TWO WAY SELECTION FOR SERUM IMMUNOGLOBULIN M (IgM) and G (IgG) LEVELS IN CHICKENS: GRAFT-VS-HOST REACTION COMPETENCE AND ANTIBODY PRODUCTION AGAINST *BRUCELLA ABORTUS* ANTIGEN

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SUMMARY

Two pairs of chicken lines were developed from individual phenotypic selection for serum immunoglobulin M and G levels at 10 weeks of age. After three generations of selection, the lines were tested for their competences to graft-vs-host reaction (GVHR) and antibody response to *brucella abortus* (BA). Serum immunoglobulin M and G levels changed significantly ($P<0.05$) during the selection period. Splenomegaly indices, used to measure GVHR competence, differed significantly ($P<0.05$) between the high IgG (HIG) and low IgG (LIG) lines. Total BA antibody titers also differed significantly ($P<0.05$) between the HIG and LIG lines at 7 day postimmunization. The selected lines showed their divergence in T and B cell responses although the selection was conducted on the basis of serum immunoglobulin isotypes.

Keywords: chicken, selection, immunoglobulin, splenomegaly indices, *Brucella abortus*

INTRODUCTION

Intensive selection is expected to change gene frequencies and modify genetic variances of the selected and correlated traits. Therefore, to improve the efficiency of immune response and to increase the disease resistance, several lines of chickens have been selected divergently against specific antigens (Steadham and Lamont 1993; Siegel and Gross 1980; Okada and Yamamoto 1987). In mice, Biozzi *et al* (1979, 1984) reported that differences in antibody production within lines selected for high (H) or low (L) antibody production to sheep red blood cell (SRBC) were associated with antigen handling by macrophages and in the multiplication rate of the B-lymphocyte. Siegel and Gross (1980) found that a high antibody production chicken line, selected against SRBC, was resistant to parasite and viruse diseases than a low line. On the contrary, selection of chickens on the basis of serum immunoglobulin isotypes represents the response to a wide variety of unknown antigens. Selection of chickens for immunoglobulin isotypes has not been paid much attention. Krieg *et al* (1978) reported that low IgG levels were associated with the mortality to myeloblastosis virus. A chicken line selected for high serum IgG level was more susceptible to Marek's disease than its low IgG counterpart (Tamaki 1981). Therefore, the purpose of the present study was to develop divergent chicken lines for serum immunoglobulin M and G levels and to characterize the humoral and cell mediated immunity of the selected lines.

MATERIALS AND METHODS

Chickens. Six chicken lines were introduced to established the base population. From the base population, each of 10 sires and 20 dams were used to construct parent stock, high IgG (HIG), low

IgG (LIG), high IgM (HIM), and low IgM (LIM) lines, respectively, according with their immunoglobulin M and G levels, at 10 weeks of age. At hatching, all the chicks were wing banded and vaccinated against Marek's disease. They were also administered Fowl pox and Newcastle disease vaccine as per schedule. Chickens of all the lines were provided tap water and a commercial diet *ad libitum.*

Quantitation of IgM and IgG. For the divergent selection, serum immunoglobulin M and G were quantitated by single radial immunodiffusion (SIRD) technique (Mancini *et al.* 1965).

Graft-vs-host reaction (GVHR). Graft-vs-host reaction was measured by splenomegaly assay at 12 weeks of age as described by Okada and Yamamoto (1987).

Administration of antigen. *Brucella abortus*, which is T cell-independent antigen (Gilmour *et al.* 1970), was injected intravenously into 20 chickens of the respective lines at 20 weeks of age. One ml of *Brucella abortus* (10^9 cells/ ml in phosphate buffered saline) was injected to each chicken. Blood samples were collected at 7 and 14 days postimmunization. Serum was separated from the blood and stored at -20 C until assayed.

Agglutination assay. Total and 2-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.

Statistical analysis. Data were analyzed by analysis of variance. Treatment means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Selection differential, selection response, and realized heritability of the selected lines are presented

Table 1. Selection differential, selection response, and realized heritability on the basis of the difference between the HIM and LIM lines

Generation	Selection differential	Selection response	Realized heritability
1	23.47	13.75	0.585
2	17.70	10.52	0.594

Table 2. Selection differential, selection response, and realized heritability on the basis of the difference between the HIG and LIG lines

Generation	Selection differential	Selection response	Realized heritability
1	74.50	29.75	0.40
2	49.84	29.74	0.60

in Tables 1 and 2. Selection differential varies during the course of selection, but the response to selection was almost constant. The realized heritability was comparatively high, which indicates the effectiveness of selection. Table 3 shows the means and standard deviation for splenomegaly indices

(SI) of the selected lines. The LIG line produced significantly higher degree of SI than the HIG line. However, the difference between the HIG and LIG lines did not differ significantly in the second generation (Sarker *et al.* 1997). From this result, it is presume that selection on the basis of serum immunoglobulin G may have changed the T cell population and subpopulations during the course of selection, which may result in significant difference between the lines in the third generation. The SI of the LIG line also differed significantly ($P<0.05$) than the HIM and LIM lines. Okada and Yamamoto (1987) reported that high IgG selected line was associated with higher degree of SI. However, in our experiment, the LIG line produced significantly ($P<0.05$) higher SI than all other lines. This may be due to the differences in genetic background of the lines. From this result, it is suggested that selection on the basis of B cell isotypes may change other immunocompetent cells as well as B cells.

