77

BARD

FINAL REPORT

PROJECT NO. US-829-84

The Role of Cell-Mediated Immunity in Genetic Resistance to Disease in Chickens

L. D. Bacon, D. Heller

1989

Date August 11, 1989

BARD
P.O. Box 6
Net Dagan, ISRAEL

-6.-8-1989

BARD Project No. US-829-84

Title of Research Project:

The role of cell-mediated immunity in genetic resistance to disease in chickens

Names of Investigators:

Principal Investigator: Larry D. Bacon

Cooperating Investigator(s): E. Dan Heller

Name and Address of Affiliated Institutions:

Principal Institution: USDA, Agricultural Research Service

Regional Poultry Research Laboratory, 3606 E. Mount Hope Rd., East Lansing, MI 48823

Cooperating Institution(s): Faculty of Agriculture, Animal Science,

Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

Project's starting date: 10/1/85

Type of Report: 1st Annual _____ 2nd Annual _____ Final X

Signatures:

Principal Investigator

Institution's Authorizing Official

Larry D. Bacon

J.C. Marten

Table of Contents

page 1	Cover Page
page 2	Table of Contents
page 3	Abstract
page 3	Objectives of Original Research Proposal
page 4	Body of Report
page 8	Description of Cooperation
page 8	Evaluation of the Original Achievements With Respect to the Aims of the Original Proposal, Emphasizing the Benefit to Agriculture
page 10	List of Publications

Abstract

In chickens several methods of testing cell mediated immunity (CMI) are known to differentiate high vs. low response in different chicken lines, and are potentially useful for selection of CMI. However, we found the height of a lines lymphocyte proliferative response to mitogens in-vitro did not correlate with the lines in-vivo tests of CMI. We therefore set out to define additional assays of CMI. Genetic differences in production of gamma interferon, and in levels of T-cells with peanut agglutinin receptors, have been identified as new measures of CMI. These may be adaptable as general methods of selection for CMI.

Chickens in an Israeli broiler strain selected at several weeks of age for high antibody response to E. coli and Newcastle disease virus (NDV) have been shown to have higher T-cell and B-cell functions than chickens of a strain selected for low response. Initial analyses for genes in the B-blood-group complex indicate the high line has different genes, or genes in different frequencies, than the low line. This and other data from special B-congenic lines in the U.S.A. suggests the B-complex genes may influence maturation of immune response. The Israeli selected strains were also studied for traits of economic importance. The selection for antibody response at an early age improved some economic traits in the high strain, e.g. it decreased mortality, but improved fertility and growth rate, in comparison to the strain selected for low immune response.

Objectives of the Original Proposal

The objectives of the original proposal were:

- A. Apply relevant existing assays and develop new assays to detect differences in cell mediated immunity between chickens of selected strains.
- B. Using a selected assay, determine if cell-mediated immunity and general antibody competence are correlated in selected Israeli strains and random commercial breeder flocks. (This objective was not achieved in the U.S. using commercial breeder flocks because a well defined assay for general cell-mediated immunity was not identified or developed in objective A.)
- C. Determine if B-haplotype influences general humoral or cell-mediated immune competence.
- D. Determine how traits of economic importance in broilers are affected by selection for protective immunity using developed Israeli strains.

Introduction

The progress for the three year period of this grant will be summarized within each of our objectives.

Methods and Materials

The methods and materials are given in the cited papers.

Results and Discussion

- A. Extensive studies were done applying relevant existing assays and developing potential new assays for cell mediated immunity (CMI). In the U.S. in-vitro mitogen and mixed lymphocyte response assays and in-vivo skin graft rejections were used to show a sex-linked gene used to sex chicks (K) did not influence CMI (1). In-vitro T-cell mitogen responses were shown to differ in some special B-congenic lines (see Table 1), and between inbred or special strains (Table 2), supporting the use of this as a possible test to use for measuring CMI in objective B. Subsequent delayed hypersensitivity tests using haptens or the mitogen PHA in the skin also showed line differences, but they did not correlate with in-vitro PHA line differences (see Tables 3,4). Therefore, we did not select any of these assays to measure general CMI in comparison to humoral immunity in commercial strains in objective B.

