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Fine Mapping Two Distinct Antigenic Sites on Simian Virus 40 (SV40) T Antigen Reactive with SV40-Specific Cytotoxic T-Cell Clones by Using SV40 Deletion Mutants

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The existence of two distinct antigenic sites at the surface of simian virus 40 (SV40)-transformed $H\text{-}2^b$ cells has been previously demonstrated (A. E. Campbell, L. F. Foley, and S. S. Tevethia, J. Immunol. 130:490–492, 1983) by using two independently isolated SV40-specific cytotoxic T-lymphocyte (CTL) clones, K11 and K19. We identified amino acids in the amino-terminal half of SV40 T antigen that are essential for the recognition of antigenic sites by these CTL clones by using $H\text{-}2^b$ cells transformed by mutants that produce T antigen truncated from the amino-terminal or carboxy-terminal end or carrying overlapping internal deletions in the amino-terminal regions of SV40 T antigen. The results show that CTL clone K11 failed to recognize and lyse target cells missing SV40 T-antigen amino acids 189 to 211, whereas CTL clone K19 lysed these cells. The cell lines missing SV40 T-antigen amino acids 220 to 223 and 220 to 228 were not lysed by CTL clone K19 but were susceptible to lysis by CTL clone K11. Two other cell lines missing amino acids 189 to 223 and 189 to 228 of SV40 T antigen were not lysed by either of the CTL clones but were lysed by SV40-specific bulk-culture CTL if sufficient amounts of relevant restriction elements were expressed at the cell surface. The SV40 T-antigen amino acids critical for the recognition of an antigenic site by CTL clone K11 were identified to be 193 to 211; 220 to 223 were identified as critical for recognition by CTL clone K19. The deletion of these amino acids from the T antigen resulted in the loss of antigenic sites specific for CTL clones K11 and K19.

Simian virus 40 (SV40)-transformed and -infected cells synthesize a tumor-specific transplantation antigen expressed on the cell surface that induces the host immune response, resulting in tumor rejection (for reviews, see references 56 and 57). Mice immunized with SV40 or with syngeneic SV40-transformed cells develop cytotoxic T lymphocytes (CTL) that lyse SV40-transformed or -infected syngeneic cells in an *H-2*-restricted manner (17, 35, 42–44) and mediate tumor rejection in vivo (18). It is now firmly established that the TSTA activity resides in a virus-encoded multifunctional protein of 94 kilodaltons (kDa), the large T antigen. T antigen purified to homogeneity immunizes mice against SV40 tumor transplantation (9, 58) and induces a cellular immune response in the immunized host (8, 58). In addition, the T antigen provides a target recognized by SV40-specific CTL (41, 61). Although the majority of T antigen is localized in the nucleus of SV40-transformed or -infected cells, a small amount can be demonstrated at the cell surface by various serological means (7, 11, 12, 27, 28, 38, 39, 46–48, 51, 52, 59). Presumably, it is this surface T antigen that is recognized by CTL.

SV40-specific monoclonal antibodies have identified multiple antigenic sites on the T antigen (3, 23, 26, 37). However, at the cell surface, only selected portions of the T antigen are accessible to these monoclonal antibodies (3, 12, 20, 46, 68). Similarly, antigenic sites recognized by CTL are present in both the amino-terminal and the carboxy-terminal halves of the T antigen. Mouse cells expressing either a 33-kDa amino-terminal T-antigen fragment (21, 61) or a 28-kDa carboxy-terminal fragment (21) are susceptible to lysis by CTL generated in bulk culture to syngeneic SV40-transformed or -infected $H\text{-}2^k$ cells which synthesize full-length T antigen. There are at least two distinct antigenic sites in the amino-terminal half of the T antigen to which clones of CTL have been generated (6). The CTL clones K11 and K19 lyse $H\text{-}2^b$ SV40-transformed cells; however, only K19 lyses both SV40- and human papovavirus BK-transformed $H\text{-}2^b$ cells. Both the CTL clones are $H\text{-}2D^b$ restricted and map within the 48-kDa amino-terminal fragment of SV40 T antigen (60).

The present studies were undertaken to define precisely the antigenic sites recognized by the CTL clones K11 and K19. We used mutants that produce various truncated T antigens. The $H\text{-}2^b$ cells expressing T antigen truncated from the amino-terminal or carboxy-terminal end or carrying overlapping internal deletions in the amino-terminal region were used as target cells against CTL clones K19 and K11 in addition to CTL generated in bulk cultures. The results show that amino acids 189 to 211 and 220 to 223 are critical to the recognition of antigenic sites by CTL clones K11 and K19, respectively, as the deletion of these specific amino acids abolished recognition of T antigen by these two CTL clones.

MATERIALS AND METHODS

Plasmids. The plasmids used in this study are shown in Tables 1 and 2. Plasmids that produce T antigens missing internal amino acids contain chimeric linker insertion and deletion mutations and were generated as described previously (32). Mutants expected to produce specific amino-terminal-truncated T antigens were constructed by joining an *Eco*RI linker added to the *Taq*I site of various mutant DNAs to the *Eco*RI site in the mutant RL18. This RL mutant

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TABLE 1. Plasmids used in this study that produce truncated T antigen

Plasmid	Maximum amino acids in truncated T antigen	Reference
pVBETK-1	1–708	45
pVBt1TK-1	1–271 (272)	45
pPVU-5-70K	109–708	This study
py2xmet128-70K	128–708	This study
py2xB9-70K	150–708	This study
p60K	176–708	This study
pRL5-S24	1–217; 251–708	This study
pRL75-RL12	1–188; 229–708	This study
pRL75-RL88	1–188; 212–708	This study
pRL75	1–188; 193–708	32
pRL75-RL115	1–188; 224–708	This study
pRL101-RL12	1–219; 229–708	This study
pRL115	1–219; 224–708	32

contains an *Eco*RI linker between nucleotides 5194 and 5186 and includes the SV40 early-region promoter but not the initiation codon for the large T antigen. The nucleotide alterations in the mutants used in these constructions are shown in Fig. 1.

The first initiation codon in the reading frame of large T antigen within the *Taq*I-*Bam*HI fragment occurs at methionine 109. The plasmid pPVU-5-70K contains the *Taq*I-*Bam*HI fragment derived from the mutant pPVU-5 (32), which contains a 7-base-pair deletion (nucleotides 4527 to 4521) 5′ to the first initiation codon. Based on nucleotide sequence, this plasmid is expected to produce a polypeptide consisting of amino acids 109 to 708.

The plasmid py2xmet128-70K contains the *Taq*I-*Bam*HI fragment of a mutant with an in-frame deletion (y2) of nucleotides 4501 to 4481 and a base change of A to T at nucleotide 4435. The y2 deletion removes the AUG codon for amino acid 109. The point mutation converts the lysine codon at position 128 to a methionine codon. The plasmid is expected to produce a T antigen consisting of amino acids 128 to 708. The plasmid py2xB9-70K contains a *Taq*I-*Bam*HI fragment bearing the y2 deletion and a G-to-A transition at nucleotide 4370. The base change introduces an initiation codon at position 150. The T antigen produced from this plasmid should contain amino acids 150 to 708. The plasmid p60K, named for the expected molecular weight of its product, contains the *Taq*I-*Bam*HI fragment from the mutant K2 (32). The G-to-A transition in this mutant converts the methionine at 109 to isoleucine. The first available in-frame methionine codon in this construct corresponds to amino acid 176 in the T antigen.

The plasmids pH-2K^b and pH-2D^b containing the murine major histocompatibility (MHC) class I genes K^b and D^b (1, 22, 50) were kindly provided by H. Allen. The plasmid pSV2-neo contains the aminoglycosyl 2′ phosphotransferase gene that confers on mammalian cells resistance to the antibiotic G418 and that has been described previously (53).

Transfection and transformation of primary C57BL/6 mouse embryo fibroblasts with plasmid DNA. Primary

TABLE 2. Other plasmids used in this study

Plasmid	Relevant gene	Reference
pSV2-neo	Neomycin	53
pH-2K^b	MHC class I K^b	1
pH-2D^b	MHC class I D^b	1

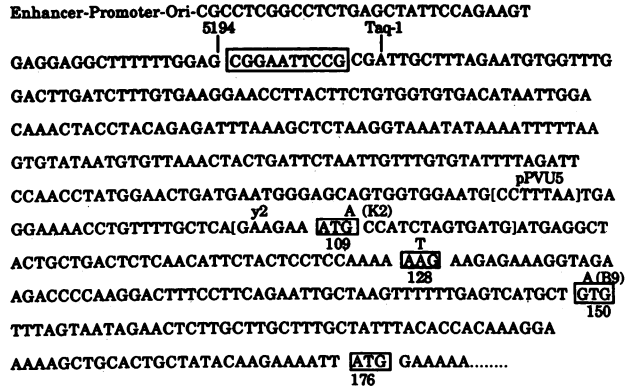

FIG. 1. Construction of plasmids encoding amino-terminal-truncated large T antigens. Mutants expected to produce T antigens missing specific amino-terminal segments were constructed by joining an *Eco*RI linker added at the *Taq*I site of various mutant DNAs to the *Eco*RI site of RL18 by exchanging the *Eco*RI-*Bam*HI fragments. The RL18 mutant contains SV40 sequences from nucleotide 270 through the enhancer-promoter origin (Ori) region to an *Eco*RI linker at nucleotide 5194. The first box encloses the *Eco*RI linker. Additional boxes enclose either natural initiation sites or codons converted to ATG in the individual mutants. The nucleotides appearing above the sequence are those altered in the mutants K2, B9, and 128 which donated early-region sequences to the plasmid constructs. The numbers below the sequence indicate the number of the first amino acid expected in the truncated T antigens. The brackets contain sequences missing in the deletions designated y2 and pPVU-5.

