Cell-Mediated and Humoral Immunity and Phagocytic Ability in Chicken Lines Divergently Selected for Serum Immunoglobulin M and G Levels

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ABSTRACT Humoral and cell-mediated immunities and phagocytic ability were examined at the third and fourth generations of selection in two pairs of chicken lines (at 10 wk of age) that were divergently selected for levels of high serum Ig M (HIM), low serum Ig M (LIM), high serum Ig G (HIG), and low serum Ig G (LIG). Cell-mediated immunity was examined by splenomegaly assay at 12 wk of age. At 20 and 23 wk of age, 20 birds from the respective lines were injected *Brucella abortus* (BA), and blood samples were collected at 7 and 14 d postprimary immunization (PPI) and postsecondary immunization (PSI). Phagocytic ability was measured by carbon clearance assay at 25 and 30 wk of age. The results showed that the LIG line had higher degree of splenomegaly indices than the HIG line in both generations. The HIM and HIG lines had significantly ($P < 0.05$) higher total antibody titers to BA than their low counterparts. Similarly, mercaptoethanol-resistant (MER) antibody titers to BA, as measured only in the fourth generation, were significantly ($P < 0.05$) higher in the HIM and HIG lines than their low counterparts. In both generations, the HIM and HIG lines had significantly ($P < 0.01$) faster carbon clearance ability than the LIM and LIG lines. The results suggest that both pairs of selected lines exhibited divergence in immunocompetence, although they had been selected for serum Ig isotypes.

(*Key words*: chicken, immunoglobulin, graft vs. host reaction, antibody titers, carbon clearance assay)

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INTRODUCTION

The immune system of poultry is a complex network of different cell types and soluble factors that give rise to an effective response to pathogenic challenges. Proper and efficient function of the immune system is directly associated with poultry health. The phagocytic ability and cell-mediated and humoral immunities combine to provide birds with a complete spectrum of resistance processes. To enhance the efficiency of their immune systems, several lines of chickens have been selected divergently against specific antigens (Siegel and Gross, 1980; Pevzner et al., 1981a,b; Okada and Yamamoto, 1987; Pitcovski et al., 1987; Steadham and Lamont, 1993; Sarker et al., 1999a,b). These selection processes must affect the major functions of the immune system, which leads to changes in the selected and correlated traits, resulting in differences in the immune responses among the selected lines.

Genetic control of cell-mediated immunity in chickens has been demonstrated (Lamont and Smyth, 1984; Cotter et al., 1987; Cheng and Lamont, 1988; Sarker et al., 1998a,b). Okada and Yamamoto (1987) found that the high graft vs. host reaction and IgG-selected lines had higher splenomegaly indices (SI) than their low counterparts. However, SI values were lower in the high leucocytozoon-selected line compared with the low line.

Several studies have demonstrated that humoral immunity is under genetic control. Dunnington et al. (1992) and Scott et al. (1994) showed that lines of chickens selected for high (HA) and low (LA) antibody responses to SRBC exhibited divergent antibody responses to *Brucella abortus* (BA). Okada and Yamamoto (1987) reported that the high IgG line was associated with high antibody response to SRBC and lipopolysaccharide and low immune response to BSA. Gross et al. (1980) demonstrated positive associations between anti-SRBC antibody production and resistance to viral and parasitic diseases but a negative association with bacterial diseases.

Several researchers have reported that phagocytic activity of chickens is genetically regulated (Lamont, 1986;

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Abbreviation Key: BA = *Brucella abortus*; HIG = high immunoglobulin G; HIM = high immunoglobulin M; LIG = low immunoglobulin G; LIM = low immunoglobulin M; PPI = postprimary immunization; PSI = postsecondary immunization; MER = mercaptoethanol-resistant; SI = Splenomegaly index.

Cheng and Lamont, 1988; Qureshi and Miller, 1991; Qureshi and Taylor, 1993). Studies with MHC *B*-congenic White Leghorn chicken lines have shown that certain chicken macrophage functions, such as phagocytosis, intracytoplasmic bacterial killing, and blood monocyte chemotaxis are influenced by allelic differences of *B*-congenic chickens (Qureshi et al., 1986, 1988).

Genetic enhancement of immunocompetence without challenging animals with disease agents may be a potential approach for improving health and production parameters as well as general disease resistance. Selection of chickens for serum Ig isotypes, which represents the response to a wide variety of unknown antigens, may be a new breeding strategy to improve resistance to infectious diseases. In this regard, genetic control of humoral and cell-mediated immunities and phagocytic ability of chicken lines selected for serum IgM and IgG has not been well investigated. In our previous report (Sarker et al., 1999a), it was shown that selection of chickens for serum Ig isotypes had considerable effect on antibody response to SRBC. This paper reports on the genetic regulation of cell-mediated and humoral immunity and phagocytic ability of the same selected chicken lines during the third and fourth generations of selection.

