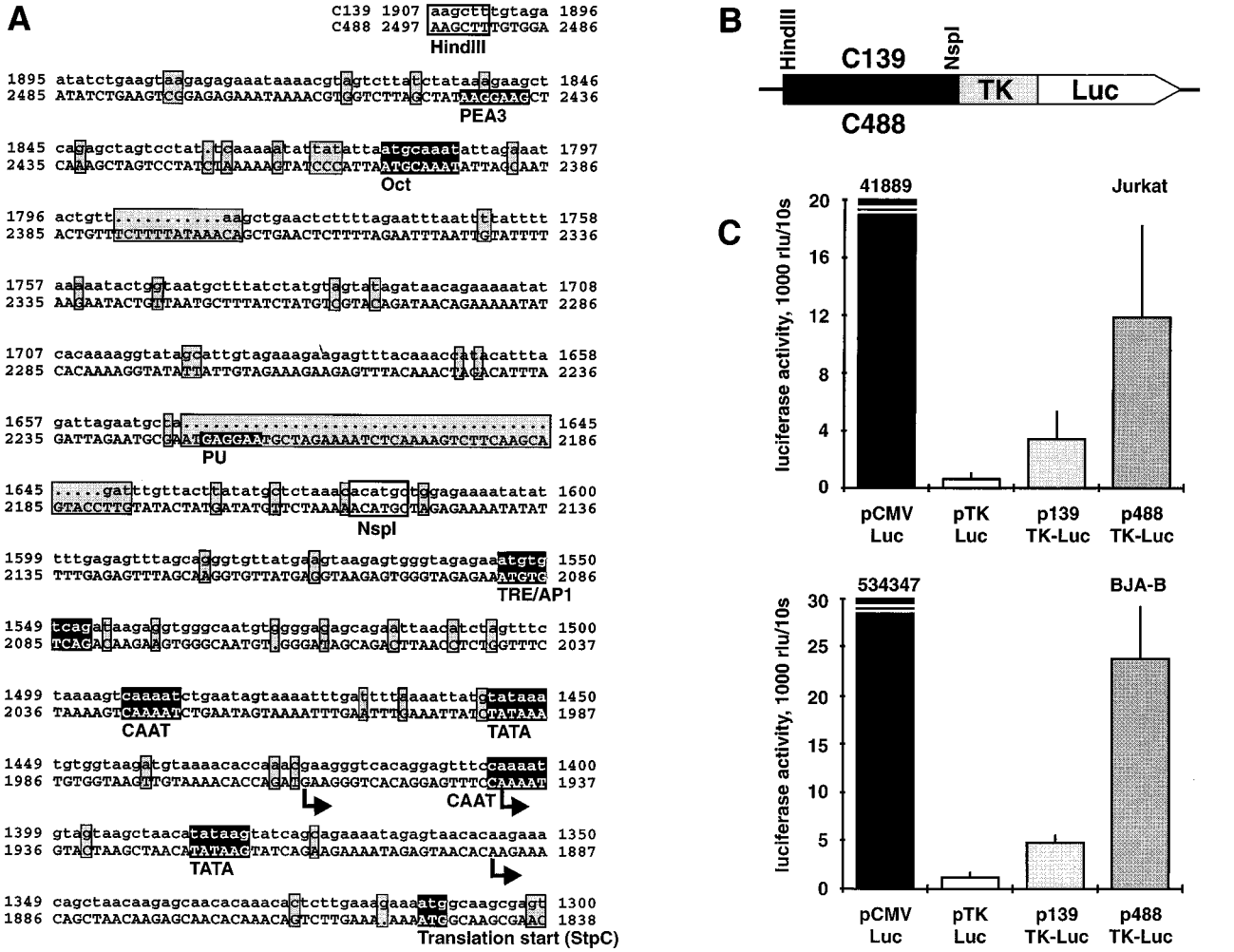


FIG. 6. Structural and functional differences in the *stpC/tip* promoter upstream regions. (A) Alignment of the *stpC/tip* promoter upstream sequences of strains C488 and C139. Differences are emphasized with grey boxes. The most extensive sequence divergence is found between the *Hin*dIII and *Nsp*I restriction sites. Potential functional elements and possible binding sites for transcription factors are marked. Within the core promoter region, the relevant elements, such as CAAT and TATA boxes and transcription and translation start sites, are depicted (23). aa, amino acids. The *Hin*dIII/*Nsp*I fragments of both strains were positioned in sense orientation at enhancer position in front of the herpes simplex virus TK promoter and the luciferase gene (Luc) (B). (C) Results of at least six luciferase transfection experiments into Jurkat and BJA-B cells, together with the standard deviation. The sequence from C139 was less active than the sequence from C488 in activating the heterologous TK promoter. rlu/10s, relative light units per 10 s.