C57BL/6 mouse embryo fibroblast cultures (B6/MEF) were prepared as described previously (55). Transfection of these cultures with plasmids or SV40 DNA was accomplished by the method of Graham and Van der Eb as modified by Wigler et al. (69) and M. Tevethia (55). Briefly, 2×10^5 B6/MEF was seeded into a 75-cm^2 plastic tissue culture flask containing Dulbecco modified Eagle medium (DMEM) with 10% fetal calf serum (FCS). After incubation at 37°C overnight, cells were transfected with 1 μg of plasmid DNA and 10 μg of carrier DNA from B6/MEF in the form of a calcium phosphate precipitate. The transfected cells containing the precipitate were incubated for 16 h at 37°C, at which time the medium was replaced with fresh DMEM containing 10% FCS. The cultures were fed every third day for a period of 3 to 4 weeks. The individual colonies of transformed cells were picked and established as cell lines.

Cell lines. The cell lines used in this study, their origins, and *H-2* haplotypes are listed in Table 3. In addition to the cell lines generated after transformation of B6/MEF by the SV40 deletion mutants, B6/WT-19, an SV40-transformed cell line of B6 origin (59), and a cell line (B6/PY) derived from a tumor induced by the polyomavirus (25) were also used. C3HSV-4 is an SV40-transformed cell line of C3H/HeJ ($H\text{-}2^k$) origin. The cell lines of $H\text{-}2^k$ origin, L/pVBETK-1 and L/pVBt1TK-1, which synthesize a full-length T antigen and a 33-kDa amino-terminal fragment of T antigen, respectively, and LTK$^-$ have been described previously (45, 61).

All the cell lines were maintained in DMEM containing either 10 or 5% FCS.

Expression of $H\text{-}2K^b$ and $H\text{-}2D^b$ genes in L/pVBETK-1, L/pVBt1TK-1, and LTK$^-$ cells. Since L/pVBt1TK-1 cells, which synthesize an amino-terminal 33-kDa fragment of SV40 T antigen, are of $H\text{-}2^k$ origin and the CTL clones (K11 and K19) used to map the antigenic sites on T antigen are of $H\text{-}2^b$ origin, the L/pVBETK-1, L/pVBt1TK-1, and LTK$^-$

TABLE 3. Cell lines used in this study

Cell line	*H-2* haplotype	Virus or DNA introduced	T-antigen amino acids missing	Reference
B6/WT-19	*b*	SV40	None	59
B6/pSV3T3 20 GV	*b*	pSV3T3 20 GV	369–708	60
B6/BKVD-1	*b*	BK virus DNA	None	6
C3HSV-4	*k*	SV40	None	This study
B6/PY	*b*	Polyomavirus	None	25
L/pVBETK-1	*k*	pVBETK-1	None	45
L/pVBETK-1/K^b	*k*/*K*^{b}	pVBETK-1	None	This study
L/pVBETK-1/D^b	*k*/*D*^{b}	pVBETK-1	None	This study
L/pVBt1TK-1	*k*	pVBt1TK-1	272–708	45
L/pVBt1TK-1/K^b	*k*/*K*^{b}	pVBt1TK-1	272–708	This study
L/pVBt1TK-1/D^b	*k*/*D*^{b}	pVBt1TK-1	272–708	This study
LTK$^-$	*k*	None		69
LTK$^-$/neo	*k*	pSV2-neo		This study
LTK$^-$/K^b	*k*/*K*^{b}	pH-2K^b		This study
LTK$^-$/D^b	*k*/*D*^{b}	pH-2D^b		This study
B6/pPVU-5-70K	*b*	pPVU-5-70K	1–108	This study
B6/y2xmet128-70K	*b*	py2xmet128-70K	1–127	This study
B6/y2xB9-70K	*b*	py2xB9-70K	1–149	This study
B6/60K	*b*	p60K	1–175	This study
B6/RL5-S24	*b*	pRL5-S24	218–250	This study
B6/RL75-RL12	*b*	pRL75-RL12	189–228	This study
B6/RL75	*b*	pRL75	189–192	This study
B6/RL75-RL88	*b*	pRL75-RL88	189–211	This study
B6/RL75-RL115	*b*	pRL75-RL115	189–223	This study
B6/RL101-RL12	*b*	pRL101-RL12	220–228	This study
B6/RL115	*b*	pRL115	220–223	This study

cell lines were transfected with plasmids which carry either the *H-2K*^{b} gene or the *H-2D*^{b} gene (1) along with the pSV2-neo plasmid as a selectable marker. Cells resistant to G418 (500 μg/ml) were developed into cell lines. The cells were shown to express the *H-2K*^{b} or *H-2D*^{b} glycoproteins as determined by flow cytometry.

Generation of SV40-sensitized lymphocytes. SV40-sensitized lymphocytes were generated as previously described (15, 59). C57BL/6 mice were immunized intraperitoneally with 1×10^7 to 2×10^7 syngeneic SV40-transformed cells. After 9 or more days, the spleens were excised, and a single-cell suspension was prepared by pressing the spleens through a 60-gauge stainless steel screen. The splenic erythrocytes were removed by hypotonic lysis with Tris-ammonium chloride. Lymphocytes (1.5×10^7) were cocultured with 2×10^5 γ-irradiated (10,000 rads), syngeneic SV40-transformed stimulator cells in RPMI 1640 supplemented with 5×10^{-5} M β-mercaptoethanol–25 μg of pyruvate per ml–10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)–0.225% $NaHCO_3$–100 U of penicillin per ml–100 μg of streptomycin per ml–0.03% glutamine–10% FCS heat inactivated at 56°C for 30 min. The cultures were maintained at 37°C in 5% CO_2 for 5 days. The phenotype of the effector cells generated by this method is Thy1$^+$ Lyt1$^-$ Lyt2$^+$ (15).

SV40-specific CTL clones. CTL clones K11 and K19 are specific for the SV40 large T antigen and are *H-2D*^{b} restricted (6). Both of these CTL clones recognize antigenic determinants present on a truncated large T antigen of amino acid residues 1 to 368 (60). CTL clone K19 cross-reacts with a large T antigen encoded by the human papovavirus BK, whereas the determinant recognized by K11 is found only on the SV40 large T antigen (6).

Cytotoxicity assay. The ^{51}Cr release assay was performed as previously described (15, 59). Target cells were labeled overnight in 75-cm^2 flasks with 250 μCi of ^{51}Cr in 10 ml of DMEM containing 15% heat-inactivated FCS. The target cells were harvested by washing the monolayer with 5 ml of 2% EDTA and adding 1 ml of 0.1% trypsin. The single-cell suspension was diluted with 9 ml of complete RPMI 1640 containing 10% FCS. The labeled target cells were washed three times with fresh RPMI 1640 containing 10% FCS. A 0.1-ml sample containing 10^4 target cells was dispensed into glass culture tubes (10 by 75 mm) and mixed with the in vitro-stimulated bulk-culture lymphocytes or with CTL clones at various lymphocyte-to-target-cell ratios. The spontaneous release of ^{51}Cr from the target cells was determined by incubating the labeled target cells in 0.2 ml of complete RPMI 1640 containing 10% FCS. The maximum amount of ^{51}Cr that could be released from the target cells was determined by lysing them with 5% sodium dodecyl sulfate. The lymphocyte-target cell suspension was centrifuged at $60 \times g$ for 5 min at 4°C and then incubated at 37°C in 5% CO_2 for 5 h. After the 5-h incubation, 0.8 ml of complete RPMI 1640 containing 10% FCS was added to each sample. All the samples were centrifuged at $250 \times g$ for 10 min at 4°C, and 0.5 ml of supernatant was aspirated and placed in a counting vial. The radioactivity in the supernatant was counted along with the remaining radioactivity in the cell pellet and supernatant in a Gamma 8500 Counter (Beckman Instruments, Inc., Palo Alto, Calif.). The percent specific lysis was determined by using the following formula: % specific lysis = [(% immune lysis − % normal lysis)/(% maximum lysis − % spontaneous lysis)] × 100.

Enhanced expression of the class I gene products in target cells by treatment with gamma interferon. Gamma interferon has been shown to induce high levels of expression of the MHC antigens (14, 67). To increase the expression of surface *H-2*^{b} on cells used as targets in the ^{51}Cr release assay, these cells were incubated with gamma interferon as described by Flyer et al. (16). The medium from subconfluent monolayers of cell cultures in 75-cm^2 flasks was removed and replaced

with 20 ml of DMEM containing 10% FCS and 2,000 U of crude gamma interferon (kindly provided by D. Murasko) for 48 h at 37°C, after which time the medium was removed and replaced with fresh medium without gamma interferon. The cells were assayed after 12 h for the expression of the class I antigens by a fluorescence-activated cell sorter (FACS) analysis and used as targets in a ^{51}Cr release assay.

FACS analysis. To determine the surface expression of the host-encoded *H-2* antigens, FACS analysis using monoclonal antibodies reactive with the *H-2K*^{b} and *H-2D*^{b} antigens was performed on viable cells (31). Cells for analysis were removed from the culture flasks by using EDTA and 0.1% trypsin and suspended with FACS buffer (phosphate-buffered saline [0.68% NaCl, 0.17% Na_2HPO_4, 0.02% KH_2PO_4, pH 7.4] with 2% FCS and 0.1% NaN_3). The cells were diluted to 10^6/ml, and 1 ml was dispensed to glass culture tubes (12 by 75 mm). The cells were centrifuged at 250 × *g* for 5 min at 4°C, and the buffer was decanted off. Monoclonal antibodies EH-144 (*H-2K*^{b} reactive) and 28-14-8 (*H-2D*^{b} reactive) (100 μl) were dispensed to the appropriate tubes. (EH-144 and 28-14-8 were kindly provided by T. V. Rajan.) The monoclonal antibodies were allowed to incubate with the cells for 45 min at 4°C. The samples were diluted by the addition of 3.0 ml of FACS buffer and centrifuged, and the diluted antibody was decanted off. The cells were suspended with 100 μl of goat anti-mouse fluorescein isothiocyanate (FITC)-conjugated antibody (Becton Dickinson and Co., Mountainview, Calif.) and incubated for 45 min at 4°C. The samples were diluted with 2.0 ml of FACS buffer, centrifuged, washed twice more with 3.0 ml of buffer, and suspended, after centrifugation, in 400 μl of FACS buffer. Propidium iodide (50 μg of propidium iodide per ml, 1 mg of RNase B per ml) was added to the samples to electronically gate out dead cells from the analysis. The samples were run on an Epics V sorter/cytometer (Coulter Electronics, Inc., Hialeah, Fla.) set at an excitation wavelength of 488 nm and 500 mW of power. The instrument was calibrated with 10-mm fluorespheres (grade II; Coulter), and the coefficients of variation were <2.5. Ninety-degree light scatter was collected with a 488-nm dichroic mirror and a neutral-density filter. Green fluorescence was collected with a 515-nm long pass filter, a 560-nm short pass filter, and a 515-nm interference filter. For each sample, 10,000 cells were analyzed at 500 cells per s by collecting the logarithm of integrated green fluorescence gated on 90° light scatter and forward-angle light scatter. The data were analyzed with the Immuno Program (Coulter).

