

# SIMIEN VIRUS 40 (SV40)-TRANSGENIC MICE THAT DEVELOP TUMORS ARE SPECIFICALLY TOLERANT TO SV40 T ANTIGEN

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Simian virus 40 (SV40)<sup>1</sup> is a DNA virus that readily infects but does not replicate in mouse cells (1). Although SV40-transformed cells form tumors in immunocompromised mice and in neonatal mice, they are generally not tumorigenic in adult syngeneic animals (2);<sup>2</sup> the ability to mount a cellular immune response to epitopes of SV40 T antigen has been implicated in the control of SV40-induced tumor cell growth in normal, immunocompetent mice (3–6). Recently, a number of SV40-transgenic mouse lines have been derived by the zygotic injection of various SV40 early-region gene constructs, and mice from many, but not all of these lines develop tumors at specific sites (7–9). Such mice offer an ideal system for investigating the potential role of immune tolerance or responsiveness to an endogenous viral oncogene to which a normal immune response has already been well characterized. Although immune tolerance to transgene products has been suggested in other systems (10), no definitive exploration of this phenomenon has yet been reported. We examined mice from two SV40 transgenic lines bearing identical SV40 plasmid constructs (7) to determine whether such mice are immunologically responsive to subsequent challenge with SV40. We find that mice from these two lines differ in tumor incidence, and that specific immune tolerance to SV40 T antigen is an invariant feature of mice from the SV40 transgenic line that consistently develops tumors expressing this viral gene product. The majority of mice from the second SV40 transgenic line that remain essentially tumor-free generate a normal cellular immune response to SV40 after immunization with the virus.

## Materials and Methods

*Mice.* The two SV40 transgenic mouse lines, 419 and 427, each contain the SVMK plasmid consisting of: a pSV3-ts58-derived Kpn I–Bam HI fragment (including the 72-

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<sup>1</sup> *Abbreviations used in this paper:* DME<sup>+</sup>, Dulbecco's modified Eagle's medium supplemented with FCS and 2-ME; SV40, simian virus 40.

<sup>2</sup> Pan, S., J. Abramczuk, and B. B. Knowles. 1986. Immune control of SV40-induced tumors in mice. Manuscript submitted for publication.

and 21-bp repeats, TATA box, and SV40 early-region coding sequence), and in the opposite orientation, the metallothionein promoter/regulator fused to the herpes simplex virus thymidine kinase gene, cloned into the Bam HI site of pBX $\Delta$  (7). The presence of the plasmid was ascertained in each mouse by dot blotting. The 419 line is homozygous for pMKSV and is of C57BL/6J origin, while the 427 line was derived from (C57BL/6J  $\times$  SJL/J) $F_2$  mice crossed *inter se*. The H-2 type of each 427-line mouse was determined by assaying the reactivity of mouse peripheral blood lymphocytes with H-2-specific monoclonal antibodies in indirect immunofluorescence. C57BL/6J and (C57BL/6J  $\times$  SJL) $F_1$  mice served as controls for these experiments.

**Antibody Response to SV40 T.** Mice were bled by cardiac puncture and sera were tested undiluted and at 1:5, 1:25, and 1:125 dilutions on  $-20^\circ\text{C}$  acetone-fixed coverslips of SV40-transformed (LB10SV) and control (BALB 3T3) mouse cell lines in indirect immunofluorescence. FITC-labeled goat IgG anti-mouse IgG (heavy and light chain-specific) served as the developing reagent (11). Pooled sera from C57BL/6J mice, repeatedly immunized with a syngeneic SV40-transformed cell line served as a positive control, and normal C57BL/6J serum served as a negative control. For detection of SV40 T antigen by indirect immunofluorescence, acetone-fixed monolayer cultures were reacted as above with culture supernatants from 503-31 cells that produce a monoclonal antibody that reacts with an epitope of SV40 T antigen (6).

