Antibody response to sheep erythrocytes in selected and unselected lines of Japanese quail

Antikörperlreaktion auf Schaf-Erythrozyten in selektierten und unselektierten Wachtellanien

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1. Introduction

Biological organisms are limited in resources available during development (SIEGEL et al., 1982; DUNNINGTON, 1990). These resources must be divided among such demands as growth, immune response, reproduction, and maintenance. Natural selection favors individuals with intermediate values for many traits. Such individuals show greater adaptability in a changing environment (e.g., HALDANE, 1954; SIEGEL and DUNNINGTON, 1987; DUNNINGTON et al., 1989). If selection favors one component of development, it may reduce resources available for other demands (DUNNINGTON, 1990). This type of imbalance can result in a negative correlation between the selected trait and an unselected trait which may affect fitness (RENDEL, 1963). For example, a negative correlation between growth and immunoresponsiveness as measured by antibody response to sheep erythrocytes has been demonstrated in several experimental lines of chickens (MARSTELLER et al., 1980; SIEGEL et al., 1982; van der ZIIPP et al., 1988) as well as in commercial broilers (SIEGEL et al., 1984; 1989).

The objectives of this study were to examine the kinetics of immune response to sheep erythrocytes in a randombred population of Japanese quail and to examine the relationship between immunoresponsiveness and selection for high body weight in this species.

2. Materials and methods

2.1 Genetic stocks

Japanese quail used in these experiments were from a line selected (22 generations) for high 4-week body weight (HW) and the randombred control line (C) which was the base population for the selected line (DARDEN and MARKS, 1988). At hatch chicks were placed in chick brooders. At 3 weeks of age, they were sexed according to breast plumage color and transferred to colony cages where they remained as sex-separated flocks until the conclusion of each trial. Feed and water were provided ad libitum throughout the experiments.

2.2 Sampling and laboratory procedures

Ten cc of blood were collected from one sheep and mixed with 20 drops of EDTA anticoagulant. The blood was then centrifuged to separate plasma and red blood cells (SRBC). Once the plasma was drawn off, physiological saline was used to wash the cells 3 times, saline was removed, and SRBC concentrations were made. Injections of antigen and bleedings were via the jugular vein. During bleedings, a sample of .5 ml of blood was drawn, transferred to a tube containing 2 drops of anticoagulant (Sequester-Sol), and refrigerated to allow the quail red blood cells to settle. If sedimentation was not complete, samples were centrifuged to separate plasma and erythrocytes.

Antibody titers were determined by the microtiter method of WEGMANN and SMITHIES (1966). Each well of a 96-well titer plate was filled with 25 µl of physiological saline. Plasma from each sample (25 µl) was added to the first well of each row on the titer plate. Then, half (25 µl) of the solution from the first well was transferred to the next well and mixed. The half dilution of the previous well was repeated for the entire row. Finally, 25 µl of sheep blood (.75 % suspension) was added to all wells. Titer plates were incubated at 37°C for 3 to 5 hours. Antibody titers were expressed as the log2 of reciprocal of the last dilution in which there was agglutination (WEGMANN and SMITHIES, 1966).

Antibodies resistant and sensitive to 2-mercaptoethanol (2-ME) were determined by microtiter methods (DELANHTY and SOLOMON, 1966). Twenty five µl of plasma and an equal amount of .15 M 2-ME were mixed and diluted in plastic titer plates. Each well in all plates contained 25 µl of normal saline prior to the addition of plasma and 2-ME. Titer plates were then incubated at 37°C for 1 hour. After incubation, 25 µl of .75 % suspension of SRBC was added to each well. Plates were then incubated for an additional 3 to 5 hours. Antibody titers were expressed as log2 of reciprocal of the last dilution in which there was agglutination (WEGMANN and SMITHIES, 1966). These titers were recorded as 2-ME resistant antibody (IgG) and differences from the SRBC total titers were recorded as the 2-ME sensitive antibody (IgM).

2.3 Trial 1

A kinetics study was conducted to determine the immunological response to SRBC for the C line. Quail were randomly assigned to one of three groups, each containing 84 individuals. At 28 days of age, quail in each group were inoculated with .1 ml of either .025, .25, or 2.50 % suspension of SRBC antigen. On the same day, prior to inocula-
within line into two groups and injected at 28 days of age with 154 MILLER et al., Antibody response to sheep erythrocytes status with no individual bled more than once.

Fig. 1. Primary and secondary antibody titer means with differing concentrations of SRBC antigen in control line quail (a b c, the different letters denote differences among means within an age P < .05). For secondary responses, each group of birds was divided and half received a .1 ml booster of 2.50 % SRBC (B) while the others were not injected (N).


Fig. 2. Primary and secondary MER titers as a percentage of total antibody titer in boosted (B) and non-boosted (N) control line quail with differing primary antigen concentrations (a b c, the different letters denote differences among means within an age P < .05)

Abb. 2. 1. und 2. MER-Titer in Prozent der gesamten Antikörper- Titer in der Kontrolle bei einmaliger (N) und wiederholter (B) Behandlung.

tion, 12 quail were bled to determine a base titer level for the line. Antibody determinations were made on 12 quail from each dosage on alternate days from day 3 to 13 following inoculation. No individual was bled more than once during this period.

