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Influence of the Bursa of Fabricius on the Pathogenesis of Marek's Disease

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A series of experiments was conducted to study the influence of embryonal bursectomy (EBX) on the early and late pathogenesis of Marek's disease (MD). The early lytic infection in the lymphoid organs normally associated with oncogenic MD virus infection in intact chickens was not seen in EBX chickens. Therefore, the damage to the immune system was minimal. EBX chickens also had lower viremia levels, higher lymphocyte responses to mitogens, and a lower or delayed MD mortality when compared with intact chickens. Furthermore, it was shown that although vaccination with SB-1 by itself did not protect against a highly virulent MD transplantable tumor, the combination of EBX and vaccination gave significant protection. All these effects could be explained by an enhanced immune response in EBX birds. In contrast, the pathogenesis of nononcogenic MD virus was not influenced by EBX. The possible mechanism(s) involved in these observed effects of EBX on MD pathogenesis are discussed.

Marek's disease (MD) is a naturally occurring, herpesvirus-induced lymphoma in domestic fowl. It is generally accepted that thymus-derived lymphocytes (T-cells) are the targets for transformation (reviewed in reference 2a). Earlier studies have addressed the possible effects of the bursa of Fabricius on the outcome of MD virus (MDV) infection. Morris et al. (20) reported that neonatal bursectomy (NBX) enhanced MD incidence; however, functional tests on the efficacy of the bursectomy were not done. More definitive studies with NBX chickens, which were monitored for the presence of immunoglobulins and antibody production, failed to show bursa-dependent influences on MD (12, 22). Chemical bursectomy by treatment of embryos with testosterone propionate (12) or by repeated injections of cyclophosphamide into newly hatched chickens (16) ameliorated MD or at least reduced the viremia levels and delayed the development of MD lesions. Both chemical treatments, however, influence the development of T-cells in addition to eliminating B-cell populations (30). Recent studies in this laboratory indicated that embryonal bursectomy (EBX) of 17-day-old embryos increased resistance to challenge with the transplantable MD tumor cell MDCT-CU8 (previously designated GA/TR-1 [36]), resulting in an enhanced rejection and reduced incidence of MD through 10 weeks of age (5). Also, EBX combined with vaccination with the nononcogenic SB-1 strain of MDV protected against challenge with the highly virulent transplant MDCT-NYM-1 (previously reported as MDT-198 [36]), whereas either treatment alone was not protective (25).

The conflicting reports on the effects of bursectomy have caused some confusion. The purpose of the present investigation was to examine the effect of EBX on the early and late pathogenesis of MD in an attempt to better understand how the bursa of Fabricius might influence the disease.

MATERIALS AND METHODS

Experimental chickens and holding conditions. Chickens of the genetically MD-susceptible P-line (6) were obtained from a specific pathogen-free flock (2). P-line chickens of the $B^{19}B^{19}C^2C^2$ haplotype (P-2) (26) were used for some of the experiments. Experimental chickens were kept on the floor or in wire-floored battery brooders.

Bursectomy. Surgical bursectomy was performed on embryos during day 17 of incubation, or on newly hatched chickens, by established procedures. The efficacy of the treatment was assessed in all experiments by gross examination for bursal remnants and in the long-term experiments (experiments 7, 8, and 9) by tests for the ability to produce antibodies against MDV and bovine serum albumin. Results from chickens with evidence of incomplete bursectomy were discarded.

Virus strains and inoculation procedures. All virus strains were cell associated and from stocks stored in liquid nitrogen (33). The nononcogenic SB-1 strain (24) was the 11th passage in chicken embryo fibroblasts. The oncogenic strains JM-10 and GA-5 have been described elsewhere (2). They were passaged up to 20 times in chicken kidney cultures. The

bird dosage, based on previous titrations, was about 500 focus forming-units (FFU). Virus was given by intraabdominal injection.