2-fold). These findings were confirmed by intracellular antibody staining and flow cytometry. In C488-transformed αβ and γδ T cells, a small proportion of cells had high levels of StpC spontaneously. After TPA stimulation, many cells expressed high levels of StpC. In contrast, StpC levels in C139-transformed T cells were low spontaneously and remained low even after TPA stimulation (Fig. 8B).

Tip levels are very low in herpesvirus saimiri C488-transformed T cells and escape demonstration by immunocytological staining or on immunoblots. Therefore, we took advantage of the specific association of Tip with $p56^{lck}$ to investigate its expression. Tip can be visualized with the very sensitive in vitro phosphotransferase assay after coprecipitation from cell lysates by using Lck antibodies. We have previously shown that TipC488 runs with an apparent molecular mass of 40 kDa in SDS-PAGE. In the two herpesvirus saimiri C139-immortalized γδ T-cell lines tested, a slightly smaller phosphoprotein of about 38 kDa was specifically associated with $p56^{lck}$ (Fig. 9C and E). As demonstrated earlier, αβ T cells transformed by herpesvirus saimiri C488 show activity of $p53/56^{lyn}$ in addition to the Src-related kinases $p56^{lck}$ and $p60^{fyn}$, which are regularly present in T cells. The same spectrum of Src family kinases was found in γδ T cells growing permanently after infection with herpesvirus saimiri C139. $p38^{tipC139}$ was associated only with $p56^{lck}$ (Fig. 9C). Primary γδ T cells did not show $p53/56^{lyn}$ activity or an Lck-associated phosphoprotein in the molecular range corresponding to the Tip variants (Fig. 9A).

Finally, transformed T-cell clones were stimulated with TPA. At different time points, $p56^{lck}$ was precipitated and subjected to an in vitro phosphotransferase assay. TPA treatment led to the appearance of the 59-kDa isoform of $p56^{lck}$ in all cell lines tested. However, only in T cells immortalized by herpesvirus saimiri C488, not in T cells transformed by C139, was there an increase in the phosphorylation of Tip and also of $p56^{lck}$ autophosphorylation (Fig. 9D and E). This likely reflects transcriptional induction of TipC488 by TPA which is followed by increased protein levels, leading in turn to the activation of Lck. In herpesvirus saimiri C139-immortalized cells, Tip signals were not enhanced. As expected, TPA treatment alone did not significantly activate $p56^{lck}$.

