IMMUNOLOGY

Genetic Line and Major Histocompatibility Complex Effects on Primary and Secondary Antibody Responses to T-Dependent and T-Independent Antigens[1]

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ABSTRACT The effects of MHC and nonMHC (background) genetics on the kinetics of primary and secondary antibody responses to T-cell-dependent (SRBC) and T-cell-independent [*Brucella abortus* (BA)] antigens were investigated. Eight genetic groups were represented, with four homozygous MHC haplotypes [B^1-$IrGAT^{low}$ (*IrGAT* = immune response to GAT locus); B^1-$IrGAT^{high}$; B^{19}-$IrGAT^{low}$; B^{19}-$IrGAT^{high}$] on two genetic backgrounds, the S1 and G lines. Birds were injected simultaneously with BA and SRBC at 4 and 7 wk of age, and blood samples were taken weekly from 4 to 10 wk of age for measurement of total agglutinating serum antibody levels. A quadratic equation and its first derivative were computed for each bird to approximate individual curve parameters: *y max*, the maximum titer; *t max*, the time required to achieve *y max*; and *c* coefficient, the rate of decline in the titer. Curve parameters of birds from different lines were analyzed separately by using the General Linear Model procedure. A second analysis that included line effect evaluated the nonMHC gene effects and their interactions with erythrocyte antigen B locus (*Ea-B*) or *IrGAT*. In the S1 line, there was an interaction ($P < 0.05$) between MHC haplotypes and sex for primary response to BA. In contrast, there were no significant main effects nor interactions in the G line background for primary and secondary responses to BA and SRBC. There was an effect ($P < 0.05$) of line background on *y max* for primary BA and for secondary SRBC responses. A positive correlation ($P < 0.05$) was found between the *c* coefficients of BA and SRBC secondary responses, suggesting that the rate of decline in the secondary response is similar between these T-dependent and T-independent responses. The overall results of this study indicate complex interactions between specific MHC alleles and the nonMHC background of the lines in which they are studied.

(*Key words*: major histocompatibility complex, antibody response, kinetics, curve parameters, quadratic equation)

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INTRODUCTION

The effect of MHC genes on resistance to disease and on other economically important traits has been extensively studied in the chicken (Bacon, 1987; Kaufman and Lamont, 1996). NonMHC genes also influence the immune response. Steadham and Lamont (1993) reported that control of immune response to GAT locus (*IrGAT*) was affected by the MHC or MHC-linked genes as well as genes not linked to the MHC. They demonstrated gene complementarity, in that mating between two unrelated lines of chickens with low antibody response to GAT resulted in progeny that excelled either of the parent lines in response to GAT immunization. Dunnington *et al.* (1992), using a White Leghorn population that had been divergently selected for high and low antibody response to SRBC antigens, determined that there is a significant background genome by MHC genotype interaction for *Brucella abortus* (BA) antibodies but not for SRBC or Newcastle disease virus (NDV) antibodies.

Genetic variation of humoral immune response in chickens has been investigated by many researchers, utilizing two general types of experimental approaches. In one approach, the level of antibody titer at a single time point after immunization was used to define the antibody response (Siegel and Gross, 1980; Pevzner *et al.*, 1978; van der Zijpp *et al.*, 1983). With this approach, the differences related to the kinetics of individual or group antibody response were not evaluated nor utilized as a selection criterion. With the second approach, which took more than three measurements, the evaluation of the level of antibody titer over time allowed determination of the

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Abbreviation Key: BA = *Brucella abortus*; *Ea-B* = erythrocyte antigen B locus; *IrGAT* = immune response to glutamic acid-alanine-tyrosine (GAT) locus; NDV = Newcastle disease virus.

individual genetic differences in the antibody reaction kinetics between chicken lines (Martin *et al.*, 1989; Kreukniet and van der Zijpp, 1990; van der Zijpp, 1983).

The humoral immune response to the first exposure of an individual to any specific antigen is termed the primary response. The secondary response is triggered by a second exposure to the same antigen. Both responses have four general characteristics: 1) a latent or lag phase, 2) an exponential production phase of antibodies during which a rapid increase in the concentration of antibody in the serum is observed, 3) a steady state during which there is a balance between the production and degradation of antibody, and 4) a declining phase in which rapid decline in the concentration of antibody in serum occurs. The major differences in the secondary response compared with the primary response are 1) the lag phase is considerably shorter in appearance of antibody in the serum, 2) antibody production is much greater, 3) the concentration of antibody detectable in the serum is much higher, and 4) the persistence of antibody production is for a longer period, with persistent levels remaining in serum for months (in mice) or even years (in humans) (Benjamini and Leskowitz, 1991). The more effective immune response upon secondary exposure is one of the underlying principles of vaccine protection against infectious disease.