MD transplantable lymphoma. MDCT-NYM-1 transplantable lymphoma was developed in G-B1 chickens (35). Tumor-bearing G-B1 chickens carrying passage 62 were kindly provided by L. W. Schierman and R. A. McBride. Cell suspensions of this passage were harvested, frozen in liquid nitrogen, and used in this study.

Tissue collection and processing for cell suspension. Spleen and thymus were collected aseptically and gently forced through a 60-μm autoclavable screen (Tetco Inc., Elmsford, N.Y.). Cells were washed once in phosphate-buffered saline (pH 7.3) and separated by centrifugation over Ficoll-Hypaque. Heparinized whole blood was similarly separated. Lymphocytes at the interface were collected, washed and counted.

Virus isolations. In vitro assays for infected lymphocytes were performed by inoculation of 1×10^6 to 2×10^6 lymphocytes onto monolayers of chicken kidney cells for oncogenic MDV and onto chicken embryo fibroblasts for nononcogenic MDV. Foci were enumerated 6 to 7 days postinoculation (p.i.). Results are reported in terms of FFU per 10^6 cells.

Examination for viral internal antigen. Viral internal antigen was detected in spleen, thymus, and skin with a direct fluorescent-antibody test with homologous fluorescein-conjugated antisera (3). Positive tissues were numerically scored to denote intensity of infection, from a rarely occurring isolated positive cell (score 1) to widespread involvement of the tissue (score 4).

Splenomegaly. The enlargement of spleens was assessed by comparing the weights of the spleens with whole body weights. Mean relative spleen weights of virus-infected groups were compared with those from controls by using Student's *t* test (32).

Mitogen stimulation. Peripheral blood lymphocytes (PBL) and spleen lymphocytes were used for mitogen stimulation with concanavalin A (ConA) (27). Stimulation was determined from the difference between mean counts per minute of stimulated and control triplicates. Differences in stimulation between control and infected groups of chickens were analyzed with Student's *t* test.

Gross examination for MD. All birds were examined for MD lesions at the end of each experiment or when tissues were collected. Lymphomas in visceral organs or at the site of inoculation, neural enlargement, and degenerative lesions of the thymus and bursa all were considered MD lesions.

Experimental design. Three aspects of the effect of EBX on MD were investigated.

(i) The influence of EBX on the early pathogenesis was studied by inoculating 2-week-old chickens with GA-5 oncogenic MDV (experiments 1 to 3) or SB-1 nononcogenic MDV (experiments 4 to 6). Neonatally bursectomized chicks were included in experiment 2. Birds were removed for sampling between days 3 and 11 p.i. The exact number of infected chickens and days of sampling are given in Tables 1 and 2. The number of control chickens was comparable to the number of infected chickens used in each experiment. Virus isolation attempts from spleen and thymus lymphocytes and PBL and fluorescent-antibody tests for viral internal antigen in spleen, thymus, and skin sections were conducted. In addition, the splenic enlargement was assessed in experiments 1 to 5. Splenic lymphocytes and PBL were used to test the response to ConA stimulation in experiment 6.

(ii) The long-term effect of EBX on the expression of MD was investigated in experiments 7 and 8. Chicks were infected with GA-5 (experiment 7) or JM-10 (experiment 8) at 2 weeks of age. JM-10 was used in the latter experiment because it is slightly less virulent than GA-5 and it was expected that small differences between intact and EBX chickens might be more apparent than with GA-5 infection. The response of PBL to ConA was investigated in experiment 7. Five chickens per treatment were bled twice a week to obtain PBL. In addition, virus isolation attempts from PBL were made weekly. In experiment 8, groups of 8 chickens per treatment were used to study the effect of EBX on virus isolation. Differences in viremia levels between the two groups were assessed with the one-tailed ranking test of Wilcoxon (32). Data were collected on MD incidence and type of lesions in dead birds and survivors at the termination of the experiments (day 43 p.i. for experiment 7 and day 51 p.i. for experiment 8). The chi-square test (32) was used to analyze differences in mortality between the groups.

