ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 8 (2): 161-165, 2009
ISSN 1682-8356

B-Complex Alleles Immunity to *Salmonella enteritidis* in Chickens

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Abstract: Six experiments were conducted during which a total of 12 congenic lines homozygous for various *B*-complex alleles, were challenged by intraperitoneal injection with either of two isolates of *Salmonella enteritidis*. Because these *B* alleles were expressed on a common genetic background and mortality differences among lines were statistically significant in three of the six trials and morbidity (body weight) differences were significant in another trial; it is suggested that *B*-complex alleles affect the degree of immunity to these isolates. When all lines and trials were compared, line 342 (*B*C/*B*C) emerged as particularly resistant, whereas lines 253 (*B*18/*B*18) and 254 (*B*15/*B*15) were more susceptible. The remainders of the lines were of neutral (intermediate) susceptibility. Sex did not appear to influence the results of the challenge, but more resistance was observed with an increase in the age at inoculation. Although the mechanism that determined this resistance is unknown it was present as early as 3 d of age and it is suggested that complement proteins, which have a known role in protection from bacterial infections and are encoded by genes located within the *B*-complex, or acute phase proteins, may account for these observations.

Key words: *Salmonella enteritidis*, B complex alleles, immunity

INTRODUCTION

The alleles of the *B*-complex have been shown to exert a major influence on the immune response to a number of infectious agents (Bacon, 1987). The establishment of such associations has been facilitated because of the availability of a number congenic lines differing among themselves principally by their MHC (*B*-complex) specified haplotypes. A series of such lines has been developed (Abplanalp *et al*., 1992) and differential immunity to several pathogenic agents has been demonstrated among them (Cotter *et al*., 1992). Given the resurgence of interest in the *Salmonella* problem in poultry, a study was designed in order to determine whether differences in resistance to *Salmonella enteritidis* challenge could be shown among these congenic lines and by inference, associated with their MHC haplotypes. The demonstration of such differences may provide some additional insight into avian salmonellosis. The method employed was to compare the morbidity and mortality observed after chicks from a number of such lines were challenged as neonates by either of two strains of *Salmonella enteritidis* previously known to be pathogenic in chickens. This strategy was similar to the one used earlier in which *B*-complex associated differences in immunity to *Staphylococcus aureus*, were detected in these lines by using the neonatal challenge method (Cotter *et al*., 1992).

MATERIALS AND METHODS

Broilers: The breeding details and some of the production characteristics of these lines as following; (Abplanalp *et al*., 1992). Briefly, inbred congenic lines were established by the use of single hens of the highly inbred Line UCD 003. These were outcrossed to males already known to carry diverse *B*-complex haplotypes. Female progeny were then repeatedly backcrossed to males of Line UCD 003. After five such backcrosses, these lines were estimated to contain 98.4% of the genetic background of Line UCD 003, while expressing variation among their *B* alleles, which were originally derived from the donor males. Thus, any differences in mortality and morbidity resulting from the challenges could reasonably be ascribed to an affect of the donated *B*-complex alleles operating within the common genetic background provided by the recurrent parent, Line UCD 003. A listing of the lines used in these trials, their origins and their *B*-complex haplotypes is given in Abplanalp *et al*. (1992).

Experiments: Six trials were conducted during a 2.5 years period involving over 1,100 chicks. From 7-12 congenic lines (haplotypes) were included in the various trials depending upon their availability. The chicks were hatched in a local farm. The chicks ranged in age from 3-6 days at the time of the challenges (five trials); however in one trial the challenge was postponed until Day 11 due to the occurrence of considerable non-specific mortality during the immediate neonatal period. The various lines were mixed randomly and reared in heated batteries. In three of the trials, the sexes were determined by vent examination at the hatchery.

