ASSOCIATION OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS OF CHICKEN MHC CLASS II GENES WITH EARLY IMMUNE RESPONSE TO E. COLI

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SUMMARY

Restriction fragment length polymorphism analyses of chicken Major Histocompetibility Complex (MHC) were performed on meat-type chickens that have been divergently selected for immune response to immunization with Escherichia coli at 10 days of age. Two lines have been selected for high early antibody titer (E) and low late (L) antibody titer along with an unselected control line (C).
Southern blotting and hybridization procedures were performed on the genomic DNA, isolated from erythrocytes, using PvuII and BglII endonuclease and MHC class II prob. RFLP analysis, reveled polymorphism at the chicken class II region with both enzymes. Two DNA restriction fragment were associated with increased antibody titer to E.coli ($P<0.05$). Analyzing 36 chickens, 11 PvuII RFLP patterns and 6 BglII patterns were identified. One pattern (BC) was associated with low antibody titer, and two others (BE,BF) were associated with high antibody titer ($P<0.01$).
The results suggest that there may be an association between chicken MHC class II and and E.coli immune response. This finding may enable a rapid selection of meat-type chickens for enhanced E.coli resistence.

INTRODUCTION

In chickens, the MHC genes and their proteins have been divided into three classes, class I, II, and IV which are designated as B-F, B-L and B-G, respectivly. Several studies confirm that genes in the chicken MHC influence resistence to disease (Bacon L.D. 1987). Most of these studies were done on Leghorn chickens, using monoclonal antibodies that have been produced to antigens of each of the three subregions of the chicken MHC (Crone et al,1985; Miller et al 1982; Pink et al, 1985). The B-L antigens (Guillemot et al. 1986) are involved in regulation of immune cell cooperation (Vainio et al.,1984). Isolation of the MHC class II(B-L)β chain sequences (Bourlet ea al 1988) provided a tool to characterize the MHC at the molecular level. Soller and Backman (1986) described the potential of RFLP application to poultry breeding. The MHC, with its established role in immune response and disease resistance should show RFLP with direct effect on immunologial traits and, therfore, be of value in breeding programs.
In this study we selectd meat-type chickens for immunological trait of early and late E.coli antibody response. By RFLP analysis, using PvuII and BglII endonuclease and MHC class II probe, we examined the association between RFLP of MHC class II and E.coli antibody levels in these meat-type selected lines.

MATERIAL AND METHODS

Animals

A commercial grandparent line was used as the based population for the selection. Eighty females were randomly mated with 20 males to produce offspring for the selected and control lines. Two lines were divergently selected for early (E) or late (L) immune response to vaccination with E.coli. The "best" (lowest in line L and highest in

line E) 15 males and 30 females were selected in each generation from about 60 birds per sex within each line. Similar number of males and females were taken at random to maintain a control line (C). Seven males and about 30 females per line were mated to produce the next generation. The same vaccination, titer determination and selection procedures were used in two cycles, producing generations S_1 and S_2.

Antibody titers

The antibody titer was determined by ELISA (Leitner et al.1989). 10 day post-immunization of chicks immunized at age of 10 days (E.coli O78; K80 $2X10^9$ CFU/ml inactevated by sonication) which was injected subcutaneously (0.5ml/chick).

DNA isolation and gene detection

Isolation of genomic DNA was from peripheral blood. Digestion by PvuII and BglII endonuclease, blotting, and hybridization with DNA probe were performed as described by Hillel et al.(1989). The class II probe was provide by Dr. Pitcovsky, Israel (Xu et al.1988).

Analysis of RFLP patterns

Molecular size of the restriction fragments were determined by comparison to size markers of HindIII digested by lambda DNA (Flanagan et al. 1988). The fragments were coded P1-P10 for PvuII and B1-B8 for BglII. Identical patterns of restriction fragments were coded as PA-PF for PvuII and BA-BH for BglII.

RESULTS

Initiation of the selection and control lines

Results in Table 1 include means of males and females from each generation, cumulative selection differentials (S) and responses to selection (R) and the estimated of realized heritability. Individual values were adjusted for differences in titer levels between generations and between hatches within generations.

Table 1. Mean Ln antibdy titer of S_1 and S_2 chicks (males+females) following vaccination with E.coli, and estimated of realized heritabilities.

