Marek's Disease: Effects of *B* Histocompatibility Alloalleles in Resistant and Susceptible Chicken Lines

Abstract. *Lines of chickens selected from a common ancestral population for either resistance or susceptibility to Marek's disease developed contrasting frequencies of particular* B *alloalleles. Comparison of inoculated sibs in backcross-families revealed that the* B *alloalleles characterizing the two lines accounted for an eightfold difference in tumor incidence. This genetic difference in tumorigenesis associated with the alloalleles of the major histocompatibility complex is probably expressed through the cell-mediated immune system.*

The *B* histocompatibility alloalleles characterizing lines of chickens selected for resistance or susceptibility to Marek's disease were evaluated for possible effects on the mortality of virus-challenged chicks of appropriate genotype. The resistant and susceptible lines utilized in this study were developed by Cole (*1*), beginning in 1965, from the highly variable Regional Cornell Randombred stock. The JM strain (*2*) of herpesvirus was used to challenge pedigreed chicks. Selection of breeders for four generations on the basis of progeny performance in challenge tests resulted in a shift in susceptibility from a level of 51.1 percent in the unselected stock to 6.5 percent in the resistant line, JM-N, and 94.4 percent in the susceptible line, JM-P (*1*). Selection for resistance or susceptibility was continued for a total of six generations; after one additional generation without selection, agglutination tests for antigens of the *B* alloalleles (*3*) were performed on erythrocyte suspensions from birds of both lines. All 313 individuals comprising line N were homozygous for the allele B^{21}, and among 420 line P individuals there existed two other alleles, B^{13} and B^{19}, with frequencies of .03 and .97, respectively. The presence of B^{21} exclusively in the resistant line N, and the high frequency of B^{19} in the susceptible line P, suggested that these two alleles may be functionally related to resistance and susceptibility to Marek's disease.

Cole (*1*) found that the resistance exhibited by line N behaved largely as a dominant trait in crosses with lines characterized by lower levels of resistance. To test the hypothesis that B^{21} favored resistance and B^{19} susceptibility, F_1 individuals ($B^{19}B^{21}$) resulting from crossing lines N ($B^{21}B^{21}$) and P ($B^{19}B^{19}$) were backcrossed to line P and the progeny were evaluated for susceptibility to Marek's disease.

Chicks were produced in eight biweekly hatches and placed in Horsfall-Bauer isolators. They were inoculated intraperitoneally at 2 weeks of age with 0.2 ml of whole blood infected with Marek's disease virus (JM strain; 2000 plaque-forming units). Blood was typed at 25 days of age to determine the *B* genotype of each individual, and the chickens were observed for the presence of Marek's disease through 20 weeks of age. Necropsies were made on all dead birds and on any birds showing clinical signs of Marek's disease at the end of the 20-week period of observation. Unfortunately, the inoculum used on the last three of the eight hatches of chicks was found later (in a separate, unrelated experiment) to have lost potency. The data from hatches one through five are presented in Table 1. Chi-square heterogeneity analysis showed the data to be homogeneous over the five hatches; therefore, the data resulting from each of the separate hatches were pooled for each of the four F_1 parents ($B^{19}B^{21}$) used in matings 628 through 631 (Table 1).

One F_1 female (NP, produced by crossing the line N male with a line P female) was backcrossed to line P (Table 1, mating 628) and produced 12 chicks of genotype $B^{19}B^{19}$, of which seven died of Marek's disease, and 17 chicks of genotype $B^{19}B^{21}$, of which two died of the disease. Similarly, a second F_1 female (PN), backcrossed to line P (mating 629), produced 25 $B^{19}B^{19}$ chicks of which 20 died, and 18 $B^{19}B^{21}$ chicks of which three died. Backcross progeny were also obtained from two F_1 (PN) males. Here again a high proportion of $B^{19}B^{19}$ and a low proportion of the $B^{19}B^{21}$ chicks died of the disease: 24 of 32 $B^{19}B^{19}$ and two of 24 $B^{19}B^{21}$ from mating 630 died while 18 of 30 $B^{19}B^{19}$ and none of 22 $B^{19}B^{21}$ from mating 631 died. Heterogeneity chi-square analysis of the total data (Table 1) revealed that the relative incidence of Marek's disease for the two genotypes in each of the four matings was essentially the same (mating × genotype interaction chi-square = .62, d.f. 3, $P = .90$). The mortality from Marek's disease for a total of 99 $B^{19}B^{19}$ and 81 $B^{19}B^{21}$ chicks from the four F_1 parents was 69.7 and 8.6 percent, respectively ($P < .001$). Thus, the incidence of the disease among chicks of genotype $B^{19}B^{19}$ was approximately eight times that among those of genotype $B^{19}B^{21}$.

