Protection Against Marek’s Disease-Derived Tumor Transplants by the Nononcogenic SB-1 Strain of Marek’s Disease Virus

K. A. SCHAT* AND B. W. CALNEK

Department of Avian and Aquatic Animal Medicine, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

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A series of experiments was conducted to study the in vivo protection against Marek’s disease-derived tumor transplants by the nononcogenic SB-1 strain of Marek’s disease virus. Intact, embryonally bursectomized (Bx), thymectomized (Tx), or cyclophosphamide (Cy)-treated chickens of four genetic lines were vaccinated with live or inactivated SB-1. JMV, a non-virus-producing transplant, and GA/Tr-1 and MDT-198, two virus-producing transplants were used for challenge. Optimal protection against JMV was present 7 days postvaccination, but there was significant protection even when SB-1 and JMV were administered together. Protection was abolished by an increase in the number of tumor cells used for challenge or by combined Tx and Cy treatment. Inactivated SB-1-infected cells were unable to induce protection against JMV challenge. Protection was also present against challenge with GA/Tr-1, but not against MDT-198, except in vaccinated, Bx chickens. It was concluded that protection against JMV was T-cell dependent and required the induction of neo-antigens not present in an inactivated SB-1 cellular preparation. The absence of protection in intact chickens against MDT-198 could not be explained.

Infection of susceptible chickens with Marek’s disease virus (MDV), a herpesvirus, may result in the formation of lymphomas with the thymus-derived lymphocyte (T cell) as the target cell for transformation. At least two sets of antigens are produced during infection and subsequent tumor formation. The first set is related to productive and restrictive infection and can be found in organs with an active viral infection (8). The same antigens have been demonstrated in the cytoplasm and nucleus of in vitro-infected cells (30) and are referred to as viral internal antigens (VIA). A surface antigen related to viral envelope proteins was found on infected cells in vitro (viral membrane antigen or VMA) (9). The second type of antigen was detected on the surface of Marek’s disease (MD) tumor cells (22, 37) and it was designated Marek’s disease tumor-associated surface antigen (MATSA) (37). Since those early reports, MATSA has been demonstrated on other MD cell lines (7) and recently on the surface of spleen lymphocytes infected 5 days earlier with oncogenic MDV (K. K. Murthy and B. W. Calnek, J. Natl. Cancer Inst., in press).

The development of live virus vaccines has virtually eliminated MD as a serious problem for the poultry industry. The most commonly used vaccine was developed from an antigenically related herpesvirus isolated from turkeys (HVT) (36). Other vaccines are a naturally apathogenic MDV (23) and HPRS-16/Att, an attenuated oncogenic MDV (10). The mechanism of protection has not been elucidated, but a two-step mechanism has been proposed, the first step directed against viral multiplication and the second against tumor cell formation (19, 21). It is interesting that HVT, SB-1, and HPRS-6/Att viruses all can protect against challenge with JMV (3, 17, 24, 31) although they are considered to be nononcogenic.

JMV is of considerable interest as a model for the study on protection against tumor cells because JMV cells carry MATSA or a MATSA-like antigen (4, 37), but MDV, VMA, or VMA can not be demonstrated (32). It is tempting to speculate, therefore, that the protection conferred by SB-1 and HVT is directed against MATSA present on the surface of JMV. Indeed, Sharma was able to detect in vitro cytotoxic activity against the MSB-1 cell line (1) by using effector cells from HVT-primed chickens (27), and we were able to demonstrate the induction of MATSA early after infection with SB-1 and HVT (K. A. Schat and B. W. Calnek, J. Natl. Cancer Inst., in press). However, all of this provides only circumstantial evidence that MATSA might be
an important factor in the immune response in vaccinated chickens, and the definitive proof is still lacking.

Another important factor for the understanding of protection by SB-1 and probably HVT is the absence of necrotobiotic damage to the bursa of Fabricius, thymus, and spleen, which is associated with oncogenic MDV infection (16). This is reflected in the ability to respond to mitogens and antigens (25, 26).