On day 17 post-primary injection (PPI), half of the quail from each dosage used in the previous section (n = 126) were injected with a .1 ml of the 2.50 % concentration of SRBC antigen. This concentration was chosen because of its tendency to result in a higher antibody titer during the measurement of the primary response. Blood was then collected from both the reinjected and non-boostered quail on alternate days from day 19 to 27 and on day 31 PPI. The sample at each bleeding consisted of 6 quail per line and reinjection status with no individual bled more than once.

2.4 Trial 2
Ninety-four HW and 64 C line quail were randomized within line into two groups and injected at 28 days of age with .1 ml of either .25 % or 2.50 % suspension of SRBC.

Eight individuals from each line were bled prior to injection to obtain base titer levels for the populations. On days 4, 7, 10 and 13 PPI blood samples were obtained from 12 HW and 8 C line quail. No individual was bled more than once during this period.

2.5 Statistical analyses
All titers were transformed to the square root of the log2 before analyses were performed using the general linear model procedure (SAS INSTITUTE, 1985). When significant differences were found for main effects having more than two means, Duncan’s multiple range test was conducted for comparisons among means.

Analysis of variance for Trial 1 was performed within sampling day with dosage concentration (.25, .25, and 2.50 %) as the main effect in the model. In Trial 2, analysis was performed within sampling day with line (HW and C) and dosage concentration (.25 and 2.50) as main effects. Interactions between the main effects were also tested.

3 Results
3.1 Trial 1
Because sexual dimorphism in antibody titers was observed for only one sample, data for sexes were pooled. At most sampling times PPI, total antibody titers to SRBC were higher for antigen concentration of 2.50 than .25 and .25 % (Fig. 1). Similarly, titers for the .25 % concentration were consistently lowest. Kinetics of the PPI response was similar for all 3 dosages with peak titers observed on day 7. Presence of MER antibodies PPI was very low (Fig. 2). Some MER was present at days 7 and 9 at the two higher concentrations (.25 and 2.50 %) and also at days 11 and 13 at the highest concentration (2.50 %). Because of the low values for MER, results for MES were essentially the same as those for total antibody.

Antibody titers increased following reinjection of SRBCs on day 17 (Fig. 1). Concentrations of SRBC used in the primary inoculation influenced secondary responses with differences among titer means for the primary injection concentrations present on day 25 (Fig. 1). Comparisons among concentrations showed highest titers at the 2.50 % dose. Levels of MER also increased after reinjection with peak PSI on days 4 and 6 (days 21 and 23 PPI). The percentage of
3.2 Trial 2

Interactions between lines and doses were not significant for this trial. Differences between lines (Fig. 3) at the concentration of 2.50 % SRBC were attributed to a lack of persistence in antibody titers in line HW with low values noted by 10 days after injection. Differences between dosages of antigen were present in line HW but not in line C. Also, quail in Line HW reacted in a dose-dependent manner to SRBC antigens, but those in Line C did not. Significant amounts of MER were not present, therefore, analysis of variance for MES antibodies yielded the same results as those for total SRBC titer.

4. Discussion

Response of Japanese quail to an intravenous injection of SRBC differed in some respects from that observed in chickens. Dosage concentrations which mask genetic differences when used in chickens (van der Zijpp, 1983; Ubos et al., 1985) were necessary to elicit an immune response in Japanese quail. Apparently quail are able to tolerate large amounts of foreign antigen (SRBC) without triggering a large humoral response.

The different pattern of MER observed between primary and secondary immune responses in quail is consistent with that reported for chickens (van der Zijpp et al., 1983; Ubos et al., 1985). Although large increases in MER proportions occurred after reinjection, results should be regarded with caution because the literature is inconsistent concerning the proportion of total titer which is due to the IgG antibodies (Yamamoto and Gluck, 1982; van der Zijpp, 1983; van der Zijpp, et al., 1983).

The inability of line HW quail to maintain high antibody levels to SRBC antigen when compared to the randombred control line from which they originated is consistent with the negative correlation between growth and immune response reported for chickens (Siegel et al., 1982; Martin et al., 1988; van der Zijpp et al., 1988). These results support the thesis, that through selection, resources may be redirected to enhance responses for the selected trait (Dunnington, 1990). This redirection has a cost in that emphasis for other important functions is reduced.

Summary

Kinetics of primary and secondary immune responses were evaluated in a randombred control (C) line of Japanese quail. Primary responses for line C were also compared with those from a line selected for high (HW) 4-week body weight which originated from line C. In the kinetics study, independent sampling of quail was made on alternate days from day 3 to 13, 19 to 27, and day 31 PPI. Half of the quail sampled on day 19 and later received an additional injection of SRBC antigen. Samplings for comparisons between line C and line HW were obtained on days 4, 7, 10, and 13 PPI. Plasma from each blood sample was examined for total, mercaptoethanol resistant (MER), and mercaptoethanol sensitive (MES) titers. At most times PPI, antibody titers were highest for antigen concentration 2.50 %. Presence of MER antibodies was very low PPI, but increased following reinjection. Persistence of antibodies to the 2.50 % SRBC antigen was less in line HW than in line C from which HW originated.

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Zusammenfassung


Stichworte:
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Literature

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