The goals of the current study were to determine the effects of MHC and nonMHC (line background genome) genetics on the kinetics of primary and secondary phase antibody response to T-cell dependent and T-cell independent antigens and also to estimate the correlation of antibody response parameters between phases and between antigens.

MATERIALS AND METHODS

Experimental Populations

The S1 White Leghorn chicken line was originated and maintained at the Iowa State University Poultry Science Research Center. This line is divided into four different MHC-haplotype sublines, based on erythrocyte antigen B (*Ea-B*) alleles (B^1B^1 or $B^{19}B^{19}$) and antibody response to the GAT locus ($IrGAT^{high}$ or $IrGAT^{low}$). The S1 line was selected on the basis of Ea-B type from 1965 to 1978. Selected birds were mated to generate homozygous and heterozygous progeny. Evidence of recombination between the serologically detectable MHC gene product (primarily B-G) and the gene or genes associated with *IrGAT* in S1-line chickens was reported (Pevzner *et al.*, 1978). After 1978, selection within each homozygous *Ea-B* type (B^1 and B^{19}) continued for *IrGAT*, resulting in four homozygous haplotype sublines: B^1B^1 $IrGAT^{high}$, B^1B^1 $IrGAT^{low}$, $B^{19}B^{19}$ $IrGAT^{high}$, and $B^{19}B^{19}$ $IrGAT^{low}$. The inbreeding coefficient for these four sublines originating from the S1 line was previously reported as approximately 52% (Nordskog and Cheng, 1988) and, in the generation used for the present studies, was approximately 65%. The four MHC haplotypes of the S1 line were also introgressed into the highly (99%) inbred G line (Knudtson and Lamont, 1989) by 10 generations of back-crossing followed by *inter se* mating to generate MHC homozygous haplotype birds that then were used to establish the new MHC-congenic lines.

Immunization and Antibody Evaluation

Birds at 4 wk of age were injected i.m. with 0.1 mL 40% SRBC diluted in PBS and 0.1 mL undiluted BA antigen. Secondary injection of the same amounts of antigens were given 3 wk after the first injection. A blood sample was obtained from each bird preceding each immunization and on Weeks 1, 2, and 3 after each immunization. Serum was stored frozen until all samples were collected.

The antibody assay procedures described by Munns and Lamont (1991) were followed with minor modifications. Complement was heat-inactivated at 56 C for 30 min. The hemagglutination assay was done by adding 0.05 mL diluent (PBS with 0.05% BSA) to each well of a 96-well microtiter plate. The initial well in each row received 0.05 mL serum, which was then serially doubly diluted. One drop (0.05 mL) of 2% SRBC in PBS was placed in each well. The plates were shaken for 1 min, incubated for 90 min at 37 C in a humidified chamber, shaken again for 1 min, incubated for 30 min at room temperature at a 45° angle, and then scored. The agglutination titer was expressed as the $\log_2$ of the highest titer giving 50% agglutination. The BA agglutination assay was similar to the hemagglutination assay, except that 0.05 mL BA (diluted 1:10 in PBS), instead of SRBC, was added to each well (McCorkle and Glick, 1980). The plates were shaken for 1 min, incubated for 2 h at 37 C in a humidified chamber, and then incubated for 24 h at room temperature in a humidified chamber before scoring.

Statistical Analysis

The primary phase was defined as the 3-wk period beginning at the first injection. The secondary phase covered the period beginning at the secondary exposure of chickens to the same antigens. For each phase, four serum samples were taken, one preimmunization and three postimmunization.

The primary and secondary humoral responses to SRBC and BA were analyzed separately by phase and by antigen. Weigend *et al.* (1997) used a nonlinear regression function to calculate the antibody maximum curve peak (*y max*) and the time (*t max*) belonging to the maximum. Their model is more appropriate for a larger number of measurements (minimum of five) for each phase. In the present study, there were four measurements in each phase (primary and secondary) for each bird. Therefore, a quadratic equation, as applied by Siegel *et al.* (1984), was used to approximate the specific antibody response curve for each phase of each bird using the titers of the four sample times within each phase. The *t max*, which was the time required to achieve the maximum titer, *y max*, was estimated by taking the first derivative of the

quadratic equation. The maximum titer, *y max*, was calculated by substituting the sample data in each individual equation for each bird. The *c* coefficient, which estimates the rate of decline in the titer, was also estimated for each bird from their defined equations.

The *y max*, *t max*, and *c* coefficients for each treatment (SRBC and BA) were evaluated as separate traits. The following analyses were based on *y max*, *t max*, and *c* coefficients. The data of each line (background) were separately analyzed.

$$y = \mu + MHC + sex + plate + MHC * sex + e$$

where y is the dependent curve traits (*y max*, *t max*, or *c* coefficient). The MHC haplotypes had four levels, defined as B^1B^1 $IrGAT^{low}$, B^1B^1 $IrGAT^{high}$, $B^{19}B^{19}$ $IrGAT^{low}$, and $B^{19}B^{19}$ $IrGAT^{high}$. Sex had two levels. Plate was varied based on the microtiter plate in which hemagglutation was done; e is the residual.