(iii) The hypothesis that EBX enhances tumor rejection (25) by preventing immunosuppressive lytic infection was tested (experiment 9). Intact or EBX chicks were vaccinated at 7 days of age with SB-1 or were left as nonvaccinated controls. All chicks were challenged 7 days postvaccination by injection of 5,000 viable NYM-1 cells into the left pectoral muscle. Tumor growth was scored by palpation of the pectoral muscle (5). All dead birds and survivors at the termination of the experiment were necropsied. The presence of tumors at the site of inoculation and elsewhere and the presence of thymus atrophy were recorded. Virtual absence of thymic tissue was considered to be evidence of thymic atrophy. Differences in mortality and patterns of progressively growing tumors between groups were analyzed with the chi-square test.

RESULTS

Early pathogenesis. Infection of intact chickens with the oncogenic GA-5 strain (experiments 1 to 3) resulted in the pattern of lytic infections described previously (3, 10) and characterized by a large amount of viral internal antigen in thymus and spleen between days 4 and 6 p.i. (Table 1). Virus was readily isolated from PBL and splenic lymphocytes (Table 1) and splenomegaly was observed starting at day 4 p.i. (Fig. 1). In contrast, MDV did not induce a lytic infection in the lymphoid organs of EBX chickens. Viral internal antigen could not be detected in the spleen and thymus (Table 1), and splenomegaly was not observed, although a slight but not statistically significant increase in relative spleen weight was seen in experiment 3 (Fig. 1) when compared to noninfected EBX

TABLE 1. *Virus isolation and virus replication in tissues from intact and EBX P-line chickens inoculated with 500 FFU of GA-5 at 2 weeks of age (experiments 1 to 3)*[a]

Expt	Days p.i.	Viral isolation: no. of birds positive/total (avg FFU/10^6 cells)						Viral internal antigen: no. of birds positive/total (avg score)			
		Spleen		Thymus		PBL		Spleen		Thymus	
		Intact	EBX	Intact	EBX	Intact	EBX	Intact	EBX	Intact	EBX
1	4	2/4 (4.5)	2/3 (1.3)	2/4 (0.7)	2/3 (1.0)	1/4 (0.2)	0/3	3/4 (1.5)	0/3	3/4 (2.4)	0/3
	7	4/4 (27)	3/3 (12)	3/4 (0.7)	1/3 (0.3)	4/4 (12)	2/3 (1.7)	0/4	0/3	0/4	0/3
	11	4/4 (117)	3/3 (12)	4/4 (2.8)	3/3 (1.7)	4/4 (43)	2/3 (3.7)	0/4	0/3	0/4	0/3
2	3	2/2 (4)	0/2	2/4 (0.7)	—[b]	—	—	4/4 (1.1)	0/4	3/4 (1.0)	0/4
	4	4/4 (18)	0/4	4/4 (4.0)	0/3	0/3	0/3	4.4 (2.2)	0/4	4/4 (2.0)	0/4
	5	3/4 (2.5)	0/4	1/4 (0.3)	0/4	3/4 (1)	0/4	1/4 (0.3)	0/4	4/4 (2.4)	0/4
	6	2/5 (0.6)	0/4	0/5	0/4	5/5 (4.4)	1/4 (0.3)	1/5 (0.2)	0/4	1/4 (0.3)	0/4
3	5	—	—	—	—	—	—	12/12 (2.3)	0/11	—	—

[a] All uninoculated control chickens were negative for virus isolation and viral internal antigens.
[b] —, Not done.