During the last 2 years of the grant extensive efforts were made in the U.S. to develop an assay for gamma-interferon as a measure of CMI. In humans an assay for gamma-interferon was shown to be more specific and sensitive for assaying T-cell activity than a proliferation assay (2). The production of interferon by chicken spleen cells activated with the T-cell mitogens CON-A or PHA was established at the RPRL by T. Fredericksen and J. Sharma using vesicular stomatitis virus (VSV) (3). The VSV was obtained from Philip Marcus and his methods have been adapted for detection of interferon (4). As summarized in Table 5, we adapted the assay to use peripheral blood lymphocytes (PBL). This was essential so chickens could be used as breeders following testing. We showed evidence for differences in the production of interferon by PBL in chickens of different lines. During the past year further changes were made in the assay. It was miniaturized so several tests on blood of each bird could be made with and without mitogen stimulation. Also, the results were analyzed photometrically as described by Skellick and Marcus (4). This version of the test again shows line differences in interferon production (Table 6). Considerable experimentation has been necessary to develop reliable methods, but final tests are underway defining line differences, and to gain support for our contention that this may be a useful assay for selection of optimal T-cell response in chickens.

In Israel in the last year, several mitogen tests were conducted in-vitro to measure ontogeny of CMI in lines imported from the RPRL: inbred line 151₅ (blood type B¹⁵B¹⁵), line 0 (B²¹B²¹) and the F₁ hybrid (B¹⁵B²¹). Also, an assay

for interferon (4)(10) involved addition of Phorbol Meristat Acetat and PHA to peripheral blood lymphocytes for 24 hours. Supernatants were collected and added to monolayer of chick embryo fibroblasts. After 20 hours of incubation Vesicular Stomatitis Virus (VSV) was added and 24 hours later the cytopathic effect was estimated by addition of neutral red. Uptake of the dye was measured using ELISA reader (O.D. 540nm). At 4 weeks of age there were no differences in the response of lymphocytes of two inbred lines to the mitogens Con-A, PHA, or PWM, or in interferon production, (Figs. 1-5), but the hybrid chickens lymphocytes responded much higher than their parents lines. At 10 weeks of age no significant difference could be observed in lymphocytes response to T cell mitogens, but PWM mitogen stimulation and interferon production were found to be significantly higher ($P < 0.05$) in line 0. At 20 weeks of age, lymphocytes from line 0 responded significantly higher ($P < 0.05$) to mitogens stimulation and interferon production. The intensity of the CMI responses were highest at 10-14 weeks of age.

In Israel one additional assay has been studied as a correlate of CMI. This method involves use of a lectine from peanuts termed peanut agglutinin (PNA). In mice the thymocytes that bind PNA (PNA+) are immature, give low responses to mitogen or in graft-vs.-host tests, and are cortisone sensitive, in contrast to PNA- thymocytes (5). Chickens of the Israeli high and low immune response lines were characterized for PNA+ cells in different lymphoid organs using FACS analysis. Chicks from the high line mating had a significantly lower percentage of PNA+ cells in PBL than chicks from a low line mating (Table 7). There were no differences in percent of PNA+ cells in spleens and thymuses of chicks of the different lines. A negative statistical correlation ($r = -0.55$) existed between PNA+ lymphocytes in the spleen and antibody titer to *E. coli*. This finding indicated that the lower the number of PNA+ cells in the spleen, the higher the titer to *E. coli* in the individual chick. A positive correlation ($r = +0.38$) was found between PNA+ cells in the thymus and the spleen and between PNA+ cells in the bursa and PBL ($r = +0.45$).

- B. No CMI - antibody correlation experiments were undertaken with commercial strains in the U.S. for reasons given above. In Israel no new methods of selection were tested during this grant period. However, the lines previously under development were further selected for early antibody response to *E. coli* and NDV, and chickens from these populations were used for work in objectives A-D.

