Susceptibility to Tumors Induced by Polyoma Virus is Conferred by an Endogenous Mouse Mammary Tumor Virus Superantigen

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Summary

A dominant gene carried in certain inbred mouse strains confers susceptibility to tumors induced by polyoma virus. This gene, designated Pyv^S, was defined in crosses between the highly susceptible C3H/BiDa strain and the highly resistant but H-2^k-identical C57BR/cdJ strain. The resistance of C57BR/cdJ mice is overcome by irradiation, indicating an immunological basis. In F1 × C57BR/cdJ backcross mice, tumor susceptibility cosegregates with Mtv-7, a mouse mammary tumor provirus carried by the C3H/BiDa strain. This suggests that Pyv^S might encode the Mtv-7 superantigen (SAG) and abrogate polyoma tumor immunosurveillance through elimination of T cells bearing specific Vβ domains. DNA typing of 110 backcross mice showed no evidence of recombination between Pyv^S and Mtv-7. Strongly biased usage of Vβ6 by polyoma virus-specific CD8$^+$ cytotoxic T lymphocytes in C57BR/cdJ mice implicates T cells bearing this Mtv-7 SAG-reactive Vβ domain as critical anti-polyoma tumor effector cells in vivo. These results indicate identity between Pyv^S and Mtv-7 sag, and demonstrate a novel mechanism of inherited susceptibility to virus-induced tumors based on effects of an endogenous superantigen on the host's T cell repertoire.

D evelopment of neoplastic disease requires that a cell first become transformed and then be able to evade the host's immune system. The importance of an intact immune system in preventing overt neoplastic disease is highlighted by the increased incidence of malignancies in immunocompromised individuals (1). Tumor cell variants that escape immunosurveillance can emerge in immunocompetent hosts, and various mechanisms of immune evasion at the level of the tumor cell have been documented (2–6). In principle, transformed cells expressing neoantigens might also escape immune recognition due to a hole in the host's T cell repertoire. Such a phenomenon involving defective tumor-specific T cell responses in otherwise immunocompetent hosts has, to our knowledge, not been reported. Results presented here on susceptibility of the mouse to polyoma virus-induced tumors provide the first evidence for a genetically dominant mechanism of this kind.

Polyoma virus is a potent oncogenic agent capable of inducing multiple solid tumors in the mouse, its natural host (7, 8). For over three decades the polyoma-mouse system has served as a model for investigations of immune responses to virus-induced cancers. Early studies using neonatally thymectomized mice, nude mice, and adoptive transfer of polyoma virus-immune splenocytes showed that resistance to polyoma tumors is mediated by T cells (9–11). Although the primary

effector T cell mediating resistance to polyoma tumors has not been fully defined, CD8$^+$ CTLs specific for syngeneic cells expressing polyoma tumor-specific transplantation antigen(s) (TSTAs)[1] are known to be induced by polyoma infection (12).

MHC molecules as well as products of non-MHC genes control susceptibility to polyoma-induced tumors. In crosses between MHC-nonidentical strains differing in susceptibility, resistance is inherited in a dominant or codominant manner determined largely by the MHC difference itself (9, 13). Interestingly, the opposite pattern of inheritance is seen in crosses between MHC-identical (H-2^k) mice where susceptibility rather than resistance is dominant (13). In crosses between the highly susceptible C3H/BiDa mouse and the highly resistant but MHC-identical C57BR/cdJ mouse, susceptibility is conferred by a single autosomal dominant gene which we have designated Pyv^S (14).

Evidence presented here strongly suggests that Pyv^S is the

endogenous superantigen encoded by the mouse mammary tumor provirus *Mtv-7*. Mouse mammary tumor virus (Mtv) superantigens (SAG) are type II transmembrane glycoproteins (15, 16) encoded by an open reading frame in the 3'LTR of the provirus (17, 18). Like bacterial superantigens, Mtv SAGs associate with class II MHC molecules and bind to the variable domain of the β-chain (Vβ) of the TCR (19). A polymorphic region in the carboxy terminus of Mtv SAGs determines its Vβ domain specificity (20). As self-proteins, Mtv SAGs alter the peripheral T cell repertoire by intrathymic deletion of Vβ-reactive T cells (21–24). Results of genetic and immunological experiments indicate Pyv^S is *Mtv-7 sag* and induces susceptibility to the oncogenic effects of the virus by deleting precursors of polyoma TSTA-specific T cells.

Materials and Methods

Mice. C57BR/cdJ, C3H/HeJ, CBA/J, CBA/CaJ, and RF/J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). C3H/BiDa mice were purchased from the Frederick Cancer Research and Development Center of the National Cancer Institute (Frederick, MD). Newborn mice (<18 h of age) were inoculated intraperitoneally with molecularly cloned and plaque-purified polyoma virus, and examined for tumors as described (8, 14).

6-wk-old C57BR/cdJ female mice received 800 or 900 rads of γ-radiation in a Gammacell 40 (Atomic Energy of Canada, Ltd., Ottawa, Canada), and, 24 h later, were inoculated intraperitoneally with virus. A cell line was established from a salivary tumor arising in one of these mice. This cell line expressed full-length small T and middle T (MT) proteins, a truncated large T (LT) protein (25), and no polyoma VP1 capsid protein.