**$^{51}\text{Cr}$ -release Assay.**  $10^6$  target cells were labeled by overnight incubation with  $100\ \mu\text{Ci}$   $\text{Na}^{51}\text{Cr}$  in 4 ml Dulbecco's modified Eagle's medium supplemented with 10% FCS and  $5 \times 10^{-5}$  M 2-ME (DME $^+$ ). The  $^{51}\text{Cr}$ -release assay was carried out in DME $^+$  by coincubation of radiolabeled target cells with effector cells, in triplicate, at the specified ratios for 4–6 h as previously described (12). Percent specific lysis was calculated as: [(experimental  $^{51}\text{Cr}$  release) – (spontaneous  $^{51}\text{Cr}$  release)]/[(maximum  $^{51}\text{Cr}$  release) – (spontaneous  $^{51}\text{Cr}$  release)]  $\times$  100, and standard deviations of triplicates were determined. Spontaneous  $^{51}\text{Cr}$  release for each target was always  $<10\%$ .

**Cell Lines.** A cytotoxic T cell line, CTB6, which recognizes an epitope of SV40 T antigen in association with H-2K $^b$  (6) was used as an effector cell in some  $^{51}\text{Cr}$ -release assays. The target panel for the  $^{51}\text{Cr}$ -release assays included the following cells: PMK79SV and PMK79SV-NT, both H-2 $^b$ , are cell lines derived from 427-line mice by the *in vitro* passage of cells from adherent thymus tissue, and by the *in vitro* cultivation of choroid plexus tumors passaged through nude mice, respectively; MC57G (H-2 $^b$ ) is a stable fibroblastic cell line derived from a methylcholanthrene-induced tumor of a C57BL/6 mouse (12); the SV40-transformed cell lines, SSSV (H-2 $^s$ ) LgC57SV and LB10SV (H-2 $^b$ ), KD $_2$ SV (H-2 $^d$ ), and (b  $\times$  s) $F_1$ SV (H-2 $^b$ ) were all derived *in vitro* by SV40 infection (12). Cultures of epithelial cells from the choroid plexus, thymus, and kidney of 419-line mice were prepared from tissue minces explanted in Eagle's MEM containing 10% FCS and 10 ng/ml epidermal growth factor (EGF culture grade; Collaborative Research, Lexington, MA).

**Cytotoxic T Lymphocytes (CTL) Response to SV40 T Antigen.** Transgenic (419- and 427-line mice) and control mice [C57BL/6J and (C57BL/6J  $\times$  SJL) $F_1$ ] were immunized in the hind footpads with  $10^7$  plaque-forming units (PFU) of SV40 (12); in some experiments, mice were immunized again 5 wk later with the same dose of SV40. Popliteal lymph node cells obtained 7 d after immunization were gently dissociated, washed, and plated at  $2\text{--}3 \times 10^6$  cells/well in 24-well plates (Linbro Chemical Co., Hamden, CT). After 4 d in culture, the cells were harvested and assayed for cytotoxicity in a  $^{51}\text{Cr}$ -release assay with the panel of target cells described above. Splenocytes removed from the same mice were restimulated *in vitro* by cocultivation with irradiated (8,000 rad) syngeneic SV40-transformed stimulator cells for 6 d at a ratio of  $10^7$  spleen cells to  $10^5$  stimulator cells (12, 13), and then harvested and tested against the full panel of  $^{51}\text{Cr}$ -labeled target cells. All cell cultures and assays were performed in DME $^+$ .

**CTL Response to Vaccinia Virus.** Transgenic (427- and 419-line mice) and control mice (C57BL/6J) were immunized with  $5 \times 10^6$  PFU vaccinia virus, administered intravenously as previously described (14). Spleens were removed 10 d later; half of these cells were infected with vaccinia virus, washed, and used to restimulate equal numbers of uninfected

TABLE I  
*Tumor Formation and Response of pSV-MK Transgenic Mice to SV40 Immunization*

Trans- genic line	Tumors*	SV40 T anti- body <sup>‡</sup>	SV40-specific CTL activity <sup>§</sup>	
			Lymph node	Spleen
427	96/96	0/12	0/9	0/9
419	1/53	3/14	10/14	10/14

\* The number of animals that develop tumors per total animals monitored.

<sup>‡</sup> Mice were bled by cardiac puncture and their sera tested for reactivity with SV40-transformed target cells in indirect immunofluorescence as described in Materials and Methods. Data represent the number of seropositive mice per total number tested. Data were analyzed by the Fisher exact probability test  $p = 0.186$ .

