KINETICS OF 2-ME ANTIBODIES TO LOW AND HIGH SRBC ANTIGEN DOES IN
CHICKENS

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Abstract
Temporal pattern of 2-ME antibody response to SRBC antigen doses following primary and secondary immunizations were measured in laying chickens. Antibody titers were measured after intramuscular injection with 0.5mls of 0.25%, 2.5% and 25%SRBC as treatment 1, 2 and 3 respectively. A booster dose 0.5mls of 2.5% SRBC was injected to birds in all treatment groups on day 25. Birds were bled on day 1.5,10,15,20 and 25 after primary immunization and on day 5,10 and 15 after secondary immunization. Kinetics of IgG and IgM responses differed significantly (P<0.05) following primary immunization and differed (P<0.01) following secondary immunization. Antibody responses to booster dose differed significantly (P<0.01) and booster effect was observed with 2.5%SRBC dose. The total and 2-ME antibody responses were dose dependent especially after primary immunization. Significance of the results in vaccination programmes was discussed.

Keywords: SRBC antigen, Antibody, IgG, IgM,

Introduction
Antigens stimulate immunological stem cells to proliferate and produce populations of antibody forming cells and reserve of immunologically activated precursors and memory cells which respond to subsequent antigen challenge (Seto,1970).

The dose of sheep red blood cells (SRBC) antigen administered and the route of administration were shown to exert varying effects on the synthesis and distribution of antibodies (vander Zijpp,1978; Donker,1989; Kreukniet and vander Zijpp,1990).The relationship between primary and secondary immune responses to SRBC doses varies in poultry (Ubosi and Abubakar,1996; Abubakar and Ubosi,2002). Kreukniet and vander Zijpp(1990) reported that dosage of primary response influenced secondary response to SRBC in chickens.

Mercaptoethanol (2-ME) is used commonly to differentiate between the different major classes of immunoglobulins (IgG and IgM) antibodies in poultry (vander Zijpp,1990). IgG is found to be most abundant in chicken (Martin,et al.,1973), pigeon and turkey (Gourdsward et al.,1977). It is also abundant in eggwhite. It is transported from hen to egg yolk to embryo (Leslie,1975).

Generally, there is an initial production of IgM antibody followed by a later production of IgG in young chickens, but could appear as early as IgM (Delhanty and Solomon,1966). Primary response of adult chicken was shown by Riha and Sviku lis (1964) to be predominantly IgM.

Differences in IgG and IgM antibody responses within and between poultry populations selected for high and low antibody productions to SRBC have been reported. (Kreukniet and vander Zijpp,1990). Macrophage activity, rate of multiplication and maturation of lymphocytes have been shown to cause some of these differences. Adequate information about the effects of high and low doses of SRBC on the pattern of IgG and IgM classes of immunoglobulins in laying chickens is lacking. The objective of this study was therefore to determine the effects of low and high doses of SRBC on synthesis and kinetics of IgG and IgM antibodies in laying chickens.
Materials and Methods

Experimental Animals and Design

A total of 90 adult laying birds raised on deep litter management system were used for the study that lasted 8 weeks. The birds were randomly divided into 3 treatment groups of 30 birds with 3 replications. The design of the experiment was randomized block design.

Preparation of SRBC antigen

Whole blood (20mls) was obtained from 2 apparently healthy Yankasa sheep. The blood was centrifuged at 4000rpm for 10 minutes and the suspended red blood cells were washed 3 times in physiological saline (0.9NaCl). The supernatant was carefully aspirated leaving the packed red blood cells. The suspended red blood cells were stored at 4°C for 7 days to increase its immunogenicity (Gross, 1979). Desired concentration was achieved and volume required were calculated using the standard formula by Nwosu (1984)

\[ X = \frac{P \times V}{H} \]

- \( P \) = % red blood cells desired
- \( V \) = volume of red blood cells desired
- \( H \) = PCV after final wash
- \( X \) = volume to be added to physiological saline

Immunization of Birds

Birds were injected (primary immunization) intramuscularly, IM, with 0.5mls of 0.25%, 2.5% and 25% SRBC as treatment 1 (T1) and treatment 2 (T2) and treatment 3 (T3) respectively. A second dose 0.5ml of 2.5%SRBC was injected intramusulary into each bird on the 25th day post-primary immunization as a booster dose (secondary immunization).