In Israel several immunological traits were examined in high and low selection lines produced on the basis of response at an early age to two antigens: *E. coli* and NDV (6). As compared with the low line, the high line was found to have: a higher response to

sheep red blood cells but not to killed Brucella abortus ; generally higher levels of immunoglobulin (Ig) G but not of total Ig; greater relative bursa weight; greater number of spleen antibody producing plaque forming cells against E. coli antigen; a higher delayed type hypersensitivity response and higher in-vitro T-cell mitogenic response; and more rapid clearance of bacteria from the blood (7). In the last year, additional mitogen assays have been performed. In the high line 18.7% of the chickens responded to Con-A stimulation in the range of $200-300 \times 10^3$ cpm, whereas in the low line none of the chickens responded in that range (Fig. 5). The same results were obtained from PHA stimulation: lymphocytes from 12% of the high line birds responded in the range of $200-300 \times 10^3$ cpm while in the low line this response level was not attained (Fig. 6). Stimulation of the B lymphocytes by Pokeweed mitogen (PWM) showed that 50% of the high line chickens responded in the range of $20-80 \times 10^3$ cpm. In the low line, 37.5% of the chickens responded in that range (Fig. 7). The results of these studies indicate that both T-cell and B-cell function changed in these selection lines as a result of the selection for high and low response to antigens at an early age.

- C. Several studies were conducted to assess B-haplotype influences on general humoral or cell-mediated immune competence. In the U.S., partially developed B-congenic chicken strains that differ mainly for the B-haplotype were used to study antibody response to sheep red blood cells, to killed Brucella abortus, or to inactivated infectious bursal disease virus (IBDV) vaccine. There was no one B-type that was clearly a best responder for all immunogens, although line differences occurred for each immunogen (8). Likewise, the lines differed by cell mediated immune measures of mitogen response in-vitro Table 1).

However, the lines did not differ as responders in mixed lymphocyte reactions (Table 8), and the lines low for in-vitro mitogen responses were not inferior for in-vivo delayed hypersensitivity responses (Table 3). These results and those of others led us to conclude in a review that we could not assume one B-type leads to best immunity to all immunogens in all strains, but that some B-types will be better than others for particular immunogens in most strains (9). Studies within strains by the breeder would determine which B-type to select for the best response to the most critical pathogen(s).

In addition to the immune competence tests conducted with B-congenic strains, alloantibodies made in these strains were taken to Israel to assess B-haplotypes in the selected Israeli strains. The selected strains differed for B blood types detectable by hemagglutination using the U.S. ⁵ antisera (Table 9). In the high immune response line the B ⁵ type was identified in 12% of birds, but it was not detected in birds of

the low line. (The types are given in " " as these results are based on reactions where the sera react to common but likely not fully identical antigenic epitopes. These provisional types need to be confirmed by histocompatibility tests and antisera made within these strains, or by DNA analysis). Type B^{0} was found in 40% of the high line, but only 13% of the low line. This group was designated as "0" as no U.S. antisera agglutinated red cells from these chickens. Type B^{13} was detected in about 10% of both populations. Types B^{15} and B^{19} were detected in 24% and 9% of low line chickens respectively, but were not detected in the high line. These initial results suggest that selection of the broiler strains for early or late response to 2 broad immunogens has resulted in a shift in the frequency of genes determining B-types. This is evidence the B-haplotype may influence maturation of immune response to the immunogens used in selection, i.e. killed *E. coli* and NDV vaccine.

- D. The Israeli broiler strains selected for protective immunity have been tested for traits of economic importance (7). Mortality data were available for generations S2 and S3. In generation S2 mortality to 20wk of age was 29% for the low line, and 19% for the high line; the difference between lines was highly significant. Differences between lines over the same age period in generation S3 were 31% and 11%, respectively.

During the course of selection, data were occasionally obtained for various nonselected traits. Since these data were not obtained in a systematic manner in all generations, differences between the selected lines should be viewed with caution. Comparisons showed that the high line chickens had significantly greater fertility (but not hatchability), percent spermatocrit, semen volume, and growth rate than low line chickens (7).

References

1. Bacon, L.D., A.M. Fadly, and L.B. Crittenden, 1986. Absence of influence on immune competence by the sex-linked gene (K) determining slow feathering in White Leghorn chickens. *Avian Dis.* 30:751-760.
2. Hao, Xi-Shan, J. Le, J. Vilcek and T.W. Chang, 1986. Determination of human T cell activity in response to allogeneic cells and mitogens. An immunochemical assay for gamma-interferon is more sensitive and specific than a proliferation assay. *J. Immunol. Methods* 92:59-63.
3. Fredericksen, T.L. and J.M. Sharma, 1987. Purification of avian T-cell growth factor and immune interferon using gel filtration high resolution chromatography. Pages 145-156 in *Avian Immunology II*. W.T. Weber and D.L. Ewert, eds. Alan R. Liss, Inc., New York, N.Y.
4. Skellick, M.J. and P.I. Marcus, 1986. Induction of high titer chicken interferon. Pages 115-126 in Vol. 119 *Methods in Enzymology*, Interferons, Part C. S. Peska, ed. Academic Press, New York, N.Y.

