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Comparison of Humoral Immune Responses and Tumor Immunity in Mice Immunized with Recombinant SV40 Large Tumor Antigen and a Monoclonal Anti-Idiotype[1]

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ABSTRACT

We compared the humoral immune responses induced in BALB/c mice by immunization with recombinant SV40 large tumor antigen (T-ag) with those induced by a monoclonal anti-idiotype (anti-Id), designated 58D, that is specific for SV40 T-ag-induced Id network components. We also challenged immunized mice with a lethal dose of SV40-transformed cells to assess *in vivo* tumor immunity. Two biweekly immunizations with either SV40 T-ag or anti-Id 58D induced humoral responses that recognized both SV40 T-ag and anti-Id 58D. Four biweekly immunizations with SV40 T-ag increased the antigen-specific antibody titers and decreased the response to anti-Id 58D, while four biweekly immunizations of anti-Id 58D increased antibody titers to both itself and SV40 T-ag. Comparison of specific T-ag epitope and idiotope specificities indicated that SV40 T-ag and anti-Id 58D immunization generated responses that recognized a similar epitope on SV40 T-ag and expressed a shared idiotope recognized by anti-Id 58D. SV40 T-ag immunized mice challenged with a lethal dose of SV40-transformed cells were completely protected and no tumors were observed. This is despite the fact that little or no SV40 T-ag-specific cytotoxic T-lymphocyte activity was detectable. In contrast, only 3 of 10 mice immunized with anti-Id 58D were protected from a lethal challenge. These results indicate that, although monoclonal anti-Id immunization can induce responses that recognize similar SV40 T-ag epitopes and express shared idiotopes associated with antibodies to SV40 T-ag, the recombinant antigen itself induces superior *in vivo* tumor immunity.

INTRODUCTION

The ability of immunoglobulin variable regions to express Id[3] constitutes the basis of a network of interactions strictly involved in the regulation of the immune response. This network is activated via the production of anti-antibodies (anti-Id) specific for idiotopes within the immunoglobulin variable region. Perhaps the most significant role of an Id is the potential involvement in the regulation of the immune response to an antigen. A number of studies have described the ability of the Id expressed on an anti-Id to modulate the immune response *in vivo*. These reports lend support for the Id network theory of immunoregulation proposed independently by Jerne (1) and Lindenmann (2). It has also been shown that certain anti-Id mimic the biological effects of the nominal antigen (Ag) and can be used as surrogate or network antigens to generate specific immune responses and induce host immunity (3).

Tumor-associated Id and anti-Id modulation have been described in a number of systems that use both human and murine tumor-associated antigens (4–6). These studies include human T-cell leukemia (7), human breast carcinoma (8), carcinoembryonic antigen (9), high-molecular-weight melanoma-associated antigen (10), mouse mammary tumor virus (11), and simian virus 40 (5, 12–14). Together, these data suggest a role for Id and anti-Id as active immunological reagents for the induction of tumor immunity (6, 15).

SV40 is a member of the papovavirus family. These tumorigenic DNA viruses include the human viruses BK and JC. Papovavirus infection has been reported to be oncogenic in rodents and able to transform cells of human and rodent origin *in vitro* (16). SV40 and JC virus have also been affiliated with progressive multifocal leukoencephalopathy, suggesting their involvement in the induction of human brain tumors; however, the role of SV40 in human brain tumor induction is still controversial (17, 18). In addition, astrocytomas, glioblastomas, and reticular cell sarcomas have been reported to harbor SV40 and BK virus genetic sequences (19–23).

Serum from animals that carry SV40-induced tumors contain antibodies that specifically bind to the SV40 gene products "large T" and "small t" antigens (24, 25). SV40 T-ag is a multifunctional protein that is essential for both the replication of the virus and for cellular transformation (26, 27). Approximately 95% of T-ag in transformed cells is located in the nucleus; however, a portion of T-ag is exposed on the surface of transformed cells (28–30). SV40 T-ag represents a virally encoded tumor-specific antigen whose transforming activity appears to be mediated in part through specific binding to certain key host proteins (31–35). For example, SV40 T-ag binds to the cellular proteins p53 (36) and retinoblastoma (Rb) (37).