RESULTS

Specificity of CTL clones K11 and K19. The CTL clones K11 and K19 were established by Campbell et al. (6) in this laboratory and were shown to be *H-2D*^{b} restricted and specific for SV40. The results in Table 4 show that the *H-2* restriction and antigen specificity of these two clones were maintained during in vitro passage and that both CTL clones lysed SV40-transformed *H-2*^{b} cells but not SV40-transformed *H-2*^{k} cells or polyomavirus-transformed *H-2*^{b} cells. The results also show that as reported previously (6), the CTL clone K19 possesses specificity for SV40 T antigen as well as for the cross-reactive T antigen present in human BK virus-transformed *H-2*^{b} cells, whereas K11 is specific for SV40 only, thus showing that CTL clones K11 and K19 recognize distinct sites on the SV40 T antigen. In addition, both clones were able to lyse B6/pSV3T3 20 GV cells (60), which synthesize a truncated T antigen of 1 to 368 amino acids, indicating that the antigenic determinants recognized by these two CTL clones lie in the amino-terminal half of SV40 T antigen. These results also suggest that the amino acid residues in the carboxy-terminal half of the T antigen do not affect the antigenic sites recognized by these CTL clones.

Localization of K11 and K19 antigenic sites between 176 and 368 amino acids of the SV40 T antigen. To resolve the epitopes recognized by CTL clones K11 and K19 which map between residues 1 and 368, B6 cell lines expressing truncated T antigens missing various lengths of the amino terminus of T antigen (Fig. 2A) were used as target cells in the ^{51}Cr release assay. The cell lines B6/PVU-5-70K, B6/y2xmet128-70K, B6/y2xB9-70K, and B6/60K synthesize T antigens which are missing amino acids 1 to 108, 1 to 127, 1 to 149, and 1 to 175, respectively, of the T antigen, and these cell lines were all lysed to a significant degree by CTL clones K11 and K19 in the cytotoxicity assay (Fig. 2B). A cell line carrying wild-type T antigen (B6/WT-19) was also positive in the assay; however, B6/PY cells, transformed by the polyomavirus, did not react with either of the CTL clones as expected (Fig. 2B). The synthesis of T antigens by cell lines B6/pVU-5-70K, B6/y2xmet128-70K, B6/y2xB9-70K, and B6/60K was confirmed by immunoprecipitation and polyacrylamide gel electrophoresis. These truncated T antigens did not react with monoclonal antibody PAb902, which recognizes a determinant mapping in the first 30 amino acids of the T antigen, but reacted with the monoclonal antibody PAb901, which recognizes a determinant in the

TABLE 4. Specificity and *H-2* restriction of CTL clones K11 and K19

Expt	Target cells	*H-2* haplotype		Transforming agent	% Specific lysis by CTL clone[a]:	
		K	*D*		K11	K19
1	B6/WT-19	*b*	*b*	SV40	82.6	65.7
	B6/pSV3T3 20 GV[b]	*b*	*b*	pSV3T3 20 GV	49.4	32.6
	B6/PY	*b*	*b*	Polyomavirus	1.0	−1.0
2	B6/WT-19	*b*	*b*	SV40	71.3	51.0
	B6/pSV3T3 20 GV[b]	*b*	*b*	pSV3T3 20 GV	50.7	34.8
	B6/BKVD-1	*b*	*b*	BK virus DNA	5.2	44.1
3	B6/WT-19	*b*	*b*	SV40	52.1	55.5
	B6/pSV3T3 20 GV[b]	*b*	*b*	pSV3T3 20 GV	58.3	59.7
	C3HSV-4	*k*	*k*	SV40	0.1	4.2

[a] Determined at an effector-to-target-cell ratio of 10:1.
[b] This cell line synthesizes amino acids 1 to 368 of T antigen.

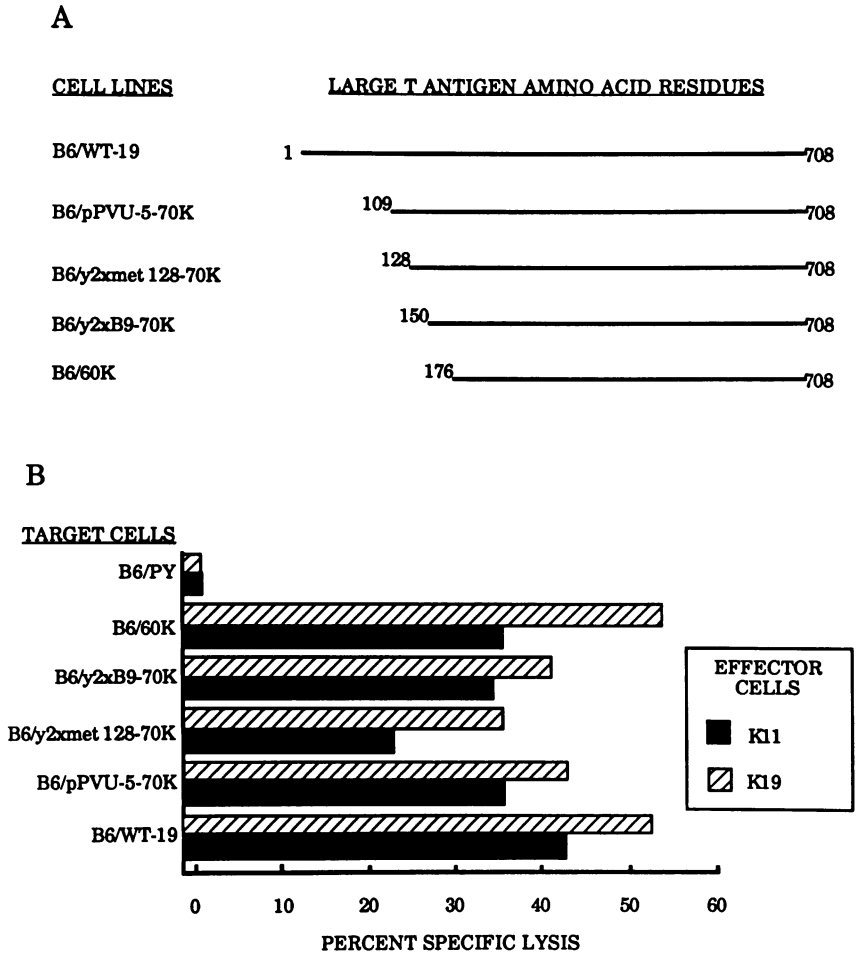

FIG. 2. Susceptibility of cell lines expressing amino-terminal-truncated T antigens by CTL clones K11 and K19. (A) Cell lines and the T antigens that they express. (B) CTL clones K11 and K19 were reacted with the ^{51}Cr-labeled cell lines shown in Fig. 1A and with B6/PY cells for 5 h at 37°C, and the percent specific lysis was calculated as described in Materials and Methods.

carboxy-terminal end of the T antigen (M. Tevethia, unpublished data). These studies indicate that the epitopes recognized by the CTL clones K11 and K19 lie between amino acids 176 and 368 of the T antigen.

Localization of epitopes recognized by CTL clones K11 and K19 between amino acids 176 and 271 of the T antigen. To further narrow the region encompassing epitopes recognized by CTL clones K11 and K19, cell line L/pVBt1TK-1, which synthesizes a truncated T antigen of 1 to 271 residues was used (Fig. 3). However, this cell line (45, 61) is of $H\text{-}2^k$ origin and was of an inappropriate haplotype for mapping CTL clones K11 and K19, which are of $H\text{-}2^b$ origin. We and others have previously shown that the L/pVBt1TK-1 cells possess epitopes that are recognized by the CTL generated in $H\text{-}2^k$ mice (21, 61). Attempts to generate a transformed cell line of $H\text{-}2^b$ origin that synthesizes a stable truncated T antigen of 33 kDa had failed. Therefore, an alternate approach was undertaken in which the $H\text{-}2^b$ class I restricting elements, the cloned $H\text{-}2K^b$ and $H\text{-}2D^b$ genes (1), were expressed in L/pVBt1TK-1 cells, so that they could provide the correct restriction element and allow these cells to be tested for reactivity against CTL clones K11 and K19. The $H\text{-}2K^b$ and $H\text{-}2D^b$ genes were transfected into L/pVBt1TK-1 cells. In addition, L/pVBETK-1 cells, which produce a 94-kDa T antigen, and the parental LTK$^-$ cells were transfected with the $H\text{-}2K^b$ and $H\text{-}2D^b$ genes to serve as positive and negative controls, respectively. Both of these cell lines are of $H\text{-}2^k$ origin (Fig. 3).