<sup>§</sup> Transgenic and control mice were immunized with  $10^7$  PFU SV40. Popliteal lymph node cells obtained 7 d later were cultured for 4 d and assayed on a panel of <sup>51</sup>Cr-labeled target cells; splenocytes removed from the same mice were restimulated by cocultivation with irradiated syngeneic SV40-transformed cells for 6 d, harvested, and tested on the panel of <sup>51</sup>Cr-labeled target cells. Data represent the number of SV40 responsive animals per total animals tested.

splenocytes by coculture in vitro for 6 d ( $10^8$  cells total). Cells were harvested and tested for cytotoxicity against syngeneic vaccinia-infected or noninfected <sup>51</sup>Cr-labeled target cells in an 8-h <sup>51</sup>Cr-release assay (14).

## Results

Two lines of SV40-transgenic mice were used in this study. Mice of the 427 line develop tumors of the choroid plexus, and approximately one-third of these mice also develop thymic hyperplasia (7, 9). Of 96 427-line mice monitored until their death, which occurs between 2 and 8 mo of age, 95 succumbed to choroid plexus tumors; the remaining mouse died with a thoracic tumor. In contrast, only one of 53 mice of the 419 line monitored for up to 1 yr developed a tumor; this tumor was also of the choroid plexus (Table I). Because the SV40 T antigen gene copy number and mRNA levels appear to be approximately the same in nonneoplastic liver tissues from mice of both transgenic lines (7), we compared them for their ability to mount an immune response to SV40.

Since antibody production to most antigens, including SV40 T antigen (11), requires T cell participation, functional immune tolerance can be manifested at the B or T cell level (15). We screened the sera from a series of 427- and 419-line mice for the presence of antibodies to SV40 T antigen to determine whether these mice reacted to their endogenous SV40 gene products. None of these animals, which ranged in age from 2 to 4 mo (427 line) or from 5 to 10 mo (419 line), exhibited visible tumors at the time of testing. No antibodies to SV40 T antigen were detected in any of the twelve 427-line mice tested; however, sera from three of the fourteen 419-line mice assayed gave the nuclear fluorescence characteristic of SV40 T antigen when reacted with SV40-transformed cell lines (Table I). These three positive sera derived from 6-, 7-, and 8-mo-old mice, respectively; samples from seven mice at 5 mo of age, three mice at 10 mo of age, one mouse aged 6 mo and one aged 8 mo were either negative or unreadable

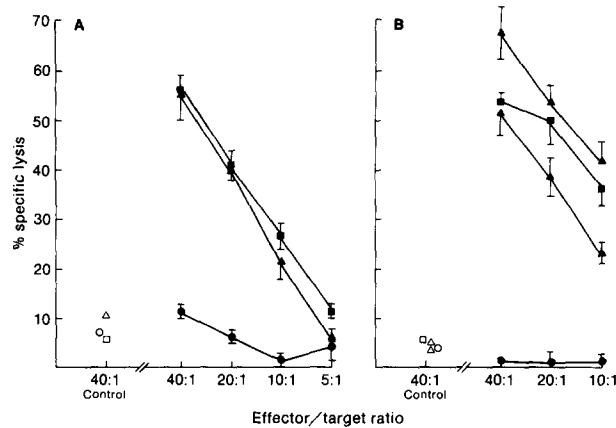


FIGURE 1. Lymph node cells from SV40-immunized 419-line but not 427-line mice lyse SV40-transformed target cells. 427-line, 419-line, and control mice [C57BL/6] or (C57BL/6)  $\times$  SJL/J)F<sub>1</sub>] were immunized with SV40. (A) <sup>51</sup>Cr-release assay of lymph node cells after a single SV40 immunization. Reactivity of lymphocytes from SV40-immunized 427-line mice with SV40 transformed LgC57SV cells (filled circles) or control MC57G cells (open circle); reactivity of lymphocytes from SV40-immunized 419-line mice with LgC57SV cells (filled triangles) or MC57G cells (open triangle); reactivity of lymphocytes from SV40-immunized control (C57BL/6J  $\times$  SJL/J)F<sub>1</sub> mice with LgC57SV (filled squares) or MC57G (open square). (B) <sup>51</sup>Cr-release assay of spleen cells following restimulation in vitro. Lymphocytes from SV40-immunized 427-line mice assayed on LgC57SV cells (filled circles) or MC57G cells (open circle); lymphocytes from SV40-immunized 419-line mice assayed on LgC57SV target cells (filled triangles) or MC57G cells (open triangle); lymphocytes from C57BL/6J assayed on LgC57SV target cells (filled squares) or MC57G cells (open square). In both A and B, results from individual animals are plotted. Similar results were obtained with the other syngeneic SV40-transformed cells (H-2<sup>b</sup> or H-2<sup>b,a</sup>) in the target panel; the allogeneic SV40-transformed cell line (H-2<sup>d</sup>) in the panel was not lysed by any effector cell shown. MC57G cells were tested at every effector/target cell ratio; data are shown for highest ratio only.