Bacterial challenge: Two clinical isolates of *Salmonella enteritidis* (Serogroup D) originally obtained from human

stool cultures were used throughout these experiments. Both isolates were previously shown to be capable of colonizing adult hens via cloacal challenges (Murphy *et al*., 1990; Cotter *et al*., 1995) and were capable of causing mortality in non-inbred chicks after neonatal intraperitoneal challenge. The bacteria were grown in trypticase soy broth to log phase and then diluted with additional tryptic soy broth to obtain the turbidity of a MacFarland No. 1 standard. Further dilation was made using tryptic soy broth so that one volume of inoculum was estimated to contain the equivalent of 3×10^5 CFU. The inoculations were made using a 1 ml tuberculin type syringe fitted with a 25 gauge needle and were delivered into the peritoneum in a volume of 0.1 ml. The dosage used in these trials was based on the results of pilot challenge studies using chicks from noninbred lines and was estimated to be equal to 1 LD50. The chickens were checked for signs of morbidity Daily and Their Day of Death (DTD) was recorded for 3 or 4 weeks following the challenges. Re-isolation of the challenge bacteria from each dead chick was not attempted due to logistical difficulties, but such re-isolations were routinely made from a few chicks during each trial. Reisolated bacteria were identified as *Salmonella* using a conventional bacteriological scheme (Cox and Williams, 1976) and were confirmed as serotype D using group specific antisera.

Statistical analysis: Haplotype and sex effects were assessed by ANOVA using DTD or body weight (experiment 4) as the independent variable. Chicks alive at the end of a trial were assigned a dummy value (typically Day 14) for their DTD so that they could be included in the haplotype analyses, whereas the analysis for a sex effect included only those chicks that actually died as a result of the challenge. Morbidity was assessed by observing gross symptoms and by comparing the body weights of *Salmonella*-challenged chicks to that of chicks injected with TSB alone. All data were analyzed using the Minitab statistical program.

RESULTS AND DISCUSSION

Neonatal challenge produced peracute morbidity in which the affected chicks demonstrated labored breathing and recumbence. Mortality began as early as 1 day post-challenge and increased steadily during the 1st week, typically reaching a plateau between Day 7 and 14. No such symptoms or deaths occurred when chicks were challenged with TSB alone. It was usual to find a chick dead in 1 or 2 days following the onset of the symptoms. However, in some cases, a symptomatic chick could remain alive for over 1 week and several survived for over 8 weeks. A few acutely ill chicks were able to recover and some were kept for further observation. They remained asymptomatic through the onset of egg production, when they were sacrificed.

A summarization of the mortality % for all the experiments is listed in Table 1. Shown are the number of chicks per experiment, the isolate used for the challenge, the age of the chicks in days at the time of the challenge, the average number of chicks per line (haplotype), the percentage mortality and the *F* value (with probability) for an effect ascribable to the MHC. The combined mortality for all experiments was 526/1,101 (47.8%); which was calculated after excluding 118 prechallenge deaths of unknown (nonspecific) cause that occurred during the third experiment. Although there was some experiment to experiment variation in the mortality rates seen during these studies, the results suggest that the *B*-complex has an influence on immunity to neonatal challenge with *Salmonella enteritidis*. Bumstead and Barrow (1993) reported variation in experiment results among inbred lines challenged with *Salmonella pullorum* suggesting that such is not unusual in this sort of experimentation. Statistically significant differences in mortality were, however, observed among haplotypes in three of six experiments, whereas in two others, the overall mortality was too low for such effects to be detected, (Table 1). There was significant body weight differences (morbidity) among the lines used in Trial 4, so that this measure of immunity was incorporated along with the mortality from the other trials to create a composite picture to indicate the global trends. Mortality was lowest when the chicks were challenged at 6 or 11 d, whereas higher mortality was obtained when they were challenged at 3 or 4 d (Table 1). Thus, haplotype differences would appear to become rapidly obscured as the chick ages. An important age-dependent role for the heterophil in resistance to *Salmonella*, consistent with the observations made here, has been described, (Stabler *et al*., 1994).