MATERIALS AND METHODS

Chickens

The lines of chickens used in the present study have been previously described (Sarker et al., 1999a). Briefly, six chicken lines were used to establish the base population. The lines were HA, LA, HG, LG, C, and GSP. Lines HA, LA, HG, and LG were White Leghorns and were established from the N strain of Hokkaido University, Sapporo, Japan, by four generations of selection for high and low competences of splenomegaly in graft vs. host reaction. Line C was a randombred control line and was established from crossbred lines of White Leghorns and White Rocks that have been maintained at the author's laboratory for 35 yr. The GSP line (Somes, 1988) was established in 1971 by pedigree breeding from the Fayoumi breed at the Nippon Institute for Biological Science, Kobuchizawa, Yamanashi, Japan. Five birds, one sire and four dams, of each line went into base population. First, lines were interbred to produce GHA (GSP ♂ × HA ♀), GHG (GSP ♂ × HG ♀), CLA (C ♂ × LA ♀), and CLG (C ♂ × LG ♀) populations. Then these GHA and CLG, and GHG and CLA were interbred reciprocally to establish the base population. From this combined gene pool, 10 sires and 20 dams were used to make each parent stock, high IgG (HIG), low IgG (LIG), high IgM (HIM), and low IgM (LIM) lines, respectively, according to their IgM and IgG levels, at 10 wk of age. At hatching, all the chicks were wing-banded and vaccinated against Marek's disease. They were also administered fowl pox vaccine at 2 wk of age and Newcastle disease vaccine at 2 and 4 wk of age. Chickens of all the lines were provided ad libitum access to water and a commercial diet.

Graft vs. Host Reaction

Graft vs. host reaction was measured by splenomegaly assay (Okada and Yamamoto, 1987) at 12 wk of age in the third and fourth generations of selection. Citrated blood (3.8% sodium citrate) was collected from 22 chickens of each line, and a lymphocyte suspension was prepared at 10^7 cells/mL PBS. Eggs from a commercial layer strain were used as recipients. At 14 d of incubation, 0.1 mL of cell suspension was injected into the chorioallontoic vein of the embryos. The same amount of PBS was injected into the control embryos. At the fifth day after injection, embryo BW and spleen weights were recorded, and splenomegaly indices (SI) were calculated dividing the spleen weight (in mg) by BW (in g). Usually six eggs were used as recipients for each donor.

Administration of Antigen

In the third and fourth generations of selection, BA, which is a T-cell-independent antigen, was injected intravenously into 20 chickens of the respective lines at 20 and 23 wk of age. *Brucella abortus* was diluted to 10^9 cells/mL PBS, and 1 mL of the diluted antigen was injected into each chicken. Blood samples were collected at 7 and 14 d postprimary immunization (PPI) and postsecondary immunization (PSI). However, in the third generation of selection, blood samples were collected only at 7 and 14 d PPI. Sera were separated from the injected birds by centrifugation at $1{,}700 \times g$ for 10 min and were stored at –20 C.

Agglutination Assay

Total and mercaptoethanol-resistant (MER) antibody titers for BA were measured as described by van der Zijpp and Leenstra (1980). Titers were expressed as the $\log_2$ of the reciprocal of the highest dilution in which agglutination occurred.

Carbon Clearance Assay

The phagocytic ability (Lamont, 1986) of the macrophages of the chicken lines was determined by carbon clearance assay at 25 and 30 wk of age in the third and fourth generations of selection. The supernatant fraction of Fount India ink[2] was injected into the wing vein of birds at 1 mL/kg body weight. One hundred microliters of blood was collected from the opposite wing before, and 3 and 15 min after, carbon injection and was transferred immediately into 2 mL of 3.8% sodium citrate. The samples were then centrifuged at $50 \times g$ for 5 min. The relative amount of carbon remaining in the supernatant of the samples was estimated spectrophotometrically at 675 nm, by using the samples collected before carbon injection as the zero value.

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TABLE 1. Splenomegaly index of high IgM (HIM) and low IgM (LIM) and high IgG (HIG) and low IgG (LIG) chicken lines[1] in the third and fourth generations of selection

Generation	Splenomegaly index			
	HIM	LIM	HIG	LIG
3	1.64 ± 0.46[a] (22)	1.37 ± 0.40[a] (21)	2.22 ± 0.85[a] (22)	2.74 ± 0.58[a] (21)
4	2.72 ± 0.67[a] (22)	2.59 ± 1.21[a] (22)	2.24 ± 0.85[a] (22)	3.42 ± 0.86[b] (22)

[a,b]Means within the row of a particular category with no common superscript differ significantly ($P < 0.01$).
[1]Numbers in parentheses indicate number of chickens used in each generation of selection.