The expression of the transfected $H\text{-}2^b$ genes was determined by reacting the cells with monoclonal antibodies EH-144 (anti-$H\text{-}2K^b$) and 28-14-8 (anti-$H\text{-}2D^b$) and analyzing these by FACS. The number of positive cells indicated on the vertical axis was plotted against the log fluorescence intensity on the horizontal axis (Fig. 4). The histograms show the background staining of the cells with the goat anti-mouse FITC-conjugated antibody and staining of the cells with either the anti-$H\text{-}2K^b$ or the anti-$H\text{-}2D^b$ monoclonal antibody. B6/WT-19, which was derived from cells of $H\text{-}2^b$ origin, was the positive control in this experiment. Binding of the anti-$H\text{-}2K^b$ or anti-$H\text{-}2D^b$ monoclonal antibody shifted the peak of fluorescence intensity to the right, indicating the cell surface expression of both the $H\text{-}2K^b$ and the $H\text{-}2D^b$ class I molecules. L/pVBt1TK-1 cells transfected with the pSV2-neo plasmid containing the G418 resistance gene alone did not stain with either the anti-$H\text{-}2K^b$ or the anti-$H\text{-}2D^b$ monoclonal antibody, ruling out any cross-reactivity with the class I molecules expressed in cells of $H\text{-}2^k$ origin. The $H\text{-}2K^b$ gene-transfected L/pVBETK-1 cell line L/pVBETK-1/K^b was stained by the anti-$H\text{-}2K^b$ monoclonal antibody but not by the anti-$H\text{-}2D^b$ monoclonal antibody.

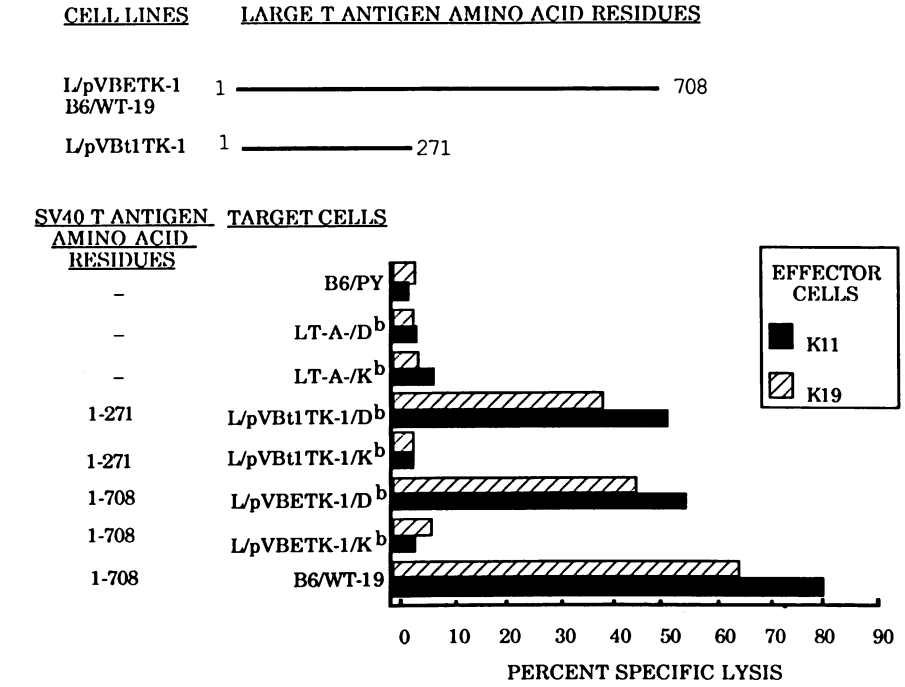

FIG. 3. Susceptibility of L/pVBETK-1 and L/pVBt1TK-1 cell lines expressing *H-2K*^{b} and *H-2D*^{b} antigens to lysis by CTL clones K11 and K19. CTL clones K11 and K19 were reacted with ^{51}Cr-labeled target cells at an effector-to-target-cell ratio of 10:1 for 5 h, and the percent specific lysis was calculated as described in Materials and Methods.

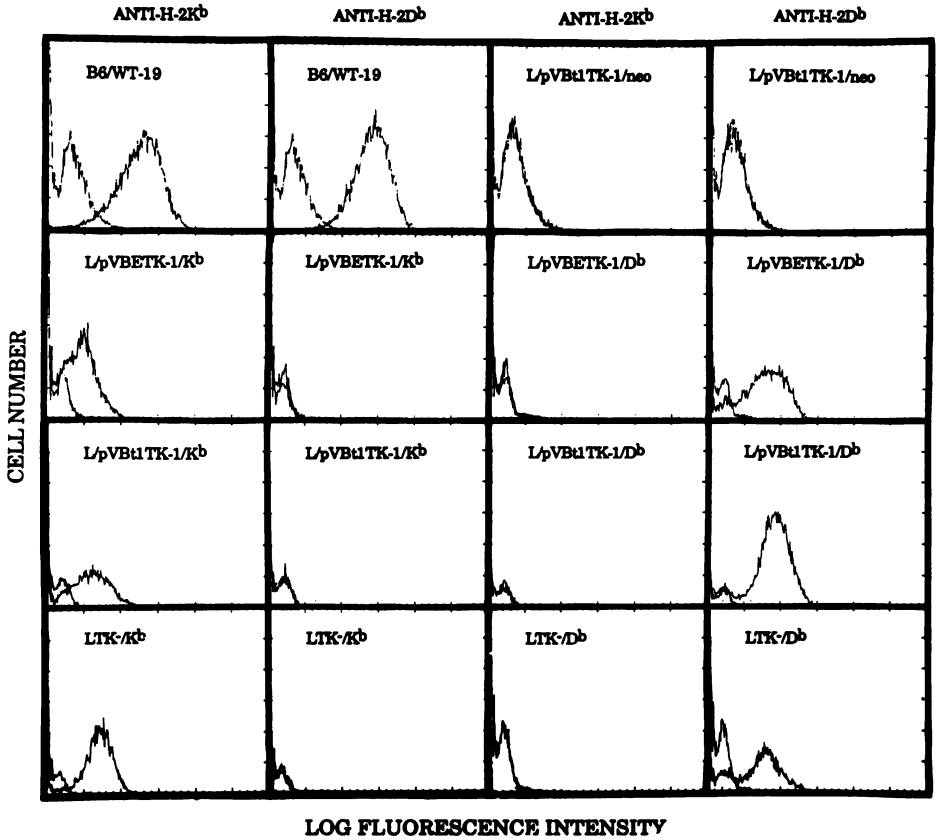

FIG. 4. *H-2*^{b} antigen expression of *H-2K*^{b}- and *H-2D*^{b}-transfected L/pVBETK-1 and L/pVBt1TK-1 cell lines. The expression of the *H-2K*^{b} molecule was determined with the monoclonal antibody EH-144. *H-2D*^{b} expression was determined with the monoclonal antibody 28-14-8. The presence of both monoclonal antibodies on the surface of the cell was revealed with goat anti-mouse antibody conjugated with FITC. The analysis was performed on a Coulter Epics V sorter/cytometer at a 488-nm excitation wavelength.

This was true also for the cell lines L/pVBt1TK-1/K^b and LTK^-/K^b. L/pVBETK-1/D^b, L/pVBt1TK-1/D^b, and LTK^-/D^b all stained with the anti-*H-2D*^{b} monoclonal antibody but not with the anti-*H-2K*^{b} antibody. The cell lines L/pVBETK-1/D^b and L/pVBt1TK-1/D^b now expressed the class I restricting element necessary for epitope mapping by SV40-specific CTL of *H-2*^{b} origin.

To determine whether the CTL clones K11 and K19 recognized a determinant present within the sequence of amino acids 176 to 271, the cell lines indicated in Fig. 3 were reacted with CTL clones K11 and K19 at an effector-to-target-cell ratio of 10:1. The results are shown in Fig. 3. The cell line B6/WT-19 (*H-2*^{b}), which served as a positive control, was lysed by both K11 and K19, while the negative control, B6/PY (*H-2*^{b}), was not lysed by either of the CTL clones. L/pVBETK-1/D^b, which expresses a full-length T antigen and the *H-2D*^{b} class I antigen, was also lysed, while the L/pVBETK-1/K^b, which synthesizes a full-length T antigen and expresses the transfected *H-2K*^{b} class I antigen, was not lysed. This is consistent with data reported by Campbell et al. (6), showing that both CTL clones are *H-2D*^{b} restricted. The results also show that the CTL clones K11 and K19 did not cross-react with the *H-2*^{k} alloantigens. The cell line L/pVBt1TK-1/D^b, which expresses the truncated T antigen of amino acids 1 to 271 and the appropriate restriction element *H-2D*^{b}, was lysed by both K11 and K19, while L/pVBt1TK-1/K^b, which does not express the appropriate restriction element, was not lysed. The cell lines LTK^-/K^b and LTK^-/D^b do not express any SV40 large T antigen and were not lysed by the CTL clones K11 and K19. Since it has been shown previously that the CTL clones lyse target cells missing T-antigen residues 1 to 175, it was concluded that these clones recognize determinants found within amino acid residues 176 to 271.

Fine mapping of antigenic sites recognized by CTL clones K11 and K19. Further mapping of the antigenic sites recognized by the CTL clones K11 and K19 required SV40 mutants that encoded T antigens with very small deletions within residues 176 to 271 of the large T antigen. A series of overlapping deletion mutants missing nucleotides corresponding to this region of large T antigen were used to finely map the epitopes recognized by the CTL clones K11 and K19. The deletion mutant plasmid DNAs (Fig. 5A) were transfected into syngeneic B6/MEF, and individual transformed cell lines were established and assayed for sensitivity to lysis by the CTL clones in a ^{51}Cr release assay at an effector-to-target-cell ratio of 10:1. CTL clones K11 and K19 were first tested against the cell lines B6/RL5-S24 and B6/RL75-RL12, which produce T-antigen molecules missing residues 218 to 250 and residues 189 to 228, respectively (Fig. 5A). The results are shown in Fig. 5B. Both CTL clones lysed B6/WT-19, the positive control, but not B6/PY, the negative control. The CTL clone K19 did not kill the cell line B6/RL5-S24 (missing residues 218 to 250), while K11 did (Fig. 5B). Neither CTL clone lysed the cell line B6/RL75-RL12, which is missing amino acids 189 to 228 (Fig. 5B). These data indicate that residues 189 to 228 are critical for recognition of antigenic sites by CTL clones K11 and K19, while residues 218 to 250 are essential for recognition of an antigenic site by CTL clone K19.