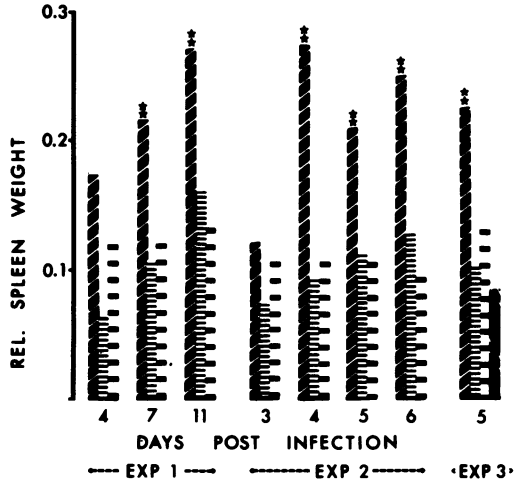

FIG. 1. *Relative spleen weights in intact or EBX P-2 chickens inoculated with 500 FFU of GA-5 at 2 weeks of age. Symbols: ▨, intact, virus infected; ■, EBX, virus infected; ▦, intact, control; ▩, EBX, control. The numbers/treatment for each experiment are given in Table 1. Statistical significance was tested with the Student's t test, comparing virus-infected groups with the proper control group. * = $P < 0.025$; ** = $P < 0.01$.*

chickens. The infection did become established in EBX chickens, as evidenced by the isolation of virus from splenic lymphocytes in experiment 1, although the number of FFU/10^6 lymphocytes was lower in EBX than intact chickens. Feather follicle epithelium became positive for viral internal antigen in both EBX and intact chickens at day 11 p.i. (experiment 1) but was at a lower level in the former. Only 4 of 22 follicles obtained from three EBX chickens were positive, whereas 23 of 24 follicles from the four intact chickens were positive.

In experiment 2, NBX chickens were used in addition to EBX chickens. The results were comparable to those obtained with EBX chickens (data not shown in tables). Viral internal antigen and splenomegaly were absent between days 3 and 6 p.i., but virus could be isolated from PBL and splenic lymphocytes.

The pathogenesis of SB-1 infection was not altered by EBX (experiments 4 to 6). Virus could be isolated from both groups, and the titers were essentially the same (Table 2). The expression of viral internal antigen in spleen and thymus was limited to a few positive cells in both intact and EBX chickens (Table 2) at day 7 p.i., and splenic enlargement was seen in both intact and EBX SB-1-infected chickens (Fig. 2). ConA stimulation of splenic lymphocytes and PBL from SB-1-infected intact and EBX chickens was severely depressed at day 7 p.i. (Table 3). Essentially no differences were observed between the two virus-infected groups, except that the depression did not reach a statistically significant level for the PBL from the EBX SB-1-infected chickens.

Long-term pathogenesis. Infection of intact chickens with GA-5 resulted in a biphasic depression on ConA-induced stimulation of PBL (experiment 7, Fig. 3). The first depression phase was transient and was noted at day 7 p.i. A permanent, and almost always statistically significant, phase of depression started at day 17 p.i. The ConA response of PBL from the infected EBX group was more variable. At day 7 p.i. there was a marked increase in response compared to that in EBX control chickens. The second phase was characterized by a slightly depressed response, but it never reached statistically significant levels. Viremia levels with JM-10 were studied with PBL obtained from intact and EBX chickens (experiment 8). The results

TABLE 2. *Virus isolation and virus replication in tissues from intact and embryonally bursectomized P-line chickens inoculated with 500 FFU of SB-1 at 2 weeks of age (experiments 4 to 6)*[a]

Expt	Days p.i.	Virus isolation: no. of birds positive/total (avg FFU/10^6 cells)				Viral internal antigen no. of birds positive/total (avg score)			
		Spleen		PBL		Spleen		Thymus	
		Intact	EBX	Intact	EBX	Intact	EBX	Intact	EBX
4	4	4/7 (2.3)	6/8 (2.1)	3/6 (10.5)	4/7 (7.1)	2/7 (0.3)	0/8	0/7	0/8
	6	1/4 (1.2)	1/2 (2.5)	3/5 (2.6)	2/3 (11)	4/7 (0.6)	5/7 (0.7)	1/7 (0.1)	2/7 (0.3)
5	5	2/4 (3.2)	5/5 (5.6)	—[b]	—	3/5 (0.6)	1/5 (0.2)	—	—
6	7	6/6 (9.5)	5/6 (8.0)	6/6 (26.5)	4/5 (17.0)	—	—	—	—

[a] All uninoculated control chickens were negative for virus isolation and viral internal antigen.
[b] —, Not done.