Previous studies have shown that mice immunized with inactivated SV40-transformed cells or purified native T-ag are protected from *in vivo* challenges with syngeneic live SV40-transformed cells, indicating SV40 T-ag is responsible for *in vivo* protective immunity (38, 39). We have examined the role of Id and anti-Id reagents in the induction of humoral tumor immunity in the T-ag system. Previously, we described anti-Id modulation and partial tumor immunity in BALB/c mice after immunization with polyclonal anti-Id preparations, even though these preparations failed to induce detectable anti-SV40 T-ag Ab3 responses *in vivo* (5, 12). We have also generated and characterized a series of monoclonal anti-Id that are capable of inducing anti-SV40 T-ag Ab3 responses in BALB/c mice (14). Recombinant SV40 T-ag produced in baculovirus and monoclonal anti-Id 58D appear to be components of an Id network induced when mice are challenged with SV40-transformed cells (13). These studies have provided the immunological reagents to examine the putative roles that Id networks and humoral immune responses to SV40 T-ag may play in protective tumor immunity.

In this study, we compared the humoral immune responses induced in BALB/c mice by immunization with baculovirus-derived recombinant SV40 T-ag to those induced by immunization with a monoclonal anti-Id designated 58D. We compared the antibody titers to SV40 T-ag and anti-Id 58D by indirect ELISA and examined SV40 T-ag epitope specificity and idiotope expression by a competitive inhibition ELISA. In addition, we challenged SV40 T-ag and anti-Id

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[3] The abbreviations used are: Id, idiotype; ELISA, enzyme-linked immunosorbent assay; BBS, borate-buffered saline; HBsAg, hepatitis B surface antigen; HRP, horseradish peroxidase; T-ag, large tumor antigen; LD_{50}, 50% lethal dose; CTL, cytotoxic T-lymphocytes.

58D immunized mice with a lethal dose of syngeneic SV40-transformed cells to examine *in vivo* tumor immunity. The humoral responses to SV40 T-ag and associated Id network components were compared and indicated that SV40 T-ag immunization is superior to anti-Id 58D immunization for the induction of productive immunity against SV40-induced tumors in mice.

MATERIALS AND METHODS

Mice. Five-week-old female BALB/c mice (Harlan Sprague Dawley, Houston, TX) were used for immunization with recombinant and antibody-derived antigens, inoculation with SV40-transformed cells, and ascites production.

Medium and Cells. The medium used for growing cells was Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 500 units/ml penicillin, 500 μg/ml steptomycin, and 10% fetal bovine serum. The transplantable SV40-transformed BALB/c mouse kidney cell line designated mKSA has been described in detail elsewhere (40).

Recombinant SV40 T-ag. Recombinant SV40 T-ag was derived from the insect cell line Sf9 using the baculovirus AcNPV expression vector system (41–43). The recombinant protein produced in insect cells is correctly modified by phosphorylation, palmitylation, and glycosylation and displays ATPase and helicase activity. SV40 T-ag was extracted from baculovirus-infected Sf9 cells and purified afterward by immunoaffinity chromatography as described previously (41, 44). The purity of SV40 T-ag was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver stain. Control baculovirus expressed HBsAg was purified by methods described previously (45, 46).

Antibody Preparations. The mouse monoclonal Ab1 PAb 405 (IgG1κ), which recognizes the carboxy terminus of SV40 T-ag; A1.2 (IgG1κ), which recognizes HBsAg; and mouse monoclonal anti-Id 58D (IgG1κ and BF4 (IgG1,k), which recognize PAb 405 and A1.2, respectively, are described elsewhere (14, 46, 47). All antibody preparations were produced as ascitic fluid in pristane- primed BALB/c mice and purified by protein A chromatography (14, 48).