Statistical Analysis

Data were analyzed with the general linear models procedure of SAS® software (SAS Institute, 1985). The model for testing the effects of line and sex was

$$Y_{ijkl} = \mu + A_i + B_j + C_{ijkl}$$

where Y_{ijkl} is any of the four observations (SI, total titer to BA, MER titer to BA, and PI) of an individual, μ is the population mean, A_i is line, B_j is the sex, and C_{ijkl} is random error. The M and G lines were analyzed separately.

RESULTS AND DISCUSSION

Least squares means for SI of the HIM, LIM, HIG, and LIG lines are presented in Table 1. In the IgM selection, a significant difference was not found between the HIM and LIM lines, although the HIM line had higher SI. In the IgG selection, the LIG line produced a significantly ($P < 0.01$) higher SI than the HIG line in the fourth generation. This difference was not significant in the third generation, although the LIG line had higher SI values than the HIG line. The reason for this difference is not clear; however, it is postulated that the frequency of MHC genes, which might be responsible for the SI, changed gradually in the IgG selection process (Sarker et al., 1999b). Genetic control of cell-mediated immunity was demonstrated by Lamont and Smyth (1984), Cotter et al. (1987), Cheng and Lamont (1988), and Sarker et al. (1998b), which is in agreement with our result. From this result, it is presumed that selection of chickens on the basis of serum IgG might have changed the T-cell populations during the course of selection. Greater proliferative activity of the T cells in the LIG line may be associated with a higher SI compared to the HIG line. The LIG line was also associated with higher antibody titers to SRBC, a T-cell-dependent antigen, than the HIG line (Sarker et al., 1999a). Okada and Yamamoto (1987) reported that high IgG selected line was associated with higher degree of SI; however, in the present experiment, the LIG line produced significantly ($P < 0.01$) higher SI than the HIG line. This discrepancy may be due to the difference in the genetic constitution and selection criteria of the lines.

Least squares means for total and MER antibody titers to BA of the HIM and LIM lines and HIG and LIG lines of the third and fourth generations are presented in Table 2. In the third generation, a significant difference ($P < 0.05$) was found between HIM and LIM lines for total antibody titers to BA at 7 d PPI. The MER antibody titers were not significantly different. A significant difference for total antibody titers to BA was also observed between the HIG and LIG lines at 7 d PPI. In the fourth generation of selection, total antibody titers to BA were significantly ($P < 0.05$) higher in the HIM line than in the LIM line at 7 and 14 d PPI and at 14 d PSI. The HIM line also had significantly ($P < 0.05$) higher MER antibody titers to BA than the LIM line at 7 and 14 d PSI. Similarly, the HIG line had higher total antibody titers to BA than the LIG

TABLE 2. Least squares means and standard devation for total titers and mercaptoethanol-resistant (MER) titers to *Brucella abortus* at 7 and 14 d postprimary (PPI) and postsecondary (PSI) immunizations in the chicken lines[1]

Generation and time of immunization	Total[2]		MER		Total		MER	
	HIM (20)	LIM (20)	HIM (20)	LIM (20)	HIG (20)	LIG (20)	HIG (20)	LIG (20)
3								
7 d PPI	8.90 ± 0.64[a]	7.90 ± 0.60[b]	5.35 ± 0.57[a]	5.50 ± 0.68[a]	8.45 ± 0.82[a]	7.55 ± 1.14[b]	5.10 ± 0.55[a]	4.72 ± 0.82[a]
14 d PPI	7.20 ± 0.52[a]	6.95 ± 0.51[a]	4.50 ± 0.68[a]	4.15 ± 0.74[a]	6.77 ± 0.64[a]	6.57 ± 0.50[a]	3.99 ± 0.63[a]	3.57 ± 0.76[a]
4								
7 d PPI	8.80 ± 1.09[a]	8.20 ± 1.13[b]	3.35 ± 0.35[a]	3.05 ± 0.48[a]	8.60 ± 0.41[a]	8.25 ± 0.55[a]	2.50 ± 0.30[a]	2.15 ± 0.50[b]
14 d PPI	6.60 ± 0.69[a]	5.55 ± 0.56[b]	2.15 ± 0.54[a]	1.80 ± 0.40[a]	6.65 ± 0.79[a]	6.25 ± 0.42[a]	2.40 ± 0.31[a]	1.50 ± 0.22[b]
7 d PSI	7.70 ± 0.94[a]	7.40 ± 0.86[a]	3.85 ± 0.66[a]	2.30 ± 0.50[b]	7.70 ± 0.86[a]	7.40 ± 1.12[a]	3.45 ± 0.66[a]	2.70 ± 0.76[b]
14 d PSI	6.85 ± 0.65[a]	6.00 ± 0.37[b]	3.35 ± 0.49[a]	2.75 ± 0.90[b]	6.85 ± 0.63[a]	6.00 ± 0.39[b]	3.00 ± 0.87[a]	2.60 ± 0.77[a]

[a,b]Means within the row of a particular category with no common superscript differ significantly ($P < 0.05$).
[1]HIM = high IgM; LIM = low IgM. HIG = high IgG; LIG = low IgG.
[2]Numbers in parentheses indicate number of chickens tested.