Polyoma tumors carried by serial passage in normal C57BR/cdJ mice were finely minced and injected subcutaneously in 0.25-ml vol into 6–8-wk-old C57BR/cdJ mice that had been inoculated at birth with polyoma virus.

Cytofluorometric Analysis. Viable mononuclear cells from spleens or lymph nodes were isolated on LSM (Organon Teknika, Rockville, MD). Spleen cells were subsequently enriched for T cells on nylon wool columns as described (26). Cells were indirectly stained with FITC-conjugated goat anti–hamster IgG or FITC-conjugated goat anti–rat IgG F(ab')$_2$ secondary antibodies (Caltag Laboratories, South San Francisco, CA) and culture supernatants of the following hybridomas: B20.6, recognizing Vβ2 (from B. Malissen, Centre d'Immunologie de Marseille-Luminy, France); KJ25, recognizing Vβ3 (from P. Marrack and J. Kappler, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO); 44-22-1, recognizing Vβ6 (from H. Hengartner, Institute of Pathology, University Hospital, Zurich, Switzerland); TR310, recognizing Vβ7 (from C. Okada, Stanford University School of Medicine, Stanford, CA); R14-2, recognizing Vβ14 (from D. Raulet, University of California, Berkeley, CA); and H57-597, recognizing $\alpha\beta$-TCR (from R. Kubo, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). PE-conjugated anti-CD4 and anti-CD8 antibodies were purchased from Caltag Laboratories. Cells were analyzed on a flow cytometer (Epics Profile II; Coulter, Hialeah, FL).

Mtv-7 Southern Hybridization and SSR Mapping. Genomic liver DNA was isolated as described (27). Eco RI-digested genomic DNA was analyzed by Southern hybridization for the presence of Mtv-7 as described (28).

D1Mit16 and D1Mit56 primers were purchased from Research

Genetics (Huntsville, AL). PCR was performed according to the specifications of the GeneAmp PCR reagent kit (Perkin-Elmer Cetus, Norwalk, CT) with the following modifications. 20 ng of genomic liver DNA was used in each 9-μl PCR reaction mixture containing 1$\times$ reaction buffer, 0.1 μl of each primer (6.6 μM stock), and 0.025 μl [α-^{32}P]dCTP (3,000 μCi/mmole; New England Nuclear, Boston, MA). Samples were first run on a programmable thermal controller (PTC-100; MJ Research, Inc., Boston, MA) according to the GeneReleaser (BioVentures, Inc., Murfreesboro, TN) thermocycling protocol. 1 μl of AmpliTaq DNA polymerase containing 0.05 U was then added to each sample, and the reaction mixtures were amplified using the thermocycling protocol described by Dietrich et al. (29). Samples were run on a 7% acrylamide-formamide gel (30). Reaction products were visualized by autoradiography.

Isolation of Polyoma Virus-specific T Cells. Newborn C57BR/cdJ mice were injected subcutaneously in footpads with 1–5 $\times$ 10^5 PFU of virus. At 8–13 d of age, 1 $\times$ 10^6 mononuclear cells from draining popliteal and inguinal lymph nodes were cocultured with 10 $\times$ 10^6 stimulator cells per well in 24-well cluster plates (Costar Corp., Cambridge, MA) in 2 ml of Iscove's modified Dulbecco's medium containing 10% Rat T-Stim without Con A (Collaborative Research, Bedford, MA), 10% heat-inactivated fetal bovine serum, 4 mM glutamine, and 50 μM 2-mercaptoethanol (complete medium). Stimulator cells were C57BR/cdJ spleen cells infected with A2 virus (multiplicity of infection = 0.01) for 24 h, and then given 2,000 rads of γ-radiation. Viable mononuclear cells were restimulated at day 10 and used at day 6–8 of culture for cytotoxicity assays and cytofluorometric analyses.

Isolation of TILs. A 5-wk-old C57BR/cdJ male inoculated at birth with A2 virus was injected subcutaneously with 13 $\times$ 10^6 cells of the C57BR/cdJ polyoma tumor cell line (see above). 15 d later, the tumor was resected, minced, and digested with 500 U/ml collagenase (ICN Biomedicals, Inc., Costa Mesa, CA) for 1 h at 37°C. Nonadherent cells were collected following two rounds of incubation in plastic petri dishes (Nunc, Roskilde, Denmark) for 1 h at 37°C and 7% CO$_2$. Viable mononuclear cells were isolated on LSM (Organon Teknika, Durham, NC), incubated for 16 h at 37°C and 7% CO$_2$ in complete medium, then stained and analyzed by cytofluorometry. No differences in CD4, CD8, or Vβ expression were found between T cells analyzed immediately after isolation from either the polyoma tumor or normal C57BR/cdJ lymph nodes and those analyzed following 16-h incubation in complete medium (data not shown). These TILs were established as a line by weekly passage with virus-infected, irradiated C57BR/cdJ spleen cells for 8 wk.