In a second analysis, effect of and interactions with line were included, and MHC influence was separated into that controlled by the two evaluated MHC loci: *Ea-B* and *IrGAT*.

$$y = \mu + line + Ea\text{-}B + IrGAT + sex + plate + line * Ea\text{-}B + line * IrGAT + Ea\text{-}B * IrGAT + line * Ea\text{-}B * IrGAT + e.$$

Line had two levels, which defines two different line background (nonMHC) genotypes (S1 and G). The *IrGAT* has two levels (high and low). Two-way interactions (line * *Ea-B*; line * *IrGAT*; and *Ea-B* * *IrGAT*) were included in the model to detect nonMHC effects or possible interactions between background genes and MHC genotypes.

Analyses were conducted by using the General Linear Model procedure (SAS Institute, 1990). The type III sums of squares were used in all analyses. Spearman's rank-order correlation coefficient, a distribution-free test, was used to compute the correlations between curve traits (*t max*, *y max*, *c* coefficient).

RESULTS

The effects of MHC- and nonMHC-linked genes on kinetics of antibody response to BA and SRBC in different phases were investigated by utilizing two different models. The first model was primarily used to evaluate MHC effects separately in the two different line backgrounds. Analysis of variance of MHC haplotypes, sex, and plate effects on primary and secondary response to BA and SRBC and sex by MHC haplotypes in the two genetic backgrounds is presented (Table 1). In the S1 background, there was no significant effect of MHC haplotype on either primary or secondary response to BA and SRBC for curve traits (*t max*, *y max*, and *c* coefficient) except the *c* coefficients of secondary response to BA and SRBC. The two-way interaction of MHC haplotypes by sex was significant ($P < 0.05$) for primary response to BA. In the G-line background, there were no significant effects detected for main

TABLE 1. Analysis of variance of MHC haplotypes, sex, and plate effects on primary and secondary antibody response to *Brucella abortus* (BA) and SRBC by response phase and by genetic background

		Primary response						Secondary response					
		BA			SRBC			BA			SRBC		
Source	df	*t max*[1]	*y max*	*c* coefficient	*t max*	*y max*	*c* coefficient	df	*t max*	*y max*	*c* coefficient	*t max*	*y max*	*c* coefficient
		P > F							*P > F*					
S1-line background														
MHC	3	0.1739	0.0448	0.0946	0.0438	0.0523	0.7914	3	0.0676	0.0744	0.0243	0.5194	0.4090	0.0066
Sex	1	0.2879	0.3301	0.6000	0.2694	0.0399	0.6279	1	0.8376	0.5764	0.5096	0.5897	0.1010	0.5574
Plate	41	0.6058	0.1213	0.2168	0.6969	0.1265	0.2152	37	0.8246	0.6798	0.0493	0.0001	0.4281	0.8825
MHC * Sex	3	0.1972	0.0230	0.0650	0.9945	0.2062	0.2044	3	0.3737	0.9778	0.1429	0.5478	0.7406	0.6488
Error	48							46						
r^2 (%)		51	65	63	48	66	53		48	51	66	89	57	52
G-line background														
MHC	3	0.8384	0.8612	0.9065	0.6846	0.0581	0.2506	3	0.5868	0.1751	0.0893	0.4837	0.5777	0.8996
Sex	1	0.2975	0.5033	0.8310	0.8341	0.1073	0.0896	1	0.7005	0.5122	0.4739	0.9132	0.0950	0.5981
Plate	38	0.0009	0.6031	0.6824	0.9749	0.4519	0.6415	34	0.9884	0.3122	0.5537	0.9511	0.1218	0.8525
MHC * Sex	3	0.6946	0.6195	0.7296	0.9434	0.6188	0.6540	3	0.7791	0.7592	0.4862	0.9141	0.5660	0.9278
Error	68							26						
r^2 (%)		87	63	59	51	70	66		40	68	59	44	71	54

[1] *t max* = time required to achieve maximum titer; *y max* = maximum titer; *c* coefficient = rate of decline in the titer.

effects nor interaction terms on primary and secondary response to BA and SRBC.

In the second model, analysis was made to additionally evaluate background gene by *Ea-B* or *IrGAT* interactions and nonMHC gene effects on the kinetics of antibody response to BA and SRBC (Table 2). There was an effect ($P < 0.05$) of line (S1 and G) on *y max* of BA in primary phases and of SRBC in secondary phases. The *Ea-B* type significantly affected the *t max* and *y max* of primary response to SRBC and the *c* coefficient of secondary response to SRBC. There was no significant effect of *IrGAT* on any of the curve traits of antibody response except *t max* of secondary response to BA. There were effects ($P < 0.05$) of two-way interactions of line by *Ea-B* on *y max* of primary response to SRBC and secondary response to BA. The three-way interaction of line, *Ea-B*, and *IrGAT* was significant ($P < 0.05$) on the following traits: *y max* of primary response to BA, *t max*, and *c* coefficient of secondary response to BA.