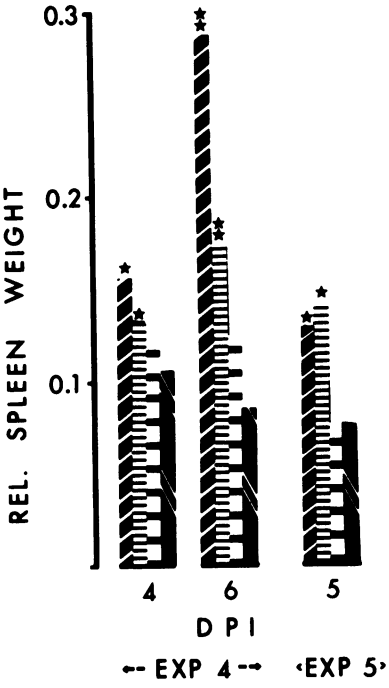

FIG. 2. *Relative spleen weights in intact or EBX P-2 chickens inoculated with 500 FFU of SB-1 at 2 weeks of age. The bars used for group identification are the same as those in Fig. 1. The numbers/treatment are given in Table 2. Statistical significance was tested with the Student's t test, comparing virus infected groups with the proper controls. * = $P < 0.025$, ** = $P < 0.01$.*

are summarized in Fig. 4. During the early period (7 to 14 days p.i.), viremia levels were slightly higher in intact than in EBX chickens, as could be expected based on experiment 1 to 3. This difference in viremia levels was maintained during the rest of the experimental period (days 17 to 38 p.i.). Statistical analysis of pooled data collected from days 17 to 38 p.i. showed that the difference was highly significant ($P < 0.01$, Wilcoxon one-tailed test).

The mortality in EBX chickens exposed to JM-10 was significantly lower than in the intact group ($P < 0.01$, chi-square test) (Table 4). A slight but not significant ($P > 0.05$) difference was also noted after GA-5 infection (experiment 7). The mean time to death with both JM-10 and GA-5 infection was slightly longer with EBX than with intact birds.

Effect of EBX and vaccination. The results of experiment 9 are summarized in Table 5. Inoculation of NYM-1 into intact and EBX chickens resulted in the formation of tumors at the site of inoculation regardless of whether or not they had been vaccinated. However, vaccination did protect against progressive tumor growth in both the intact and EBX chickens ($P < 0.01$). The presence of progressively growing tumors appeared to be correlated with severe damage to the thymus. A possible exception was with the vaccinated intact chickens. Vaccination alone did not protect against MD (total incidence), but the combination of bursectomy and vaccination protected at significant levels ($P < 0.01$).

DISCUSSION

EBX on day 17 of incubation removes the bursa just before the migration of B-cells to the spleen and other organs (14) and is therefore a specific and efficient way to impair the humoral immune response. Neonatal treatment with cyclophosphamide also results in severe and permanent damage to bursa-dependent cell functions (17). However, cyclophosphamide also has a temporary effect on thymus-derived lymphocytes (19, 32).

The results obtained in this study clearly demonstrated that the bursa of Fabricius can influence the pathogenesis of MD. Removal of the bursa resulted in a delay of the development of the disease. The effects on the early pathogenic events were especially dramatic; the lymphoid organ lytic infection typical of MD in intact chickens was absent. Thus, the important organs

TABLE 3. *ConA stimulation of spleen lymphocytes and PBL from intact and EBX P-line chickens inoculated with 500 FFU of SB-1 at 2 weeks of age (experiment 6)*[a]

Treatment	SB-1 infection	No. of chickens tested		Incorporation of [^{3}H]thymidine (cpm ± SEM)[b]	
		Spleen	PBL	Spleen	PBL
None	−	5	6	19,764 ± 7,363	16,436 ± 4,644
	+	6	6	248 ± 123*	270 ± 119*
EBX	−	5	4	15,337 ± 5,003	17,206 ± 10,270
	+	6	5	6 ± 38**	2,454 ± 2,052

[a] Sampled at 7 days p.i.