The magnitude of the differences in the influence of *B* alloalleles previously reported was considerably lower than that obtained under the conditions of this study. Hansen *et al.* (*4*) reported that the mortality of single-cross chicks inheriting paternal alleles B^{21} or B^{19} accompanied by various *B* alleles (designated $B^{?}$) from a group of maternal lines showed that the mortality of $B^{19}B^{?}$ was only about twice that for $B^{21}B^{?}$ chicks. This relative difference in mortality was expressed both at 8 (or 12) weeks of age, following inoculation with the virus at

Table 1. Incidence of Marek's disease (MD) among backcross sibs differing in *B* genotype. The chicks resulted from backcrossing F_1 PN or NP (produced by mating line P males to line N females or N males to P females, respectively) to line P. The F_1 parents were of the genotype $B^{19}B^{21}$ and the line P backcross parents were $B^{19}B^{19}$.

Mating		Genotype of progeny						Chi-square analysis*	
		$B^{19}B^{19}$			$B^{19}B^{21}$				
Code	Sire and dam	No. inoculated	No. with MD	Percentage with MD	No. inoculated	No. with MD	Percentage with MD	d.f.	Chi-square
628	P × NP	12	7	58.3	17	2	11.8	1	7.17†
629	P × PN	25	20	80.0	18	3	16.7	1	16.87‡
630	PN × P	32	24	75.0	24	2	8.3	1	24.51‡
631	PN × P	30	18	60.0	22	0	0.0	1	20.19‡
Total		99	69	69.7	81	7	8.6	4	68.70‡
Pooled over matings ($B^{19}B^{19}$ compared to $B^{19}B^{21}$)								1	68.08‡
Interaction (mating × genotype)								3	0.62

*Chi-square for each mating by method of fourfold contingency table; test for interaction by heterogeneity chi-square. †$P < .01$. ‡$P < .001$.

hatching, and at 22 weeks of age, when the chicks had been grown on farms contaminated with virus. The lower relative difference for these single-cross chicks between $B^{21}B^{?}$ and $B^{19}B^{?}$ compared to $B^{21}B^{19}$ and $B^{19}B^{19}$ found in this study was undoubtedly due in part to the presence in some chicks of unknown *B* alloalleles resistant to Marek's disease ($B^{?}$) received from the maternal lines; these resistant alleles would be expected to act as dominants (*1*), thereby increasing the survival of those chicks inheriting B^{19} from the paternal side of the cross. In another study, Brewer *et al.* (*5*) found that the relative mortality effects of five *B* alleles ranged from 0 to 14 percent under natural exposure and 3.8 to 8.2 percent when the chicks were artificially exposed to the GA (Georgia) isolate of Marek's disease virus. In a third study (*6*), high mortality resulting from a natural outbreak of Marek's disease depleted differentially sisters from single-crosses inheriting particular *B* alleles from their heterozygous sires. The rate of survival of $B^{6}B^{19}$ or $B^{7}B^{19}$ pullets was 2.5 times that of their respective $B^{13}B^{19}$ sisters.

In a previous report (*7*) of the data in Table 1, a family of 44 full-sib chicks was included to give data on the relative susceptibility of B^{13} and B^{21} to Marek's disease. The mother of this family was the last remaining bird of the genotype $B^{13}B^{21}$ in line N (generation 6). On encountering this bird in the initial typing of the line at the Northern Illinois University laboratory, she was mated (at the East Lansing Laboratory) to a line P male of the genotype $B^{19}B^{19}$. After inoculation, blood typing, and observation for 20 weeks as described above, the incidence of Marek's disease was 92.6 percent among 27 $B^{19}B^{13}$ and 11.8 percent among 17 $B^{19}B^{21}$ chicks ($P < .01$), resulting in roughly the same relative increase (eightfold) in susceptibility of $B^{19}B^{13}$ (as found above for $B^{19}B^{19}$) over that of $B^{19}B^{21}$. More recently, in the studies following the identification of the *B* alleles in the Regional Cornell Randombred population (*8*), similar determinations of susceptibility to Marek's disease revealed at least three additional *B* alleles which exhibit a high degree of susceptibility, comparable to that of B^{13} and B^{19} (*9*). Thus, in the original population from which N and P lines were derived, there must have existed at the time of the initial selections at least five alleles imparting a high degree of susceptibility, expressed in the recessive state or in combination with each other, and at least one allele (B^{21}) imparting a high degree of resistance, expressed either in heterozygous or homozygous state—that is, dominant to any of the susceptible alleles.