To identify the smallest possible deletion that could destroy CTL recognition, several additional deletion mutants missing amino acids ranging from residues 189 to 228 were chosen. Transformed cell lines were established and assayed for sensitivity to lysis by K11 and K19 in a ^{51}Cr release assay at an effector-to-target-cell ratio of 10:1 (Fig. 5C). Both K11 and K19 lysed the positive control B6/WT-19, whereas neither clone lysed B6/PY, the negative control. Both CTL clones lysed B6/RL75, which synthesizes a T antigen missing residues 189 to 192. CTL clone K11 did not lyse B6/RL75-RL88 (missing residues 189 to 211), while CTL clone K19 lysed these cells, indicating that amino acids 189 to 211 are critical for the recognition of the antigenic site by CTL K11 but not by K19. B6/RL101-RL12 (missing amino acids 220 to 228) and B6/RL115 (missing amino acids 220 to 223) were not lysed by CTL clone K19 but were killed by CTL clone K11, indicating that amino acids 220 to 223 are critical for the recognition of the antigenic site by K19. The cell lines B6/RL75-RL12 and B6/RL75-RL115, which syn-

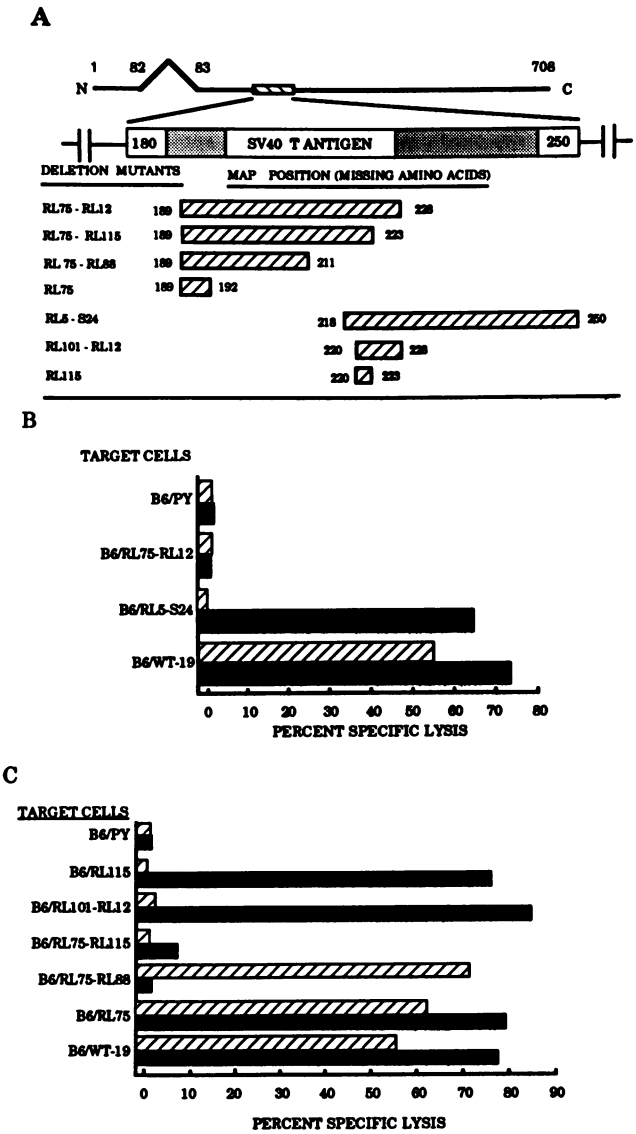

FIG. 5. Susceptibility of B6 cells expressing T antigen carrying internal deletions of various lengths to lysis by CTL clones K11 and K19. (A) Deletion mutants used in this experiment. (B and C) Susceptibility of deletion-mutant-transformed *H-2*^{b} cells to lysis by CTL clones K11 (▬) and K19 (▨). Transformed cells were labeled with ^{51}Cr and reacted with CTL clones at an effector-to-target-cell ratio of 10:1. The percent specific lysis was calculated as described in Materials and Methods.

thesize T antigens missing amino acids 189 to 228 and 189 to 223, respectively, were not lysed by either of the CTL clones. This result was expected, since amino acids 189 to 223 include amino acids critical for the recognition of antigenic sites by CTL clone K11 (189 to 211) and by K19 (220 to 223).

Effect of deletion of amino acids 189 to 228 and 189 to 223 on antigenic sites recognized by CTL clones K11 and K19. The results of the preceding experiments showed that amino acids 189 to 211 are critical for the recognition of an antigenic site by CTL clone K11, whereas amino acids 220 to 223 are critical for the recognition of an antigenic site by CTL clone K19. When cell lines expressing SV40 T antigen carrying a deletion of amino acids 189 to 228 (B6/RL75-RL12) or 189 to 223 (B6/RL75-RL115) were tested against CTL clones K11 and K19, they were found to be nonreactive in the ^{51}Cr release assay in repeated experiments. Results obtained so far support the conclusion that deletions of amino acids 189 to 223 and 189 to 228 affect antigenic sites recognized by both the CTL clones (K11 and K19). For the mapping studies to be valid, it must be demonstrated that the mutant SV40 T antigen is expressed at the cell surface and is able to associate with class I antigens. Preliminary results indicate that the two cell lines B6/RL75-RL12 and B6/RL75-RL115 were not sensitive to lysis by a heterogeneous bulk culture of SV40-specific CTL generated in B6 mice by using syngeneic SV40-transformed cells carrying wild-type T antigen.

To determine whether a decreased level of *H-2* expression is responsible for the inability of SV40-specific CTL to lyse B6/RL75-RL12 and B6/RL75-RL115 cells, and to finally confirm the CTL mapping studies described earlier, it was necessary to enhance the level of expression of the class I molecules. Treatment of cells with gamma interferon has been shown to enhance the expression of the MHC antigens (14, 16, 67). In these experiments, the cells were treated with gamma interferon and first analyzed for the expression of the $H\text{-}2^b$ molecules and then for sensitivity to lysis by the CTL clones K11 and K19. B6/WT-19, B6/RL75-RL12 (missing residues 189 to 228), B6/RL75-RL115 (missing residues 189 to 223), and B6/PY were treated with 100 U of gamma interferon per ml of DMEM containing 10% FCS for 48 h, after which the medium was removed and fresh medium without gamma interferon was added for 12 h. Treated and untreated cells were analyzed for the expression of the $H\text{-}2K^b$ and $H\text{-}2D^b$ molecules by flow cytometry (Fig. 6). Untreated B6/RL75-RL12 and B6/RL75-RL115 cells showed a very low level of $H\text{-}2K^b$ and $H\text{-}2D^b$ expression, while B6/WT-19 expressed a normal level. Treatment of these same cell lines with gamma interferon raised the percentage of cells expressing both of the *H-2* molecules $H\text{-}2K^b$ and $H\text{-}2D^b$ to over 90 in each case. With the high level of expression of the $H\text{-}2K^b$ and $H\text{-}2D^b$ antigens, these cell lines should be sensitive to lysis by heterogeneous cultures of CTL if the truncated T antigen is expressed on the cell surfaces. The same cell lines were treated with gamma interferon and assayed for sensitivity to lysis by CTL clones K11 and K19 and by B6/WT-19 immune lymphocytes, prepared as described above, in a ^{51}Cr release assay (Table 5). Gamma interferon-treated and untreated B6/WT-19 cells were sensitive to lysis by all the effectors, while B6/PY cells were not susceptible to lysis. The cell lines B6/RL75-RL12 and B6/RL75-RL115 were sensitive to lysis by bulk-culture anti-SV40 immune lymphocytes only after treatment with gamma interferon. Gamma interferon-treated and untreated B6/RL75-RL12 and B6/RL75-RL115 cells were not sensitive to lysis by the CTL clones K11 and K19. These results show that the T antigens that carry deletions are expressed on the surfaces of B6/RL75-RL12 and B6/RL75-RL115 cells and are not recognized by CTL clones K11 and K19. Therefore, residues 189 to 223 are essential for recognition of the large T antigen by CTL clones K11 and K19.

The fine mapping data shown earlier indicate that residues 193 to 211 are essential for recognition by the CTL clone K11 and residues 220 to 223 are important for recognition by the CTL clone K19. The amino acid sequence of T antigens which includes amino acids critical to the recognition of antigenic sites by CTL clones K11 and K19 is shown in Fig. 7.

DISCUSSION

The SV40 tumor-specific transplantation antigen, although described in 1963 (10, 24, 33, 36), remained an elusive antigenicity present at the surface of SV40-transformed cells. It was not until 1977 that Anderson et al. (2) successfully used crude preparations of SV40 T antigen to specifically immunize BALB/c mice against a challenge by syngeneic SV40 tumor cells, thereby associating tumor-specific transplantation antigen with SV40 early-gene products. Later studies using T antigen purified to homogeneity led to the conclusion that the antigenicity for tumor-specific transplantation antigen resides in T antigen (9, 58). A similar conclusion was also reached by using SV40 T antigen fragments produced by the adenovirus type 2-SV40 hybrid viruses (30). Several additional lines of evidence indicated that T antigen is the target protein for the CTL that recognize SV40 tumor-specific transplantation antigen in association with MHC class I antigens. Purified T antigen could prime mice for the generation of SV40-specific CTL (8, 58). In addition, immunogenetic evidence demonstrated that T antigen is reprocessed in vivo by antigen-presenting cells. SV40-transformed allogeneic and xenogeneic cells induce the generation of pre-CTL, which can be stimulated in vitro only with SV40-transformed cells which share *H-2* class I antigens with the pre-CTL (19). Finally, the reactivity of a single SV40-specific $H\text{-}2K^b$-restricted CTL clone can be abrogated by a monoclonal antibody directed to the T antigen (41). However, the precise region of SV40 T antigen to which either the CTL clone or the monoclonal antibody is directed was not determined.