Figure 1 shows that birds from the S1 line have a higher *y max*, indicating the level of antibody production against foreign antigens (BA or SRBC) is higher in S1-line than in G-line birds for both primary and secondary response. Overall, birds that have the B^1 $IrGAT^{high}$ allele are the highest antibody producers in the primary phase. However, birds that have the B^{19} $IrGAT^{low}$ allele produced antibody in the shortest time period compared with others, except for the G-line response to SRBC. The decline in antibody production, the *c* coefficient, did not exhibit trends similar to *t max* or *y max*.

Means and standard errors from two-way interactions of line by *Ea-B*, line by *IrGAT*, and *Ea-B* by *IrGAT* are presented in Table 3. Chickens from the S1-B^1B^1 genotype required significantly more time to reach maximum response to SRBC antigen in the primary phase (Table 3). However, these chickens had a significantly higher maximum titer compared with other chickens. Decline in maximum titer, as estimated by the *c* coefficient, for either BA or SRBC in the primary phase was not affected by line by *Ea-B* interaction (Table 3). Means in the secondary response phase showed significant differences in all parameters (*t max*, *y max*, and *c* coefficient) for both antigens except *t max* of BA. The most rapid declines in antibody titers were observed in two G-line genotypes (Table 3).

The two-way interaction of *Ea-B* by *IrGAT* was significant for *y max* and *c* coefficients of both BA and SRBC in the primary phase (Table 3). Chickens from B^1B^1 $IrGAT^{high}$ and $B^{19}B^{19}$ $IrGAT^{low}$ were ranked the same for maximum antibody response (*y max*) and decline in maximum titer (*c* coefficient) for both antigens in the primary phase. The range of titer differences between the highest and lowest responders was 0.79 to 1.36 for BA and SRBC, respectively. The most rapid declines in antibody titer were observed for groups with the highest maximum titers to BA and SRBC in the primary phase.

Spearman rank correlation coefficients were calculated between *t max*, *y max*, and *c* coefficients of antibody response to BA and SRBC 1) within phases between antigens (BA and SRBC), and 2) between phases within anti-

TABLE 2. Analysis of variance of line, *Ea-B* (erythrocyte antigen B locus), and *IrGAT* [immune response to glutamic acid-alanine-tyrosine (GAT) locus] effects and two-way and three-way interactions on primary and secondary antibody response to *Brucella abortus* (BA) and SRBC

		Primary response						Secondary response					
		BA			SRBC			BA			SRBC		
Source	df	*t max*[1]	*y max*	*c* coefficient	*t max*	*y max*	*c* coefficient	df	*t max*	*y max*	*c* coefficient	*t max*	*y max*	*c* coefficient
		P > F							*P > F*					
Line	1	0.1172	0.0229	0.7481	0.1647	0.1832	0.3724	1	0.1409	0.3985	0.0791	0.1076	0.0117	0.0260
Ea-B	1	0.8136	0.8671	0.8300	0.0002	0.0096	0.3808	1	0.2470	0.8649	0.2227	0.2965	0.1243	0.0014
IrGAT	1	0.2972	0.4893	0.7018	0.6022	0.1527	0.3452	1	0.0143	0.3855	0.3341	0.7988	0.8146	0.6743
Sex	1	0.8790	0.8835	0.5267	0.6680	0.6610	0.2213	1	0.6533	0.2517	0.2767	0.1508	0.0015	0.3793
Plate	52	0.0023	0.0690	0.0442	0.8264	0.1079	0.1267	46	0.9986	0.0876	0.5808	0.8132	0.0100	0.9925
L * *Ea-B*	1	0.2201	0.9200	0.9342	0.7603	0.0205	0.0999	1	0.8279	0.0110	0.1881	0.4880	0.9660	0.1805
L * *IrGAT*	1	0.7365	0.5983	0.7591	0.8115	0.2617	0.2891	1	0.7341	0.1723	0.3237	0.9109	0.2083	0.8940
E * *IrGAT*	1	0.1448	0.0003	0.0132	0.9711	0.0002	0.0184	1	0.7337	0.8771	0.2704	0.4713	0.3647	0.0624
L * *Ea-B* * *IrGAT*	1	0.0727	0.0395	0.0509	0.0131	0.9506	0.0287	1	0.5581	0.0058	0.0077	0.6707	0.1985	0.2542
Error	105							100						
r^2 (%)		51	51	49	37	50	44	27	48	38		31	53	37

[1] *t max* = time required to achieve maximum titer; *y max* = maximum titer; *c* coefficient = rate of decline in the titer.