[b] Values are the arithmetic mean ± standard error of the mean (SEM) derived from counts on triplicate samples from each bird. * and** indicate significance (Student's t test) at $P < 0.025$ and $P < 0.01$, respectively, when compared with noninfected controls.

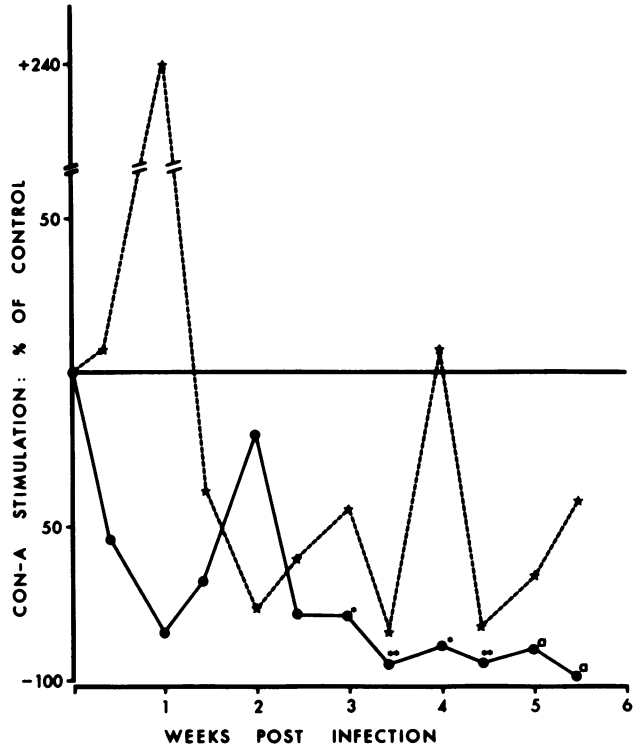

FIG. 3. *ConA response of PBL from intact and EBX P-line chickens inoculated with 500 FFU of GA-5 at 2 weeks of age. Values represent the percentage of change from the proper control groups. Symbols: ●, intact; ★, EBX; a, results from one surviving chicken.*

for cell-mediated immune responses (thymus and spleen) were spared. The effect of the sparing of immunocompetence could explain the results of the studies on the late pathogenic events such as a lower viremia level (experiment 8), prolonged normal blastogenic response to ConA (experiment 7), or lower (experiment 8) or delayed (experiment 7) MD mortality. Those results are comparable to the effects of cyclophosphamide treatment on viremia levels (16, 18), blastogenic response to ConA (16, 18), and lower mortality (4, 5, 16, 18). Sharma (29) reported that EBX did not alter the outcome of MD infection. However, he used line 6 chickens, which are genetically resistant to MD, and thus there was little chance for observing enhanced resistance.

The sparing of the immunocompetence also provides an explanation for the increased resistance against challenge with MDCT-CU8 in EBX chickens (5) and against challenge with MDCT-NYM-1 in vaccinated EBX chickens (25; experiment 9). Both transplants are virus producers; hence, chickens will undergo a viremia after inoculation with the transplantable tumor cells, resulting in a lytic infection of the lymphoid organs and immunosuppression. The absence of lytic infection in EBX chickens would leave the

