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Effect of Selection for Delayed Amelanosis on Immune Response in Chickens: 1. Antibody Production

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Abstract

Studies were conducted to compare the antibody production of the Delayed Amelanotic (DAM) line of chickens with that of the line from which it originated, the Brown line (BR), and a distantly related environmental control, the Light Brown Leghorn (LBL). Total agglutinating antibody titers following immunization with sheep red blood cells (SRBC) or *Brucella abortus* (BA), were determined at 4, 6, 8, 10, 12, 16, and 20 weeks. The SRBC titers of DAM line birds were significantly higher than those of LBL birds but not BR birds at young ages (4 to 8 weeks) and at 20 weeks. The BA titers of DAM and BR lines were significantly higher than those of the LBL line at 4 and 6 weeks. We conclude that the DAM line exhibits hyperreactivity in B-cell function, which may be related to the line-associated pigmentation destruction. These studies also confirm that age is an important factor in making comparisons of antibody production between different genetic stocks.

Keywords

delayed amelanosis, immune response, antibody production, DAM line chicken

Disciplines

Agriculture | Animal Sciences | Poultry or Avian Science

Comments

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Effect of Selection for Delayed Amelanosis on Immune Response in Chickens. 1. Antibody Production[1,2]

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ABSTRACT Studies were conducted to compare the antibody production of the Delayed Amelanotic (DAM) line of chickens with that of the line from which it originated, the Brown line (BR), and a distantly related environmental control, the Light Brown Leghorn (LBL). Total agglutinating antibody titers following immunization with sheep red blood cells (SRBC) or *Brucella abortus* (BA), were determined at 4, 6, 8, 10, 12, 16, and 20 weeks. The SRBC titers of DAM line birds were significantly higher than those of LBL birds but not BR birds at young ages (4 to 8 weeks) and at 20 weeks. The BA titers of DAM and BR lines were significantly higher than those of the LBL line at 4 and 6 weeks. We conclude that the DAM line exhibits hyperreactivity in B-cell function, which may be related to the line-associated pigmentation destruction. These studies also confirm that age is an important factor in making comparisons of antibody production between different genetic stocks.

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INTRODUCTION

The Delayed Amelanotic (DAM) line of chickens has been developed by selection for spontaneous loss of feather pigmentation and blindness (Smyth *et al.*, 1981). We have demonstrated that the bursa-dependent compartment of the immune system plays an important role in the development of the disease pathology in this line (Lamont and Smyth, 1981). Neonatal surgical bursectomy significantly decreases both the incidence and severity of feather amelanosis. Additionally, there is a within-line association between elevated antibody responses to foreign antigens and the incidence and severity of pigmentary and visual defects in the DAM line (Lamont *et al.*, 1982). To investigate further the apparent antibody-mediated immune hyperreactivity of the DAM line, the present studies were conducted. Antibody production to sheep red blood cells (SRBC) and *Brucella abortus* (BA) was measured from 1 to 5 months of age in the DAM line and the line from which it originated, as well as in a distantly related environmental control line. The second paper in this series examines cell-mediated immunity in the same three lines (Lamont and Smyth, 1984).

MATERIALS AND METHODS

Genetic Stocks. Three lines of chickens maintained at the University of Massachusetts Poultry Research Center were used in these studies. The Light Brown Leghorn (LBL) and Brown (BR) lines have been described in detail previously (Smyth and Somes, 1965). Briefly, the LBL line was kept as a closed population for over 25 years, always producing typical offspring of uniform LBL coloration. The first BR line birds were observed to segregate from an F2 mating of LBL and White Plymouth Rocks. The brown segregates formed the base of the synthesized BR line and have been kept as a closed population for over 20 years.

The DAM line was initiated in 1971 with a progenitor hen from the BR line, which was observed to lose normal pigmentation and become blind (Smyth *et al.*, 1981). This amelanotic and blind hen was mated to four non-pedigreed males of the BR line. The progeny of this mating were mated *inter se* to related birds of the BR line and to three unrelated stocks that segregated for a number of plumage-color genes. Backcross and F2 populations were produced, and only amelanotic birds were

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combined with intra-BR line G3 progeny to produce the genetic basis of the DAM line. The DAM line birds used in the present studies were from the tenth generation selected for loss of normal feather pigmentation. All three lines were housed together in floor pens with food and water available *ad libitum*.

Immunization with Sheep Red Blood Cells and Hemagglutination Assay. Groups of 8 to 14 birds were injected with .3 ml of 10% washed SRBC in saline. Injections were given intramuscularly at 1, 2, 3, 5, 7, 9, 11, 15, and 19 weeks of age. Birds were bled from the wing vein 7 days after each immunization, beginning at 4 weeks. The blood was allowed to clot at room temperature, and serum was separated by centrifugation. The sera were tested for anti-SRBC antibody activity within 4 hr of bleeding by a direct microhemagglutination assay (Wegman and Smithies, 1966). Titers are expressed as the log 2 of the reciprocal of the highest serum dilution showing visible agglutination.