The present study was initiated to obtain additional information on the in vivo protection by SB-1 against JMV and to study possible protection against two other MD transplants.

(This work was done in partial fulfillment of the requirements for the Ph.D. degree.)

MATERIALS AND METHODS

Birds and holding conditions. Four specific pathogen-free flocks were sources of experimental chickens; S-strain, N- and P-lines, and G-B1. The first three lines have been described before (5, 6); the syngenic G-B1 line (33) was recently obtained from fertile eggs kindly supplied by L. W. Schierman. Blood samples from the N- and P-line flocks were tested for antigens specified by B-locus (major histocompatibility antigen); N-lines were homozygous for the B\(^{\text{II}}\) allele, whereas the B\(^{\text{II}}\) allele predominated (with some B\(^{\text{II}}\) “contamination”) in the P-lines (W. E. Briles, personal communication). The B\(^{\text{II}}\) genotype confers resistance to MD (3). Experimental chickens were held in isolation units.

Viruses and viral preparations. Passage 11 of SB-1 (24) in chick embryo fibroblasts (CEF) was used throughout this study as a vaccine. Uninoculated CEF were prepared in parallel as control inocula. Low-passage GA-5 and JM-10 (6), cultivated in chick kidney cultures, served as oncogenic challenge viruses. All virus-infected and control cells were stored in liquid nitrogen. A special batch of SB-1 was prepared for inactivation with glutaraldehyde. CEF were harvested and divided into two batches when more than 75% of the cells were affected by the virus. One batch was stored in liquid nitrogen, whereas the second was inactivated as described by Powell (20) with some minor modifications. Briefly, 10\(^5\) cells/ml were incubated for 1 h at 22°C in a solution of 0.125% glutaraldehyde in phosphate-buffered saline (PBS), pH 7.3. Afterwards, the cells were washed three times in PBS, resuspended in portions of 22 x 10\(^5\) cells/ml and stored at -70°C (referred to as SB-1 inactivated). Control CEF were prepared in parallel for a control inoculum (inactivated CEF). Viable cells could not be detected by trypan blue exclusion, nor did the cells grow out when resuspended in growth medium and incubated at 38°C in 5% CO\(_2\). Virus could not be recovered after inoculation into CEF cultures.

MD transplantable tumors. JMV transplantable leukemia cells were propagated by passage in chickens from material originally obtained from M. Sevoian, Amherst, Mass. Two recently established MD tumor transplants were also used. GA/Tr-1 was developed in N-line chickens from a tumor originally induced by GA-5 in an N-line chicken (6). Bird passage 9 of GA/Tr-1 was employed in this study. The second transplant was MDT-198, developed in G-B1 chickens (33). Tumor-bearing G-B1 chickens, carrying passage 62 were kindly supplied by L. W. Schierman and R. A. McBride. Cell suspensions of this passage were harvested and used in this study. MDT-198 can cross the histocompatibility barrier and grow progressively in allogenic chickens (Schierman, personal communication; J. Fabricant et al., unpublished data). Single cell suspensions of all three transplants were stored under liquid nitrogen. Cells were thawed before use and viable cell counts were made by the trypan blue exclusion technique.

Immunosuppressive treatments. The techniques for embryonal thymectomy (Bx) performed on 17-day-old embryos, neonatal thymectomy (Tx), cyclophosphamide (Cy) treatment, or combined thymectomy and cyclophosphamide (Tx-Cy) treatment are discussed in detail elsewhere (6). The efficacy of the treatment was assessed only by gross examination for bursa or thymus remnants.