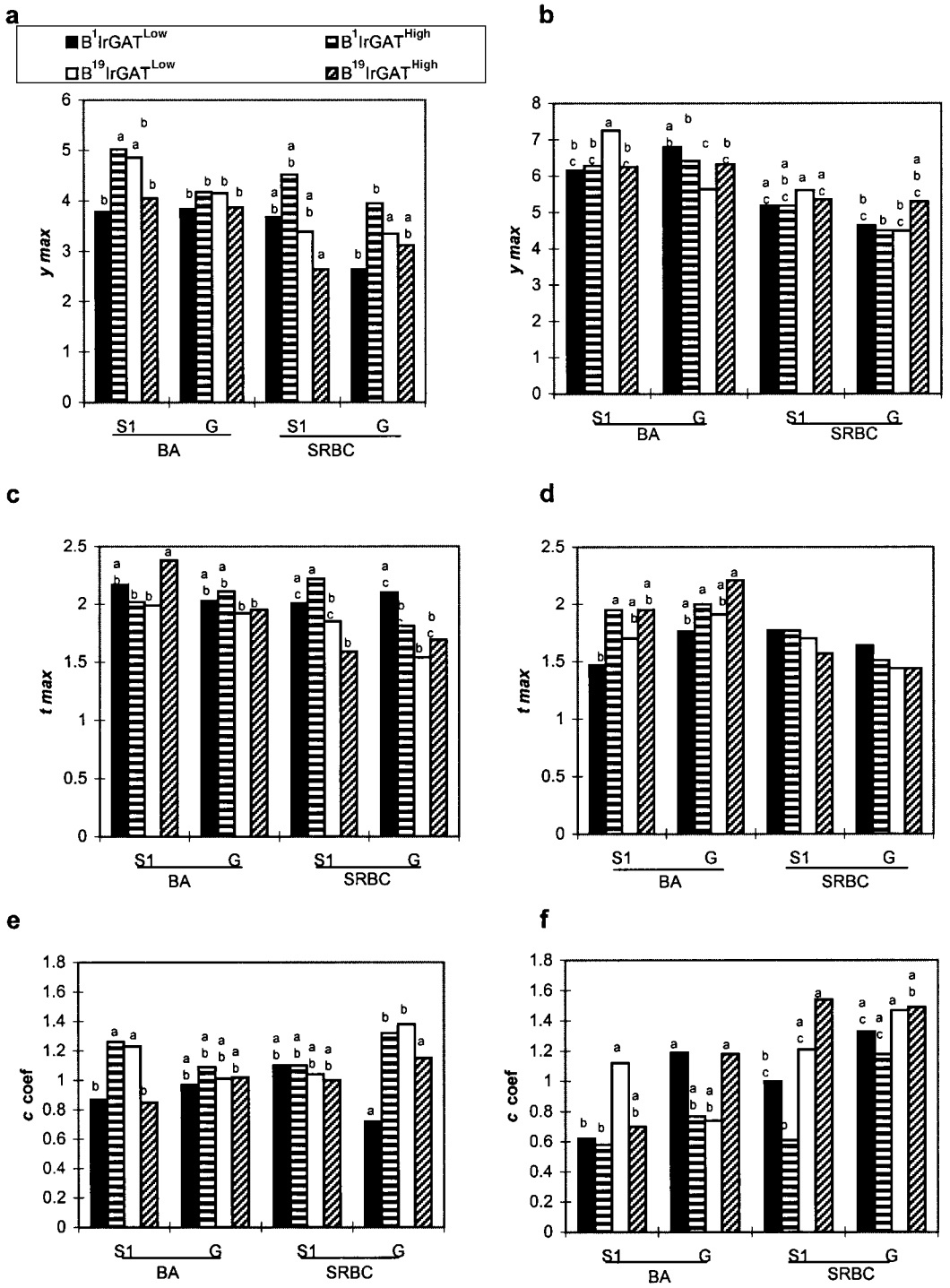

FIGURE 1. Histogram of line, B blood group, and *IrGAT* effects on kinetic parameters of primary and secondary antibody response to *Brucella abortus* (BA) and SRBC. Primary responses are presented in the left column (a, c, e); secondary responses are presented in the right column (b, d, f). The parameters shown are *y max* (maximum titer) (a,b), *t max* (time required to achieve *y max*) (c,d), and *c* coefficient (rate of decline in the titer) (e,f). Parameters with the same letter do not differ from each other compared within antigen, phase, and line at $P \leq 0.05$.

gens. Correlations within antigens between phases revealed that there is a significant positive correlation between *y max* of primary and secondary response to BA but not to SRBC (Table 4). The *c* coefficients of these two traits were also positively correlated. There were correlations ($P < 0.05$) between *t max* of BA and SRBC within both phases. A significant positive correlation of 0.27 was found between the *c* coefficients of secondary phases of both BA and SRBC response.