5. Reisner, Y., M. Linker-Israeli, and N. Sharon, 1976. Cell. Immunol. 25:124-129.
6. Pitcovsky, J., D.E. Heller, A. Cahaner and B.A. Peleg, 1987. Selection for early responsiveness of chicks to Escherichia coli and Newcastle disease virus. Poultry Sci. 66:1276-1282.
7. Pitcovski, J., E.D. Heller, A. Cahaner, B.A. Peleg and N. Drabin, 1987. Immunological traits of chicks selected for early and late immune response to E. coli and Newcastle disease virus. Pages 295-305 in Avian Immunology II. W.T. Weber and D.L. Ewert, ed. Alan R. Liss, Inc. New York, N.Y.
8. Bacon, L.D., N. Ismail and J.V. Motta, 1987. Allograft and antibody responses of 15I₅-B-congenic chickens. Pages 219-233 in Avian Immunology II. W.T. Weber and D.L. Ewert, ed. Alan R. Liss, Inc. New York, N.Y.
9. Bacon, L.D., 1987. Influence of the major histocompatibility complex on disease resistance and productivity. Poultry Sci. 66:802-811.
10. Rubinstein, S., P.C. Familletti, and S. Pestka, 1981. Convenient assay for interferon. J. Virol. 37:753-758.

Description of Cooperation

In March of 1986 Dr. Bacon went to Israel and used 27 antisera specific for B-locus antigens to study the B-types in the Israeli selected strains. Additional RPRL antisera were sent to Israel on four other occasions. Dr. Bacon also described additional procedures that would need to be done with sibling chicks of the Israeli strains, tentatively identified for B-locus antigens using antisera provided by the RPRL. The procedures involved how to produce monospecific alloantisera in conjunction with skin grafting and cellular response tests.

The two labs also had a chance to plan and exchange data while presenting data at the Avian Immunology Conference in Philadelphia in July 1986, and again in October, 1987, when Dr. Heller visited Dr. Bacon's lab. After that meeting both presented data at the MHC Conference held in Ames, Iowa. Most useful were discussions on blood-typing, on methods for lymphocyte purification and analysis, and on tests of gamma-interferon production. Also, fertile eggs from two highly inbred lines at the RPRL were sent to Israel so the lines could be used by Dr. Heller and others doing basic immunology and genetic studies.

Evaluation

During the assessment of some of the current assays of CMI in chickens we demonstrated some repeatable strain differences in response to mitogens in-vitro and in-vivo. However, we were perplexed that there was not a good correlation in strain differences using different assays. Rather

than propose using one of these as a parameter for comparison with humoral immunity or selection in Objective B, we decided to work at defining an assay for gamma-interferon in the U.S., and on an assay using peanut agglutinin in Israel, to determine if one of these would be a better correlate of general CMI function. We can show line differences in gamma-interferon production in-vitro with and without mitogen stimulation, and see a possible correlation between production following mitogen stimulation and Marek's disease resistance. However, the original assay needed mineralization, adaptations for peripheral blood, and work on cell-culture methods, to be highly repeatable and potentially useful. Our gamma-interferon assay is still undergoing refinements to make it practical as a method for selection of cell mediated immunity by poultry breeders.

The possible effect on immune competence of the slow-feathering (SF) gene in White Leghorns was studied using several assays of CMI and several assays of humoral antibody response. The assay using responsiveness in mixed lymphocyte response was unique. The slow-feather trait did not have a detrimental influence on general immune competence, a fact of great importance to general poultry breeders who want to use this gene to sex chicks at one day of age. A detrimental effect of the SF gene on specific immunity to avian leucosis virus, and on general productivity of ALV infected chickens, has been clarified by the discovery at the RPRL of an endogenous viral gene linked to the gene determining the SF trait.