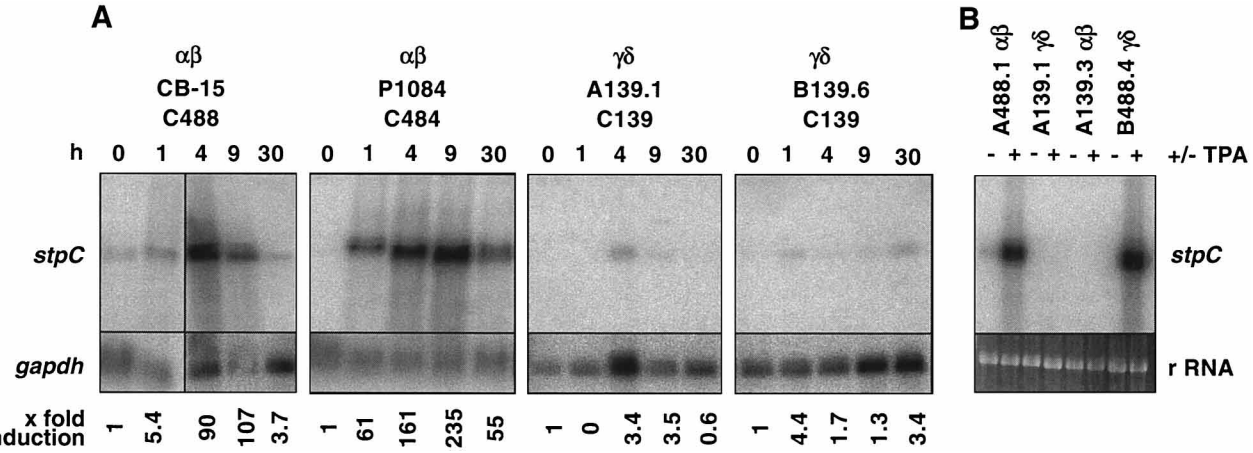


FIG. 7. Transcriptional activity of the *stpC/tip* gene in different types of T cells transformed by different virus strains. (A) Time course experiment in which *stpC/tip* gene activity was measured on the RNA level. In T cells transformed by C488 or C484, there was an early and transient induction of gene expression after addition of TPA (2 ng/ml). In contrast, C139-immortalized cells did not significantly react to TPA. Signal intensities are given normalized on cellular *gapdh* expression. (B) Northern analysis of prototype cell lines with respect to TcR and transforming virus strain, with or without TPA stimulation for 6 h. The ethidium bromide-stained rRNA bands served as a control.

## DISCUSSION

**Transformation of various types of human T cells by different subgroup C virus strains.** Tumor formation by subgroup A and C strains of herpesvirus saimiri has been observed in New World primates and New Zealand White rabbits. These strains are also able to transform rabbit and marmoset T lymphocytes in vitro (48, 49, 60). Herpesvirus saimiri isolate C488 readily immortalizes human T cells in cell culture (4, 8, 15, 65). Here, we demonstrate that three isolates of herpesvirus saimiri subgroup C, C488, C484, and C139, were able to transform human T cells in vitro. The resulting T-cell clones exhibited differences in their functional properties. We tested the efficiency of transformation by the different herpesvirus saimiri subgroup C strains under limiting conditions. In contrast to T cells trans-