Indirect ELISA for the Detection of Antibodies to SV40 T-ag and Anti-Id 58D. The ability of serum from immunized mice to recognize either SV40 T-ag or anti-Id 58D was determined by indirect ELISA (13, 14). In this assay, 100 ng of purified SV40 T-ag in 50 μl of BBS (pH 8.2) was coated onto 96-well flat-bottomed microtiter plates for 1 h at 37°C. For monoclonal anti-Id 58D, 200 ng of pepsin-derived $F(ab')_2$ fragments were adsorbed to the solid phase. Nonspecific binding sites were blocked with 200 μl of 10% normal goat serum in BBS for 30 min at 37°C, and the plates were washed with BBS that contained 0.05% Tween 20. A total of 50 μl, diluted in 10% normal goat serum which contained various 4-fold dilutions of mouse sera, was added to the wells and incubated for 1 h at 37°C. Unbound serum was removed by washing with BBS containing Tween 20, and 50 μl of HRP-conjugated goat anti-mouse IgG (Fc specific) diluted in 10% goat serum was added for 1 h at 37°C. Unbound goat anti-mouse IgG-HRP was removed by washing with BBS-Tween 20, and the assay was developed by the addition of 75 μl of 0.3 mg/ml 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and 0.17% H_2O_2 in 0.1 M citrate buffer (pH 4.0). The enzyme-substrate reaction was terminated by the addition of 75 μl/well of 5% sodium dodecyl sulfate, and the absorbance at 410 nm was determined using an automatic ELISA plate reader. The anti-SV40 T-ag end point titers represent the reciprocal dilution of sera that resulted in an absorbance value 3 times above that obtained with a 1:10 dilution of preimmune serum less background. In most instances, the absorbance value obtained with the preimmune serum was negligible; therefore a cutoff value of 0.1 was used as the end point.

Inhibition ELISA for SV40 T-ag Epitope and PAb 405 Idiotope Analysis. Mouse serum recognized epitopes associated with the carboxy terminus of SV40 T-ag as shown by the ability to inhibit the SV40 T-ag-PAb 405 interaction (13, 14). In this assay, a 1:50 dilution of mouse serum was used to block a concentration of PAb 405 (20 ng) labeled with HRP from binding to solid phase adsorbed SV40 T-ag (49). The concentration of HRP-labeled antibody used in the assay was on the linear portion of the SV40 T-ag binding curve. Similarly, antibodies in the mouse serum recognized an antigen combining site-related idiotope as shown by their ability to inhibit the PAb 405-anti-Id 58D reaction. A 1:50 dilution of serum was used to block PAb 405-HRP from binding to solid phase adsorbed anti-Id 58D. To examine possible nonspecific reactivity, we compared results from similar assays using HBsAg, HRP-labeled monoclonal antibody to HBsAg, A1.2, and anti-Id BF4. The percentage of inhibition (I) of the SV40 T-ag-PAb 405, PAb 405-anti-Id 58D, and irrelevant control reactions was calculated as

$$\% \text{ of I} = 100 \times 1 - \frac{A_{410\,nm} \text{ with inhibitor-background}}{A_{410\,nm} \text{ without inhibitor-background}}$$

***In Vivo* Administration of Antigens and Tumor Challenge.** Groups of 10 BALB/c mice each received 2 biweekly injections of alum- precipitated SV40 T-ag or 2 biweekly injections of anti-Id 58D (10 μg/injection). Control groups of mice received either alum precipitate alone, alum-precipitated PAb 405, or alum-precipitated anti-Id BF4 in a similar manner. All monoclonal preparations were coupled to keyhole limpet hemocyanin prior to precipitation in alum. Animals were bled prior to the immunizations (preimmune stage) and 7 days after the second inoculation. *In vivo* tumor immunity induced by SV40 T-ag immunization was determined using i.p. injections of SV40-transformed cells, and survival time was used as a parameter of tumor immunity. Inoculation of a lethal dose of tumor and subsequent assessment of survival time were based on previously published data which demonstrate the efficient use of mKSA cells and BALB/c mice in this manner (12). The tumor cells were titrated to give a dose of transformed cells that resulted in no survivors in untreated mice after approximately 30 days (2 LD_{50}). The *in vivo* titration of SV40-transformed cells and determination of the LD_{50} has been described elsewhere (13).