Vaccination and challenge procedures. One-day-old (experiments 1, 2, 4, 6, and 7), 7-day-old (experiments 3), or 14-day-old (experiment 8) chickens were vaccinated by intraabdominal (i.a.) or intramuscular (i.m.) injection with 500 focus-forming units (FFU). In experiment 5, SB-1 inactivated was used to vaccinate chickens. In trial 1, 1-day-old birds received a single dose equivalent to 14,300 FFU of live SB-1. For trial 2, 7-day-old chickens received the same dose of SB-1 inactivated, first emulsified in complete Freund adjuvant and then mixed 1:1 with 2% Tween 80. A second injection was given without adjuvant 3 weeks later. Control chickens in all experiments received an equivalent number of uninactivated CEF.

Challenge was performed by i.a. or i.m. inoculation with the tumor transplants JMV (experiments 1–5), GA/Tr-1 (experiment 6), or MDT-198 (experiments 7 and 8), or with the MDV strains JM-10 and GA-5 (experiment 5). Experiments involving JMV challenge were terminated between 14 and 17 days postchallenge, except in experiment 4 (24 days postchallenge). The other experiments were terminated at 6 or 10 weeks postchallenge.

The genetic lines and the exact numbers (excluding nonspecific mortality) of chickens used in the experiments are shown in the tables. Statistical analysis. The degree of protection was expressed in a protective index (PI) by the formula, PI = (C - V/C) / 100, where C = percent mortality in the control and V = percent mortality in the vaccinated group (34). The \(x^2\) test for independence (29) was used to calculate the level of significance of the differences in specific mortality between vaccinated and control groups. Mean scores of the size of pectoral muscle tumors were subjected to the median test (4). If ties occurred at the median, the split was made at the median plus one half. Fisher’s exact test was applied to the resulting 2 x 2 table.

RESULTS

Onset of protection against JMV challenge. Chickens were challenged with 10\(^3\) viable JMV cells at days 0, 3, 7, and 14 postvaccination.
(PV). Significant protection occurred even when vaccine and challenge doses were administered simultaneously (Table 1). Protection was optimal at day 7 PV and thereafter. Based on these results, challenge at day 7 PV was selected for the other experiments.

**Degree of protection against JMV.** Protection was less against higher challenge doses of JMV (Table 2). Challenge with $10^4$ cells resulted in a PI = 73; the PI was only 47 when the challenge dose was $1 \times 10^5$ cells. The PI of 47 constituted a significant difference ($P < 0.05$) from the PI of 73. Chickens were not significantly protected against challenge with $6 \times 10^5$ cells (PI = 19).

**Effect of immunosuppression on protection against JMV.** In experiment 3, the effect of immunosuppression on vaccine protection was studied to gain information on the effector systems involved. Vaccinated and control intact Tx, Bx, and Tx-Cy chickens were challenged with $10^3$ or $10^4$ JMV cells by i.a. or i.m. inoculation. The results are summarized in Table 3.

The data in trial 1 seem to indicate that Bx decreased the level of protection (PI = 75 for the intact versus PI = 56 for the Bx chickens). However, the difference was not significant, and the mortality in the Bx group was perhaps influenced by bacterial infections resulting from the i.a. inoculations. Therefore, an additional trial was conducted, using larger numbers of chickens, in which vaccination and challenge inocula were administered by i.m. injection (data not presented in a table). Bacterial infection problems were not encountered in this trial. Differences were not observed between intact and Bx-vaccinated chickens; the mortality due to JMV challenge was 5 out of 40 for the intact (PI = 87.5) and 4 out of 34 for the Bx chickens (PI = 89).

**Tx was not very effective in eliminating vaccine protection (Table 3, trial 2).** Specific mortality was somewhat higher in the Tx than in the intact group (PI = 90 versus 72), but the observed difference was not significant ($\chi^2 = 3.44$). The combination of Tx/Cy, however, caused a marked decrease in protection; seven out of eight vaccinated chickens in this group died after challenge (Table 3). Cy alone did not affect protection. There was one drawback in this experiment. Although both the Tx-Cy and the Cy group were initiated with 20 chicks, the final number of chickens in each was rather low, due to the toxicity of the Cy treatment.