We have previously demonstrated that CTL-reactive sites are located in the amino-terminal region of the T antigen, since a cell line synthesizing SV40 T antigen of 1 to 271 amino acids is susceptible to CTL generated in response to transformed cells synthesizing wild-type T antigen (61). These studies were later confirmed and extended, demonstrating that the CTL-reactive sites also were located in the carboxy-terminal region of the molecule (21). Campbell et al. (6) were the first to provide evidence that there are two distinct antigenic sites on T antigen with which CTL react by establishing two CTL clones designated as K11 and K19. The reactivity of these clones was later localized to the amino-terminal region of the T antigen between amino acids 1 and 368 (60).

We have now more precisely defined amino acids critical to the recognition of antigenic sites by CTL clones K11 and K19 to a small stretch of amino acids extending from positions 193 to 228. By using deletion mutants, we have shown that amino acids 193 to 211 are critical for maintenance of the K11 antigenic site, whereas amino acids 220 to 223 are critical for maintenance of the K19 antigenic site. Although there is no direct evidence as yet that amino acids

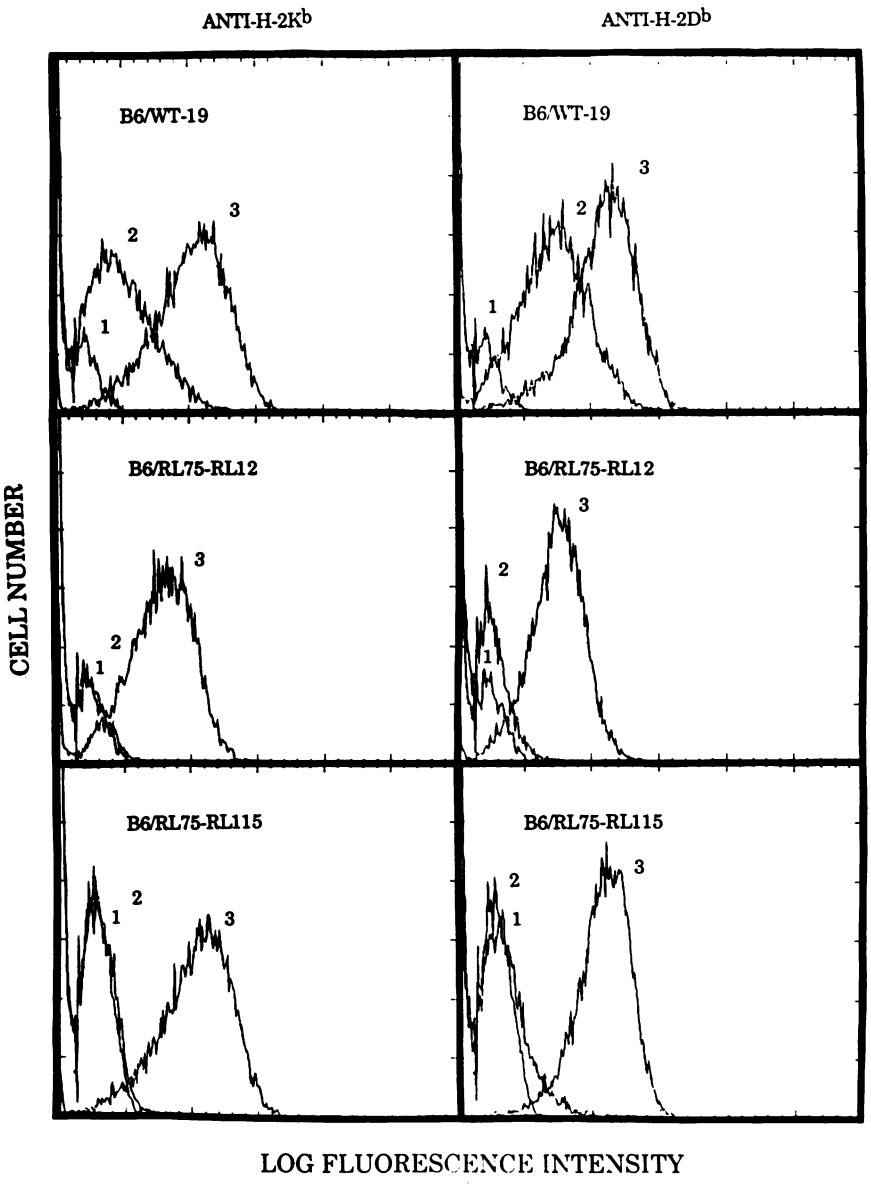

FIG. 6. *H-2^b* antigen expression on B6/RL75-RL12 and B6/RL75-RL115 after gamma interferon treatment. B6/WT-19, B6/RL75-RL12, and B6/RL75-RL115 were treated with gamma interferon as described in Materials and Methods. The FITC-conjugated control (peak 1) was compared with the untreated (peak 2) and gamma interferon-treated (peak 3) cells. The expression of the *H-2K^b* molecule was monitored with the monoclonal antibody EH-144. *H-2D^b* expression was monitored with the monoclonal antibody 28-14-8. The presence of monoclonal antibodies on the surface of the cell was detected with goat anti-mouse antibody conjugated with FITC. The analysis was performed on a Coulter Epics V sorter at a 488-nm excitation wavelength.

193 to 211 and 220 to 223 directly contribute to the antigenic sites recognized by K11 and K19, respectively, indirect evidence suggests this possibility. Deletion of amino acids 193 to 211 abolished only the site for K11 and not for K19. Similarly, a deletion of residues 220 to 223 abolished the site for K19 but not for K11. Thus, these sites appear to be independent of each other. Finally, a deletion of amino acids common to both the sites, 189 to 223, prevented killing by both CTL clones. Although we know the boundaries of the region required for recognition of an antigenic site by K11 (189 and 217) and one boundary for the K19 site (amino acid 212), we do not know the carboxy boundary of the antigenic site recognized by K19. A boundary is taken to be the position at which the antigenic site is regained. The results show that T antigen amino acids 1 to 192 do not contribute to the K11 and K19 sites, as these amino acids can be deleted without affecting recognition of these sites. Similarly, amino acids beyond 271 also do not contribute toward these antigenic sites, since the cell line L/pVBt1TK-1/D^b was lysed by CTL clones K11 and K19.

The close proximity of the antigenic sites for K11 and K19 was surprising. The data indicate that these antigenic sites are distinct, as the removal of one by deleting relevant amino acids did not affect the site for the other. As we have previously shown (6), K11 and K19 can be distinguished on the basis of their cross-reactivities to human BK virus-transformed *H-2^b* cells. Human BK virus encodes a serologically cross-reactive T antigen (54). While CTL clone K19

reacts with BK and SV40 T antigens, K11 is specific only for SV40 T antigen. The cross-reactivity of K19 with SV40 and BK virus T antigens can be explained on the basis that amino acids 220 to 223 in SV40 T antigen, which are critical for recognition of an antigenic site by K19, are identical to the amino acids in BK virus T antigen. However, amino acids Tyr-211 to Ala-212 in SV40 large T antigen, which is recognized by CTL clone K11, are replaced in the BK virus large T antigen, which is not recognized by the CTL clone K11, by Phe-211 to Cys-212. In addition, leucine at position 195 in SV40 T antigen is replaced by isoleucine in BK virus T antigen (62). It is possible that these amino acids may directly confer specificity to the antigenic site recognized by CTL clone K11.

Recognition of the antigenic site by CTL requires self-recognition of MHC class I antigen. Both K11 and K19 CTL clones are *H-2D*^{b} restricted (6). For mapping studies, the restriction elements, either *H-2K*^{b} or *H-2D*^{b}, must be expressed in sufficient quantities to provide self-recognition. This requirement was evident from our results with cell lines missing amino acid residues 189 to 223 or 189 to 228. These cell lines did not express sufficient amounts of *H-2D*^{b} to be lysed by SV40 CTL clones K11 and K19 or by SV40 CTL generated in bulk culture. Treatment of the cell lines with gamma interferon restored *H-2* expression, and the cells became susceptible to SV40 CTL generated in bulk culture but not to clones K11 and K19. The requirement for corecognition of the MHC class I antigens and the antigenic site by antigen-specific CTL may be partly responsible for the limited number of antigenic sites recognized by CTL. Since the T-cell receptor must recognize class I antigens in association with an antigen site on the T antigen, the residues which become accessible to the T-cell receptor may be limited. The site on SV40 T antigen that associates with MHC class I antigen is not yet known.

Since there are no known stretches of hydrophobic residues in the SV40 T antigen to explain its presence in the cell membranes, Sharma et al. (49) have proposed that T antigen is not transported to the cell membranes by an exocytotic pathway. Regardless of the mechanism, SV40 T antigen is present in the intact form in the plasma membranes. Both carboxy- and amino-terminal ends of cell surface T antigen are accessible to binding by SV40-specific monoclonal antibodies (3, 13, 20, 48, 68), and the T antigen can be immunoprecipitated intact from the plasma membrane (46, 48, 51, 52, 59). There is evidence that cell surface T antigen is acylated (34) and glycosylated (29). T antigen is not the only nuclear protein that is recognized by CTL in the plasma membrane. The nucleoprotein (NP) of influenza virus is also recognized by antiviral MHC class II-restricted CTL (63, 64, 66, 70). In addition, influenza virus polymerases and a nonstructural protein have been shown to provide a target for CTL (4, 5). Townsend et al. (65) have suggested that the influenza virus NP is proteolytically processed and expressed on the infected-cell surface, much as an antigen-presenting cell would process and express antigen. In support of this notion, short stretches of synthetic peptides corresponding to regions of influenza virus NP, when adsorbed to cells provide a target for NP-specific CTL (63). However, these investigators were not able to detect any effect of chloroquine, an agent that raises lysosomal pH and inhibits the lysosomal degradation of protein, on the susceptibility of virus-infected or NP-gene-transfected cells to lysis by NP-specific CTL. Therefore, proteolytic processing of the target antigen must occur in a nonlysosomal compartment of the target cell. Morrison et al. (40) have suggested that CTL restricted by MHC class I molecules recognize viral antigen in their native conformation, while class II-restricted CTL may recognize an antigenic determinant on processed protein. For SV40 T antigen, the question as to whether CTL recognize the intact T antigen or processed T antigen at the cell surface remains open.