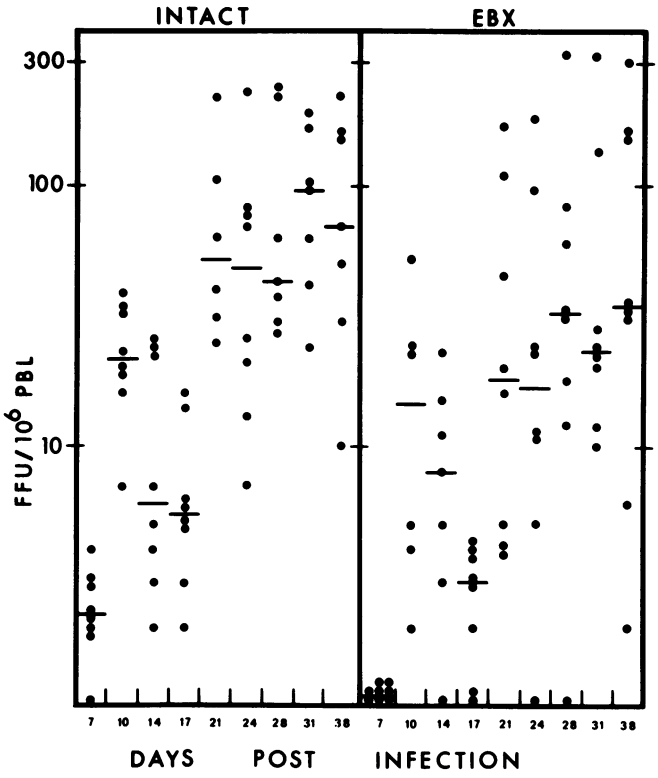

FIG. 4. *Effect of EBX on viremia levels in P-line chickens inoculated with 500 FFU of JM-10 at 2 weeks of age. Dots represent the number of FFU/10^6 PBL for each individual chicken. The lines are the median values.*

TABLE 4. *MD incidence in EBX or intact P-line chickens inoculated with 500 FFU of MDV at 2 weeks of age*

Expt	Treatment		Total no. of birds	MD incidence[a]			Mean days to death ± SEM[b]
	Virus	EBX		Dead birds	Total (dead + surviving)	%	
7	GA-5	−	7	6	7	100	33.7 ± 2.2
		+	6	3	5	83	35.6 ± 4.9
8	JM-19	−	21	12	21	100	44.8 ± 1.8
		+	31	9	22	71[c]	47.4 ± 1.5

[a] The experiments were terminated at 43 (GA-5) and 51 (JM-19) days p.i.

[b] Based on dead birds only. SEM, Standard error of the mean.

[c] Statistically significantly different from intact JM-10-infected chickens: $P < 0.01$.

cell-mediated immune response intact, and an enhanced rejection of the tumor would be the result. In the study on transplant rejection with MDCT-CU8, it had been shown that administration of MDV antibodies also enhanced rejection. Subsequent studies (Calnek, unpublished data) showed that this effect was probably mediated through the known ability of passively administered antibodies to prevent severe damage from lytic infection (1). Yet further support for this hypothesis comes from the fact that the non-virus-producing MD transplant JMV (34) is not influenced by EBX (25). Taken altogether, these various observations suggest that the enhanced rejection of CU8 and NYM-1 is mediated through a sparing of the immunocompetence rather than by a direct effect against the tumor cell.

It is interesting that SB-1 vaccination of intact chickens resulted in an enhanced rejection of tumors at the site of inoculation. The total incidence of MD, however, was comparable to that in the control group. Similar results were obtained by Schierman and McBride (28) when allogeneic chickens were vaccinated with HVT and challenged with NYM-1. It could be that NYM-1 has a highly virulent virus that can break through the protection offered either by vaccination or by EBX. The combination of vaccination and EBX, however, might offer protection against this infection.

The mechanisms involved in the alteration of the pathogenesis in EBX chickens remain to be

TABLE 5. *Effect of EBX and vaccination with SB-1 on the incidence of thymic atrophy, palpable tumors, and MD induced by the MD transplant NYM-1 in P-line chickens*

Prechallenge treatment		Total MD incidence 6 weeks post-challenge[a]	Incidence of palpable tumors		No. with thymic atrophy/total no. of birds	No. with progressive tumors/no. with thymic atrophy	No. with regressive tumors/ no. with thymic atrophy	No. without tumors/no. with thymic atrophy
EBX	SB-1		Total	Progressive tumors/total				
−	−	15/15	15/15	14/15	10/15	10/10	0/10	0/10
−	+	12/15	15/15	5/15[b]	3/15[b]	1/3	2/3	0/3
+	−	14/15	13/15	12/13	13/15	11/13	0/13	2/13
+	+	5/14[c]	10/14	3/10[b]	3/14[b]	3/3	0/3	0/3