due to high nonspecific nuclear reactivity of the serum sample. Detection of antibody reactivity at a given time in these serum samples is a function of the time of expression of SV40 T antigen in the mouse and the level of circulating antibody, a parameter itself dependent on both restimulation and/or absorption of antibody by antigen-expressing cells. Therefore these data indicate that SV40 T antigen is expressed in some mice of the 419 line, and these mice are immunoresponsive to this protein.

The immunologic potential of mice from both transgenic lines was further evaluated by comparing their ability to generate CTL specific for SV40 T antigen upon immunization with SV40. Lymph node cells from 427-line mice were unable to lyse syngeneic SV40-transformed target cell lines before immunization (not shown), or after primary in vivo SV40 immunization (Fig. 1 A). In contrast, immune lymph node cells from 419-line mice lysed syngeneic SV40-transformed target cells with the same high efficiency as control C57BL/6 mice (Fig. 1 A). Spleen cells from these animals were restimulated in vitro by coculture with irradiated SV40-transformed cells to enrich for a population of SV40-specific T cells. When tested for cytotoxicity against the panel of target cells, splenic lymphocytes from 427-line mice were unable to lyse syngeneic SV40-transformed targets (Fig. 1 B). Restimulated splenocytes from 419-line and control C57BL/6J mice, however, demonstrated comparably high levels of H-2-

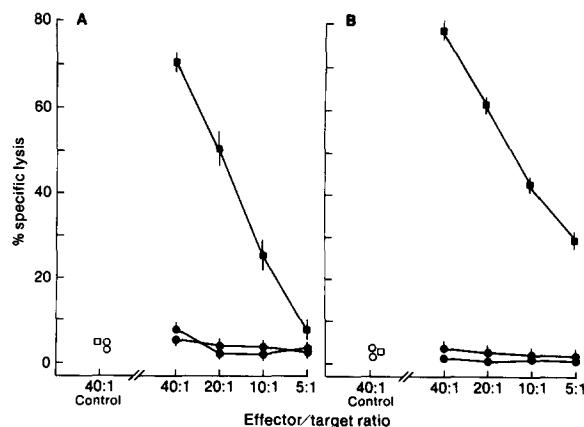


FIGURE 2. Mice of the 427 line do not generate CTL in response to multiple SV40 immunization. (A) Lymph node-derived cells from 427-line ( $H-2^b$ ) mice immunized twice with SV40 assayed on  $^{51}\text{Cr}$ -labeled LgC57SV (filled circles) or MC57G (open circle) cells, or from similarly immunized C57BL/6J mice, assayed on LgC57SV (filled squares) or MC57G (open square)  $^{51}\text{Cr}$ -labeled target cells. (B) Splenocytes from animals immunized twice with SV40 were removed 7 d after the second SV40 immunization, and cocultured in vitro with irradiated PMK79SV cells for 6 d before assay on  $^{51}\text{Cr}$ -labeled target cells. Splenocytes from 427-line ( $H-2^b$ ) mice assayed with LgC57SV (filled circles) or MC57G (open circles); splenocytes from C57BL/6J mice assayed with LgC57SV (filled squares) or MC57G (open square). Data shown are from individual mice; similar results were obtained with all syngeneic SV40-transformed cells in the complete target panel; neither allogeneic SV40-transformed cell lines nor MC57G cells were lysed at any of the effector/target cell ratios tested.

restricted SV40-specific target cell lysis (Fig. 1B). Although we could not demonstrate immune responsiveness in every 419-line animal tested (Table I), we found no correlation between immune responsiveness and the sex of the 419-line mice or their age at immunization; two of the four unresponsive mice were males, two were females, each was 5 mo old, and the age of the SV40-responsive mice ranged between 5 and 10 mo.