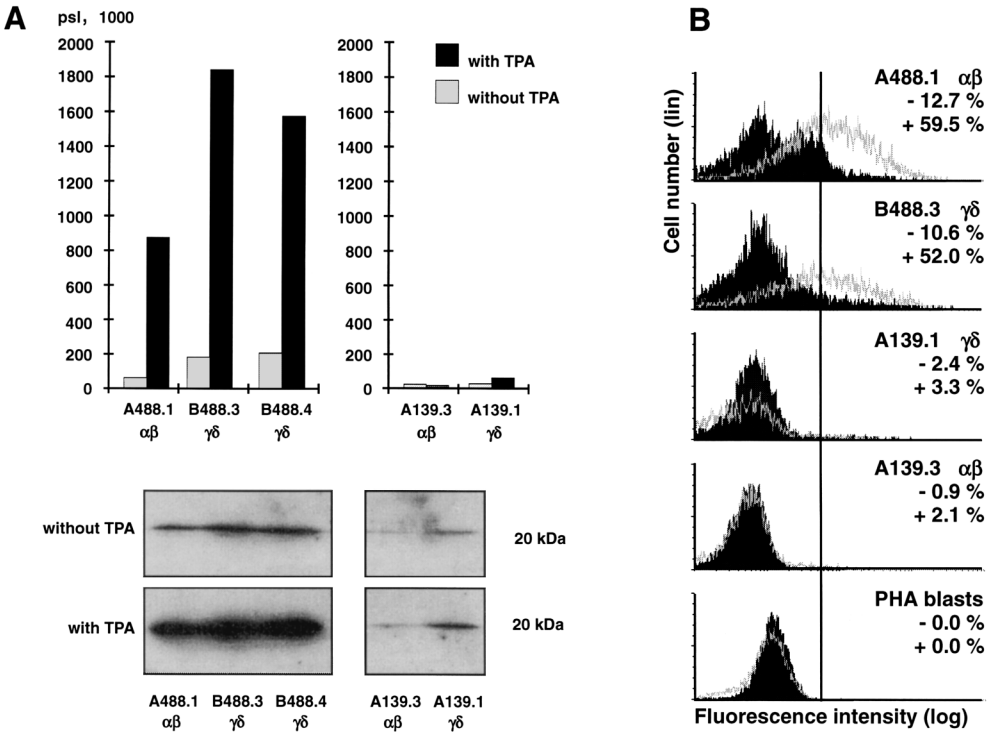


FIG. 8. StpC protein abundance and inducibility in transformed T cells. StpC protein expression was measured by immunoblot analysis (A) and intracellular flow cytometry (B). Protein extracts from untreated and TPA-treated (12 h) T cells of different types were separated by SDS-PAGE and transferred to membranes, which were analyzed by using an StpC-specific rabbit antiserum and iodinated protein A. The radioactive signal intensities are shown in the histograms. These findings were confirmed by permeabilization and intracellular StpC staining of stimulated (open graphs) and unstimulated cells (closed graphs). The percentages of cells expressing high amounts of StpC without (−) or with (+) TPA induction for 12 h are given on the right. StpC is heterogeneously expressed in C488-transformed T cells and can be strongly induced. In contrast, C139-immortalized T cells exhibit low StpC expression, which is not inducible. psl, photostimulated luminescence.

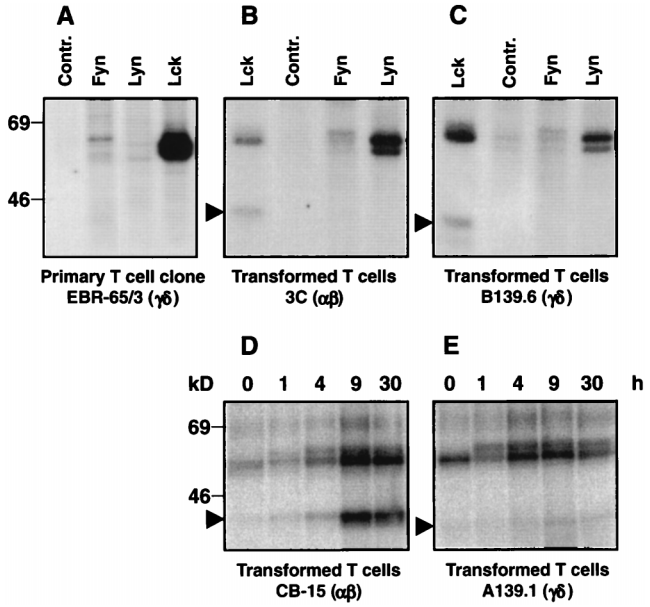


FIG. 9. T-cell tyrosine phosphotransferases and virus-associated Tip proteins. The activities of Src-related tyrosine kinases and of the Lck-associated viral protein Tip were analyzed by immunoprecipitation and subsequent in vitro phosphotransferase reactions. A primary γδ T-cell clone (EBR-65/3; A), a CD8-positive, polyclonal αβ T-cell line (3C; B), and a transformed γδ T-cell line (B139.6; C) were subjected to immunoprecipitation with various Src kinase-specific antisera. Activity of $p53/56^{lyn}$ was detected only after herpesvirus transformation. $p40^{tipC488}$ was specifically detected in Lck precipitates from C488-transformed T cells. In C139-transformed lymphocytes, Tip appeared slightly smaller, at about 38 kDa. Weak expression of Fyn was found in all cases. Lyn is not expressed in primary cells but is expressed in all virus-transformed cells tested. Tip was further analyzed in a kinetic experiment using Lck precipitates before and 1 to 30 h after TPA stimulation. In an αβ T-cell line (CB-15; D), $p40^{tipC488}$ and Lck signal intensities clearly increased. In a transformed γδ T-cell line (A139.1; E), there was no significant increase in signal intensities. However, as shown in panel D, Lck signals were partially shifted to the 59-kDa position after TPA stimulation. $p38^{tipC139}$ signals were found at constant low intensity. Contr., control.