TABLE 5. Susceptibility of B6/RL75-RL12 and B6/RL75-RL115 to lysis by CTL clones K11 and K19 and bulk cultures of SV40-specific CTL after treatment with gamma interferon[a]

Target cells	T-antigen amino acids missing	Gamma interferon	% Specific lysis by:		
			K11	K19	Anti-SV40
B6/WT-19	None	−	72.0	53.1	75.3
B6/WT-19	None	+	59.8	42.3	69.8
B6/RL75-RL12	189–228	−	−1.8	−1.2	13.3
B6/RL75-RL12	189–228	+	−1.2	0.4	70.5
B6/RL75-RL115	189–223	−	−0.8	−0.3	9.6
B6/RL75-RL115	189–223	+	−4.0	−0.4	67.3
B6/PY		−	−2.1	−0.6	3.7
B6/PY		+	−2.0	−2.5	12.6

[a] The cell lines were treated with gamma interferon as described in Materials and Methods. SV40-specific bulk-culture CTL were generated in C57BL/6 mice by secondary stimulation of in vivo-primed splenocytes. The percent lysis was determined at an effector-to-target-cell ratio of 10:1 for the CTL clones and 30:1 for the bulk cultures of SV40-specific CTL by incubating ^{51}Cr-labeled targets with the CTL clones for 5 h.

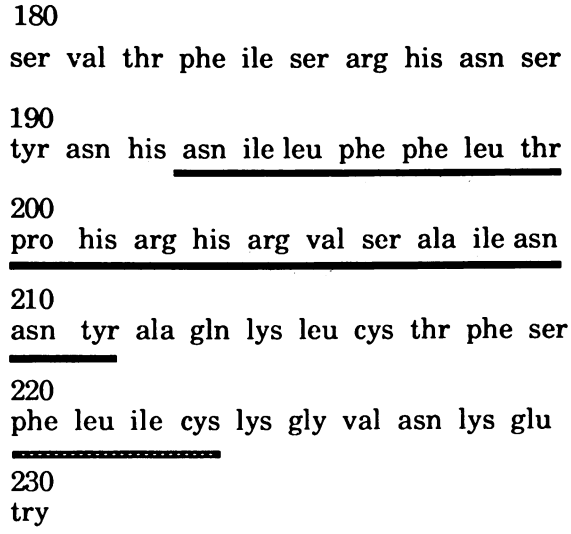

FIG. 7. T-antigen amino acid sequence showing residues (underlined) critical to recognition of antigenic sites by CTL clones K11 (▬) and K19 (▭).

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LITERATURE CITED

1. **Allen, H., D. Wraith, P. Pala, B. Askonas, and R. A. Flavell.** 1984. Domain interactions of H-2 class I antigens alter cytotoxic T-cell recognition sites. Nature (London) **309**:279–281.
2. **Anderson, J. L., R. G. Martin, C. Chang, P. T. Mora, and D. M. Livingston.** 1977. Nuclear preparations of SV40-transformed

cells contain tumor-specific transplantation antigen activity. Virology **76**:420–425.
3. **Ball, R. K., B. Siegel, S. Quellhorst, G. Bradner, and D. G. Braun.** 1984. Monoclonal antibodies against simian virus 40 nuclear large T tumor antigen: epitope mapping, papova virus cross-reaction and cell surface staining. EMBO J. **3**:1485–1491.
4. **Bennink, J. R., J. W. Yewdell, and W. Gerhard.** 1982. A viral polymerase involved in recognition of influenza virus-infected cells by a cytotoxic T-cell clone. Nature (London) **296**:75–76.
5. **Bennink, J. R., J. W. Yewdell, G. L. Smith, and B. Moss.** 1987. Anti-influenza virus cytotoxic T lymphocytes recognize the three viral polymerases and a nonstructoral protein: responsiveness to individual viral antigens is major histocompatibility complex controlled. J. Virol. **61**:1098–1102.
6. **Campbell, A. E., L. F. Foley, and S. S. Tevethia.** 1983. Demonstration of multiple antigenic sites of the SV40 transplantation rejection antigen by using cytotoxic lymphocyte clones. J. Immunol. **130**:490–492.
7. **Chandrasekaran, K., D. J. Winterbourne, S. W. Luborsky, and P. T. Mora.** 1981. Surface proteins of simian-virus-40-transformed cells. Int. J. Cancer **27**:397–407.
8. **Chang, C., R. Chang, P. T. Mora, and C.-P. Hu.** 1982. Generation of cytotoxic lymphocytes by SV40-induced antigens. J. Immunol. **128**:2160–2163.
9. **Chang, C., R. G. Martin, D. M. Livingston, S. W. Luborsky, C.-P. Hu, and P. T. Mora.** 1979. Relationship between T-antigen and tumor-specific transplantation antigen in simian virus 40-transformed cells. J. Virol. **29**:69–75.
10. **Defendi, V.** 1963. Effect of SV40 virus immunization on growth of transplantable SV40 and polyoma virus tumors in hamsters. Proc. Soc. Exp. Biol. Med. **113**:12–16.
11. **Deppert, W., and R. Pates.** 1979. Cell surface location of simian virus 40-specific proteins on HeLa cells infected with adenovirus type 2-simian virus 40 hybrid viruses Ad2$^+$ND1 and Ad2$^+$ND2. J. Virol. **31**:522–536.
12. **Deppert, W., and R. Pates.** 1979. Simian virus 40 specific proteins on surface of HeLa cells infected with adenovirus 2-SV40 hybrid virus Ad2$^+$ND2. Nature (London) **277**:322–324.
13. **Deppert, W., and G. Walter.** 1982. Domains of simian virus 40 large T antigen exposed on the cell surface. Virology **122**:56–70.
14. **Eager, K. B., J. Williams, D. Breiding, S. Pan, B. Knowles, E. Appella, and R. P. Ricciardi.** 1985. Expression of histocompatibility antigens H-2L, -D, and -L is reduced in adenovirus-12-transformed mouse cells and is restored by interferon gamma. Proc. Natl. Acad. Sci. USA **82**:5525–5529.
15. **Flyer, D. C., R. W. Anderson, and S. S. Tevethia.** 1982. Lyt phenotype of H-2^b CTL effectors and precursors specific for the SV40 transplantation rejection antigen. J. Immunol. **129**:2368–2371.
16. **Flyer, D. C., S. J. Burakoff, and D. V. Faller.** 1985. Retrovirus-induced changes in major histocompatibility complex antigen expression influence susceptibility to lysis by cytotoxic T lymphocytes. J. Immunol. **135**:2287–2292.
17. **Gooding, L. R.** 1979. Specificities of killing by T lymphocytes generated against syngeneic SV40-transformants: studies employing recombinants within the H-2 complex. J. Immunol. **122**:1002–1008.
18. **Gooding, L. R.** 1982. Characterization of a progressive tumor from C3H fibroblasts transformed *in vitro* with SV40 virus: immunoresistance *in vivo* correlates with phenotypic loss of H-2K^k. J. Immunol. **129**:1306–1312.
19. **Gooding, L. R., and C. B. Edwards.** 1980. H-2 antigen requirements in the *in vitro* induction of SV40-specific cytotoxic T lymphocytes. J. Immunol. **124**:1258–1262.
20. **Gooding, L. R., R. W. Geib, K. A. O'Connell, and E. Harlow.** 1984. Antibody and cellular detection of SV40 T antigenic determinants on the surfaces of transformed cells, p. 263–269. *In* A. J. Levine, G. F. Vande Woude, W. C. Topp, and J. D. Watson (ed.), Cancer cells: the transformed phenotype. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Gooding, L. R., and K. A. O'Connell.** 1983. Recognition by cytotoxic T lymphocytes of cells expressing fragments of the SV40 tumor antigen. J. Immunol. **131**:2580–2586.
22. **Grosveld, F. G., T. Lund, E. J. Murray, A. L. Mellor, H. H. M. Dahl, and R. A. Flavell.** 1982. The construction of cosmid libraries which can be used to transform eukaryotic cells. Nucleic Acids Res. **10**:6715–6732.
23. **Gurney, E. G., S. Tamowski, and W. Deppert.** 1986. Antigenic binding sites of monoclonal antibodies specific for simian virus 40 large T antigen. J. Virol. **57**:1168–1172.
24. **Habel, K., and B. E. Eddy.** 1963. Specificity of resistance to tumor challenge of polyoma and SV40 virus-immune hamsters. Proc. Soc. Exp. Biol. Med. **113**:1–4.
25. **Habel, K., and R. J. Silverberg.** 1960. Relationship of polyoma virus and tumor *in vivo*. Virology **12**:463–476.
26. **Harlow, E., L. V. Crawford, D. C. Pim, and N. M. Williamson.** 1981. Monoclonal antibodies specific for simian virus 40 tumor antigens. J. Virol. **39**:861–869.
27. **Henning, R., J. Lange-Mutschler, and W. Deppert.** 1981. SV40-transformed cells express SV40 T antigen-related antigens on the cell surface. Virology **108**:325–337.
28. **Ismail, A., E. A. Baumann, and R. Hand.** 1981. Cell surface T antigen in cells infected with simian virus 40 or an adenovirus-simian virus 40 hybrid, Ad2$^+$D2. J. Virol. **40**:615–619.
29. **Jarvis, D. L., and J. L. Butel.** 1985. Modification of simian virus 40 large T antigen by glycosylation. Virology **141**:173–189.
30. **Jay, G., F. T. Jay, C. Chang, R. M. Friedman, and A. S. Levine.** 1978. Tumor-specific transplantation antigen: use of the Ad2$^+$ND1 hybrid virus to identify the protein responsible for simian virus 40 tumor rejection and its genetic origin. Proc. Natl. Acad. Sci. USA **75**:3055–3059.
31. **Jennings, S. R., P. L. Rice, E. D. Kloszewski, R. W. Anderson, D. L. Thompson, and S. S. Tevethia.** 1985. Effect of herpes simplex virus types 1 and 2 on surface expression of class I major histocompatibility complex antigens on infected cells. J. Virol. **56**:757–766.
32. **Kalderon, D., and A. E. Smith.** 1984. *In vitro* mutagenesis of a putative DNA binding domain of SV40 large-T antigen. Virology **139**:109–137.
33. **Khera, K. S., A. Ashkenazi, F. Rapp, and J. L. Melnick.** 1963. Immunity in hamsters to cells transformed *in vitro* and *in vivo* by SV40: tests for antigenic relationship among papovaviruses. J. Immunol. **91**:604–613.
34. **Klockmann, U., and W. Deppert.** 1983. Acylation: a new post-translational modification specific for plasma membrane associated simian virus 40 large T-antigen. FEBS Lett. **151**:257–259.
35. **Knowles, B. B., M. Koncar, K. Pfizenmaier, D. Solter, D. P. Aden, and G. Trinchieri.** 1979. Genetic control of the cytotoxic T cell response to SV40 tumor-associated specific antigen. J. Immunol. **122**:1798–1806.
36. **Koch, M. A., and A. B. Sabin.** 1963. Specificity of virus-induced resistance to transplantation of polyoma and SV40 tumors in adult hamsters. Proc. Soc. Exp. Biol. Med. **113**:4–12.
37. **Lane, D. P., and W. T. Hoeffler.** 1980. SV40 large T shares an antigenic determinant with a cellular protein of molecular weight 68,000. Nature (London) **288**:167–170.
38. **Lange-Mutschler, J., and R. Henning.** 1982. Cell surface binding affinity of simian virus 40 T-antigen. Virology **117**:173–185.
39. **Luborsky, S. W., and K. Chandrasekaran.** 1980. Subcellular distribution of simian virus 40 T antigen in various cell lines: the 56K protein. Int. J. Cancer **25**:517–527.
40. **Morrison, L. A., A. E. Lukacher, V. L. Braciale, D. P. Fan, and T. J. Braciale.** 1986. Differences in antigen presentation to MHC class I and class II-restricted influenza virus-specific cytolytic T lymphocytes. J. Exp. Med. **163**:903–921.
41. **Pan, S., and B. B. Knowles.** 1983. Monoclonal antibodies to SV40 T antigen blocks lysis of cloned cytotoxic-T cell line specific for SV40 TASA. Virology **125**:1–7.
42. **Pfizenmaier, K., S.-H. Pan, and B. B. Knowles.** 1980. Preferential H-2 association in cytotoxic T cell responses to SV40 tumor-associated specific antigens. J. Immunol. **124**:1888–1891.
43. **Pfizenmaier, K., G. Trinchieri, D. P. Solter, and B. B. Knowles.** 1978. Mapping of H-2 genes associated with T cell-mediated cytotoxic responses to SV40-tumor-associated specific antigens. Nature (London) **274**:691–693.
44. **Pretell, J., R. S. Greenfield, and S. S. Tevethia.** 1979. Biology of