DISCUSSION

Kinetic differences in antibody production in the primary and secondary phases were evaluated by using

TABLE 3. Selected two-way interaction effects (mean ± SEM) on primary and secondary antibody production to SRBC and *Brucella abortus* (BA)

		Primary response						Secondary response					
		BA			SRBC			BA			SRBC		
Line	*IrGAT*[1]	*t max*[2]	*y max*	*c* coefficient	*t max*	*y max*	*c* coefficient	*t max*	*y max*	*c* coefficient	*t max*	*y max*	*c* coefficient
		(mean ± SE)											
Background line by *IrGAT* interaction effects													
S1	Low	2.08 ± 0.08	4.31 ± 0.17^{ab}	−1.05 ± 0.08	1.93 ± 0.08	3.53 ± 0.19	−1.07 ± 0.08	4.59 ± 0.12^{b}	6.70 ± 0.18	−0.87 ± 0.10	4.74 ± 0.10	5.39 ± 0.18^{a}	−1.11 ± 0.11
S1	High	2.20 ± 0.08	4.54 ± 0.16^{a}	−1.06 ± 0.08	1.90 ± 0.09	3.58 ± 0.20	−1.05 ± 0.09	4.95 ± 0.12^{a}	6.26 ± 0.19	−0.64 ± 0.11	4.67 ± 0.10	5.21 ± 0.19^{a}	−1.07 ± 0.11
G	Low	1.97 ± 0.09	4.00 ± 0.18^{b}	−0.99 ± 0.09	1.82 ± 0.10	2.99 ± 0.22	−1.05 ± 0.10	4.83 ± 0.14^{ab}	6.21 ± 0.21	−0.96 ± 0.12	4.54 ± 0.11	4.56 ± 0.21^{b}	−1.40 ± 0.13
G	High	2.03 ± 0.10	4.02 ± 0.22^{ab}	−1.06 ± 0.11	1.75 ± 0.11	3.54 ± 0.24	−1.23 ± 0.11	5.11 ± 0.15^{a}	6.36 ± 0.23	−0.97 ± 0.13	4.47 ± 0.12	4.89 ± 0.24^{ab}	−1.34 ± 0.14
Ea-B[3] by *IrGAT* interaction effects													
1	Low	2.10 ± 0.08	3.81 ± 0.18^{b}	−0.92 ± 0.09^{b}	2.05 ± 0.09^{a}	3.16 ± 0.21^{b}	−0.91 ± 0.09^{b}	4.61 ± 0.13^{b}	6.47 ± 0.20	−0.91 ± 0.12	4.71 ± 0.11	4.91 ± 0.21	−1.16 ± 0.12^{ab}
1	High	2.06 ± 0.08	4.60 ± 0.16^{a}	−1.18 ± 0.08^{a}	2.01 ± 0.09^{a}	4.24 ± 0.20^{a}	−1.21 ± 0.09^{a}	4.97 ± 0.12^{a}	6.34 ± 0.18	−0.67 ± 0.11	4.64 ± 0.09	4.79 ± 0.18	−0.89 ± 0.11^{b}
19	Low	1.96 ± 0.08	4.51 ± 0.16^{a}	−1.12 ± 0.08^{ab}	1.69 ± 0.08^{b}	3.37 ± 0.19^{b}	−1.21 ± 0.08^{a}	4.81 ± 0.11^{ab}	6.44 ± 0.17	−0.93 ± 0.10	4.57 ± 0.09	5.04 ± 0.18	−1.34 ± 0.11^{a}
19	High	2.17 ± 0.10	3.96 ± 0.21^{b}	−0.94 ± 0.11^{ab}	1.64 ± 0.11^{b}	2.88 ± 0.24^{b}	−1.08 ± 0.11^{ab}	5.08 ± 0.15^{a}	6.29 ± 0.22	−0.94 ± 0.13	4.50 ± 0.13	5.32 ± 0.24	−1.51 ± 0.14^{a}
Line by *Ea-B* interaction effects													
S1	1	2.10 ± 0.07	4.40 ± 0.15	−1.07 ± 0.08	2.11 ± 0.08^{a}	4.10 ± 0.19^{a}	−1.10 ± 0.08	4.71 ± 0.12	6.22 ± 0.18^{bc}	−0.60 ± 0.11^{b}	4.77 ± 0.09^{a}	5.13 ± 0.18^{a}	−0.80 ± 0.11^{b}
S1	19	2.19 ± 0.08	4.45 ± 0.17	−1.04 ± 0.09	1.72 ± 0.09^{bc}	3.01 ± 0.21^{b}	−1.02 ± 0.10	4.83 ± 0.12	6.75 ± 0.18^{a}	−0.91 ± 0.11^{a}	4.64 ± 0.10^{ab}	5.47 ± 0.20^{a}	−1.38 ± 0.12^{a}
G	1	2.07 ± 0.09	4.00 ± 0.19	−1.03 ± 0.09	1.95 ± 0.10^{ac}	3.29 ± 0.22^{b}	−1.02 ± 0.10	4.88 ± 0.14	6.60 ± 0.21^{ac}	−0.98 ± 0.13^{a}	4.57 ± 0.11^{ab}	4.57 ± 0.21^{b}	−1.25 ± 0.13^{a}
G	19	1.94 ± 0.10	4.01 ± 0.22	−1.02 ± 0.11	1.62 ± 0.11^{b}	3.24 ± 0.25^{b}	−1.26 ± 0.11	5.06 ± 0.15	5.98 ± 0.23^{b}	−0.96 ± 0.14^{a}	4.44 ± 0.13^{b}	4.89 ± 0.24^{ab}	−1.48 ± 0.14^{a}