Cytotoxicity Assays. The CytoTox 96 nonradioactive assay (Promega, Madison, WI) was used with TCMK-1 (H-2^k) target cells (American Type Culture Collection, Rockville, MD) to assay cytotoxic activity by the tertiary culture of polyoma-immune T cells (see Fig. 3). The assay was carried out according to manufacturer's directions using 1 $\times$ 10^4 TCMK-1 cells per well of 96-well U-bottom microtiter plates, except that the assay medium was phenol red-free AIM V medium (GIBCO BRL, Gaithersburg, MD). TCMK-1 cells were either left uninfected or infected with virus (multiplicity of infection = 10) for 20 h before use in the assay. Spontaneous release of lactate dehydrogenase was ~10% for uninfected and infected cells over a 4-h assay at 37°C and 7% CO$_2$.

To assay cytotoxic activity by the TIL line (see Fig. 5), ^{51}Cr-release assays were performed as described by Gooding (31), using C57BR/cdJ polyoma tumor cells and SV40-transformed (H-2^k) PSC3H cells as targets. These target cells were incubated with 100 U/ml recombinant mouse interferon-γ (Genzyme, Cambridge, MA)

for 48 h before use in the assay. Spontaneous release of ^{51}Cr was 16% for PSC3H cells and 26% for polyoma tumor cells over a 6-h assay at 37°C and 7% CO_2.

Percent specific lysis for each assay was determined from quadruplicate samples. Standard errors of the mean value were always <5% of mean values and are omitted.

Results

Resistance of the C57BR/cdJ Mouse to Polyoma Tumors Has an Immunological Basis. C57BR/cdJ mice are Pyv^s-negative and highly resistant to tumors induced by polyoma virus, while C3H/BiDa mice are Pyv^s-positive and highly susceptible. Despite this difference, neonatally infected mice of both strains develop disseminated infections, and cells from the resistant C57BR/cdJ mouse are readily transformed by polyoma T antigens in vitro (14). It thus appears that Pyv^s is not essential for replication or transformation by the virus, but rather acts in some systemic manner to allow development of tumors. One model consistent with these observations is that Pyv^s acts to prevent an effective immune response to polyoma tumors. Such a model would imply that the resistance of C57BR/cdJ mice has an immunological basis and that these mice would become susceptible if immunosuppressed. Results shown in Table 1 confirm this expectation.

Tumors developed in 100% of adult C57BR/cdJ mice irradiated before virus inoculation, while the same dose of virus induced tumors in less than 5% of unirradiated newborn C57BR/cdJ mice. This difference is due to irradiation and not to the age difference between the two groups, since newborn mice of even the most susceptible strains are known to become resistant as adults (7, 32). Irradiated mice uniformly developed bilateral salivary gland tumors at 7–8 wk postinfection; mammary gland tumors, thymic epitheliomas and an ameloblastoma were also seen in this group of animals. Peripheral blood smears taken at necropsy showed severe leukopenia, and immunoblots of tumors were clearly positive for viral T antigens and capsid proteins, confirming that the tumors arose in immunosuppressed hosts and were virus-induced.

The two positive animals in the nonirradiated group each developed a single gross tumor. This contrasts with the development of multiple gross tumors per animal typically seen in Pyv^s-positive mouse strains such as C3H/BiDa (8, 33). These C57BR/cdJ tumors, both of mammary gland origin, were composed of sheets of spindle-shaped cells and differed histologically from the comedo-type intraductal carcinoma typically seen in C3H/BiDa mice (34). It seemed possible that these unusual tumors arising in immunocompetent mice were variants that somehow escaped immunosurveillance. To test this possibility, the tumors were injected subcutaneously into adult C57BR/cdJ mice that had been inoculated at birth with polyoma virus. Both tumors transplanted readily to these polyoma-immune hosts, indicating that they were indeed immune escape variants. As a control, a tumor that arose in an irradiated C57BR/cdJ mouse (and was therefore not subject to immune selection) was tested; this tumor was rejected by polyoma-immune C57BR/cdJ mice as expected, but did

Table 1. *Immunological Resistance of C57BR/cdJ Mice*

a. Effects of irradiation on the tumor response of C57BR/cdJ mice*

	Unirradiated	Irradiated
Fraction of mice with tumor(s)	2/47	17/17

b. Transplantation properties of tumors arising in unirradiated and irradiated C57BR/cdJ mice‡

Tumor Donor	Fraction of polyoma-immune mice developing tumor
Unirradiated C57BR/cdJ	4/4
Unirradiated C57BR/cdJ	5/5
Irradiated C57BR/cdJ	0/8

* Unirradiated C57BR/cdJ mice were inoculated at birth with polyoma virus. Adult C57BR/cdJ mice were γ-irradiated (800–900 rads) and inoculated with polyoma virus 24 h later. Mice were necropsied when moribund, or at 6 mo after infection, and tissue sections examined histologically.
‡ Adult C57BR/cdJ mice that had been inoculated with polyoma virus at birth were injected subcutaneously with minced tumor suspensions. Tumors from both of the unirradiated and from one of the irradiated polyoma-infected C57BR/cdJ mice (a) were maintained by serial passage in C57BR/cdJ mice before transplantation. Mice were scored for gross tumors 2 wk after transplantation.

transplant readily to nonimmune C57BR/cdJ mice. These results show that the C57BR/cdJ mouse is capable of mounting an effective immune response to polyoma tumors, and that rare tumors which arise in this strain are variants that escape immune recognition.