**Local tumors caused by JMV.** Vaccinated chickens in experiment 3 developed tumors in the pectoral muscle when JMV was administered at that site. This was observed when they

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**Table 1. Onset of protection in P-line chickens, vaccinated at 1 day of age against challenge with $10^6$ JMV transplantable tumor cells (experiment 1)**

<table>
<thead>
<tr>
<th>Trial</th>
<th>SB-1 (500 FFU)</th>
<th>Specific mortality at PV day:*</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>ND</td>
<td>3/6(47) c</td>
<td>2/20(90)d</td>
<td>2/20(89)d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>ND</td>
<td>16/17</td>
<td>18/18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>20/33(40)d</td>
<td>15/30(46)d</td>
<td>2/21(89)d</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>33/33</td>
<td>29/31</td>
<td>16/18</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* Number dead from JMV challenge per number challenged. Numbers in parentheses indicate PI (see text).

b ND, not done.

c Statistical significance ($\chi^2$ test), $P < 0.025$.

d Statistical significance ($\chi^2$ test), $P < 0.001$.

**Table 2. Degree of protection in P-line chicks, 7 days post SB-1 vaccination, against increasing doses of JMV transplantable tumor cells (experiment 2)**

<table>
<thead>
<tr>
<th>Group</th>
<th>SB-1 (500 FFU)</th>
<th>No. of JMV challenge cells</th>
<th>Specific mortality</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Compared to group</td>
</tr>
<tr>
<td>I</td>
<td>−</td>
<td>$10^4$</td>
<td>17/19*</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>+</td>
<td>$10^4$</td>
<td>13/53</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>+</td>
<td>$10^5$</td>
<td>23/49</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>+</td>
<td>$6 \times 10^5$</td>
<td>31/43</td>
<td></td>
</tr>
</tbody>
</table>

* Number dead from JMV challenge per number challenged.
were examined 14 days postchallenge. The growth and regression of local JMV tumors were studied in experiment 4; $5 \times 10^4$ JMV cells were inoculated in the right pectoral muscle on day 7 PV. Starting at day 6 postchallenge, tumors were scored three times per week by palpation as follows: 0, No tumor; 1+, a tumor up to 0.5 cm; 2+, 0.6 to 1.0 cm; 3+, 1.1 to 2.0 cm and 4+, larger than 2 cm. All CEF-inoculated chickens died within 6 days postchallenge without tumors at the site of inoculation. The first local tumors in the vaccinated chickens appeared on day 8, and a maximum mean tumor size (mean score, 2.5) was reached on day 16 postchallenge. All eight surviving chickens developed local tumors. Two of those chickens died with progressive local tumors on days 18 and 22 postchallenge, respectively. In the remaining six chickens, rapid tumor regression started on day 18 and all tumors were gone by day 22 postchallenge.

Protection by inactivated SB-1 against JMV and MDV. The results of experiment 5 are summarized in Table 4. Trial 1 showed that a single injection of inactivated SB-1 did not protect against viral challenge with GA-5 or against tumor cell challenge with JMV. This was in contrast with results from live SB-1, which gave significant protection against GA-5 (PI = 70) and JMV (PI = 81). The vaccination procedure was modified for trial 2 by the use of CFA and Tween 80 to make an emulsified SB-1-inactivated mixture for primary vaccination followed by a second inoculation of inactivated SB-1 without CFA. Chickens vaccinated by this method also were unable to resist challenge with JMV, but a low degree of protection was present against JM-10 (PI = 42, $P < 0.025$).

Protection against GA/Tr-1. SB-1-inoculated N-line chickens rejected the GA/Tr-1 faster than did CEF-inoculated controls (Table 5). The mean tumor size (scored as outlined before) was significantly lower for the vaccinated chickens than for the controls at days 11, 14, and 17 postchallenge. Protection was also established against host-derived tumors resulting from the viral infection originating from the inoculated tumor cells (PI = 100).