The means by which the *B* histocompatibility complex affects the incidence of Marek's disease is unknown; however, it is clear that the influence does not result from histocompatibility interactions between inoculum and host. For practical reasons, cell-associated inoculums are widely used in experiments on controlled exposure to Marek's disease (*10–12*). The cells in these inoculums serve to "transfer" the virus to host cells, after which the donor cells are presumed to be eliminated by host-versus-graft reaction. The inoculum used in this study consisted of a cell-associated virus (*2*) from infected birds of inbred line RPRL 7, uniformly homozygous for the B^{2} alloallele whose antigenic product is serologically distinct from B^{13}, B^{19}, and B^{21} (*8*) and thus histoincompatible with all inoculated chicks. Tumors resulting from inoculations of chicks with gonadal tumor cells from chicks of the opposite sex have karyotypes that match the phenotypic sex of the host chickens (*13*); that is, the cells in the resulting lymphomas were genetically that of the host. This is in agreement with the formation of tumors typical of Marek's disease after the inoculation of chicks with cell-free herpesvirus (*14*).

The herpesvirus of Marek's disease and antibodies against it persist throughout the lives of most or all chickens (*15*). Immunoglobulins produced by N and P lines injected with Marek's disease inoculum were studied both qualitatively and quantitatively by Higgins and Calnek (*10*). They observed a somewhat less favorable antibody response in the susceptible line but considered that this resulted secondarily from lymphoid tissue damage caused by the disease. Others (*11*) have shown that removal of the bursa of Fabricius, either by surgical procedure or hormonal treatment, does not affect the incidence of Marek's disease. Thus, the genetic differences between lines N and P in susceptibility to the disease do not appear to result from primary differences in the humoral immune system. On the other hand, different levels of susceptibility do appear to result from functional differences in the cell-mediated immune system. An "age resistance" for Marek's disease is developed by older chickens and is expressed through lesion regression (*16*). Calnek (*17*) found that the ability to develop increasing resistance with age was well expressed in two genetically resistant lines (including line N) but was only very weakly expressed in the one susceptible line studied (line P). The expression of age resistance by chickens genetically resistant to Marek's disease can be blocked by neonatal thymectomy (*12*). Further, when thymectomized chickens of N and P lines received thymic transplants from individuals of the opposite line, and 10 days later were inoculated with JM virus, the level of virus developed at 4 and 6 weeks after inoculation followed that characteristic of the donor line—low level of virus for line N and high for line P (*18*).

The major histocompatibility complex in each of several mammals (mice, guinea pigs, rats, and monkeys) can influence individual immune responses to simple synthetic polypeptides as well as complex antigens (*19, 20*). In the mouse, immune response differences are due to *Ir* genes which map within the H-2 complex (*21*). These genes code for cell surface structures on B and T cells and play significant roles in humoral and cell-mediated immune responses, depending on the particular immunogen (*20–22*). In man, certain antigens of the major histocompatibility system, HLA, have been shown to be associated with particular diseases, presumably through the action of histocompatibility-linked *Ir* genes (*23*).

The major histocompatibility complex of the chicken (*24*), originally identified as the *B* blood group system of alloalleles (*3*), also has been found to affect immune responses to synthetic polypeptides (*25*), a bacterial antigen (*26*), and tuberculin (*27*); further, the *B* locus influences the pathology of autoimmune thyroiditis and antibody titer in the Obese strain of chickens (*28*). These effects strongly suggest that the observed relationship between particular alleles of the *B* system and the degree of resistance to Marek's disease is due to differences in immune response—probably through histocompatibility-linked *Ir* genes or possibly through antigen recognition phenomena controlled primarily by the B histocompatibility region itself. Of special interest with regard to this B region is the discovery of Doherty and Zinkernagel and co-workers (*29*) that in the mouse the H-2 exerts a restriction on the specificity of cytotoxic T cells against virus-infected cells. For maximum response the immune T cell and the virus-infected target cell must share at least one set of H-2 specificities (either K or D). In view of the nature of the inoculum used in our present study, the Doherty-Zinkernagel phenomenon per se does not appear to be directly related to the observed association of *B* alleles with resistance to Marek's disease; however, T cell sensitization through recognition of altered self-

antigens or a complex of virus with self-components, as hypothesized in accounting for this phenomenon (*29*), could well underlie the tumor regression occurring in chickens genetically resistant to Marek's disease.