During this grant the Israeli poultry strains selected for antibody to E. coli and NDV at an early age were studied for CMI function. Both T-cell and B-cell function improved in the line selected for high antibody compared with the line selected for low-antibody, and this should have general applicability for agricultural animals.

Several types of experiments have shown that the MHC (B-complex of chickens) has general as well as specific influences on humoral and CMI competence. Examples of general influences included differences in mitogen response(s) (in-vitro and in-vivo) between B-congenic lines, as well as evidence that the selection for two broad immunogens in poultry in Israel resulted in strains having different frequencies of several B-complex genes. However, it is stressed that the B-complex determines specific control as well and this explains why one B-type is not the best responder in antibody production to all immunogens, and why there are so many forms of MHC genes present in many strains of chickens. This evidence for general (or broad) as well as specific influences of the MHC on immune response is likely a general rule for domestic animals.

The Israeli poultry strains selected for antibody at an early age were studied for several traits of economic importance. It is of importance that chickens in the strain selected for high immunity had less total mortality due to all causes than chickens of the low immune line after the second and third generations of selection. The high line also had better fertility, percent spermatocrit, semen volume and growth rate in some generations. Thus, the selection for antibody at an early age may actually improve some economic traits in comparison to strains selected

for low immune response, and this could be relevant for other domestic animals in addition to chickens. Data were not collected on a control unselected strain, and thus we are uncertain if positive selection actually increased values of the economic traits.

List of Publications

1. Bacon, L.D., A.M. Fadly, and L.B. Crittenden, 1986. Absence of influence on immune competence by the sex-linked gene (K) determining slow feathering in White Leghorn chickens. *Avian Dis.* 30:751-760.
2. Pitcovsky, J., D.E. Heller, A. Cahaner and B.A. Peleg, 1987. Selection for early responsiveness of chicks to Escherichia coli and Newcastle disease virus. *Poultry Sci.* 66:1276-1282.
3. Pitcovski, J., E.D. Heller, A. Cahaner, B.A. Peleg and N. Drabin, 1987. Immunological traits of chicks selected for early and late immune response to E. coli and Newcastle disease virus. Pages 295-305 in *Avian Immunology II*. W.T. Weber and D.L. Ewert, eds. Alan R. Liss, Inc. New York, N.Y.
4. Bacon, L.D., N. Ismail and J.V. Motta, 1987. Allograft and antibody responses of 15I₅-B-congenic chickens. Pages 219-233 in *Avian Immunology II*. W.T. Weber and D.L. Ewert, eds. Alan R. Liss, Inc. New York, N.Y.
5. Bacon, L.D., 1987. Influence of the major histocompatibility complex on disease resistance and productivity. *Poultry Sci.* 66:802-811.

Table-1. T-cell mitogen responses in 15.B congenic chickens.^c

Line	No. Birds	PHA-P				CON-A			
		12.5 mg	25 mg ($\bar{x}$ cpm X 1000)	50 mg ($\bar{x}$ cpm X 1000)	max	21 mg	4 mg ($\bar{x}$ cpm X 1000)	8 mg ($\bar{x}$ cpm X 1000)	max high responders (%)
.6-2 (5)	8	3.0 ^A	6.4 ^A	7.3 ^{AB}	7.6 ^{AB}	5.9 ^A	8.0 ^{AB}	5.8 ^B	12.5% ^b
.7-2 (5)	7	2.2 ^A	9.2 ^{ABC}	8.8 ^{AB}	10.1 ^{ABC}	6.0 ^{AB}	9.6 ^{AB}	8.5 ^{AB}	28 %
.15I-5 (4)	9	2.9 ^A	6.1 ^A	7.0 ^{AB}	7.5 ^A	1.0 ^A	2.8 ^A	2.2 ^A	0 %
.C-12 (2)	8	6.8 ^B	16.6 ^C	10.9 ^B	17 ^C	28.3 ^C	33.5 ^B	23.8 ^B	88 %
.P-13 (4)	8	4.6 ^{AB}	7.3 ^{AB}	7.5 ^{AB}	8.2 ^{AB}	26.9 ^{BC}	30.3 ^B	18.1 ^B	62 %
.P-19 (5)	7	2.4 ^A	9.9 ^{ABC}	7.1 ^{AB}	10.1 ^{ABC}	24.6 ^C	26.5 ^B	17.5 ^B	86 %
.N-21 (5)	8	2.3 ^A	6.1 ^A	6.1 ^A	6.9 ^A	0.7 ^A	1.6 ^A	1.6 ^A	0 %
15I-5-15	8	4.1 ^{AB}	12.7 ^{BC}	10.4 ^B	13.4 ^{BC}	2.1 ^{AB}	7.0 ^A	5.8 ^{AB}	14 %

^aArithmetic means of triplicate wells were converted to log_e and tested by Games t-test. Values within a column followed by different letters are significantly different (P > .05).