formed by herpesvirus saimiri C488 from bulk cultures of human peripheral and cord blood cells, which were polyclonal and expressed different types of αβ TcR (24), the T-cell lines obtained from the infection of small cell numbers appeared monoclonal: the cultures had either αβ or γδ TcRs. Vβ expression on individual T-cell lines was monospecific and homogeneous. In contrast to the broad diversity of cord blood γδ TcRs (54), most transformed γδ T-cell lines obtained in this study carried the Vδ1 chain; the Vγ9 chain was not observed (Table 1; Fig. 1). Approximately 1 of $10^5$ cord blood cells was transformed to permanent growth by herpesvirus saimiri C488 and C139. Herpesvirus saimiri C484 was at least 10-fold less efficient; a more accurate estimate is not possible, as we obtained only a single transformed culture out of 120 samples. The low initial cell number and the absence of exogenous IL-2 during the first weeks after infection selected quickly and strongly for growth transformation. Therefore, our estimate is conservative; the transformation efficiency may well be higher following the standard transformation procedure (22) in which cells are infected at high density in the presence of saturating concentrations of exogenous IL-2. While transformation of bulk cultures with herpesvirus saimiri C488 regularly gave rise to αβ T-cell lines (24), either αβ or γδ T-cell clones were obtained when presumably only one T cell per well was transformed. Immortalized αβ T cells appeared to grow faster than γδ T cells initially. With herpesvirus saimiri C488, two of seven proliferating clones expressed the γδ TcR, whereas six of nine did so after infection with herpesvirus saimiri C139. We further compared virus strain C488 with strains C139 and C484 with regard to transformation of peripheral blood mononuclear cell bulk cultures from adult blood. We found high efficiency consistently with strain C488 and in only a few cases with strains C484 and C139 (data not shown). Taken together, the data suggest that there may be subtle differences in the transformation tropism and efficiency of herpesvirus saimiri subgroup C strains in T-cell subpopulations.

**Differences in cytokine production and reactivity to CD2 ligation between T cells transformed by different subgroup C virus strains.** It is well documented that infection with herpesvirus saimiri C488 shifts the cytokine pattern toward a Th1 profile in $CD4^+$ αβ T-cell clones. The transformed cells generated increased amounts of IL-2 and especially of IFN-γ; at the same time, the secretion levels of IL-4 and IL-5 were reduced in Th2 clones (15, 16). The cord blood T cells used in this study were most likely naive and undifferentiated (54). After transformation, the clones could be induced to secrete high amounts of IL-2 and IFN-γ (Fig. 3). Interestingly, $CD4^+$, $CD8^+$, αβ, and γδ T cells produced comparable amounts of these cytokines after maximal stimulation with the lectin PHA and stimulator cells. There were no large differences between cells transformed by different virus isolates. Only one T-cell clone secreted small amounts of IL-4. Therefore, the bias toward a Th1-like phenotype is a general property of human T cells transformed by herpesvirus saimiri and not restricted to a T-cell subpopulation or to a particular strain of herpesvirus saimiri. The Th1 cytokine secretion phenotype is also dominant in untransformed human γδ T-cell clones, while the Th2 type is found less frequently (10, 12, 55).

Hyperreactivity to ligation of the CD2 epitope T11.1, which is also engaged by the natural ligand CD58, is a striking feature of αβ T cells immortalized by herpesvirus saimiri C488, where it leads to cytokine secretion, increased proliferation, and cytotoxic activity (52). In contrast, untransformed T cells do not respond to the isolated ligation of CD2 (T11.1) by monoclonal antibodies or by CD58, but they require engagement of a second epitope on CD2 for stimulation (51). The transformed T cells express at the same time CD2 and its ligand CD58/LFA3; thus, they are able to stimulate each other via cell-to-cell contact to IL-2 production. This has been invoked as the driving force of an autocrine loop essential for the continuous growth stimulation of herpesvirus saimiri-transformed T cells (52). Our data confirm these findings and demonstrate in addition that the extent of CD2 hyperreactivity depends on the particular strain of herpesvirus saimiri but not on the T-cell subpopulation. Both αβ and γδ T cells transformed by herpesvirus saimiri C488 and C484 responded to ligation of CD2 (T11.1) with the secretion of maximal amounts of IL-2. However, this was not the case for T cells immortalized by herpesvirus saimiri C139. Only low levels of IL-2 were inducible via CD2 in C139-transformed T cells, which were in principle able to produce comparably large quantities of IL-2 (Fig. 3). Obviously, the small amounts of IL-2 which can be secreted by the cells in response to CD2-CD58 interaction are sufficient for growth stimulation of T cells immortalized by herpesvirus saimiri C139. Interestingly, the proliferation of C139-transformed T cells was less sensitive to inhibition by cyclosporin A, which interferes with IL-2 production (Fig. 4). Thus, human T cells transformed by herpesvirus saimiri C139 are less dependent on IL-2. Other growth factors which, like IFN-γ, do not show a significant difference in the response to CD2 ligation between virus strains may also play a role.