To address the possibility that 427-line mice were not tolerant to SV40 but instead possessed SV40-specific T cells in a frequency too low to be detected by our immunization protocol, we immunized 427-line and control C57BL/6J mice twice in vivo over a 5-wk period. Splenocytes from these animals were further restimulated in vitro with irradiated SV40-transformed cells to provide the opportunity for maximal expansion of any SV40-specific T cells present. When tested for cytotoxicity against the target panel, both lymph node cells (Fig. 2A) and restimulated splenocytes (Fig. 2B) from these immunized 427-line mice were unable to lyse SV40-transformed target cell lines at any effector/target cell ratio tested; C57BL/6J control mice demonstrated efficient SV40-specific target cell lysis.

To determine whether mice of the 427 line are specifically tolerized to SV40 or are incapable of generating functional CTL to any antigen, we immunized transgenic (427- and 419-line mice) and control mice (C57BL/6J) with vaccinia virus and evaluated their cytotoxic response to vaccinia-infected target cells in an 8-h  $^{51}\text{Cr}$ -release assay. 427-line mice were fully capable of generating splenic CTL that efficiently lysed syngeneic vaccinia-infected target cells (Fig. 3). The

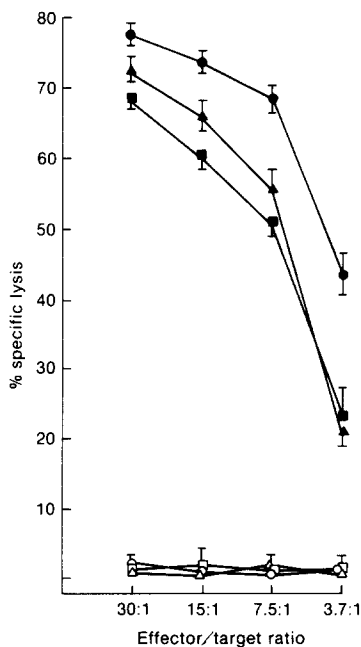


FIGURE 3. Mice of the 427 and 419 transgenic lines and C57BL/6J control mice are equally responsive to vaccinia virus immunization. Splenocytes, from mice immunized in vivo and restimulated in vitro with vaccinia virus, assayed on  $^{51}\text{Cr}$ -labeled LB10SV cells which were (closed symbols) or were not (open symbols) infected with vaccinia virus: 427-line mice (circles); 419-line mice (triangles); C57BL/6J mice (squares). All immunized mice were H-2<sup>b</sup>; LB10SV is an H-2<sup>b</sup> SV40-transformed cell line.

degree of lysis of infected target cells was comparable to that achieved by 419 line and C57BL/6 immunized splenocytes; uninfected target cells were not lysed by any effector group (Fig. 3). It is noteworthy that the vaccinia-infected and noninfected cell lines used as targets are the same SV40-transformed cell lines used in the usual SV40 target cell panel, indicating that immune cells from 427-line mice are capable of lysing these cells if directed to an antigen other than SV40.

To determine whether SV40 T antigen is expressed in the tolerant 427-line mice in an immunologically recognizable form, we isolated cells from two mice of the 427 line and assayed them after they became established transformed cell lines. Both of these cell lines express serologically detectable SV40 T antigen (Fig. 4A), as do transgenic tissues examined by Western blot analysis (7). We also tested whether these isolated cell lines could be lysed by CTL specific for SV40 T antigen. Both cell lines are lysed by SV40-immune splenocytes from C57BL/6J mice (Fig. 4B) and by CTB6, a CTL clone that recognizes an epitope of SV40 T antigen expressed at the cell surface in association with the H-2K<sup>b</sup> molecule (Fig. 4C). The range of specific lysis of these target cells in multiple experiments is comparable to that achieved using target cell lines conventionally transformed by SV40; furthermore, they are not lysed by H-2-incompatible SV40-specific effector cells (data not shown). We have also prepared primary cultures from a 419-line mouse to determine whether cells from these mice could express SV40