Tumor Susceptibility and Mtv-7 Cosegregate in Backcross Mice. Given that Pyv^s determines susceptibility in F1 hybrids between C3H/BiDa and C57BR/cdJ mice, it must act in some way to negate the immune response contributed by the C57BR/cdJ parent. We hypothesize that Pyv^s encodes an Mtv SAG that deletes T cells required for protection against polyoma tumors. Comparison of the Mtv proviruses carried by these strains suggests two candidates, Mtv-6 and Mtv-7, based on their presence in C3H/BiDa and absence in C57BR/cdJ mice (Table 2). Mtv-17 is not considered because a mutation in its LTR most likely prevents expression (45), and no deletion of T cells bearing the expected Vβ specificity has been found (44). Anti-polyoma T cells in the resistant C57BR/cdJ mouse would therefore be predicted to express either Vβ3 if Pyv^s were Mtv-6 sag, or Vβ6 and/or Vβ7 if Pyv^s were Mtv-7 sag. Expression of other Vβ domains targeted by Mtv-6 sag (Vβ5) or Mtv-7 sag (Vβ8.1 and Vβ9) is excluded since the coding regions for these segments are absent from the germline in C57BR/cdJ mice (37).

To determine if tumor susceptibility cosegregates with either Mtv-6 or Mtv-7, 35 backcross (F1 × C57BR/cdJ) mice

Table 2. *Mtv Proviruses and Associated TCR Vβ-specific deletions in C3H/BiDa and C57BR/cdJ Mice*

Mtv Provirus	C3H/BiDa	C57BR/cdJ	Vβ Deletion	References
6(16)	+	−	**3, 5**	38, 39
7(1)	+	−	**6, 7, 8.1, 9**	40
8(6)	+	+	11, 12	41
9(12)	+	+	5, 11, 12	41–43
11(14)	−	+	11, 12	41, 42
14(4)	+	+	?	
17(4)	+	−	none	44
29(6)	−	+	?	

Mtv provirus distributions in C3H/BiDa and C57BR/cdJ mice are taken from Frankel et al. (28), Kozak et al. (35), and Scherer et al. (19). Numbers in parentheses indicate chromosomal locations of Mtv proviruses (35, 36). Bold-faced Vβs are encoded in germlines of both C3H/BiDa and C57BR/cdJ mice; genes of all other listed Vβs are deleted in C57BR/cdJ mice (37).

were examined for Vβ usage in their peripheral T cells by cytofluorometry using absence of Vβ3 and Vβ6 as markers for *Mtv-6* and *Mtv-7*, respectively. The same mice were also typed for *Mtv-7* by Southern hybridization. Of 18 mice which failed to develop tumors, 15 had high levels of Vβ6$^+$ T cells, similar to the levels seen in C57BR/cdJ mice; none of these animals inherited the Mtv-7 provirus (Table 3). Conversely, 15 of 17 mice which developed tumors deleted Vβ6$^+$ T cells, as in C3H/BiDa mice; all 15 of these animals inherited *Mtv-7*. No correlation was seen between Vβ3 usage and presence or absence of tumors (Table 3). Thus, in 30 of 35 mice, the presence (or absence) of tumors was correlated with the

Table 3. *Correlation of Vβ Usage, Inheritance of Mtv-7, and Susceptibility to Tumors in Backcross Mice*

	Tumor-free			Tumor-bearing	
Vβ3	Vβ6	Mtv-7	Vβ3	Vβ6	Mtv-7
0	19	−	0	1	+
0	18	−	0	0	+
0	20	−	0	1	+
0	19	−	0	1	+
1	17	−	0	1	+
9	18	−	1	2	+
4	17	−	6	2	+
7	19	−	6	1	+
7	17	−	6	1	+
6	19	−	5	2	+
nd	16	−	7	0	+
nd	18	−	7	0	+
nd	18	−	5	1	+
nd	18	−	7	0	+
nd	14	−	6	1	+
nd	1	+	0	21	−
10	1	+	9	20	−
11	3	+			

Vβ expression by splenic T cells from 35 (C3H/BiDa × C57BR/cdJ) × C57BR/cdJ backcross mice inoculated at birth with polyoma virus was determined by cytofluorometric analysis. Values are % total αβ-T cells expressing either Vβ3 or Vβ6. nd indicates not determined. As reference, values for the parental strains are: for Vβ3, 1.0 ± 0.6 in C3H/BiDa and 7.0 ± 1.2 in C57BR/cdJ; and for Vβ6, 0.3 ± 0.3 in C3H/BiDa and 16.2 ± 0.8 in C57BR/cdJ. Results of Mtv-7 genotyping by Southern hybridization are given for each animal.

presence (or absence) of *Mtv-7*. The remaining five animals showed the expected concordance between inheritance of *Mtv-7* and absence of Vβ6$^+$ T cells, but were clearly nonconcordant with respect to *Mtv-7* and tumor phenotype.

The analysis was extended to an additional 84 backcross mice which were typed for *Mtv-7*. The combined data for all 119 mice are presented in Table 4. The association between *Pyv*^{S} and *Mtv-7* is highly significant, with 102 animals (86%) showing concordance between *Mtv-7* and *Pyv*^{S}. Chi-square analysis gives a probability of $\ll$ 0.005 that these results would arise by chance, assuming no association between these genes. 17 backcross mice (14%) were nonconcordant. Eight of these were Mtv-7$^-$ but tumor-bearing. Interestingly, only single tumors were found in these mice, as was the case with the rare C57BR/cdJ parental mice that developed tumors. This contrasts with the majority of the Mtv-7$^+$ backcross mice which developed multiple tumors (Table 4). The finding of 14% nonconcordant mice raises two possibilities: (a) *Pyv*^{S} and *Mtv-7 sag* are separate but linked genes, or (b) *Pyv*^{S} is identical to *Mtv-7 sag*, and incomplete penetrance or other mitigating factors are involved.