RESULTS

Detection of Antibodies to SV40 T-ag. To determine if mice immunized with either SV40 T-ag or anti-Id 58D produce antibodies that are reactive with SV40 T-ag, immune sera were assayed by indirect ELISA (Table 1). Groups of mice that received either two or four injections of SV40 T-ag or anti-Id 58D produced antibodies reactive with SV40 T-ag. Antibodies to T-ag were not observed in sera collected from mice prior to immunization, and anti-SV40 T-ag reactivity was specific since no binding to HBsAg was observed (data not shown). Control groups of mice immunized with alum alone, PAb 405, and the control monoclonal anti-Id BF4 demonstrated no significant antibodies to SV40 T-ag (end point titers less than 50). Following two injections, mice immunized with SV40 T-ag had 48-fold greater antibody titers than did mice immunized with anti-Id 58D. Not all mice immunized with anti-Id 58D produced detectable levels of antibodies reactive with SV40 T-ag. Ten mice were immunized with anti-Ids 58D and 9 produced T-ag- specific antibodies (end point titers >50; Table 2). After 4 immunizations, SV40-T-ag immunized mice induced 324-fold greater antibody titers than mice immunized with anti-Id 58D. The two additional immunizations increased the mean end point titer of the SV40 T-ag-immunized group 9-fold. Following 4 immunizations with anti-Id 58D, 6 of 10 mice produced antibodies

Table 1 *Anti-SV40 T-ag and anti-Id 58D end point titers in immunized mice*

Group[a]	No. of injections	Anti-SV40 T-ag titer[b]	Anti-58D titer
Alum	2	<50	<50
	4	<50	<50
405	2	<50	<50
	4	<50	<50
58D	2	365 (<50–800)	5,120 (3,200–12,800)
	4	525 (<50–3,200)	158,720 (51,200–204,800)
BF4	2	<50	<50
	4	<50	<50
T-ag	2	17,600 (3,200–51,200)	7,520 (800–12,800)
	4	170,240 (12,800–204,800)	4,805 (<50–12,800)

[a] Each immunization group consisted of 10 mice/group.
[b] Values represent the mean of the individual reciprocal dilutions of end point titers. Numbers in parentheses, range.

Table 2 *Individual anti-SV40 T-ag and anti-58D end point titers in SV40 T-ag and anti-Id 58D-immunized mice*

SV40 T-ag-immunized				Anti-Id 58D-immunized			
58D titers		T-ag titers		T-ag titers		58D titers	
2[a]	4	2	4	2	4	2	4
800[b]	800	3,200	204,800	200	200	3,200	204,800
3,200	3,200	12,800	204,800	<50	800	3,200	204,800
3,200	800	3,200	204,200	200	800	3,200	204,800
12,800	3,200	12,800	204,800	200	<50	3,200	204,800
12,800	800	12,800	12,800	800	3,200	12,800	51,200
12,800	12,800	51,200	204,800	200	50	3,200	204,800
12,800	12,800	12,800	204,800	800	<50	3,200	204,800
12,800	800	12,800	204,800	800	<50	12,800	204,800
800	50	3,200	51,200	200	<50	3,200	51,200
3,200	12,800	51,200	204,800	200	200	3,200	51,200

[a] Mice received either 2 or 4 injections of either SV40 T-ag or anti-Id 58D.
[b] Values represent the individual reciprocal dilutions of end point titers.

that recognized SV40 T-ag (Table 2). The overall mean anti-SV40 T-ag titer increased 1.4-fold after the two additional immunizations in the anti-Id 58D group (Table 1). However, within individual mice, the anti-SV40 T-ag titers decreased in 5 of 10 mice, with 4 of these 5 demonstrating no specific T-ag antibodies (end point titers less than 50) following the 2 additional anti-Id 58D immunizations (Table 2).

Detection of Antibodies to Anti-Id 58D. To determine if mice immunized with SV40 T-ag or anti-Id 58D produced antibodies reactive with anti-Id 58D, serum was assayed by indirect ELISA (Table 1). Mice immunized 2 or 4 times with either SV40 T-ag or anti-Id 58D produced antibodies reactive with anti-Id 58D. Antibodies to anti-Id 58D were not observed in serum collected prior to immunization (data not shown). Control alum-immunized, PAb 405-immunized, and anti-Id BF4 immunized groups demonstrated no significant anti-58D titer. After 2 injections, the SV40 T-ag inoculated mice exhibited a 1.4-fold higher anti-Id 58D titer than mice immunized with anti-Id 58D. In addition, sera from all 10 individuals in the SV40 T-ag and anti-Id 58D treatment groups recognized anti-Id 58D. Following 4 injections, the anti-Id 58D-immunized group exhibited a 33-fold greater mean anti-Id 58D titer than did mice immunized with SV40 T-ag. The two additional immunizations of anti-Id 58D increased the mean anti-Id 58D titer 31-fold. Within the 10 individual mice, an increase in the anti-Id 58D titer was observed following the 2 additional injections (Table 2). Interestingly, 2 additional immunizations of SV40 T-ag decreased the anti-58D titer. Furthermore, 2 of 10 mice that received 4 injections of SV40 T-ag no longer produced detectable levels of anti-Id 58D antibodies (Table 2). These data indicate that mice immunized with either SV40 T-ag or anti-Id 58D produced antibodies that specifically recognized the anti-Id 58D. However, additional injections of SV40 T-ag decreased the antibody response to Id 58D and, in 2 of these mice, anti-Id 58D responses were no longer detectable.