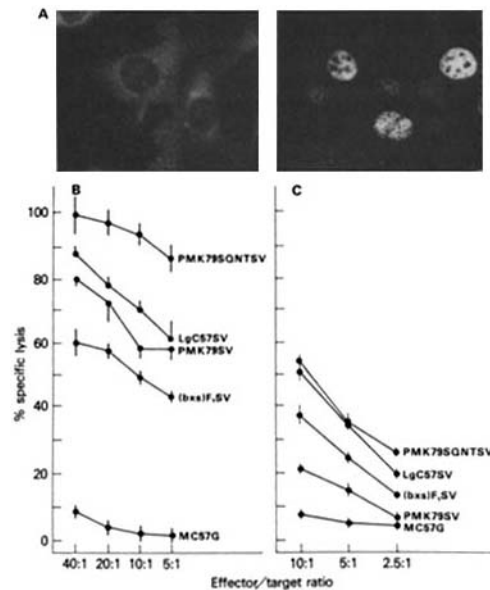


FIGURE 4. Expression of SV40 T antigen in cell lines derived from SV40-transgenic tissues. (A) SV40 nuclear T antigen detected by indirect immunofluorescence of cell line PMK79SV (*right*). BALB 3T3 cells incubated under the same conditions served as a negative control (*left*). (B) SV40-immune splenocytes specifically lyse  $^{51}\text{Cr}$ -labeled target cell lines from 427-line SV40-transgenic mice. Splenocytes from a C57BL/6J mouse immunized with SV40 were restimulated in vitro and tested for cytotoxic activity against a panel of  $^{51}\text{Cr}$ -labeled target cell lines derived from normal and SV40-transgenic mice at the effector/target cell ratios indicated. (C) A cytotoxic T cell clone, CTB6, which recognizes an epitope of SV40 T antigen in association with H-2K<sup>b</sup>, was tested against the same panel of  $^{51}\text{Cr}$ -labeled target cell lines.

T antigen in vitro. A few of the cells arising in these primary cultures express SV40 T antigen. A cell line of epithelial morphology was established from the outgrowth of a 419 kidney explant. This cell line is susceptible to lysis by SV40-specific CTLs (data not shown). Thus, cell lines obtained from tissues of both 427-line and 419-line mice express SV40 T antigen in an immunologically recognizable form.

### Discussion

We have evaluated a panel of mice from two distinct SV40 transgenic lines for their ability to mount an immune response to SV40. Mice from both the 427 and 419 lines bear identical plasmid constructs in approximately the same gene copy number per cell as in normal tissues; however, these mice differ markedly both in the incidence of spontaneous tumors, which express detectable levels of SV40 T antigen, and in their ability to generate antibodies and CTL specific for this viral gene product.

Mice tested from the tumor-prone 427 line are completely unresponsive to immunization with SV40. These mice respond normally to immunization with vaccinia virus, indicating that they are functionally immunocompetent but are specifically tolerant to SV40 T antigen. In contrast, the majority of mice from the 419 line mount a normal immune response to SV40 T antigen compared with nontransgenic control mice.

Immune tolerance is a somatically acquired trait (16) thought to result from events occurring in the thymus during T cell maturation (17, 18). Thus the developmental timing of initial expression of SV40 T antigen, the tissue distribution of cells bearing the antigen, and the amount of protein synthesized may all contribute to the functional outcome, immune tolerance or responsiveness to SV40 T antigen, in these transgenic mice. The observation that the thymus is one of the target organs for SV40 T antigen expression in these mice (7, 9), and that cells derived from a 427-line thymus can be lysed by SV40 T antigen-specific CTL (Fig. 4, *B* and *C*), suggests ready exposure of differentiating thymocytes to the viral protein in these animals. Their profound immune tolerance to SV40 T antigen may be mediated by the active participation of antigen-specific suppressor T cells (19, 20), or alternatively, may be the result of elimination of antigen-specific T cells (17, 21) during selection of the T cell repertoire in the thymus early in development. Expression of SV40 T antigen during fetal development is still under active investigation in both the 427 and 419 lines. Such an analysis may help delineate the cellular mechanisms involved in the induction of immune tolerance to SV40 in 427-line mice, and may define the differences that allow most 419-line mice to respond normally to immunization with SV40. SV40 T antigen is immunohistochemically detectable within sporadic cells of the thymic epithelium in 14-d-old 427-line mice and within the choroid plexus and kidney by 28 d of age (A. Messing, University of Wisconsin, Madison, WI, personal communication). SV40 T antigen has not been previously detected in 419-line mice before 32 wk of age. At this time, low levels of T antigen were found in the kidney, but not the brain or thymus, by immunoprecipitation (22). Our antibody data demonstrates that SV40 is expressed and immunologically recognized within some 419-line mice by 6 mo of age. The sensitivity of detection of minimal numbers of SV40 T antigen-positive cells by these various techniques differs widely. Low levels of SV40 T antigen detected by immunoprecipitation from an intact organ can be ascribed to the presence of but a few SV40 T antigen-expressing cells in the intact organ, or of a majority of cells each expressing at a low level. By the former interpretation, low-level expression in the seemingly normal tissues of 419-line mice relative to that in transgenic tumor tissue could result from active elimination of SV40 T antigen-positive cells as they arise, or from an inherent inability of these cells to progress *in vivo*. Cell lines derived from 419-line mice express ample levels of SV40 T antigen, and this molecule is not defective (22).