**Variable and conserved functions of transforming strains of herpesvirus saimiri.** Looking for an explanation for the differ-

ent behavior of T cells transformed by herpesvirus saimiri C488, C484, and C139, we cloned and sequenced the transformation-associated terminal L-DNA regions and compared the protein sequences of Tip and StpC as well as the 5′ regulatory regions of these genes. There were only minor differences between the StpC protein sequences (Fig. 5A). Three amino acid exchanges between C488 and C139 in the Tip sequence might be more relevant (Fig. 5B). These are located in two motifs, a Src kinase homology region and a proline-rich SH3 binding region which in TipC488 are both necessary and sufficient for binding of the molecule to the T-cell kinase $p56^{lck}$ (6, 35). However, binding of TipC139 to $p56^{lck}$ was grossly intact (Fig. 9C and E). Prominent sequence differences were localized in the N-terminal region of Tip, the function of which is unknown. The N-terminal region of TipC484 carries an insertion of nine amino acids (Fig. 5B). Further studies are required to determine whether this insertion is involved in the low transformation frequency of this strain. Transcription and transcription regulation of *stpC/tip* differed greatly between herpesvirus saimiri C488- and C139-immortalized T cells (Fig. 6 and 7). We have previously shown that in T cells transformed with herpesvirus saimiri C488, the bicistronic 1.7-kb transcript encoding Tip and StpC is regulated like a T-cell activation gene (23). Surprisingly, this was not the case in T cells immortalized by herpesvirus saimiri C139; here *stpC/tip* transcript levels were low and remained almost constant. This finding correlated with variations in the 5′ upstream promoter sequences. They were almost identical in strains C488 and C484, in which *stpC/tip* mRNA levels can be strongly induced, but exhibited significant alterations in strain C139, where *stpC/tip* mRNA levels were constantly low (Fig. 6 and 7). The differences in the *cis*-activating functions of these sequences (high and inducible in strains C488 and C484; low and not inducible in strain C139) identify transcriptional activation as one important mechanism. However, a contribution of posttranscriptional mechanisms such as mRNA stabilization cannot be ruled out. With strain C484M, which is closely related to strain C139 on the sequence level, expression of the StpC counterpart was similarly low or even absent in transformed human T cells, but induction has not yet been analyzed (47).

StpC protein expression paralleled the findings regarding the *stpC/tip* mRNA level (Fig. 8A). The basal expression level of the StpC139 oncoprotein was lower than that of StpC488. After stimulation of the T cells with TPA, this difference was even more pronounced, since the abundance of StpC488 was strongly induced whereas expression of StpC139 remained stable. It remains possible that sequence variations between StpC488 and StpC139 influence binding of the antisera to StpC139 and thereby simulate low abundance. However, this is unlikely because StpC protein levels correlated with the RNA levels, with a delay of several hours. In addition, results for two different rabbit antisera, one directed against an N-terminal peptide and the other directed against full-length StpC488 fused to GST, were identical. In T cells transformed by either strain, StpC expression in single cells was heterogeneous, as determined by intracellular fluorescence analysis. At a given time point, only a few cells strongly expressed StpC protein. The number of StpC-positive cells was strongly increased by TPA stimulation, but only in C488 transformed T cells (23) (Fig. 9B).