simian virus 40 (SV40) transplantation rejection antigen (TrAg). V. *In vitro* demonstration of SV40 TrAg in SV40 infected nonpermissive mouse cells by the lymphocyte mediated cytotoxicity assay. Virology **97**:32–41.

45. **Reddy, V. B., S. S. Tevethia, M. J. Tevethia, and S. M. Weissman.** 1982. Nonselective expression of simian virus 40 large tumor antigen fragments in mouse cells. Proc. Natl. Acad. Sci. USA **79**:2064–2067.
46. **Santos, M., and J. S. Butel.** 1982. Association of SV40 large tumor antigen and cellular proteins on the surface of SV40-transformed mouse cells. Virology **120**:1–17.
47. **Santos, M., and J. S. Butel.** 1982. Detection of a complex of SV40 large tumor antigen and 53K cellular protein on the surface of SV40-transformed mouse cells. J. Cell. Biochem. **19**:127–144.
48. **Santos, M., and J. S. Butel.** 1984. Antigenic structure of simian virus 40 large tumor antigen and association with cellular protein p53 on the surfaces of simian virus 40-infected and -transformed cells. J. Virol. **51**:376–383.
49. **Sharma, S., L. Rodgers, J. Brandsma, M.-J. Gething, and J. Sambrook.** 1985. SV40 T antigen and the exocytotic pathway. EMBO J. **4**:1479–1489.
50. **Soberon, X., F. Corarrubias, and F. Bolivar.** 1980. Construction and characterization of new cloning vehicles. IV. Deletion derivatives of pBR322 and pBR325. Gene **9**:287–305.
51. **Soule, H. R., and J. S. Butel.** 1979. Subcellular location of simian virus 40 large tumor antigen. J. Virol. **30**:523–532.
52. **Soule, H. R., R. E. Lanford, and J. S. Butel.** 1980. Antigenic and immunogenic characteristics of nuclear and membrane-associated simian virus 40 tumor antigen. J. Virol. **33**:887–901.
53. **Southern, P. J., and P. Berg.** 1982. Transformation of mammalian cells to an antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. J. Mol. Appl. Genet. **1**:327–341.
54. **Takemoto, K. K., and M. F. Mullarkey.** 1973. Human papovavirus, BK strain: biological studies including antigenic relationship to simian virus 40. J. Virol. **12**:625–631.
55. **Tevethia, M. J.** 1984. Immortalization of primary mouse embryo fibroblasts with SV40 virions, viral DNA, and a subgenomic fragment in a quantitative assay. Virology **137**:414–421.
56. **Tevethia, S. S.** 1980. Immunology of simian virus 40, p. 581–601. *In* G. Klein (ed.), Viral oncology. Raven Press, New York.
57. **Tevethia, S. S.** 1986. Heterogeneity of CTL reactive sites on SV40 tumor antigen, p. 182–186. *In* D. L. Notkins and M. B. A. Oldstone (ed.), Concepts in viral pathogenesis, vol. 2. Springer-Verlag, New York.
58. **Tevethia, S. S., D. C. Flyer, and R. Tjian.** 1980. Biology of simian virus 40 (SV40) transplantation antigen (TrAg). VI. Mechanism of induction of SV40 transplantation immunity in mice by purified SV40 T antigen (D2 protein). Virology **107**: 13–23.
59. **Tevethia, S. S., R. S. Greenfield, D. Flyer, and M. J. Tevethia.** 1980. SV40 transplantation antigen: relationship to SV40-specific proteins. Cold Spring Harbor Symp. Quant. Biol. **44**:235–242.
60. **Tevethia, S. S., A. J. Lewis, A. E. Campbell, M. J. Tevethia, and P. W. J. Rigby.** 1984. Simian virus 40 specific cytotoxic lymphocyte clones localize two distinct TSTA sites on cells synthesizing a 48 kD SV40 T antigen. Virology **133**:443–447.
61. **Tevethia, S. S., M. J. Tevethia, A. J. Lewis, V. B. Reddy, and S. M. Weissman.** 1983. Biology of simian virus 40 (SV40) transplantation antigen (TrAg). IX. Analysis of TrAg in mouse cells synthesizing truncated SV40 large T antigen. Virology **128**:319–330.
62. **Tooze, J.** 1980. Molecular biology of tumor viruses. *In* J. Tooze (ed.), DNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
63. **Townsend, A. R. M., F. M. Gotch, and J. Davey.** 1985. Cytotoxic T cells recognize fragments of the influenza nucleoprotein. Cell **42**:457–467.
64. **Townsend, A. R. M., A. J. McMichael, M. P. Carter, J. A. Haddleston, and G. G. Brownlee.** 1984. Cytotoxic T cells recognition of the influenza nucleoprotein and hemagglutinin expressed in transfected mouse L cells. Cell **39**:13–25.
65. **Townsend, A. R. M., J. Rothband, F. M. Gotch, G. Bahadur, D. Wraith, and A. J. McMichael.** 1986. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. Cell **44**:959–968.
66. **Townsend, A. R. M., J. J. Skehel, P. M. Taylor, and P. A. Palese.** 1984. Recognition of influenza A virus nucleoprotein by an H-2 restricted cytotoxic T cell clone. Virology **133**:456–459.
67. **Wallich, M., D. Fellows, and M. Revel.** 1982. Preferential effect of gamma interferon on the synthesis of HLA antigens and their mRNAs in human cells. Nature (London) **299**:833–836.
68. **Whittaker, L., A. Fuks, and R. Hand.** 1985. Plasma membrane orientation of simian virus 40 T antigen in three transformed cell lines mapped with monoclonal antibodies. J. Virol. **53**:366–373.
69. **Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, and L. Chasin.** 1979. DNA-mediated transfer of the adenine phosphoribosyltransferase locus into mammalian cells. Proc. Natl. Acad. Sci. USA **76**:1373–1376.
70. **Yewdell, J. W., J. R. Bennink, G. L. Smith, and B. Moss.** 1985. Influenza A virus nucleoprotein is a major target antigen for cross-reactive anti-influenza A virus cytotoxic T lymphocytes. Proc. Natl. Acad. Sci. USA **82**:1785–1789.