The expression of SV40 T antigen in 419- and 427-line mice is likely a function of sequences contained within the inserted construct and of flanking cellular sequences. Tissue-targeted expression of several genes has been shown to depend on specific regulatory enhancer sequences (8, 23–25); the targeting of SV40 tumor formation to a specific tissue in the transgenic mice used in this study is influenced by the regulatory sequences of the gene construct used (9). The 72-bp SV40 enhancer appears to target abnormalities to the choroid plexus and perhaps kidney and thymus (7, 9). The gene construct used to generate both the 427- and 419-lines contains this enhancer sequence. Since the chromosomal site of gene insertion is random in the founder mice of each line, flanking cellular sequences may contribute to the differences in SV40 T antigen expression,



tumor formation, and immune responsiveness to SV40 observed between the two transgenic lines.

The relationship between cellular immunity to SV40 T antigen and the control of SV40-transformed cell growth in the mouse has been suggested previously (2-5). SV40 injection into young immunocompetent animals results in tumor appearance late in life in low-responder but not high-responder H-2-congenic strains (3). SV40-transformed cell lines are usually nontumorigenic when injected into syngeneic adult animals, yet they form tumors when injected into neonatal mice before 24 h of birth (1). In addition, loss of expression of the H-2 restriction element on passaged cell lines positively correlates with the ability of these cell lines to form tumors in adult mice (2, 4). The demonstration that (a) expression of endogenous SV40 early region genes can occur in the absence of overt tumor formation (reference 7 and this report), and (b) that those animals succumbing to spontaneous tumors are specifically tolerized to SV40 T antigen and cannot respond when immunized with the virus suggests that the immune response can have a controlling influence on the development of tumors associated with this viral transforming gene product.

### Summary

The ability to mount an immune response to simian virus 40 (SV40) T antigen was evaluated using mice from two distinct SV40 transgenic lines derived from injection of the same gene construct. Our studies demonstrate functional immune tolerance to SV40 T antigen in a SV40 transgenic line that consistently develops tumors of the choroid plexus by 7 mo of age. Antibodies to SV40 T antigen are undetectable in the serum of these animals; furthermore, mice from this line are unable to generate SV40-specific CTL after primary or secondary immunization with the virus, although they mount a normal CTL response to vaccinia virus when appropriately immunized. In contrast, we find that mice from a second transgenic line of low tumor incidence can mount a humoral response to SV40 T antigen, and upon immunization they generally respond with a vigorous cytotoxic T cell response to SV40 T antigen. These data suggest that specific immune tolerance to the product of an integrated viral oncogene may be induced, and is likely a reflection of the time in development at which the gene product first appears. Immune tolerance or responsiveness to the endogenous oncogene product may in turn play a role in the tumorigenic potential of such genes.

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### References

1. Tooze, J. 1980. *Molecular Biology of the Tumor Viruses—DNA Tumor Viruses*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 207.
2. Pan, S., and B. B. Knowles. 1983. Interaction between SV40 T/TSTA and the H-2 K/D molecules. *L'Igiene Moderna*. 80:1112.
3. Abramczuk, J., S. Pan, G. Maul, and B. B. Knowles. 1984. Tumor induction by