[a] Total MD includes progressive tumors, visceral tumors, and dead birds with tumors.
[b] Statistical significance different from proper control groups ($P < 0.01$).
[c] Statistical significance different from all groups ($P < 0.01$).

elucidated. Kermani-Arab et al. (16) and Lu et al. (18) speculated that the effect of cyclophosphamide on the pathogenesis was mediated by a selective, prolonged suppressive effect on certain populations of T-cells. These might normally form the transformable subset, or they could be cells which carry infection (as a viremia) to the transformable cells. However, it is more likely that the mechanism involved in the alteration of the pathogenesis after cyclophosphamide treatment is identical to that involved with EBX. This would then indicate that it is the removal of B-cells in each case which is crucial for the enhanced resistance against MD.

Several possible explanations can be offered for the requirement of B-cells in the normal pathogenesis of MD. (i) Oncogenic MDV strains may need an early multiplication in B-cells, which then carry infection to transformable T-cells. Studies on the early pathogenesis of MD do not support this concept. Thymus tissue was generally infected to a greater extent than bursal tissue judged by fluorescent antibody test scores (3, 10). Also, Sharma and Witter (31) reported that treatment with cyclophosphamide at hatching and challenge at 8 to 9 weeks of age did not influence the development of MD. The loss of B-cells is permanent with cyclophosphamide treatment (17).

(ii) Absence of blocking antibodies in EBX chickens might permit an unusually rapid immune response by T-lymphocytes. In previous studies we failed to demonstrate the presence of blocking antibodies after challenge with MDCT-CU8, although the studies were not exhaustive. Moreover, it was possible to prevent the enhanced rejection in EBX N-line chickens after transfer of B-lymphocytes, but antibodies could not be demonstrated against MDV and bovine serum albumin (Schat, unpublished data). Thus, the cells which are responsible for the effect are not likely to be antibody-producing cells.

(iii) Removal of suppressor T-cells by EBX. Droege (7–9) hypothesized that thymus-derived suppressor cells were formed under the influence of the bursa of Fabricius, since NBX diminished the suppressive activity of thymus cell preparations. Moticka (21) showed also that suppressor cells were especially prevalent in young and intact chickens. Antigen-inexperienced cells were able to suppress cell-mediated and humoral immune responses. This could explain the rapid suppression of the early infection of MD. On the other hand, the existence of bursa-dependent suppressor T-cells remains to be clarified. Suppression of antibody response has been demonstrated with spleen cells obtained from agammaglobulinemic chickens (13, 15).

(iv) Rudczynski and Mortensen (23) described the presence of suppressor cells with characteristics of B-cells in the mouse. As far as known to us, such cells have not yet been described in the chicken, although Bauer (unpublished data, quoted in H. Bauer and B. Fleischer, *in* J. Blasecki, ed., *Mechanism of Immunity to Virus-Induced Tumors*, in press) speculated on the existence of such cells in Japanese quail. The slower growth of Rous sarcoma virus-induced tumors in bursectomized chickens (19) could be explained by the existence of a suppressor B-cell. Also, consistent with the hypothesized presence of a suppressor B-cell were unpublished observations from our laboratory in which repopulation of EBX N-line chickens with B-cells, but not with T-cells from either intact or EBX donors, resulted in delayed rejection of MDCT-CU8. Further studies, in which EBX chickens will be repopulated with subpopulations of bursa and spleen cells, might help to solve the question of which one of the alternative hypotheses is responsible for the observed effect of EBX on the pathogenesis of oncogenic MDV infection.

The difference between the pathogenesis of SB-1 and that of oncogenic MDV in EBX chickens is difficult to explain. A possible explanation is that there is little or no early lytic infection in

SB-1-infected chickens (5). Then there is no immune response towards antigens associated with lytically infected cells. Therefore, the presence of either blocking antibodies or one of the types of suppressor cells in intact chickens will not influence the pathogenesis. Identification of the mechanism involved with oncogenic MDV might help to clarify the effect on SB-1 infection.

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