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DIFFERENCES IN HUMORAL IMMUNITY BETWEEN LEGHORN, WHITE BALADI AND THEIR CROSSES

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SUMMARY

Antibody response to primary and secondary immunization with Newcastle disease virus (NDV) and sheep red blood cells (SRBC) antigen was studied in male of Leghorn, White Baladi and their Cross chicks. At 7and 22 days of age, the chicks were vaccinated against NDV by Hitchner B1 and Lasota strain vaccine, respectively. At 35 and 56 days of age, chicks were injected with 0.2 ml of 10% SRBC suspension via brachial vein. To detect primary and secondary anti-NDV and SRBC antibody titer, blood samples were drown at 3,6,9 and 12 days post first and second immunization with each antigen. Correlation coefficients were calculated between primary and secondary antibody response. In general, Leghorn chicks had significantly superior primary and secondary anti-NDV antibody titer over both White Baladi and cross chicks, but no significant differences were observed between antibody titer of White Baladi and cross chicks. On the other hand, no differences were observed in pooled means of primary antibody titer against SRBC between genotype. However, White Baladi chicks had significantly the highest secondary anti-SRBC antibody level as compared to Leghorn or cross chicks. Cross chicks occupied second with significantly difference than Leghorn chicks. The results of this experiment show that correlated response between primary and second response were not always observed.

Keywords: Newcastle disease virus, sheeep red blood cells, antibody response

INTRODUCTION

Genetic variation among hosts in the response to avian infectoin disease is well established (Jeffers and Shirley, 1982, Clare *et al*, 1989). It is well documented that genetic variation in the antibody producing ability bears a relation to resistance to infection (Inooka *et al.*, 1984). Moreover a correlation has been described between genetic variation and antibody producing ability to several related antigens (Adams and Sobey, 1962; Sellei and Rendel, 1968 and Parmentier *et al.*, 1998). The B blood group system, discovered by Briles and Cowerkers (1950) and established as the marker for chicken major histocompatibility complex (Schierman and Nordiskog, 1961). This has been shown to control immune response and disease resistance (Caron *et al.*, 1997). Immune response has also been linked to alloantigen systems (non-B system) (Lepage *et al.*, 2000).

In most studies sheep red blood cells (SRBC) or Newcastle disease virus (NDV) were chosen as the immunizing agent. The SRBC is a complex, multideterminant natural antigen provoking a T-B cell dependent antibody response (Van der Zijpp *et al.*, 1983). NDV is a contagious viral disease caused by an avian paramyxo virus. It causes a significant economic loss, to poultry producers, as it leads to high mortality or impadity. NDV is a pathogenic agent that has a provoking T-B cell dependent antibody response as well as cell mediated immune response.

The aim of the present work was to compare the primary and secondary immune responses against both SRBC and NDV antigens between Egyptian native fowl (White Baladi), Leghorn and their crosses.

MATERIALS AND METHODS

This study was carried out at the Poultry Research Center, Department of Animal Production, Faculty of Agriculture, Cairo University. One day old White Baladi , Leghorn and their Crosses (Leghorn males X White Baladi females) chicks were used in this study.

General Management

At hatch, chicks were wing banded and weighed to the nearest gram. Chicks were placed intermingled in floor brooder pens which contained wood shaving litter.Hanging tube feeders and plastic drinkers were used. All chicks were exposed to continuous light during the first three days of age and to natural day light (about 14 hrs) thereafter. Chicks were fed a commercial starter containg 20% crude protein and 2800 k.cal ME/kg. Both feed and water were provided *ad libitum.*

Immunization and titeration against newcastle disease virus

All chicks were vaccinated against (NDV) at day 7 of age by Hitchner B_1 strain using dipping method as a route of vaccine administration. Chicks were revacinated by Lasota strain at day 22 of age using drinking water as a route of administration method. Blood samples were collected from 15 male chicks of each genotype at 3, 6, 9 and 12 days post primary (dppi) and secondry (dpsi) immunization to detect primary and secondary antibody titer, respectively. The microtitter procedure described by Beard (1980) was used.

Immunization and titeration against sheep red blood cells

Fifteen male chicks from each genotype were randomly chosen to detect primary and secondary antibody titers against SRBC. At 35 day of age, chicks were immunized intravenously with 0.2 ml of 10% SRBC suspension via brachial vein. Twenty-one days after primary immunization (at day 56 of age), chicks were reimmunized with the same antigen to detect the secondary antibody response. Blood samples were drawn at 3, 6, 9 and 12 dppi and dpsi to detect primary and secondary antibody titer. The microtitter procedure described by Van Der Zijpp and Leenstra (1980) was used.