Expression of TipC488 and also of TipC139 was too low for detection on immunoblots. We and others have shown that TipC488 and the Tip homolog of strain C484M tightly associate with $p56^{lck}$ (6, 43), a nonreceptor tyrosine kinase which is pivotal for signaling in T cells (7, 58). Since Tip is an excellent substrate for Lck (6, 35, 36, 66), it can be visualized after coprecipitation with Lck followed by an in vitro phosphotransferase reaction. Bands of approximately 56 kDa reflect the autophosphorylation activity of Lck, and 38- to 40-kDa bands result from the tyrosine phosphorylation of Tip by Lck. After stimulation of the T cells with TPA, a higher-molecular-weight isoform of $p56^{lck}$ which is due to serine phosphorylation appeared (Fig. 9D and E) (45, 64). Only in cells transformed with herpesvirus saimiri C488 was Lck strongly activated in response to TPA. This is an unexpected finding, since TPA is known to activate the serine protein kinase C and is not expected to influence the activity of Src family kinases which act upstream of protein kinase C in the signaling cascade. However, as we have previously shown, binding of TipC488 to Lck can activate the kinase (66). Therefore, increased amounts of TipC488, which is translated from the same bicistronic mRNA species as StpC488, can best explain the enzymatic activation of Lck. Activation of Lck would not be expected in T cells transformed by herpesvirus saimiri C139, where *stpC/tip* mRNA was not induced by TPA (Fig. 9E). Indeed, Lck from C139-immortalized T cells responded to TPA, as demonstrated by the decreased electrophoretic mobility of Lck. However, Lck did not exhibit enzymatic activation in response to TPA. Though it is unlikely, we cannot exclude the possibility that TipC139 protein, independent of its concentration, is unable to stimulate phosphotransferase activity of Lck. It has recently been reported that Lck-mediated signaling was impaired in Jurkat cells overexpressing TipC488 (36). Our results for C488-immortalized T-cell clones confirm that Lck can be activated by TipC488 (66). The sequence differences in the regulatory regions of the *stpC/tip* genes of different subgroup C strains cause differences in promoter activity and consequently of Tip protein expression. This can explain the differences in the inducibility of Lck activity in transformed T cells.

$p56^{lck}$ has been found in complex with CD2, and the enzyme becomes activated after cross-linking of CD2 (14, 44). Therefore, activation of $p56^{lck}$ by Tip could enhance the T-cell response to CD2 binding. Increased expression of Tip following T-cell activation, e.g., by TPA or CD2 ligation, would then result in a positive feedback and prolong or further enhance the T-cell activation. This positive feedback can take place in T cells transformed by herpesvirus saimiri C488 but not by herpesvirus saimiri C139. This might explain the lower IL-2 responses to CD2 triggering in T cells immortalized by herpesvirus saimiri C139 (Fig. 3). Thus, C139-transformed T cells appear to be less regulated and possibly in a more autonomous state of proliferation compared with C488-immortalized T lymphocytes.

Infection of αβ T cells with herpesvirus saimiri C488 leads to the expression of the Src family kinase $p53/56^{lyn}$, which is not active in normal T cells but is found in human T-cell lymphotropic virus type 1-transformed producer cells (7, 66, 67). We tested γδ T cells transformed by herpesvirus saimiri C139 to see whether Lyn regulation was affected by the T-cell subtype or by the differences in transcription and translation of the viral gene encoding StpC and Tip. We found that expression of Lyn is a general feature of herpesvirus saimiri-transformed T cells, independent of the virus strain used and of the T-cell subtype (Fig. 9A to C).

Surprisingly, virus strain C139 was found to persist episomally at low copy number, in contrast to all previous studies on herpesvirus saimiri in transformed T cells (Fig. 2B). Probably C139 carries a further sequence divergence in an L-DNA region highly conserved among transforming and nontransforming virus strains. A possible origin of plasmid replication has been recently localized to a polymorphic region upstream of the dihydrofolate reductase gene in virus strain C484M (41).

This sequence is not conserved in virus strains A11 and C488. It is also unlikely that the herpesvirus origin of plasmid replication is located in the transformation-associated terminal L-DNA with its extensive sequence variability among virus strains. The DNA sequences analyzed in this study cannot explain the low copy number of herpesvirus saimiri C139 persistence.

Taken together, our results provide an example for the fine-tuning of the virus-host relationship in the case of herpesvirus saimiri and human T cells, where small sequence alterations in transforming viral genes lead to significant differences in cellular functions.

## ACKNOWLEDGMENTS

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