Pyv^S *and Mtv-7 Show No Evidence of Recombining.* If *Pyv*^{S} and *Mtv-7 sag* are linked genes, then the nonconcordant backcross mice would be expected to have inherited a recombinant chromosome 1 from their F1 parent. To test this directly, simple sequence repeat (SSR) polymorphisms (29) were sought as markers to detect recombination on either side of *Mtv-7*. SSR probes known to map to the distal region of chromosome 1 (map obtained from Research Genetics, Inc., Huntsville, AL) were screened, and two appropriate markers identified.

The DNAs from 110 backcross mice were typed for the SSR markers by PCR and for *Mtv-7* by Southern hybridization. Based on their frequencies of recombination with *Mtv-7*, the SSR markers are located ∼10 cM on either side of the provirus (Fig. 1). Significantly, the fraction of mice that was recombinant in each of these intervals was roughly the same for the nonconcordant and concordant groups, i.e., in the range of 6–12% (Table 5). These results do not support the possibility that *Pyv*^{S} and *Mtv-7* are separate genes, since this would require that the nonconcordant mice show a much higher frequency of recombination (∼70% if the genes were 14 cM apart) in one of the two intervals.

Figure 1. Mapping of D1Mit16 and D1Mit56 SSRs with respect to Mtv-7 on chromosome 1. 110 (C57BR/cdJ × C3H/BiDa)F1 × C57BR/cdJ backcross mice were typed for presence of Mtv-7 and for D1Mit16 and D1Mit56 SSR polymorphisms. Autoradiographs show length polymorphisms of these SSRs for parental and F1 mice. Numbers indicate location (in cM) of D1Mit16, D1Mit56 and Mtv-7 on chromosome 1, based on the Jackson Laboratory genetic map placing Mtv-7 at 75.0 cM.

Vβ6 Is Used Preferentially by Polyoma-specific T Cells. If *Pyv*^{S} is *Mtv-7 sag*, then its action in determining susceptibility to polyoma tumors is expected to be based on the elimination of T cells bearing either Vβ6 or Vβ7 domains. Likewise, resistance in the C57BR/cdJ mouse should be mediated by T cells bearing one, or possibly both, of these Vβ domains. To test this prediction, T cells taken from lymph nodes of polyoma virus-infected C57BR/cdJ mice were stimulated twice in vitro with virus-infected, irradiated syngeneic spleen cells, and then analyzed for Vβ usage as well as for expression of CD4 and CD8. As shown in Fig. 2, the majority of these T cells expressed Vβ6 (76%) and were CD8$^+$ (95%); no other Vβ type was elevated, including Vβ7. T cells from lymph nodes of normal C57BR/cdJ mice were only 15% Vβ6$^+$. This experiment was repeated with separate cultures from eight C57BR/cdJ mice, with the same general results. Vβ6-usage ranged from 33 to 100%, while usage of Vβs 2, 3, 7, and 14 remained low (≤10%). A tertiary culture composed of >98% CD8$^+$Vβ6$^+$ T cells lysed polyoma virus-infected but not uninfected H-2^k target cells (Fig. 3).

Biased usage of Vβ6 was also observed in polyoma tumor-

Table 4. *Association of Mtv-7 with Development of Tumors in Backcross Mice*

Mtv-7	Backcross mice	
	Tumor-bearing	Tumor-free
+	63 (73%)	9
−	8 (0%)	39

119 (C3H/BiDa × C57BR/cdJ) × C57BR/cdJ backcross mice were genotyped for Mtv-7 by Southern hybridization. Numbers in parenthesis indicate % mice with multiple tumors.
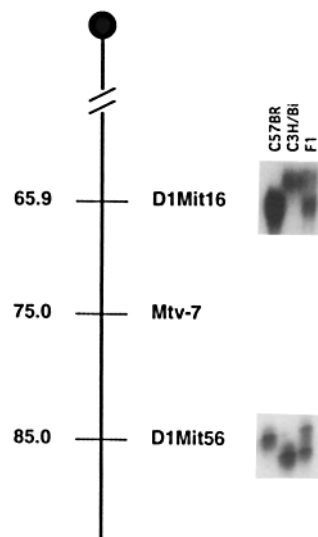
Table 5. *Recombination Analysis of Backcross Mice*

Backcross Mice	Recombination frequencies between Mtv-7 and flanking SSRs	
	D1Mit16	D1Mit56
Concordant	8.6% (8/93)	10.8% (10/93)
Nonconcordant	11.8% (2/17)	5.9% (1/17)