[a,b,c]Means in the same column with different superscripts are different ($P < 0.05$).

[1]*IrGAT* = immune response to glutamic acid-alanine-tyrosine (GAT) locus.

[2]*t max* = time required to achieve maximum titer; *y max* = maximum titer; *c* coefficient = rate of decline in the titer.

[3]*Ea-B* = erythrocyte antigen B locus.

chickens of four MHC haplotypes in two different background lines. Immunization of birds with both BA and SRBC allowed investigation of not only time course and level of antibody production, but also the underlying correlations between phases and between antigen-specific responses. A quadratic equation method was used to approximate curve traits (*y max*, *t max*, and *c* coefficient) to define the individual kinetics of antibody response.

TABLE 4. Correlation coefficients of antibody response A) by phase and B) by antigen type

A	BA1–BA2	SRBC1–SRBC2
t max[1]	−0.01	−0.04
y max	0.17*	0.14
c coefficient	0.22*	0.05
B	BA1-SRBC1	BA2-SRBC2
t max	0.35*	0.36*
y max	0.13	0.06
c coefficient	0.08	0.27*

[1]*t max* = time required to achieve maximum titer; *y max* = maximum titer; *c* coefficient = rate of decline in the titer.

*$P \leq 0.05$.

The four MHC haplotypes, analyzed within line, had little effect on primary and secondary antibody response to either antigen. There were indications of differences between lines for significance of main effects, in that the probability values for the same parameters, although not significant, sometimes differed up to 10-fold in magnitude between lines. In general, *P* values were higher in the highly inbred G line than in the moderately inbred S1 line (35 out of 48 comparisons; Table 1). Although significant main effects were not detected, the model used for analysis explained a high proportion of variance in response to both antigens.

To investigate the genetic effects in more detail than the first model could reveal, a statistical model was applied that included line and also separated the individual main effects of *Ea-B* and *IrGAT*, two loci within the MHC haplotype. There was not a consistent picture for the significance of the main sources of variation in this model. However, many of the two-way interactions were significant, especially when *Ea-B* was a component of the interaction (Table 2). There were significant line by *Ea-B* interactions for the following traits: *y max* and *c* coefficient of

primary response to SRBC and *y max* of secondary response to BA. The significant effect of *Ea-B* by *IrGAT* interaction was only observed in the primary response for *y max* of BA and SRC and the *c* coefficient of SRBC (Table 2). The least squares means of traits that were under significant two-way interaction of *Ea-B* support the ANOVA results (Table 3). Chickens from B^1 $IrGAT^{high}$ and B^{19} $IrGAT^{low}$ yielded a higher *y max* than other haplotypes for primary response to BA. Overall, chickens of B^1 $IrGAT^{high}$ had higher *y max* for both BA and SRBC. However, the *c* coefficient of these birds was also significantly higher than other types.

Several main effects are apparent from the analyses of variance presented in Tables 1 and 2. The MHC, as a main effect, shows significance ($P < 0.05$) in only three of the 12 tests applied in the S1 line and none in the G-line background (Table 1). These results indicate the importance of MHC by nonMHC interactions, which is supported by the many two- and three-way interactions, including line, that are presented in Table 2. Sex, which is often a source of variation in antibody production, does have a significant effect on antibody level (*y max*) in this study (Tables 1 and 2), but only for SRBC response, for which it is sometimes significant and sometimes approaches significance in effect. In general, the other antibody response parameters (*t max* and *c* coefficient) seem unaffected by sex. The birds in this study were juveniles (4 to 7 wk of age during the experiments), and perhaps a greater differentiation between the sexes would be expressed at later ages. The individual microtiter plate in which each sample was assayed reflects several important environmental factors in the antibody measurements (assay date, room temperature and humidity, order of assay) and is, therefore, included in the model. As expected, it is a significant source of variation in several instances, illustrating the importance of including this factor in the analysis (Tables 1 and 2).