Protection against MDV-198. Vaccination of N-line, S-strain, and G-B1 chickens with SB-1 did not result in protection against the development of tumors in the pectoral muscle when challenged with $5 \times 10^5$ cells of MDV-198. All

### Table 3. Effect of immunosuppression in P-line chickens, vaccinated at 7 days of age, against challenge with JMV transplantable tumor cells (experiment 3)

<table>
<thead>
<tr>
<th>Trial</th>
<th>SB-1 (500 FFU)</th>
<th>No. of JMV challenge cells</th>
<th>Specific mortality (PI) after treatment by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>$10^4$</td>
<td>6/30(75)$^c$</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>$10^4$</td>
<td>22/27</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>$10^4$</td>
<td>1/19(90)$^c$</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>$10^4$</td>
<td>19/20</td>
</tr>
</tbody>
</table>

$^a$ Each trial contains pooled data from two identical smaller trials.

$^b$ See Table 1, footnote a.

$^c$ Statistical significance ($\chi^2$ test), $P < 0.001$.

$^d$ Statistical significance ($\chi^2$ test), $P < 0.01$.

$^e$ ND, Not done.

### Table 4. Protection by vaccination with live or inactivated SB-1 infected cells (experiment 5)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Chicken line</th>
<th>Challenge</th>
<th>Incidence of MD after challenge of birds vaccinated with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Live CEF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>P</td>
<td>JMV tumor cells</td>
<td>15/15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA-5 virus</td>
<td>18/19</td>
</tr>
<tr>
<td>2</td>
<td>N</td>
<td>JMV tumor cells</td>
<td>17/26</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>JM-10 virus</td>
<td>19/26</td>
</tr>
</tbody>
</table>

$^a$ Trial 1: Vaccination (single dose) at 1 day of age; challenge at 8 days of age. Trial 2: Vaccination (2 doses) at 7 and 28 days; challenge at 35 days.

$^b$ See Table 1, footnote a.

$^c$ Statistical significance ($\chi^2$ test), $P < 0.001$.

$^d$ Statistical significance ($\chi^2$ test), $P < 0.02$. 
chickens in experiment 7 had developed palpable tumors by day 10 postchallenge. These progressed rapidly to 4+ tumors. S-strain and G-B1 chickens died soon afterwards, whereas the N-line chickens survived for a longer period. Significant differences in mean time to death (MTD) were not observed between vaccinated and control groups (Table 6). Only two vaccinated chickens (one S-strain and one N-line) had regressive tumors. Two-week-old rather than 1-day-old P-line chicks were used in experiment 8 to enhance the possible influence of maturation of the immune response. However, there was no increase noted in rate of tumor rejection over that seen in younger birds in nonvaccinated intact or Bx chickens; once a tumor developed the result was almost always death (Table 7). The MTD was comparable to that with N-line chickens in experiment 7. One difference from the previous experiment was that not all challenged chickens developed a tumor at the site of inoculation. However, these chickens died with massive visceral tumors before termination of the experiment. Vaccination with SB-1 affected the pattern of tumor development and regression only slightly in the case of intact chickens, but the low number of chickens which developed tumors at the site of inoculation (four out of eight) makes conclusions impossible. Of the four birds with local tumors, one had a regressive tumor and, of the remaining three, one died late in the experimental period. The four chickens without tumors at the site of inoculation survived the experimental period. All survivors had small tumors in the kidneys and, in some instances, elsewhere. The type of tumors suggested metastases of the original inoculum, but confirmation was not attempted. The combination of bursectomy and vaccination gave interesting results. Birds so treated were protected \( (P < 0.05) \) when compared with nonvaccinated intact or Bx birds, or with intact vaccinated birds for total incidence of MD (Table 7). Four of the seven tumors which developed at the site of inoculation regressed completely, and three of the four chickens with tumors in regression did not have any tumors in the internal organs at 6 weeks postchallenge. One in this group did not develop either type of tumor.