Table 3. Means and standard deviation for splenomegaly index of donor lymphocytes from different chicken lines

Line	No. of donor	Splenomegaly index (SI)
HIG	22	2.24 ± 0.85^{a}
LIG	22	3.42 ± 0.86^{b}
HIM	22	2.72 ± 0.67^{a}
LIM	22	2.59 ± 1.21^{a}

[a-b] Means within the column with no common superscripts differ significantly ($P<0.05$).

Mean total antibody titers to BA in each selected line are presented in Table 4. Between the HIG and LIG lines, significant difference was found at 7 day postimmunization. Whereas, when all the lines were compared, the HIG, HIM and LIM lines produced significantly ($P<0.05$) higher total BA antibody titers than the LIG line at 7 day postimmunization. However, on the 14 day postimmunization, only the HIM line produced significantly ($P<0.05$) higher than the LIG line. Higher total BA antibody titers in the HA chicken was also reported (Scott *et al.* 1994), however there is no published data on IgM selected lines. The HIM line also showed significantly higher total antibody titers against SRBC, which is T cell dependent antigen, in the second generation (Sarker *et al.* 1997). Intra line difference was not observed for MER antibody titers. MER antibody titers were significantly ($P<0.05$) higher in the HIM and LIM lines than the LIG line at 7 and 14 days of postimmunization. The efficiency of antibody production depends upon the influence of antigen and the genetic constitution of the immunized animal . In this study, higher antibody titers were observed in the HIM line, which helps to postulate that overall multiplication and differentiation of the B cells as well as T cells of the the HIM line may be more efficient to react to BA promptly than other lines. Another possibility is that the antigen processing and presentation by macrophages in the the HIM line may be more equipped than other lines. *Brucella abortus* is a type-1 T-independent antigen, which 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). Heller *et al* (1992) also reported in chickens that their HC line had faster carbon clearance ability than LC line.

However, further study with the accessory cell component will test the hypothesis. Evaluation of T

Table 4. Total and MER antibody titers to (mean±SD) *brucella abortus* at 7 and 14 days of postimmunization in the selected chicken lines

Line	No.	Postimmunization			
		7		14	
		Total	MER	Total	MER
HIG	20	8.45±0.82[a]	5.10±0.55[ab]	6.77±0.64[ab]	3.99±0.63[ab]
LIG	18	7.55±1.14[b]	4.72±0.82[b]	6.57±0.50[a]	3.57±0.76[b]
HIM	20	8.90±0.64[a]	5.35±0.67[a]	7.20±0.52[b]	4.50±0.68[a]
LIM	20	8.50±0.60[a]	5.50±0.68[a]	6.95±0.51[ab]	4.15±0.74[a]

[a-b] Means within the column with no common superscripts differ significantly ($P<0.05$).
MER= Mercaptoethanol resistant (IgG) antibody titers.

cell subsets and T cell receptors as well as B cell numbers of these lines is needed to better understand the contribution of these cell types to the differences of immune response among lines.

REFERENCES

Biozzi, G., Mounton, D. Sant'Anna, O. A. Passos, H. C. Gennari, M. Reis, M. H. Ferreria, V. C. A. Heiman, A. M. Bouthillier, Y. Ibanez, O. M. Stiffel, C. and Siquera, M. (1979) *Cuur. Top. Microbiol. Immunol.* **85**: 31-98.

Biozzi, G., Mounton, D. Stiffel, C. and Bouthillier, Y. (1984) *Adv. Immunol.* **36** : 189-234.

Gilmour, D. G., Thies, G. A. and Thorbecke, G. J. (1970) *J. Exp. Med.* **132** : 134-147.

Heller, E. D., Leitner, G. Friedman, A. Uni, Z. Gutman, M. and Cahaner, A. (1992) *Vet. Immunol. Immunopathol.* **34** : 159-172.

Krieg, R. D., Hagen, von. dem. Hartman, W. and Loliger, H. Ch. (1978) *Arch. Fur. Gelfun.* **42** : 1-7.

Mancini, G., Carbonara, A. and Hermans, J. F. (1965) *Immunohistochemistry* **2**: 235-239.

Moiser, D. E., and Subbaro, D. (1982) *Immunol. Today* **38** : 217-223.

Okada, I., and Yamamoto, Y. (1987) *Poult. Sci.* **66** : 769-773.

Sarker, N., Tsudzuki, M. and Yamamoto, Y. (1997) in: Proceedings of the International Conference on Animal Biotechnology. Beijing, China : 45-47.

Scott, T. R., Dunnington, E. A. and Siegel, P. B. (1994) *Poult. Sci.* **73** : 346-349.

Siegel, H. S., and Gross, W. B. (1980) *World Poult. Sci. J.* **59** : 1-5.

Steadham, E. M., and Lamont, S. J. (1993) Poult. Sci. **72** : 67-75.

Tamaki, Y. (1981) Annu. Rep. Nat. Inst. Anim. Ind. Tsuku. Jpn. **20** : 97-104.

van der Zijpp, A. J., Leenstra, F. R. (1980) *Poult. Sci.* **59** : 1363-1369.