110 (C3H/BiDa × C57BR/cdJ) × C57BR/cdJ backcross mice inoculated at birth with polyoma virus were scored for tumors and their DNAs genotyped for Mtv-7 and SSR polymorphisms. Concordant mice were either Mtv-7$^+$ and tumor-bearing, or Mtv-7$^-$ and tumor-free. Nonconcordant mice were either Mtv-7$^+$ and tumor-free, or Mtv-7$^-$ and tumor-bearing.
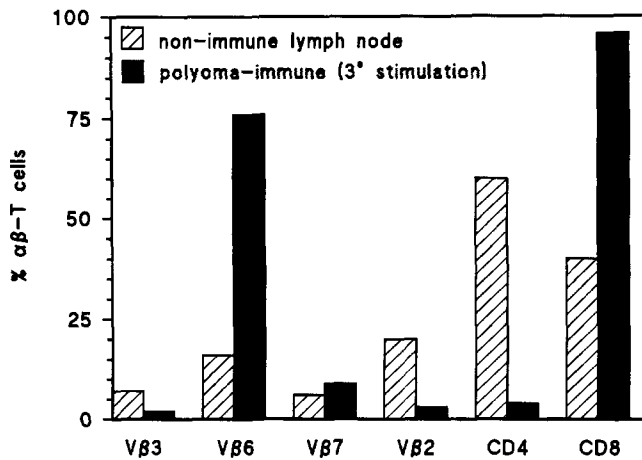
Figure 2. Polyoma virus-specific T cells are predominantly $V\beta6^+$ and $CD8^+$. T cells from polyoma virus-immune C57BR/cdJ lymph node cells were stimulated twice in vitro with virus-infected, irradiated C57BR/cdJ spleen cells. T cells from inguinal lymph nodes pooled from four C57BR/cdJ mice served as controls. T cells were indirectly stained with monoclonal antibodies to $\alpha\beta$-TCR and to the indicated $V\beta$ specificities, or directly stained with PE-conjugated anti-CD4 and PE-conjugated anti-CD8.

Figure 4. $V\beta6$ usage by T cells infiltrating a polyoma tumor. TILs recovered from a C57BR/cdJ-derived polyoma tumor in a polyoma virus-immune C57BR/cdJ host were indirectly stained with monoclonal antibodies to the $\alpha\beta$-TCR or to $V\beta6$; anti-$V\beta6$-stained cells were double-labeled with PE-anti-CD4 or PE-anti-CD8 antibodies. Cells were quantitated by cytofluorometry. Data are presented as log fluorescence intensity. Numbers are % $\alpha\beta$-T cells.

usage of $V\beta6$ by polyoma-specific CTLs in the resistant C57BR/cdJ mouse, and support the conclusion that Pyv^S is indeed Mtv-7 sag acting to delete $V\beta6^+$ T cells in the susceptible C3H/BiDa mouse.

infiltrating lymphocytes (TILs), as follows. A C57BR/cdJ mouse immunized at birth with virus was injected subcutaneously with a large dose (13 $\times$ 10⁶) of cells from a polyoma tumor that arose in an irradiated C57BR/cdJ animal (see Table 1 b). Two weeks later, the tumor was harvested and the infiltrating T cells were recovered and analyzed. Roughly 80% of the TILs were $CD8^+$ and nearly half of these expressed $V\beta6$ (Fig. 4). In contrast, only 14% of $CD4^+$ TILs expressed $V\beta6$. A line was established from TILs by repeated passage in vitro in the presence of infected, irradiated stimulator cells. This line, containing >95% $CD8^+V\beta6^+$ cells, exhibited cytolytic activity against C57BR/cdJ polyoma tumor cells but not against an SV40-transformed H-2^k cell line (Fig. 5). These results confirm the expectation of preferential
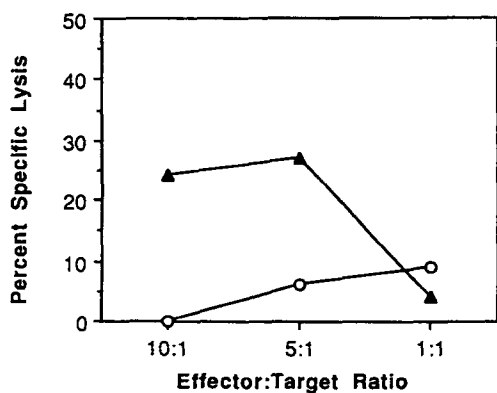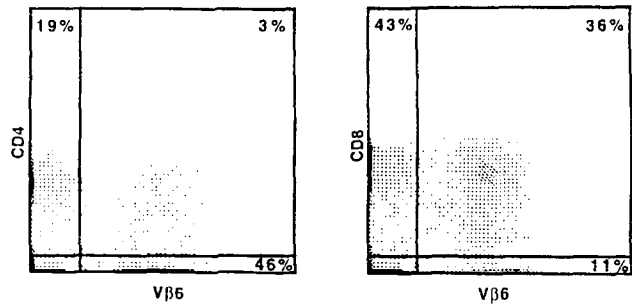
Discussion

The polyoma tumor susceptibility gene Pyv^S acts by preventing the host from mounting an effective immune response to virus-induced tumors. In a majority of mice from a cross in which Pyv^S and the mouse mammary tumor provirus Mtv-7 are segregating, tumor development cosegregates with the provirus. The known role of Mtv-7 SAG in deleting $V\beta6^+$ T cells provides a plausible mechanism of action for Pyv^S.