	Line mean		Realized heritability (R/S)		
Line	S_1	S_2	S_0-S_1	S_1-S_2	S_0-S_2
L	8.56	8.10	0.38	0.24	0.33
E	9.57	9.80	0.30	0.66	0.43

Basic structure of B-L broiler chicken RFLP patterns

1. PvuII restriction endonuclease: RFLP analysis, after digestion with the restriction endonuclease PvuII reveled polimorphism at the chicken class II region. Ten restriction fragments were presented while 2 were common to all chickens (P1 and P3), the other 8 showed differential presence . (Table 2). Out of 36 chickens that were examined , 11 PvuII RFLP patterns were identified No significant antibodies differences were found between the different patterns.

Table 2. Restriction fragments, their size and frequency in selected lines (E ,L and C) using restriction endonuclease Pvu II

Number	Fragment size (Kilobase)	Frequency (%) line E	line L	line C
P1	11.5	100	100	100
P2	7.8	66.6	70.2	68.8
P3	4.7	100	100	100
P4	4.2	0	5	3
P5	3.2	0	5	3
P6	2.8	44.4	35.3	39.5
P7	2.3	77.5	82.1	78.6
P8	1.7	100	82.1	89.2
P9	1.5	100	76.4	85.6
P10	1.2	77.4	70.6	68.5

2. BglII restriction endonuclease: Eight restriction fragments were identified using BglII endonuclease. Five fragments were common to all chickens and the other 3 showed diversity (Table 3). Screening 36 chickens, 6 different BglII RFLP patterns have been identified. Patterns distribution in the selected lines E and L are presented in Table 4. Patterns BE,BF were detected only in line E and were characterized by high antibody titer. Pattern BC was detected only in the line L and associated with low antibody titer. ($P<0.01$)

Table 3. Restriction fragments, their size and frequency in the selected lines (E, L and C) using restriction endonuclease Bgl II

Number	Fragment size (Kilobase)	Frequency (%) E line	L line	C line
B1	14.0	100	100	100
B2	11.2	100	100	100
B3	8.1	100	100	100
B4	6.8	100	100	100
B5	6.0	20	0	8
B6	4.3	36	20	30
B7	3.0	45.5	46.6	60.3
B8	2.7	100	100	100

Relationship between DNA restriction fragments and selection trait

1.Pattern analysis:

Table 4. RFLP patterns, using BglII enzyme, their mean antibody levels±S.E. and distribution in lines E,L,and C

Pattern	E. coli abtibody level	statistical grouping*	Frequency (%) line E	line L	line C
BA	9.36±1.3	bc	25	31.5	12.5
BB	9.22±1.2	bc	37.5	43.7	50
BC	7.81±0.3	b	0	18.6	8.2
BD	7.52±0.8	b	0	6.2	10
BE	10.42±0.3	c	12.5	0	12.5
BF	10.28±0.4	c	25	0	6.8

*Different letters designated statistical difference($P<0.01$).

2.Fragments analysis:
Whithin each B and P fragment group, multiple regression analysis were carried out and selection was priformed using stepwise procedure. Two fragments (B5,P9) were found to be positively associated with E.coli antibody leveles (Table 5).

Table 5. Effect of DNA restriction fragments on E.coli antibody levels

Restriction fragment	P9	B5
Fragment effect	1.08*	1.6**
Standart error	0.63	0.7

**P<0.05, *P<0.01

Discussion

This study used an MHC class II chicken DNA probe to demonstrate variation among meat-type chickens selected for early and late E.coli homural immune response. Digestion of DNA with PvuII and BglII generated RFLP patterns. Two of the BglII patterns were associated with high antibody titer to E.coli and were presented only in the E line. One BglII pattern was associated with late antibody response and was presented only in the L line. PvuII digestion reveled greater polymorphism. The small number of individuals in each line examined (N=36) prevented us from finding clear Pvu patterns that are significantly associated with either high or low antibody response to E.coli. Looking at individual bands it was obvious that two restriction fragments were associated with increased antibody titer. Those results indicated that this method, though with some limitations, can be applied in genetic selection plans. Certain DNA restriction fragments or certain pattern for the chicken MHC class II genes appear to be associated with the selection trait. It may be possible, therefore to screen animals early in life for presence or absence of a particular pattern or restriction fragment associated with class II B-L genes, and than select on that basis to improve E.coli resistance.

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