In view of the immunological significance of the major histocompatibility complex across species, and the probable nature of immunological resistance to Marek's disease, our finding suggests that the high degree of resistance to herpesvirus tumorigenesis in individual chickens possessing the B^{21} alloallele results from cell-mediated immunity.

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Glia Maturation Factor: Effect on Chemical Differentiation of Glioblasts in Culture

Abstract. *A protein factor from the adult brain increases the concentrations of adenosine 3',5'-monophosphate and S-100 protein in glioblasts in culture. Such changes are correlated with the outgrowth of cell processes.*

The correlation of morphological and chemical differentiation at the cellular level is basic to the understanding of life forms and processes. We recently detected in adult brains a protein factor which is capable of inducing histotypic maturation of glioblasts in culture (*1*, *2*). The morphological transformation results from the retraction of cell bodies and the active extrusion of processes. We now attempt to relate the morphological change with the chemical evidence of differentiation.

Brain cells were dissociated from 17-day fetal rats (Sprague-Dawley) and seeded in Falcon plastic culture flasks as previously described (*2*). After the cells were carried into the second passage, the glioblasts, now free of neuroblast contamination, were exposed to a medium consisting of F_{10} nutrient (*3*), 5 percent fetal calf serum, and 100 μg of the glia maturation factor per milliliter [glia maturation factor was prepared from pig brain by step III of the published procedure (*2*)]. The cells were grown at 37°C in an atmosphere of 5 percent (by volume) CO_2 in air and 100 per-

Table 1. Effect of glia maturation factor on concentrations of cyclic AMP and S-100 protein in glioblasts. Control and experimental cells were paired cultures of glioblasts grown in the absence and presence, respectively, of the factor. Falcon flasks having a surface area of 75 cm² were used. The days indicate the periods after exposure to the factor. For cyclic AMP determination, the incubation medium was decanted from the flasks and the monolayer was rinsed for 2 seconds with ice-cold 0.15*M* NaCl in 0.02*M* tris-HCl, *p*H 7.4 (tris-saline), and immediately mixed with 5 ml of cold 5 percent trichloroacetic acid (TCA). The cells were scraped off with a rubber policeman and homogenized with a ground glass homogenizer. Subsequent steps and cyclic AMP assay were as described by Gilman (*5*). Protein was determined (*6*) in the TCA precipitate after dissolving it in 0.1*N* NaOH. Duplicate flasks were pooled and results from four pools were averaged and presented as mean ± standard deviation (S.D.). For S-100 determination, the monolayer was rinsed twice with ice-cold tris-saline. The cells from two flasks were combined and scraped into 2.5 ml of tris-saline containing 0.1 m*M* EDTA. The cell suspension was homogenized and subsequently sonicated with two 5-second bursts at 50 watts an output. After centrifugation at 30,000*g* for 45 minutes, the supernatant was dialyzed against two changes of tris-saline. The extracted S-100 was assayed by the microcomplement fixation test, using pure S-100 protein and its antiserum prepared by one of us (B.W.M.). Protein was determined (*6*) in the supernatant. Results from four pools were averaged and presented as mean ± S.D.

Days	Cyclic AMP (pmole/mg protein)		S-100 protein (ng/100 μg protein)	
	Control	Experimental	Control	Experimental
0	22 ± 2	22 ± 1	7.6 ± 0.1	7.5 ± 0.1
1	25 ± 3	24 ± 2	5.0 ± 0.1	5.2 ± 0.2
4	24 ± 2	70 ± 13*	6.0 ± 0.7	35.3 ± 0.8*
7	20 ± 5	66 ± 7*	5.4 ± 0.5	50.3 ± 1.2*

*Significantly different from controls, $P < .001$.