^bA bird was considered a high responder to CON-A if the maximal cpm to CON-A was greater than the maximal cpm to PHA-P.

^cThe in vitro test uses .1 ml of whole blood diluted 1:20 and .1 ml of mitogen. Cells harvested after 72 hrs. at 39.5°C.

Table 2. Chicken lines differ in response to mitogens PHA and CON-A.

Line	No.	CON-A response					PHA response				
		none	8 μ g	4 μ g	2 μ g	max.	none	50 μ g	25 μ g	12.5 μ g	max.
P	10	1.0 ^a	5.8	4.2	2.7	6.3	0.3	5.2	7.2	5.5	7.2
N	10	1.1	3.5	1.8	0.9	3.5	0.4	19.0	11.6	6.1	19.3
72	10	0.4	18.4	10.5	8.0	18.4	0.2	11.4	12.8	8.1	13.4
63	10	0.3	3.8	2.6	1.0	3.8	0.3	4.9	4.4	1.5	5.2

^a Arithmetic (CPM $\times 10^3$) means of triplicate wells for 10 birds. Harvest 48 hours.

Table-3. Response of 15.E congenics to PHA in wattle.

Line	PHA-P injection			PHA-M injections		
	Number Chickens	Mean thickness increase (mm)		Number Chickens	Mean thickness increase (mm)	
		24 Hrs.	48 Hrs.		24 Hrs.	after 2 injections
.6-2(5)	8 ¹	89 ^{B2}	14 ^{AB}	6 ³	8 ^{AB}	2.7 ^{A4}
.7-2(5)	8	82 ^B	10 ^A	6	5 ^A	3.7 ^{AB}
.151-2(4)	8	81 ^{AB}	16 ^{AB}	6	10 ^A	2.7 ^A
.C-12(2)	8	83 ^B	8 ^A	6	8 ^{AB}	6.5 ^B
.P-13(4)	8	70 ^A	11 ^A	6	5 ^A	3.3 ^{AB}
.P-19(5)	5	94 ^B	23 ^B	6	9 ^{AB}	2.3 ^A
.N-21(4)	9	80 ^{AB}	13 ^{AB}	6	6 ^{AB}	4.0 ^{AB}
.P-15/15(10)	8	77 ^{AB}	21 ^B	not done	6 ^{AB}	not done

¹ Male chickens injected at 8 weeks of age with 0.1 ml of saline in the left wattle and 0.1 ml (500 ug) of PHA-P in the right wattle. The saline injected wattles did not swell significantly, and saline injected wattles were equal in thickness at all 3 times of measurement.

² The increment in thickness at 24, 48, and 72 hours after injection. Values within a column followed by a common superscript letter are not significantly different ($P < .05$).

³ Female chickens injected at 13 weeks of age with 0.1 ml (10 ug) of PHA-M in the right wattle, and challenged 1 week later with the same amount. No swelling occurred at 24 or 48 hours after the first injection and test groups injected with saline, or with PHA-M, did not differ statistically. Wattles were only measured at 24 hours after the second injection.

Table 4.. Response of young adult females to PHA in wattles^a.

Line	No.	wattle mean thickness increase
P	9	3.1
N	4	1.3
72	10	2.1
63	10	3.1

^a Hens were injected with .1 ml saline in the left wattle and .1 ml (500 mg) PHA P in right wattle. Saline injected wattles did not swell.

Table 5. Capacity of PHA stimulated PHL of 3 chickens of 6 lines to proliferate and produce interferon on days 2, 3, 4, and 5 of culture.