Immunization with Brucella abortus Antigen and Agglutination Assay. Groups of 8 to 14 birds were injected with .1 ml of BA antigen (Difco) in saline. Immunization and bleeding schedules and sera isolation were the same as described for SRBC antibody testing. Sera were tested for anti-BA activity by a microagglutination assay (McCorkle and Glick, 1980). Titers are expressed as for SRBC antibodies.

Statistical Analysis. Data were subjected to Harvey's Least Squares analysis, and means were separated by *t* test.

RESULTS

Sheep Red Blood Cells Hemagglutination Titers. The antibody titers to SRBC of each of the three lines tested are presented in Table 1. Saline-injected controls did not produce antibodies above background levels (titer $\leq$ 2, data not shown). At 4, 6, and 8 weeks of age, the mean SRBC antibody titer of the DAM line birds was significantly ($P<.05$) higher than that of the LBL line. The BR antibody response was intermediate to that of the other two lines. The antibody response did not differ between lines at 10, 12, or 16 weeks, but at 20 weeks both DAM and BR line titers were significantly higher ($P<.05$) than those of the LBL line.

Brucella abortus Antigen Titers. The antibody titers to BA of each of the three lines tested are presented in Table 2. At 4 and 6 weeks, the mean BA antibody titers of both the DAM and BR lines were significantly greater ($P<.05$) than those of the LBL line. The same differences approached significance ($.05<P<.10$) at 8 weeks. There were no differences between line responses to BA measured at 10 to 20 weeks.

DISCUSSION

In the present studies, antibody production differed between the three lines examined at young ages, but this difference did not persist with time. The DAM line was selected for spontaneous feather pigmentation loss and blindness. These line-associated traits have previously been positively correlated with intraline elevated antibody responses to SRBC and BA (Lamont *et al.*, 1982). It is hypothesized that DAM chickens exhibit a generalized state of hyperimmunity, which may predispose them to reaction against self-antigens. The results of the present studies support the hypothesis that DAM line birds have a hyperactive humoral immune system. These studies show a significantly higher antibody response, at young ages, by DAM line than control (LBL) line birds. This hyperactivity may be reflective of differences in the rate of development of humoral immune competence between the lines, since the significant differences appeared at young ages. Similar results were found by Kite *et al.* (1979) in comparisons of the SRBC antibody response of the Obese strain and Cornell C strain. At young ages the Obese strain chickens, which develop severe spontaneous autoimmune thyroiditis, had higher SRBC titers than C strain birds.

While the line ranking remained generally constant over time (DAM>LBL), the ability to statistically separate the mean titers disappeared after 8 weeks. A statistical difference again appeared at 20 weeks for SRBC response but did not reappear for BA response throughout the remainder of the testing period. Differences between line responses, which were separable at younger ages, were no longer discernible using older birds. Thus, in order to accurately profile differences in antibody production between different stocks, it may be necessary to test the response at a variety of ages. McCorkle and Glick (1980) demonstrated a decline in SRBC antibody production with age but no decline in BA antibody levels. The present studies differ in that both antibody

TABLE 1. *Antibody titers to sheep red blood cells (least squares means ± standard errors)*

	Age in weeks					
4	6	8	10	12	16	20
5.05^{a} ±.54	3.72^{a} ±.54	5.71^{a} ±.49	6.57 ± .49	5.53 ± .54	4.35 ± .68	3.
6.13^{ab} ±.46	4.00^{ab} ±.46	6.50^{ab} ±.50	6.25 ± .46	5.50 ± .46	3.82 ± .68	5.
7.02^{b} ±.40	5.18^{b} ±.36	7.07^{b} ±.35	6.07 ± .35	5.93 ± .35	5.13 ± .36	5.

the same column with different superscripts are significantly different ($P<.05$).

t Brown Leghorn; BR, Brown; and DAM, Delayed Amelanotic.

TABLE 2. *Antibody titers to Brucella abortus (least squares means ± standard errors)*

	Age in weeks					
4	6	8	10	12	16	20
5.85^{a} ± .36	5.57^{a} ± .36	5.13 ± .33	5.40 ± .36	5.79 ± .36	5.50 ± .33	5.
7.71^{b} ± .32	7.13^{b} ± .33	5.94 ± .36	5.63 ± .33	6.85 ± .36	6.34 ± .36	5.
7.82^{b} ± .31	7.07^{b} ± .28	6.35 ± .28	6.00 ± .25	6.50 ± .25	6.66 ± .26	5.

the same column with different superscripts are significantly different ($P<.05$).

t Brown Leghorn; BR, Brown; and DAM, Delayed Amelanotic.

levels declined slightly after a peak at 1 to 2 months.

It is possible that the difference in antibody response between the lines is due to random genetic drift rather than phenotypic selection for spontaneous loss of feather pigmentation. The initial few generations in the development of the DAM line did consist of relatively small populations. However, the previously cited (Lamont *et al.*, 1982) association of amelanosis and elevated antibody production with the DAM line does not support the argument of genetic drift as the sole basis for hyperactive humoral immunity in the DAM line.

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