TABLE 3. Phagocytic ability of carbon of high IgM (HIM) and low IgM (LIM) and high IgG (HIG) and low IgG (LIG) chicken lines[1] in the third and fourth generations of selection

			Phagocytic index			
Generation	Age[2]	n[3]	HIM	LIM	HIG	LIG
3	25	12	0.013[b]	0.020[a]	0.016[b]	0.023[a]
	30	12	0.011[b]	0.022[a]	0.017[a]	0.020[a]
4	25	12	0.014[b]	0.020[a]	0.016[b]	0.023[a]
	30	12	0.010[b]	0.021[a]	0.013[a]	0.015[a]

[a,b]Means within the row of a particular category with no common superscript differ significantly ($P < 0.01$).

[1]HIM = high IgM; LIM = low IgM; HIG = high IgG; LIG = low IgG.

[2]Age of the birds (wk).

[3]Number of chickens used in each generation of selection.

line; however, a significant ($P < 0.05$) difference was found only at 14 d PSI. The HIG line also had significantly ($P < 0.05$) higher MER antibody titers to BA than the LIG line at 7 and 14 d PPI and at 7 d PSI.

Higher BA antibody titers in the chicken line selected for high antibody titers to SRBC were reported by Scott et al. (1994) and Dunnington et al. (1992). They observed that the HA chicks had higher total antibody titers to BA than the LA chicks. In this study, higher antibody titers to BA were observed in the HIM and HIG lines, which supports the hypothesis that overall multiplication and differentiation of the B cells of the HIM and HIG lines may be more efficient than the LIM and LIG lines. Biozzi et al. (1979, 1984) demonstrated that differences in antibody production within lines of mice selected for high or low antibody production to SRBC were associated with antigen handling by macrophages and in the multiplication rate of the B lymphocytes. *Brucella abortus* is a Type-1, T-independent antigen that stimulates B cells with little assistance from T-helper cells but does require macrophage-like adherent accessory cells to induce antibody formation (Moiser and Subbarao, 1982). A similar mechanism might have been associated with our results. Additionally, the HIM and HIG lines were selected on the basis of serum IgM and IgG levels; therefore, these lines may have greater surface Ig to produce antibodies against T-cell-independent antigens.

Table 3 shows the phagocytic index of the selected lines. The HIM line had a significantly ($P < 0.01$) higher degree of phagocytic ability than the LIM line at 25 and 30 wk of age. In contrast, the HIG line had a significantly ($P < 0.01$) greater phagocytic ability than the LIG line only at 25 wk of age.

Genetic control of phagocytosis has been reported to involve non-MHC genes (Powell et al., 1983; Lamont, 1986; Chu and Dietert, 1989) and MHC-associated genes (Qureshi et al., 1988; Puzzi et al., 1990a,b). Similarly, in our study, genetic control of phagocytosis was observed. The greater phagocytic ability of the HIM and HIG lines might have influenced the antibody production of the HIM and HIG lines to BA. Heller et al. (1992) reported that for chickens that their HC line had faster carbon clearance ability than the LC line, which supports our results. These results suggest that the antigen processing and presentation ability of the macrophages of the HIM and HIG lines are more efficient than their low counterparts. Macrophages initiate cellular and humoral immune responses by being antigen-presenting cells (Powell, 1987; Vainio et al., 1988). Macrophage monokines, such as interleukin-1 (IL-1), also mediate activation and differentiation of B and T lymphocytes. A similar mechanism may be responsible for higher antibody titers to BA in the HIM and HIG lines.

Cytokines are useful for studying the mechanisms of immune response and disease resistance. Some cytokines are involved in Ig class switching and growth and differentiation of B and T lymphocytes. Moreover, macrophage monokines mediate activation and differentiation of B and T lymphocytes. Therefore, measurement of cytokines of the selected chicken lines would be more helpful to better understand the cells involved in immune response and disease resistance. From these results, it is suggested that high levels of IgM and IgG emphasize the difference in levels of IgM and IgG between selected high and low responders after four generations. These levels were 2- and 1.6-fold, respectively, (Sarker et al., 1999b) and were associated with greater carbon clearance ability and higher antibody production to BA. However, low IgG level was associated with a higher degree of SI. It is also postulated that divergent selection of chickens for serum Ig isotypes may change other immunocompetent cells in the selected lines, leading to significant line differences in their immunocompetences.

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