We have argued against the possibility of linkage and in favor of identity between Pyv^S and Mtv-7 sag on two grounds. The first is genetic, based on the fact that phenotypically nonconcordant backcross mice — those that are either
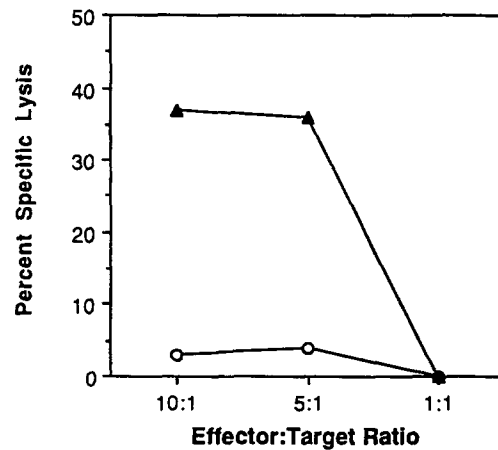

Figure 3. $CD8^+$ $V\beta6^+$ T cells possess polyoma virus-specific cytotoxic activity. An in vitro tertiary culture of polyoma virus-immune C57BR/cdJ cells derived as in Fig. 2 and composed of >98% $CD8^+$ $V\beta6^+$ T cells, was assayed for lytic activity against uninfected (—O—) and polyoma virus-infected (—▲—) H-2^k target cells.

Figure 5. Specific lysis of polyoma tumor cells by a $CD8^+V\beta6^+$ TIL line. A TIL-derived T cell line composed of >95% $CD8^+V\beta6^+$ T cells was assayed for lytic activity against 51Cr-labeled C57BR/cdJ polyoma tumor cell (▲) and 51Cr-labeled SV40-transformed H-2^k cells (—O—).

tumor-free and Mtv-7$^+$, or tumor-bearing and Mtv-7$^-$ — are not selectively recombinant in the region of chromosome 1 flanking Mtv-7. Recombinant backcross mice were found at equal frequencies in the nonconcordant and concordant groups, and not preferentially in the former group as would be expected if Pyv^s and Mtv-7 were separate linked genes. The second is immunological, and is based on deletion of Vβ6$^+$ T cells by Mtv-7 sag in the susceptible C3H/BiDa mouse coupled with strongly biased usage of Vβ6 by polyoma-specific T cells in the resistant C57BR/cdJ mouse. T cells from polyoma-primed C57BR/cdJ mice stimulated in vitro, as well as those recovered from a polyoma tumor carried in a virus-immune C57BR/cdJ host, are predominantly CD8$^+$Vβ6$^+$. These cells possess virus-specific cytolytic activity.

Several factors may be cited to explain the appearance of nonconcordant mice, all consistent with Pyv^s being Mtv-7 sag. Approximately 7% of backcross mice were scored as tumor-free through they clearly inherited Mtv-7. These mice can be explained, at least in part, by the fact that Pyv^s is less than 100% penetrant; in repeated experiments with groups of ∼30 C3H/BiDa mice, the frequency of tumor induction is between 95 and 100%. In addition, the chance of misphenotyping backcross mice as tumor-free is elevated because the average number of tumors per affected animal, termed the tumor frequency index (TFI), is lower in these mice compared to (C3H/BiDa × C57BR/cdJ)F1 mice. The TFI for backcross mice was 2.9 (131 tumors among 45 mice) and for F1 mice 4.6 (207 tumors among 45 mice). There could also be other effects attributed to the C57BR/cdJ genetic background which might, for example, cause a slower deletion of T cells by Mtv-7 sag. Delayed T cell deletion by an endogenous superantigen may allow specific antigen to block deletion of antigen-specific T cells bearing superantigen-reactive Vβ domains (46). Any or all of these factors could result

in the failure of Mtv-7$^+$ backcross mice to be scored as tumor-bearing. With respect to the 7% of backcross mice which developed tumors without inheriting Mtv-7, the most plausible explanation is that these tumors represent immune escape variants as has been shown for the rare tumors in C57BR/cdJ mice (Table 1). This interpretation is supported by the fact that only single tumors were found in these nonconcordant backcross mice (Table 4).

While the identification of Pyv^s as Mtv-7 sag has been based on analyses of crosses between C3H/BiDa and C57BR/cdJ mice, additional support comes from the known distributions of Mtv proviruses and polyoma tumor susceptibilities among other mouse strains. Historically (7) and in our own hands (13), mice possessing the highest susceptibility to tumor induction by polyoma all possess the H-2^k haplotype. Not all H-2^k mice are highly susceptible, however. As shown in Table 6, the correlation of high susceptibility with Mtv-7 and with no other Mtv provirus is clear. The combination of H-2^k and Mtv-7 as interactive codeterminants of susceptibility also reflects the hierarchy of presentation of Mtv-7 SAG by class II MHC molecules of different haplotypes: k > d > b > > q (22, 47).

Our results implicate CD8$^+$Vβ6$^+$CTL as critical anti-polyoma effector T cells in H-2^k Mtv-7$^-$ mice inoculated with virus as newborns. However, H-2^k Mtv-7$^+$ mice, which are susceptible as newborns, are known to develop resistance soon after birth (7). This implies that non-Vβ6-bearing T cells are involved in polyoma tumor immunity in C3H/BiDa mice inoculated as young adults.