Statistical Analyses

Statistical analyses were based on a two-way ANOVA, using the general linear model procedure of SAS (SAS Institute, 1988). Both genotype and days post immunization were used as main effects. Means were separated using the Duncan's multiple range test. The relationship between primary and secondary antibody response, at different days post immunization with each antigen (NDV or SRBC), was assessed by correlation analysis.

RESULTS

NDV antibody titers

As shown in Table (1), no significant differences were observed between primary anti-NDV antibody titer of the three genotypes at 3 and 12 dppi, while at 6 dppi cross chicks had significantly lower titers than Leghorn. At 9 dppi, the cross chicks had significantly lower antibody titer than the White Baladi chicks.

Table 1. Primary and secondary antibody levels ($\overline{X}$ SE) at different days post immunization with NDV antigen

	Primary antibody titer								Means
	Days post immunization								
	3		6		9		12		
Leghorn	4.9	0.23 ^{a*}	6.1	0.32 ^{a}	3.7	0.19 ^{ab}	3.9	0.34 ^{a}	4.6 0.14 ^{A**}
Baladi	4.3	0.23 ^{a}	5.2	0.32 ^{ab}	4.0	0.19 ^{a}	3.2	0.34 ^{a}	4.1 0.14 ^{B}
Cross	4.3	0.23 ^{a}	4.6	0.32 ^{b}	3.3	0.19 ^{b}	3.2	0.34 ^{a}	3.8 0.14 ^{B}
Means	4.5	0.17^{B}	5.3	0.17^{A}	3.6	0.17^{C}	3.4	0.17^{C}	
Secondary antibody titer									
Leghorn	7.4	0.18 ^{a}	5.2	0 .45 ^{a}	5.6	0.66 ^{a}	4.8	0.56 ^{a}	5.7 0.34 ^{A}
Baladi	6.4	0.18 ^{b}	5.9	0.45 ^{a}	3.6	0.66 ^{a}	2.6	0.56 ^{b}	4.6 0.34 ^{B}
Cross	6.7	0.18 ^{b}	5.4	0.45 ^{a}	4.0	0.66 ^{a}	2.6	0.56 ^{b}	4.6 0.34 ^{B}
Means	6.8	0.3 ^{A}	5.5	0.3 ^{B}	4.4	0.3 ^{C}	3.3	0.3 ^{D}	

* values followed by different superscript within days post immunization and within type of immunization are significantly different ($P < 0.05$)

** pooled means within each response or days post immunization followed by different superscript are significantly different ($P < 0.05$)

n = 15

The Leghorn chicks produced significantly higher secondry antibody levels at 3 and 12 dpsi than that of both Baladi and the croos chicks. On the other hand, no differences were observed between White Baladi and the cross chicks at any dpsi. Irrespective of sampling time, Leghorn chicks had significantly superior pooled primary and secondary antibody titer over both White Baladi and Cross chicks. On the other hand, there were no significant differences between pooled means of primary or secondary antibody titers of the cross and White Baladi chicks.

SRBC antibody titers

Primary and secondary anti-SRBC antibody titers at different days post immunization are shown in Table 2. The results demonstrate that cross chicks produced the lowest level of antibody. These differences were significant at 3 and 6 dppi from the White Baladi chicks.While the difference from Leghorn chicks was significantl at 3 dppi. On the other hand, Leghorn chicks produced intermediate antibody level. No differences were observed in pooled of primng antibody titer between genotypes.

Table 2 Primary and secondary antibody level ($\bar{X}$ SE) at different days post immunization with SRBC antigen

	Primary antibody titer								Mean	
	Days post immunization									
	3		6		9		12			
Leghorn	3.1	0.40 ^{a*}	7.1	0.43 ^{ab}	4.3	0.42 ^{a}	2.7	0.37 ^{a}	4.6	0.29 ^{A**}
Baladi	3.4	0.43 ^{a}	8.1	0.47 ^{a}	4.4	0.44 ^{a}	2.5	0.41 ^{a}	4.3	0.31 ^{A}
Cross	2.9	0.46 ^{b}	6.6	0.50 ^{b}	3.6	0.40 ^{a}	2.1	0.45 ^{a}	3.8	0.33 ^{A}
Mean	3.1	0.24 ^{C}	7.4	0.24 ^{A}	4.1	0.24^{B}	2.4	0.24 ^{C}		
Secondary antibody titer										
Leghorn	1.7	0.41^{b}	5.2	0.28 ^{b}	3.8	0.40 c	2.9	0.36 ^{b}	3.4	0.26 ^{C}
Baladi	5.6	0.43 ^{a}	7.2	0.29 ^{a}	7.1	0.42 ^{a}	6.1	0.38 ^{a}	6.5	0.28 ^{A}
Cross	4.6	0.46 ^{a}	7.0	0.31 ^{a}	5.6	0.46 ^{b}	5.5	0.41 ^{a}	5.6	0.30 ^{B}
Mean	3.9	0.3 ^{C}	6.4	0.3 ^{A}	5.4	0.3^{B}	4.7	0.3 ^{B}		