Preparation of Serum

On 0, 5, 10, 15, 20, 25 days post-primary immunization, 9 birds from each treatment group were bled from the brachial vein. Different set of birds from each group were further bled on 5, 10 and 15day post-secondary immunization. Syringe was rinsed in Ethylene diethylenetetramin (EDTA) solution before bleeding to prevent clotting of blood in syringe. Blood was transferred into a non-EDTA tube to allow for clot formation. Clotted blood was centrifuged at 4000rpm for 10 minutes to obtain serum.

2-ME treatment

Part of serum obtained was treated with 0.5M70 2-ME prior to haemagglutination Assay. Treatment of the serum with 2-ME was done by mixing equal volumes of serum (50ul) and 0.5M 2-ME in microtitre plate well. The mixture was covered and incubated at 37°C for 1hour prior to serial dilutions.

Haemagglutination Assay

Haemagglutination titer was obtained for both the treated and untreated serum. The titer was measured by the haemagglutination method of vander Zipp and leenstra (1980). Briefly, serial dilution of the serum was made in microtitre plate wells. Using initially 50ul serum and 50ul physiological saline. An aliquot 50ul of the suspended SRBC was pipetted and titrated in the plate well against the diluted serum. The microtitre plate was covered and incubated at 37°C for 4hrs. Antibody titre were expressed as log2 of the highest dilution giving complete agglutination.

The titers obtained for treated serum were termed 2-ME resistant titers (IgG). The reduction of the total antibody titer due to 2-ME treatment is called 2-ME sensitive (IgM) antibody titer (Delhanty and Solomon, 1966).

Statistical Analysis

Analyses of variance were used to test for differences among treatment groups (Steel and Torrie, 1980). When differences were found means were separated by Least Significant Difference (LSD)
Results

Responses of the three SRBC dose levels are shown in Tables 1-2 and Fig.1-3. Antibody production commenced immediately and increased rapidly reaching a peak at day 10 and lasted for about 15 days after which it declined.

Table 1. Primary and Secondary Total titers of chickens immunized with different doses of SRBC.

<table>
<thead>
<tr>
<th>Doses of SRBC</th>
<th>Primary response, Days after immunization</th>
<th>Secondary response, Days after immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>0.25%</td>
<td></td>
<td>1.30b</td>
</tr>
<tr>
<td>2.5%</td>
<td></td>
<td>1.30b</td>
</tr>
<tr>
<td>25%</td>
<td></td>
<td>1.30b</td>
</tr>
<tr>
<td>SED</td>
<td></td>
<td>0.39</td>
</tr>
</tbody>
</table>

Table 2. Primary and secondary 2-ME titers of chickens immunized with different doses of SRBC.

<table>
<thead>
<tr>
<th>Doses of SRBC</th>
<th>primary response, Days after immunization</th>
<th>secondary response, Days after re immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>0.25%</td>
<td></td>
<td>1.20b</td>
</tr>
<tr>
<td>2.5%</td>
<td></td>
<td>1.10b</td>
</tr>
<tr>
<td>25%</td>
<td></td>
<td>2.00c</td>
</tr>
<tr>
<td>SED</td>
<td></td>
<td>1.33</td>
</tr>
</tbody>
</table>

Total antibody titer

On day 5 PPI, the primary titer for 2.5% and 25% SRBC were significantly higher (P<0.01) than that for 0.25% SRBC. On day 10PPI, peak titers for 0.25% SRBC were attained. The primary titer for 25% and 0.25% SRBC was significantly higher (P<0.001) than those for 2.5% and 0.25% SRBC. On day 15PPI, peak titer for 25%SRBC was obtained and was significantly higher (P<0.01) than the titer for 0.25%SRBC only. On day 20PPI, 25% SRBC induced significantly higher (P<0.01) titer than for 2.5% and 0.25% SRBC. On day 25PPI, which was the day for secondary immunization, no significant differences were recorded.