**DISCUSSION**

In vivo investigation of vaccine protection

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**TABLE 5. Protection in N-line chickens vaccinated at 1 day of age against i.m. challenge with the virus-producing MD transplantable lymphoma GA/Tr-1 (experiment 6)**

<table>
<thead>
<tr>
<th>No. of chickens vaccinated with SB-1 (500 FFU)</th>
<th>Mean tumor size (% chickens positive) at postchallenge day</th>
<th>% MD at 10 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>19</td>
<td>0 (0)</td>
<td>0.55 (45)</td>
</tr>
<tr>
<td>21</td>
<td>0 (0)</td>
<td>0.19 (19)</td>
</tr>
</tbody>
</table>

* Tumors were scored from 0 to 4+; the mean sizes of vaccinated and controls were compared with the median test.
* Positive birds = dead birds and survivors at 10 weeks with MD lesion.
* Statistical significance (median test), \( P < 0.05 \).
* Statistical significance (median test), \( P < 0.001 \).
* Statistical significance (median test), \( P < 0.025 \).
* Statistical significance \( (\chi^2 \text{ test}) \), \( P < 0.001 \).

**TABLE 6. Protection in SB-1-vaccinated chickens against i.m. challenge 7 days PV with MTD-198 (5 \times 10^6 cells) (experiment 7)**

<table>
<thead>
<tr>
<th>Chicken</th>
<th>No.</th>
<th>SB-1 (500 FFU)</th>
<th>No tu- Regressive tu- Progressive tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No tumor</td>
</tr>
<tr>
<td>G-B1</td>
<td>6</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>N-line</td>
<td>10</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

* Experiments were terminated for G-B1 at 17 days, for S-strain at 21 days, and for N-line at 42 days postinoculation.
* Mean time to death is based on the mortality of birds with progressive tumors.
* Regressed from 1+ tumor.
* Regressed from 4+ tumor.
against MD tumor cells requires MD transplantable lymphomas or cell lines, but not all are suitable for such studies. MSB-1 does not produce tumors from transplantation of donor cells, and any apparent protection against MSB-1 could well be directed against the concomitant viral infection instead of the tumor cells (11). JMV is the only transplantable MD tumor cell used extensively for the study of protection in vaccinated chickens (17, 31, 34). The experiments reported here confirm earlier observations (24) that SB-1 induces immunity against JMV.

Protection depends on at least three factors. First is the time required between vaccination and challenge. Optimal protection is seen 7 days PV, although chickens were protected even when SB-1 and JMV were injected simultaneously. The immune response is apparently induced within a few days so that JMV cell proliferation is stopped before a critical (lethal) level of cells is reached. It is known that a rapid generation of effector cells is possible; Japanese quail inoculated with Rous sarcoma virus produced by cytotoxic spleen cells as early as 3 to 5 days postinoculation, even before palpable tumors were detectable (15).

A second factor is the challenge dose. A high level of tumor cells can overwhelm the immune response as demonstrated in experiment 2. Similar results have been obtained in mice vaccinated with glutaraldehyde-treated tumor cells and subsequently challenged with live tumor cells (14). The third important factor for the immune response is an intact T cell system (reviewed in reference 19) and our data support a central role for T cells in MD immunity. The combination of Tx-Cy prevented an effective immune response against JMV, whereas either Tx or Cy alone or Bx did not ameliorate the immune response. It had been shown that a functional bursa-dependent immune system was not required for age-related resistance to MD (28) or vaccinal immunity against viral challenge (13). In fact, there is some evidence that a functional B-cell-dependent immune response might enhance MD tumor growth. This became obvious during earlier studies on the rejection of tumor transplants; embryonally bursectomized chickens were able to reject GA/Tr-1 transplants earlier than were intact controls (6). The observation that Bx, SB-1-vaccinated P-line chickens rejected challenge with MDT-198 better than did intact vaccinated birds (Table 7) is in agreement with those results. Recently, McBride et al. (18) reported the same type of response with RSV when tumors were shown to grow more slowly in Bx chickens than in controls. It was speculated both by McBride and co-workers and our group, that this effect could be due to the absence of suppressor T cells in Bx chickens. It has been hypothesized that suppressor T cells are formed under influence of the bursa (12) and Bx would thus prevent their formation.