Recognition of SV40 T-ag Epitopes and Idiotope Expression. We used a competitive inhibition ELISA to determine if serum from immunized mice contains antibodies that specifically recognize the epitope defined by PAb 405 and are therefore associated with the carboxy terminus of SV40 T-ag (Table 3). The SV40 T-ag-PAb 405 interaction was inhibited by serum samples from mice immunized 2 times with SV40 T-ag or anti-Id 58D by mean inhibition levels of 59 and 11%, respectively. Following 4 injections, the SV40 T-ag and anti-Id 58D inoculated groups inhibited the SV40 T-ag-PAb 405 interaction by mean inhibition levels of 89 and 15%, respectively. Although the anti-Id 58D immunized groups inhibited the SV40 T-ag-PAb 405 reaction by mean inhibition levels of 11 and 15% after 2 and 4 injections, respectively, the inhibition observed within the anti-SV40 T-ag-responding mice were specific with levels of inhibition approaching 55% in some mice. The lower levels of inhibition represented by the mean values reflect that some of the individual mice

Table 3 *Analysis of SV40 T-ag epitope recognition and idiotype expression in mice immunized with SV40 T-ag, 405 (Id), or 58D (anti-Id)*

Group[a]	No. of injections	% of inhibition[b]	
		SV40 T-ag-405	405–58D
Alum	2	<5[c]	<5
	4	<5	<5
405	2	26 (15–50)	30 (25–52)
	4	86 (56–94)	89 (78–93)
58D	2	11 (0–31)	77 (65–84)
	4	15 (0–55)	88 (84–90)
BF4	2	<5	<5
	4	<5	<5
T-ag	2	59 (27–77)	57 (8–76)
	4	89 (48–96)	80 (55–90)

[a] Each immunization consisted of 10 mice/group.
[b] Percentage inhibition was determined at 1:50 dilution of serum.
[c] Values represent the mean of the individual percentage inhibition values. Numbers in parentheses, range.

failed to respond with T-ag antibodies, and little inhibition of the SV40 T-ag-PAb 405 interaction was observed in these individuals. The level of inhibition of the T-ag-PAb 405 reaction correlated with the T-ag antibody titers induced in the individual mice by anti-Id 58D immunization. All samples from SV40 T-ag immunized mice appear to recognize the carboxy terminal epitope defined by PAb 405. Serum from these two groups of mice failed to inhibit the control reaction HBsAg-A1.2 (data not shown). Serum from groups of mice immunized with alum alone or the control anti-Id, BF4, failed to inhibit the SV40 T-ag-PAb 405 reaction. In mice immunized with PAb 405, this reaction was inhibited by 26 and 86% following 2 and 4 injections, respectively. This was expected and most likely reflects the production of polyclonal anti-Id responses in the serum that detect combining site-related idiotopes on PAb 405.

To further examine the antibody response to SV40 T-ag and anti-Id 58D immunization, we used a competitive inhibition ELISA to determine whether these sera recognized PAb 405-like idiotopes defined by anti-Id 58D (Table 3). Serum obtained from mice immunized twice with either SV40 T-ag or the anti-Id 58D inhibited the interaction by a mean level of 57 and 77%, respectively. After 4 injections, the interaction was inhibited by mean values of 80 and 88%, respectively. These data suggest that anti-SV40 T-ag responses induced by either SV40 T-ag or anti-Id 58D immunization express idiotopes that are defined by anti-Id 58D. Serum from mice immunized with either alum or the control anti-Id BF4 failed to inhibit the PAb 405-anti-Id 58D reaction. In serum obtained from mice immunized with PAb 405, the reaction was inhibited by mean inhibition levels of 30 and 89% following 2 and 4 injections, respectively. Again, this inhibition most likely reflects the production of polyclonal anti-Id responses that recognize PAb 405 idiotopes similar to those recognized by anti-Id

58D. The control reaction A1.2-anti-Id BF4 was not inhibited by serum from either SV40 T-ag or anti-Id 58D-immunized mice (data not shown).