* Values followed by different superscript within days post immunization and within type of immunization are significantly different (P< 0.05)

** Pooled means within each response or days post immunization followed by different superscript are significantly different (P < 0.05)

n = 15

The results also showed that secondary antibody response (anamonstic) to SRBC were affected by genotype (Table 2). During the entire post secondary immunization period, White Baladi and cross chicks had higher anamonstic titer as compared to Leghorn chicks. The only significant difference between White Baladi chicks and cross chicks was observed at 9 dpsi

The pooled secondary antibody titer was signficantly higher for the White Baladi chicks than both the Leghorn and Cross chicks.

Correlation coefficients

No apparent significant phenotypic correlation trends were observed between primary and secondary immunization. However, most of the correlations were negative (although not significant) (Tables 3 and 4).

Table 3. Correlation coefficients of primary and secondary NDV antibody titers of Leghorn, White Baladi and cross male chicks

		d PPI			
	d PSI	3	6	9	12
Strain					
Leghorn	3	- 0.39	- 0.06	- 0.25	- 0.11
	6	- 0.36	- 0.25	- 0.23	0.12
	9	- 0.19	0.12	- 0.73 *	0.66 *
	12	- 0.26	- 0.08	- 0.75 *	- 0.63 *
Baladi	3	0.36	0.28	0.32	0.41
	6	- 0.39	- 0.46	- 0.50	0.03
	9	0.38	- 0.20	- 0.19	0.63 *
	12	0.29	- 0.19	- 0.12	- 0.15
Cross	3	- 0.42	- 0.28	- 0.72 *	- 0.30
	6	0.25	0.06	0.58	0.63 *
	9	0.13	0.00	0.22	0.08
	12	0.06	- 0.24	- 0.11	- 0.06

n = 15
* $p < 0.05$
d PPI: days post primary immunization
d PSI: days post secondary immunization

Table 4: Correlation coefficients of primary and secondary SRBC antibody titers of Leghorn, White Baladi and cross male chicks

		d PPI			
	d PSI	3	6	9	12
Strain					
Leghorn	3	0.09	0.08	0.04	0.17
	6	- 0.04	- 0.29	- 0.17	- 0.15
	9	0.29	0.29	0.17	0.17
	12	- 0.16	0.09	0.23	0.14
Baladi	3	0.12	- 0.18	0.08	0.01
	6	- 0.37	- 0.14	- 0.12	0.06
	9	- 0.25	0.13	0.14	0.34
	12	- 0.25	0.10	0.16	0.13
Cross	3	- 0.38	- 0.17	0.23	0.32
	6	- 0.35	- 0.36	0.48	0.71 **
	9	- 0.39	- 0.21	0.52 *	0.63 *
	12	- 0.26	- 0.69 **	0.14	0.27

n = 15
* $p < 0.05$
** $p < 0.01$
d PPI: days post primary immunization
d PSI: days post secondary immunization

DISCUSSION

The presnt results explains the genetic variation as well as the contradicting response to both NDV and SRBC antigens. The superiority of White Baladi chicks to produce primary and secondary antibody titers against SRBC antigen was coupled with its inferiority for producing antibody titers against NDV. Legohrn chicks produced the highest antibody level against NDV with the lowest level against SRBC. This phenomenon may indicate that White Baladi chicks possess an active innate immunity (anatomic, physiological, and phagocytic barriers). These barriers interfere with the entry of NDV to reach the target cells and proliferate within them. This may result in reducing virus numbers, that can react and stimulate acquired immune response (humoral and cell mediated). On the other hand, immunization with SRBC using i.v. injection as route of administration makes this antigen by pass most innate barriers. Thus it reaches directly the secondary lymphoid organs, and reacts with the immunecompetant cells. This interpretation may indicate also that White Baladi chicks may possess a genetic make up that qualify their immune system to react against some antigens like SRBC with high effeciency.