The sex of chicks was known in three of the six experiments, which included 267/1,101 (24%) of the total challenged. In experiment 4, chicks were weighed on the day of the challenge and again at, 16 and 27 days, which corresponded to Days 0, 10 and 21 Post-challenge (DPC). The weights of the salmonella-challenged chicks were compared to those from a synthetic control group. The latter was challenged with TSB alone and was constructed by combining eight chicks from several of the congenic lines. The results suggesting that there were haplotype associated morbidity differences (Table 2). The mortality results of experiments 2, 5 and 6, where there were statistically significant differences among the lines (haplotypes) (Table 3).

There were only 7 deaths among the 60 (12%) of *Salmonella*-challenged chicks available during experiment 4. Although the mortality was low, morbidity differences could be demonstrated by using body weight measurements taken on DPC 10 and 21. From these observations it was clear that the bacterial challenge

Table 1: Summarized mortality results of MHC congenic chicks challenged on various days of age with $3x10^5$ CFU *Salmonella enteritidis*

Exper.	no.	isolate	age	Lines No.	Chicks/line	Mortality%	F_{MHC}	P
1	110	736	6d	9	12	45	1.33	0.26
2	226	736	3d	12	19	100	3.59	0.0
3	327	736	11d	12	27	7	1.49	0.14
4	60	736	6d	7	10	17	NS	NS
5	190	736	4d	9	21	70	4.09	0.0
6	188	737	3d	10	19	49	2.59	0.01

NS, non significant

Table 2: Mean body weights and number of Deaths by 21 d Postchallenge (DPC) in lines challenged at 6 d by *Salmonella enteritidis* compared with a synthetic control challenged with Trypticase Soy Broth (TSB) alone

			Days post-challenges			
Line	B alleles	No.	0	10	21	deaths
Control	-	8	40	108[a]	196	0
104	O	7	40	90[ab]	191	1
312	24	16	39	88[ab]	166	2
316	14	4	44	88[ab]	150	0
331	3	9	42	80[b]	170	2
335	19	10	40	82[b]	171	2
336	Q	10	38	91[ab]	165	0
342	C	4	42	109[a]	190	0

ANOVA	DPC	F	P
	0	1.5	0.22
	10	2.3	0.04
	21	1.9	0.08

[a,b]Means with no common superscript differ significantly ($p \leq 0.05$).

Table 3: Mean DTD (No. died/No. challenged) in experiments 2, 5 and 6

		Experiments		
Line	Alleles	(2)	(5)	(6)
003	17[2]	2.4	4.6[b]	7.2[a]
104	O	2.1[bc]	NS	NS
253	18	2.4[b]	4.8[b]	4.2[b]
254	15	2.0[c]	4.7[b]	7.1[ab]
312	24	2.0[c]	7.0[a]	8.8[b]
313	3	2.1[bc]	2.2[b]	4.9[b]
316	14	2.8[ab]	NS	NS
330	21	2.1[bc]	9.1[a]	9.8[a]
331	2	2.8[ab]	NS	NS
335	19	2.3[b]	10.0[a]	7.8[a]
336	Q	2.1[bc]	4.2[b]	10.9[a]
342	C	3.1[a]	9.0[a]	8.8[a]

[a-c]Means within a column with no common superscript differ significantly ($p \leq 0.05$).

retarded growth, Table 2. These results was most evident at DPC 10, but by DPC 21 the difference between the weights of chicks challenged with bacteria and the synthetic control group were less apparent; suggesting some recovery had occurred.