Comparison of SV40 T-ag and Anti-Id 58D Immunization for the Induction of Tumor Immunity. To assess *in vivo* tumor immunity, the various groups of mice received lethal i.p. injections of live SV40-transformed mKSA cells, and survival times were compared to determine tumor immunity. Since previous studies have correlated SV40 T-ag responses with *in vivo* tumor immunity, we selected two immunizations for this experiment. Additional injections (up to five) with anti-Id 58D resulted in a decrease in the T-ag titers with some mice who responded at two injections becoming nonresponsive with additional immunizations. Groups were immunized twice and challenged with approximately 2 LD_{50} of mKSA cells (Table 4).

The survival time for the control groups ranged from 28 to 35 days (mean, 30.8) for alum-immunized mice, from 28 to 33 days (mean, 30.7) for PAb 405-immunized mice, and from 28 to 35 days (mean, 31.5) for control anti-Id BF4- immunized mice. In all groups of control mice, no survivors were observed 35 days following challenge. In the anti-Id 58D-immunized group, the mean survival (46.5 days) time was longer than that observed in the control groups. Three of 10 anti-Id 58D immunized mice survived greater than 150 days. These three mice exhibited the highest anti-SV40 T-ag titers among the anti-Id 58D- immunized group (data not shown). The SV40 T-ag immunized group exhibited mean survival times of greater than 150 days with all 10 mice surviving. These data indicate that SV40 T-ag induces complete tumor immunity and that anti-Id 58D immunization protects some BALB/c mice from SV40-transformed cells. The tumor immunity observed in the anti-Id 58D-immunized group of mice appears to correlate with the induction of high levels of anti-SV40 T-ag based on end point titers.

DISCUSSION

This study compares humoral immune responses, as well as tumor immunity, induced in BALB/c mice immunized with baculovirus-derived recombinant SV40 T-ag and in mice immunized with the monoclonal anti-Id 58D. Studies conducted more than 10 years ago indicate that BALB/c mice challenged with SV40- transformed cells develop tumors with little or no SV40 T-ag-specific CTL activity (38). Previous studies have demonstrated that SV40 T-ag-immunized mice fail to generate detectable SV40 T-ag-specific CTL responses, even after a 6-day CTL *in vitro* priming of splenic-derived effector cell populations (50). Therefore, it is possible that humoral immune mechanisms may play an important role in tumor protection. We are presently examining the potential mechanism(s) for the observed tumor immunity. Putative humoral immune mechanism(s) being examined include passive transfer experiments and whether T-ag antibodies mediate the lysis of SV40-transformed cells via complement-indicated and/or antibody-dependent cellular cytotoxicity.

Table 4 *Effect of SV40 T-ag and monoclonal anti-Id 58D immunization on SV40-induced tumor formation*

Group[a]	No. of survivors	Mean survival time (days)[b]
Alum	0	30.8 (28–35)
405	0	30.7 (28–33)
58D	3	46.5 (31–>150)
BF4	0	31.5 (28–35)
SV40 T-ag	10	>150

[a] Each group contained ten mice immunized twice and challenged with 2 LD_{50} of transformed cells.

[b] Values represent the mean of the individual survival times. Numbers in parentheses, range.

Since SV40 T-ag is a nonreplicating entity that enters antigen-presenting cells exogenously, it is anticipated that various SV40 T-ag epitopes are processed in the endosomal compartment and presented on the cell surface in association with MHC class II antigens. This activates CD4+ T helper cells which are required for the production of antibodies. Because no CTL activity was observed in SV40 T-ag-immunized mice, it is unlikely that CD4+ class II-restricted CTL were induced. Alternatively, endogenously processed antigens undergo intracellular replication, processing in the endoplasmic reticulum, and presentation on the cell surface with MHC class I antigens. This latter antigen presentation pathway may be required to induce CTL activity and CD8+ class I-restricted CTL against SV40 T-ag (51). Since cell-mediated immune responses appear to play a major role in tumor surveillance and tumor immunity, our results suggest that within the SV40 T-ag-BALB/c mouse system, humoral immune parameters can also play a role in tumor immunity. In this light, we are presently determining whether CD4+ T_H1 subpopulations that produce γ-interferon may play a role in tumor immunity (52).