In general, the current experiment showed that primary antibody titer against NDV and SRBC antigens increased and peaked significantly after 6 dPPI and then reduced gradually (Tables 1&2). Similar observation had been reported by Benjamini and Leskowitz (1991) and Kuby (2000). They stated that the first contact of an individual with an antigen generates a primary antibody response. This response is characterized by a latent (lag) period lasts 3-4 days followed by an exponential increase to a maximum titer which attained by 5-7 days, then antibody level eventually plateaue and thereafter decline. Secondary antibody titers against NDV peaked at 3 days after secondary immunizarion (rather than 6d peak following primary inoculation). This pattern resulted in a negative correlation between each primary and secondary titers for Leghorn chicks. However, these correlations were not significant and was not always observed in Wighte Baladi or the Cross chicks. Both primary and secondary antibody titers of SRBC peaked at 6d post each inoculation. Negative correlations between both responses were not always observed. Similar results were reported by Boa-Amponsem *et al.* (1999). They found that correlated responses in immunologic memories were not always observed. This suggests that the two types of responses might be under different genetic control. However Biozzi *et al.* (1979), indicated that both primary and secondary responses to SRBC in mice were at least partially under the same genetic control.

The benefits of repeated inoculations with the same antigen is to maintain high level of antigen-specific antibody (Boa-Amponsem *et al.*, 1999). Accelerated responses associated with re-exposure to antigens are principally due to increases in the frequency of antigen-specific immune cells (memory B and T cells) that are of higher affinity than those involved in primary responses (Roitt, 1994; Ahmed and Gary, 1996 and Kuby, 2000). Coligan *et al.* (1994) showed that the pattern of secondary response also depends on the presence of residual antigen specific antibody at the time of repeated antigen administration, which thus neutralized a proportion of the new antigen.

Ubosi *et al.* (1985) studied the kinetics of primary and secondary immunization of a pair of chicken lines divergently selected for antibody response to SRBC. They reported that antibody peaked at 3days after secondary immunization rather than

5days following primary inoculation. An anamnestic response to the secondary inoculation was, however, observed only in the low antibody response line. Secondary anti-SRBC antibody response of these lines were also studied by Martin *et al.* (1989). They found memory response only in the low response line. The influence of genetic make up on the immune response against NDV or SRBC have been documented (Takahashi *et al.*, 1984; Gyles *et al.*, 1986). Also the superiority of Egyptian native breed to produce antibody titer against SRBC, over foreign breed was also reported (Atta, 1990; El-Kaiaty, 1993; Siam, 1994; Mohamed, 1998). The variation in antibody production between strains may be attributed to the differences in immune competence activity (Kreutnient *et al.*, 1994; Scott *et al.*, 1994; Atta *et al.*, 1996).

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الاختلافات فى الاستجابة المناعية المصلية بين كتاكيت اللجهورن و البلدى الأبيض وخليطهما

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تم فى هذه الدراسة تقدير مستوى الأجسام المناعية الأولية والثانوية الناجمة عن تطعيم كتاكيت اللجهورن البلدى الأبيض الخليط بينهما بفيروس النيوكاسل وأنتجين كرات الدم الحمراء للغنم. عند عمر ٧ و ٢٢ يوم تم تطعيم الكتاكيت ضد مرض النيوكاسل باستخدام عترة هتشنر ب١ واللاسوتا على التوالى بينما فى عمر ٣٥ و ٥٦ يوم تم تطعيم الكتاكيت ٠,٢سم^3 معلق ١٠% من كرات الدم الحمراء للغنم وذلك بالحقن فى الوريد العضدى بالجناح.

ولتقدير الاستجابة المناعية الأولية والثانوية أخذت عينات دم من الطيور بعد ٣،٦،٩و١٢ يوم من التطعيم الأول والثانى بكلا الأنتيجين ، كما تم تقدير معامل التلازم بين المناعة الأولية والثانوية.

بوجه عام توضح النتائج أن كتاكيت اللجهورن أنتجت بصورة معنوية أعلى استجابة مناعة مصلية أولية وثانوية ضد فيروس النيوكاسل بالمقارنة بكتاكيت البلدى الأبيض أو الخليط ولكن لم تلاحظ اختلافات بين الكتاكيت البلدى الأبيض والخليط. من جهة أخرى لم تلاحظ أى اختلافات معنوية فى الاستجابة المناعية الأولية ضد أنتيجين كرات الدم الحمراء للغنم ولكن كتاكيت البلدى سجلت بصورة معنوية أعلى مستوى للأجسام المناعية الثانوية بينما احتلت الاستجابة المناعية الثانوية لكتاكيت الخليط المرتبة الثانية حيث أنها كانت أعلى معنوياً عن مثيلاتها فى كتاكيت اللجهورن. وأوضحت النتائج أن التلازم بين المناعة الأولية والثانوية لم يلاحظ بصفة دائمة.