When mortality was considered, the performance of a line was based on its particular DTD compared with the average DTD for that experiment. Body weight on DPC 10 was used similarly to evaluate morbidity (experiment 4). So the resistance of a line may include both measures and its global performance was estimated by summing across the rows. A +4 (Line 342), suggests a resistant line, whereas a -3 suggests that the lines are susceptible (Lines 253 and 254). An exactly neutral line is represented by a zero (Lines 003 and 335), whereas the others were arbitrarily assumed to represent neutrality also. It is important to note that, using this method, Line 003, from which the background genes for all the other lines were derived, was included among the lines scored as neutral. Thus, the effects observed here must be due to an immune mechanism influenced by the *B*-complex haplotype as this was the major source of the line differences. It is also clear, using this sort of global comparison, that there is evidence for consistency among the various lines across trials. Line 342, which was judged as resistant based on the grounds of relatively low mortality, Table 4, also showed the least affects on body weight. Although the mechanism responsible for the MHC associated resistance demonstrated by these data remains unknown, it must be of a type already present at or near birth. Chicks are known to acquire resistance to *Salmonella* very early, as was evident in these studies. Additionally, complement proteins that are known to have an anti-bacterial function are already present in the embryo (Gewurz *et al*., 1966) and they may be important in this regard as well. Support for the latter possibility comes from the observation that differential levels of complement have been associated with the MHC haplotypes. Moreover, serum hemolytic complement levels increased rapidly during the first 4 weeks after hatching in some lines and crosses, whereas another line homozygous for the *B4*/*B4* haplotype had a much slower rate of increase. These results could account for the observation that most chickens rapidly acquire resistance to *Salmonella* with age, whereas others remain susceptible for longer periods. The results obtained in these studies contrast with those of Bumstead and Barrow (1988) who challenged neonatal chicks with *Salmonella typhimurium*.

Those authors found wide differences in susceptibility using that system, but these differences could not be associated with the MHC haplotypes characteristic of the chicks used in their study, perhaps because the haplotypes used by them would fall within the "neutral" resistance category. Assuming that the resistance mechanism is similar for both species of *Salmonella*,

Table 4: Global resistance using mortality and morbidity (body weight, experiment 4) in *B*-congenic lines and a summarization sum of the challenge results across all trials

		Experiments						
Line	Alleles	1	2	3	4	5	6	Sum
003	17[2]	+[2]	0	0	NS	0	0	0
104	O	NS	-	0	0	NA	NA	-1
253	18	NS	-	0	NS	-	-	-3
254	15	0	-	0	NS	-	-	-3
312	24	-	-	-	0	+	0	-2
313	3	NS	-	+	NS	-	-	-2
316	14	-	+	+	0	NA	NS	+1
330	21	+	-	0	NS	+	+	+2
331	2	-	+	0	-	NA	NS	-1
335	19	0	0	0	0	+	-	0
336	Q	-	-	0	0	-	+	-2
342	C	NS	+	0	+	+	+	+4
P		NS	0.0	NS	0.04	0.0	0.01	

higher (+), lower (-), or showed neutral (0) resistance when compared to the other lines in a given trial

as was suggested by the observations of Bumstead and Barrow (1993) genes outside the *B*-complex could interact with genes located within the MHC to determine the overall response to *Salmonella*. Pevzner *et al*. (1981) suggested that genes both inside and outside the MHC controlled immunity to *Salmonella pullorum*. These same authors showed that 10 weeks old female chickens of haplotype *B*1/*B*1 produced lower agglutinating antibody titers after immunization with an *Salmonella pullorum* bacterin than other haplotype combinations containing either the *B*2 or *B*19 alleles. The physiological mechanism underlying the early onset of resistance in the *S. pullorum* system has long been known to be associated with thermoregulation (Hutt and Crawford, 1960). More recently, soluble factors similar to mammalian cytokines, which have a thermoregulatory capacity, have been identified in chickens (Klasing, 1994). These substances are known to stimulate the production of acute phase proteins that are capable of acting as opsonins that accelerate phagocytosis of bacteria, Janeway and Travers (1994). The data presented here demonstrating the early onset of resistance are consistent with these observations. Perhaps differential antibody levels are more important in the maintenance of resistance than they are in its establishment. Desmidt *et al*. (1997) reported that intact 4-wk-old chickens were better able to clear *Salmonella enteritidis* when compared with chickens bursectomized using testosterone.

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