Based on end point titer analysis, SV40 T-ag immunization produced higher antibody responses than did equivalent immunizations with anti-Id 58D. This most likely reflects the multideterminant nature of SV40 T-ag compared to anti-Id 58D. It was of interest to note that not every mouse immunized with anti-Id 58D induced a detectable anti-SV40 T-ag response. In fact, additional injections with anti-Id 58D resulted in a decrease in the individual mouse T-ag responses. Only 6 of the 10 mice that were immunized 4 times exhibited detectable levels of antibodies to SV40 T-ag. Additional immunizations with anti-Id 58D and/or inclusion of other adjuvants, such as Freund's incomplete adjuvant, did not appear to increase the anti-SV40 T-ag response and in several instances decreased the T-ag titers within individual mice (data not shown). Based on previous studies, we selected the optimal concentration, adjuvant formulation, and immunization schedule for the induction by anti-Id of antigen-specific antibody responses (14, 53).

To determine whether humoral responses induced by SV40 T-ag or anti-Id 58D immunization can protect mice from a lethal challenge of syngeneic SV40-transformed cells, the various groups were inoculated with an *in vivo* titrated tumorigenic dose of live SV40-transformed mKSA cells (2 LD_{50}). The mean survival time of control groups was 31 days with no survivors observed 35 days after challenge. The SV40 T-ag immunized group of mice were completely protected from the tumor challenge. No fatalities have been observed in this group of mice for at least 150 days following challenge. The anti-Id-immunized group survived the tumor challenge considerably longer (46.5 days) than the control-immunized groups. Seven mice succumbed to tumors in this group, while 3 mice survived the challenge past 150 days. The superior level of tumor immunity induced by SV40 T-ag most likely reflects its multideterminant nature. The monoclonal anti-Id induces responses that recognize a more limited SV40 T-ag epitope repertoire than recombinant protein. Anti-SV40 T-ag responses produced by anti-Id immunization focus on epitopes detected by PAb 405. Thus, multiple SV40 T-ag epitopes (other than those recognized by PAb 405) may also play a role in SV40 T-ag-induced tumor immunity. We have previously generated and characterized monoclonal anti-Id to anti-SV40 T-ag preparations that recognize different epitopes than PAb 405 (14).[4] These monoclonal anti-Id fail to induce anti-SV40 T-ag responses when used to immunize mice. Thus, inclusion of these other available monoclonal anti-Ids into a cocktail for immunization would most likely yield little relevant in-

[4] Unpublished data.

formation regarding Id-based tumor immunity in the SV40 system and may effectively dilute the T-ag responses induced by anti-Id 58D.

The data presented herein indicate that the Id defined by anti-Id 58D may be regulatory and may represent a focal point for inducing SV40 T-ag responses that result in tumor immunity. In addition, BALB/c mice can be immunized with a recombinant tumor-specific antigen and produce a totally protective response to a lethal challenge with SV40-transformed cells. The Id expressed on anti-Id 58D, which is associated with SV40 T-ag-related Id network components, can also be used to generate protective tumor-specific immunity to an *in vivo* challenge with SV40-transformed cells in some mice but was not as effective as immunization with the recombinant tumor antigen. Since the monoclonal anti-Id utilized in this study may not represent an internal image, the potential exists for the induction of anti-SV40 T-ag via the stimulation of specific B- cells that exhibit complementary Id receptors (54). Thus, anti-Id 58D may function as an anti-clonotypic reagent to induce T-ag-specific responses via Id matching between the anti-Id and complementary receptors expressed on B- cells.

Although this represents somewhat disappointing results for idiotype-based vaccines, the possibility of using a pool or cocktail of different monoclonal anti-Id that mimic several SV40 T-ag epitopes is indicated from these studies. This model tumor system is of interest since tumor immunity is produced despite the fact that BALB/c mice fail to generate detectable SV40 T-ag- specific CTL responses. This represents a potential mechanism of cell-mediated responses which are thought to play a major role in tumor immunity. The studies described herein indicate that humoral immune mechanisms may have a role in tumor immunity. This system may allow for the dissection of humoral immune parameters within a viral-induced tumor model in mice.

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