Most Mtv SAGs, including Mtv-7 SAG, negatively select CD8$^+$ as well as CD4$^+$ thymocytes despite the requirement for association of Mtv SAGs with class II MHC molecules (23). Clonal deletion of CD4$^+$CD8$^+$ thymocytes has been put forward to explain Mtv SAG-mediated deletion of Vβ-

Table 6. *Polyoma Tumor Susceptibility of H-2^k Mouse Strains Correlates with the Presence of Mtv-7*

Strain	Susceptibility	Mtv Proviruses											
		1	3	6	7	8	9	11	14	17	23	29	30
C3H/BiDa	high	−	−	+	+	+	+	−	+	+	−	−	−
C58	high	−	+	−	+	−	−	−	−	+	−	−	+
CBA/J	high	−	−	+	+	+	+	−	+	+	−	−	−
AKR	high	−	−	−	+	+	+	−	−	+	+	−	+
RF/J	high	+	−	−	+	+	−	−	−	+	+	+	+
C57BR/cdJ	low	−	−	−	−	+	+	+	+	−	−	+	−
C3H/HeJ	low	+	−	+	−	+	−	+	+	−	−	−	−
CBA/CaJ	low	−	−	−	−	+	+	−	+	−	−	−	−
B10.BR	low	−	−	−	−	+	+	−	−	+	−	−	+
BALB.K	low	−	−	+	−	+	+	−	−	−	−	−	−

The distribution of Mtv proviruses are taken from Frankel et al. (28), Ignatowicz et al. (44), Kozak et al. (35), Pullen et al. (39), and Scherer et al. (19). High susceptibility strains have a >95% tumor incidence and develop multiple tumors of diverse types; low susceptibility strains have a lower tumor incidence and generally develop single tumors per animal (7, 13, and unpublished data).

reactive peripheral CD8$^+$ T cells (23, 48, 49). However, the finding that Mtv-7$^+$ mice expressing a transgene encoding a class I MHC-restricted Vβ8.1-TCR delete mature single CD8$^+$ but not CD4$^+$CD8$^+$ thymocytes indicates that Mtv-7 SAG can act in thymocyte maturation at a point beyond the CD4$^+$CD8$^+$ stage (50). In addition, Mtv-7$^+$ CD4-knockout mice delete CD8$^+$ T cells bearing Vβ6, 8.1, and 9, but not Vβ7 which has the lowest affinity for Mtv-7 SAG (51), implying that CD4 expression may be required for clonal deletion only when Mtv SAG-TCR affinity is low (52).

In another system, a novel Mtv-SAG contributes to development of spontaneous B cell lymphomas in SJL mice. In this case, the Mtv-SAG stimulates CD4$^+$Vβ16$^+$ T cells to release cytokines required for tumor growth (53, 54). While endogenous Mtv-SAGs are required for tumor development in both this and the polyoma system, the strategies are clearly different, involving stimulation of CD4$^+$ T cells in one case and deletion of CD8$^+$ T cells in the other.

Biased usage of Vβ6 by polyoma-specific CTL suggests that these T cells recognize few, and perhaps a single, viral peptide(s) associated with a particular H-2^k class I molecule. A growing body of evidence demonstrates limited Vβ as well as Vα domain usage by class I- and class II-restricted T cell clones and hybridomas recognizing specific antigen–MHC complexes (reviewed by Casanova and Maryanski [55], and references therein). The peptide(s) recognized by the Vβ6$^+$ anti-polyoma CTL are most likely derived from the MT and/or LT protein(s) of polyoma virus. Recombinant vaccinia viruses carrying MT or LT coding sequences as well as synthetic MT or LT peptides immunize rodents against transplanted polyoma-induced tumors (56, 57). Polyoma tumors invariably express MT, consistent with the essential role this protein plays in cellular transformation (58, 59), tumor induction (60), and virion assembly (61). LT is also generally expressed, though often in a truncated form, while viral capsid proteins are either not expressed or expressed non-uniformly in polyoma tumors (25). Because the polyoma tumor target cells used here (Fig. 5) express only T proteins, the latter must provide the TSTAs for the polyoma-specific CD8$^+$Vβ6$^+$ CTLs in C57BR/cdJ mice (Tables 3 and 5). Attempts are underway to identify the T antigen peptide(s) recognized by these CTLs.

The identification of Pyv^s as Mtv-7 sag represents a third manifestation of the function of this gene, having first been discovered as encoding the minor lymphocyte stimulating antigen Mls-1^a (28, 40). A number of interesting questions can be raised concerning the biological significance of these nonessential but widely distributed proviral genes which encode superantigens. Though clearly a susceptibility factor vis-à-vis experimental infection with polyoma virus, Mtv SAGs work in the opposite way, as determinants of resistance, with respect to infection by exogenous Mtvs. These viruses are lymphotropic and depend on SAG-mediated T cell activation in their life cycle (62). Mice carrying a particular Mtv provirus undergo deletion of those T cells required for replication by the homologous Mtv (63). While the presence of germline proviruses may reflect an evolutionary mechanism for protection against horizontal transmission of Mtvs, it also appears to carry a potential risk of increasing susceptibility to unrelated infectious agents. A larger question concerns the possibility that endogenous superantigens other than Mtv SAGs exist, and in species other than the mouse, and that such genes may act in a similar manner to influence susceptibility to disease.

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