The inability of inactivated SB-1 to protect against the transplantable JMV tumor cells while it was able to induce a low degree of immunity against JM-10 virus infection is of considerable interest. It can be inferred that protection against the non-virus-producing JMV tumor cell by live SB-1 but not by inactivated SB-1 is the result of in vivo induction of antigen(s) (MATSA?) not present on in vitro infected cells. It has been proposed that the immune response to MD is a two-phase process, in which the first step is directed against viral multiplication by immunity to VIA and eventually VMA, whereas the second step is aimed at tumor cells by immunity against MATSA.

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**Table 7. Effect of Bx in P-line chickens on SB-1 vaccine protection against MDT-198 transplantable lymphoma cells (experiment 8)**

<table>
<thead>
<tr>
<th>Prechallenge treatment</th>
<th>Total incidence of palpable tumors*</th>
<th>No. of tumor-positive birds with:</th>
<th>MTD† (days)</th>
<th>Total incidence of MD at 6 weeks postchallenge:‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bx SB-1 vaccination</td>
<td>Regressive tumors</td>
<td>Progressive tumors</td>
<td>Surviving at 6 weeks</td>
<td>Dead</td>
</tr>
<tr>
<td>- -</td>
<td>7/10</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>- +</td>
<td>4/8</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>+ -</td>
<td>6/7</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>+ +</td>
<td>7/8</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

*Number positive per number challenged.
†MTD, Mean time to death of birds that died with progressive tumors.
‡Number positive per number challenged. Includes birds that did not develop palpable muscle tumors or had regressive tumors but had lymphomas in visceral organs.
§Incidence statistically different (P < 0.05) from that in intact, nonvaccinated controls.
The early response against JMV in SB-1-immunized chickens, together with the apparent necessity for the induction of a neoantigen for this protection, suggests that the two steps could go together rather than in sequence. The use of the GA/Tr-1 and MDT-198 transplants to test SB-1 protection produced confusing results. On the one hand, SB-1 protected against GA/Tr-1, evidenced not only by enhanced tumor rejection, but also by the absence of metastases and virus-induced tumors. On the other hand, protection was not seen against MDT-198 in intact syngenic and allogenic chickens.

Several possible explanations for the different responses to GA/Tr-1 and MDT-198 should be considered. (i) The Conn B virus associated with MDT-198 is exceptionally immunosuppressive, and thus the ability of the host to reject the tumor is impaired. This explanation is not too plausible since GA-5 infection is probably equally immunosuppressive. (ii) MDT-198 tumor cells are only weakly antigenic and do not stimulate a vigorous immune response; this could be due to weak or absent viral induced neoantigens or due to loss of histocompatibility antigens, either of which might occur following the selective pressures of passage. The observation that MDT-198 crossed the histocompatibility barrier only after many passages (L. W. Schierman, personal communication) supports this possibility. (iii) MDT-198 elicits a stronger response in terms of blocking factors (2) or, perhaps, stimulates suppressor T-cell activity more strongly than does GA/Tr-1. Either could inhibit an immune response from SB-1 infection, and our data with Bx chickens could support both possibilities. More comparative studies are necessary to unravel this perplexing question.

The reason for solid tumor formation induced at the site of inoculation with JMV in SB-1-vaccinated birds is unknown. This fact is curious since vaccinated birds clearly rejected proliferation of JMV cells in the usual sites (visceral organs).

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LITERATURE CITED