The results of line by *Ea-B* interaction revealed that S1-line birds with B^1 had the highest *y max* in response to SRBC (Table 3c). However, chickens from the S1 line with B^{19} had the highest *y max* in response to BA in the secondary stage. Although main effects of line, *Ea-B*, and *IrGAT* are not very significant, one-half of the three-way interactions are significant, and the significance levels were higher for two-way and three-way interactions than for the corresponding main effects (Table 2). This result reveals that these antibody kinetics traits are under very complex genetic control that is explained better by two- and three-way interactions than by simpler sources of variation.

A significant interaction of *Ea-B* and *IrGAT* was detected for several traits of primary response but not traits of secondary response (Table 2). This finding is in agreement with the phenotypes generated by the IrGAT locus. The contrast in antibody levels between homozygous $IrGAT^{low}$ and $IrGAT^{high}$ birds can be more readily detected after primary immunization, when $IrGAT^{low}$ birds produce almost no GAT antibody but $IrGAT^{high}$ birds produce normal levels compared with levels after secondary immunization (Pevzner *et al.*, 1978). The *IrGAT* locus may be either directly controlling or linked to other genes controlling the primary antibody response to a greater extent than the secondary response. With the ability to conduct molecular typing, the opportunity exists to more accurately estimate the underlying genetic basis of two- and three-way interaction at the DNA level.

The r^2 values range from 27 to 53% for the specific individual traits as tested across lines (Table 2). These lower values, as compared with those presented in Table 1 (40 to 89%), are to be expected when the two background lines are combined for analysis rather than analyzed separately. Because these r^2 values are computed for the ANOVA of very different specific traits, they vary widely. However, the individual curve trait parameters were estimated from a linear regression with a mean r^2 of 93%, which compares favorably with mean r^2 values for the quadratic equations in a comparable study, which were 62 to 87% (Siegel *et al.*, 1984).

The measurement of antibody response to two different antigens and for both primary and secondary response phases allowed the estimation of correlation between phases within antigen and between antigen responses within phase (Table 4). Based on the between-phases correlation results, the primary phase BA response level and rate of decline are predictive for the same traits in the secondary phase, but this does not hold true for SRBC response. Correlations of the time needed to reach maximum response in primary and secondary phases are close to zero for both antigens (Table 4). The rapidity, therefore, of the primary response is not predictive of the speed of the secondary response to the same antigen. For both primary and secondary response, however, the *t max* was significantly positively correlated between antigens. This result suggests that the rate of antibody response is consistent across the two antigens, as investigated within each response phase. There was a significant positive correlation between antigens in the rate of antibody decline in the secondary phase only.

In the present study, the decline in *c* coefficient (maximum titer) for both BA and SRBC in the primary phase was not affected by *B* type. However, LePage *et al.* (1996) observed a more rapid antibody titer decline from the peak in chickens trisomic and tetrasomic for the *B* complex than in disomic birds. Those researchers suggested that disomic (*B* complex) chickens might have longer antigen persistency, which does not significantly increase the peak antibody titer but does prevent the faster decline from peak titer level.

Other selection experiments have evaluated humoral response to the same antigens used in the current study with contrasting results, likely because of the original genetic material used and minor procedural differences. Haddad *et al.* (1994), using the Arkansas Regressor and Progressor chickens, reported that there were significant differences between the two lines in their humoral immune response to SRBC and GAT. Chicks from the Regressor line showed an earlier antibody response to SRBC than did chicks of the Regressor line. Scott *et al.* (1994)

reported that chickens selected for antibody responsiveness to SRBC did not differ in ability to produce antibody responses against BA. Parmentier *et al.* (1998), who studied SRBC-selected lines, reported that antibody responses to SRBC and BA were significantly affected by line by treatment by time interaction. Those researchers observed that levels of antibodies to SRBC and BA were significantly higher in the H (high) line than either the C (control) or L (low) line.

The current study administered the two antigens simultaneously. The decision to administer antigens singly or in combination may effect the antibody response but is considered to be an appropriate model for approximating field conditions. Single or simultaneous administration of SRBC, NDV, and BA did not show interaction with background genome or MHC genotype (Dunnington *et al.*, 1992). Those researchers reported that higher response to BA was obtained when given alone or with SRBC. However, higher response to SRBC was obtained when SRBC was given alone or with NDV.

In summary, this study evaluated the kinetics of both primary and secondary antibody response to SRBC (a T-cell dependent antigen) and BA (a T-cell independent antigen). The complexity of the genetic control of the immune response is suggested by the interaction effects, which were more frequently significant than were the main effects. The correlations indicate that primary phase antibody response level and rate of decline to BA, but not SRBC, is predictive of secondary response level and rate of decline. Also, speed of reaching maximum response levels was positively correlated between the two antigens in both the primary and secondary phases, suggesting that genetic selection for rate of antibody response may have similar effects in other immune response phases and to other antigens. Collection of antibody response data at multiple time points allows the opportunity to more accurately define the total humoral response, including the important persistency phase, providing valuable criteria for a genetic selection program in antibody enhancement, especially in breeder hens.

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