On day 5PSI, peak titer for all the SRBC dose levels were recorded, but there was no significant difference between them. A booster effect was observed only with 0.25%SRBC. On day 10PSI, no significant differences were observed. On day 15PSI, no significant differences were observed.

2-ME antibody titer

No IgG antibodies were detected on day 0 of primary immunization. On day 5 PPI, IgG titers for 0.25% and 2.25% were significantly lower (P<0.05) than that for 25%SRBC. On day 10PPI, peak titers for 0.25% and 2.25%SRBC were obtained. All the titers were significantly different (P<0.05) from one another. On day 15PPI, peak titer for 25%SRBC was attained. On day 20PPI, titer for 25%SRBC was significantly (P<0.05) higher than for 2.5% and 0.25%SRBC. On day 25PPI, titers for 25% and 2.5%SRBC were significantly (P<0.05) higher than that of 0.25%SRBC.

On day 5PSI, secondary peak titers for all the dose levels were attained. On day 10PSI, titer for 25%SRBC was significantly higher (P<0.05). On day 15PSI, both 0.25% and 2.5%SRBC had significantly higher (P<0.05) titers than for 25%SRBC.
On day 5 PSI, secondary peak titers for all the dose levels were attained. On day 10 PSI, titer for 25% SRBC was significantly higher (P < 0.05). On day 15 PSI, both 0.25% and 2.5% SRBC had significantly higher (P < 0.05) titers than for 25% SRBC.

Fig 1: Total Antibody, IgG and IgM responses to 0.25% SRBC in Chicken

Fig 2: Total Antibody, IgG and IgM responses to 2.5% SRBC in Chicken
Discussion

The dose levels used in this study did induce humoral immune response. It is believed that early immune response contributes immensely to development of disease resistance. Higher antibody titers produced by the group that received higher antigen doses agreed with earlier observations of van der Zijpp and Leenstra (1980), Ubosi (1984), Donker (1989) and Kreukniet and van der Zijpp (1990) that a very high antigen dose of SRBC antigen produced the maximal attainable response in chickens. However, initial dosage of 0.25% developed peak titer earlier in this study. Significant interaction between dose and period of antibody production, post immunization, was also observed in the study. Indicating that primary antibody response was greatly influenced by primary immunization dose. This agreed with the results of Boa-Ampsonsem et al. (2001) and Koenen et al. (2002) in selected lines of chickens.

IgG and IgM responses following secondary immunization showed that priming with 25% or 2.5% SRBC could result in poor secondary response when the booster dose was 2.5%. A booster effect was seen when the priming dose was lower (0.25%). This might mean that the optimal dose or threshold for the breed of chickens used was perhaps lower than 0.25% dose. This could also mean that the breed of the experimental chickens were perhaps selected and bred to respond to low priming antigen challenges. This could favour vaccination against infectious diseases.

The route of immunization is believed to influence the rate of response. Appearance of IgG was relatively late in this study when compared with the report of Martin et al. (1989) and Boa-Ampsonsem et al. (2001). Although the IgG antibodies significantly persisted within dose, as was observed by van der Zijpp et al. (1980), 0.25% and 2.5% SRBC dose level did not differ significantly. This was because relatively high dose of SRBC was known to favour IgG production (Ubosi et al., 1985). IgM synthesis preceded that of IgG and by the time IgG was detectable, IgM level was reducing persistently in the response period. (Fig 1-3). Even when IgM titers peaked IgG titers were detected. The significant interaction between dose and day observed in this study agreed with the observation of Donker (1989).

Anamnestic response (booster effect) was only seen with the 0.25% SRBC antigen dose because this dose was lower than the booster dose. Therefore, a higher re-immunization dose is therefore necessary to elicit or evoke anamnestic effect of IgG and IgM responses in chickens.
In conclusion, early immune response especially proportion in the production of IgG and IgM and other immune mechanisms may all contribute to development of infectious disease resistance in chickens. Temporal patterns in response to various levels or doses of antigen challenges are important in poultry vaccination programmes. A rapid, reliable, consistently high response to a low antigenetic challenge is desirable for an effective protection of chickens against infectious diseases.

References