- simian virus 40 in mice controlled by long-term persistence of the viral genome and the immune response of the host. *J. Virol.* 49:540.
4. Gooding, L. R. 1982. Characterization of a progressive tumor from C3H fibroblasts transformed in vitro with SV40. Immuno-resistance in vivo correlates with phenotypic loss of H-2K<sup>k</sup>. *J. Immunol.* 129:1306.
  5. Tevethia, S. S., J. W. Blaseck, G. Waneck, and A. L. Goldstein. 1974. Requirements of thymus-derived  $\theta$ -positive lymphocytes for rejection of DNA virus (SV40) tumors in mice. *J. Immunol.* 113:1417.
  6. Pan, S., and B. B. Knowles. 1983. Monoclonal antibody to SV40 T-antigen blocks lysis of cloned cytotoxic T-cell line specific for SV40 TASA. *Virology.* 125:1.
  7. Brinster, R. L., H. Y. Chen, A. Messing, T. VanDyke, A. J. Levine, and R. D. Palmiter. 1984. Transgenic mice harboring SV40 T-antigen genes develop characteristic brain tumors. *Cell.* 37:376.
  8. Hanahan, D. 1985. Heritable formation of pancreatic  $\beta$ -cell tumors in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. *Nature (Lond.)* 315:115.
  9. Palmiter, R. D., A. Messing, H. Y. Chen, and R. L. Brinster. 1985. SV40 enhancer and large-T antigen are instrumental in development of choroid plexus tumors in transgenic mice. *Nature (Lond.)* 316:457.
  10. Chisari, F. V., C. A. Pinkert, D. R. Milich, P. Filippi, A. McLachlan, R. D. Palmiter, and R. L. Brinster. 1985. A transgenic model of the chronic hepatitis B surface antigen carrier state. *Science (Wash. DC)* 230:1157.
  11. Knowles, B. B., S. R. Ford, and D. P. Aden. 1977. Reactivity to SV40 T antigen in athymic (nude), antithymocyte serum-treated and normal mice. *J. Immunol.* 119:79.
  12. 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 antigen. *J. Immunol.* 122:1798.
  13. Gooding, L. R. 1977. Specificities of killing by cytotoxic lymphocytes generated in vivo and in vitro to syngeneic SV40-transformed cells. *J. Immunol.* 118:920.
  14. Korngold, R., J. R. Bennick, and P. C. Doherty. 1981. Early dominance of irradiated host cells in the responder profiles of thymocytes from P  $\rightarrow$  F<sub>1</sub> radiation chimeras. *J. Immunol.* 127:124.
  15. Weigle, W. O. 1973. Immunological unresponsiveness. *Adv. Immunol.* 16:61.
  16. Billingham, R. E., L. Brent, and P. B. Medawar. 1953. Actively acquired tolerance of foreign cells. *Nature (Lond.)* 172:603.
  17. Nossal, G. J. V., and B. J. Pike. 1981. Functional clonal deletion in immunological tolerance to major histocompatibility complex antigens. *Proc. Natl. Acad. Sci. USA.* 78:3844.
  18. Wood, P. J., and J. W. Streilein. 1982. Ontogeny of acquired immunological tolerance to H-2 alloantigens. *Eur. J. Immunol.* 12:188.
  19. Dorsch, S., and B. Roser. 1977. Recirculatory, suppressor T cells in transplantation tolerance. *J. Exp. Med.* 145:1144.
  20. Stockinger, B. 1984. Cytotoxic T-cell precursors revealed in neonatally tolerant mice. *Proc. Natl. Acad. Sci. USA.* 81:220.
  21. Gruchalla, R. S., and J. W. Streilein. 1982. Analysis of neonatally induced tolerance of H-2 alloantigens. II. Failure to detect alloantigen specific T lymphocyte precursors and suppressors. *Immunogenetics.* 15:111.
  22. Van Dyke, T., Finlay, C., and A. J. Levine. 1985. A comparison of several lines of transgenic mice containing the SV40 early genes. *Cold Spring Harbor Symp. Quant. Biol.* 50:671.
  23. Banerji, J., L. Olson, and W. Schaffner. 1983. A lymphocyte specific cellular enhancer

is located downstream of the joining region in immunoglobulin heavy chain genes. *Cell*. 33:729.

24. Gillies, S. D., S. L. Morrison, V. T. Oi, and S. Tonegawa. 1983. A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. *Cell*. 33:717.
25. Gillies, S. D., V. Falsam, and S. Tonegawa. 1984. Cell type specific enhancer element associated with a mouse MHC gene, *E $\alpha$* . *Nature (Lond.)*. 310:594.