Line	with PHA					no PHA				
	(CPM X 10 ³)/log ₂ interferon titer					(CPM X 10 ³)/log ₂ interferon titer				
	day = 2	3	4	5		2	3	4	5	
N	53.3/0 ^a	44.9/0.1	98.6/5.2	29.2/4.1		11.1/0	2.6/0	11.0/0	5.9/0	
63	47.1/0	22.0/0	106.3/4.2	64.2/4.3		6.1/0	2.4/0	2.7/0	8.8/0	
1515	41.0/0	19.0/0	31.0/3.7	44.8/1.7		2.6/0	0.7/0	1.1/1.3	1.8/0	
MLC	217.6/2.7	155.1/1.2	296.2/9.7 ^b	61.4/7.1		2.7/0	1.2/0	12.6/4.0 ^b	2.3/0	
F	117.3/3.6	193.5/1.0	219.4/9.2 ^b	36.0/7.8		2.4/0	2.2/0	21.5/5.7 ^b	2.0/0	
72	220.5/5.3	154.2/4.0	133.1/9.4 ^b	36.3/5.4		5.1/3.3	0.9/0	8.3/7.0 ^b	9.6/0.3	

^a Each value is based on the mean of 3 tests with PHA (and 1 without PHA) for each bird using 3 chickens per line. Separate chickens were used as peripheral blood donors for each days experiment.

^b High background interferon from cultures with no PHA.

Table 6. Log₂ gamma-interferon titer in supernatants of PBLs after 3 days culture in vitro.

Chicken line	No.	Controls			PHA- sup		PHA+ sup	
		cell	virus	INF	titer		titer	
		$\bar{X}$ OD ^a	$\bar{X}$ OD ^b	titer ^{c,d}	Indiv.	$\bar{X}$	Indiv.	$\bar{X}$
RLC	1	.74±.06	.25±.11	6	(0,0,1)	0.3	(10,10,9)	9.7
		.75±.02	.19±.08	6				
	2	.75±.04	.28±.15	6	(0,0,0)	0	(9,9,8)	8.7
		.84±.04	.21±.06	6				
	3	.73±.02	.25±.10	6	(0,0,0)	0	(8,6,9)	7.7
		.55±.02	.23±.13	5				
	4	.75±.08	.24±.11	6	(0,0,1)	0.3	(0,0,1)	0.3
		.80±.01	.24±.14	6				
	5	.78±.01	.15±.07	6	(0,0,0)	0	(6,6,6)	0.6
		.76±.02	.16±.06	6				
Mean		.75±.07	.22±.04	5.9		.12±.15		6.48±3.32
7 ₂	6	.83±.03	.20±.09	6	(4,4,3)	3.7	(1,2,3)	2.0
		.83±.03	.22±.13	6				
	7	.80±.04	.21±.13	6	(4,6,5)	5.0	(5,3,4)	4.0
		.83±.04	.16±.10	7				
	8	.81±.04	.24±.11	5	(5,4,6)	5.0	(7,5,4)	5.3
		.81±.03	.21±.10	6				
Mean		.81±.01	.20±.03	6		4.57±.61		3.77±1.36

^a The optical density (OD) grand mean of the cell controls for 20 plates = .777. Coefficient of variation (CV) = 12.7.

^b The grand mean OD for supernatants from wells infected with vesicular stomatitis virus only = .212; CV = 5.17.

^c The grand mean titer for sups from wells given a standard interferon supernatant before VSV = 6.15; CV = 10.8.

^d The titer was the last well where supernatants had an OD > 50% of the cell control = .777/2 = .388. Each bird was tested using 3 wells with and 3 wells without PHA on a 24 well plate. The supernatant from each well was tested in duplicate for interferon in adjacent wells on a 96 well plate (doubling dilutions in adjacent rows for 11 wells). The titer was based on the mean of both wells when the results were not identical.

Table 7. Percent of lymphocytes binding PNA in different organs.

	<u>High line</u>	<u>Low line</u>
PBL	33.3-3.1 A	48.9-3.2 B
Spleen	50.1-2.7 A	51.2-2.8 A
Thymus	88.3-2.0 A	87.8-2.2 A
Bursa	77.7-2.3 A	78.9-2.2 A

All chicks were 3.5 weeks old. High line = 38 chicks, low line = 34 chicks.

Different letters indicate statistical differences ($P < 0.01$).

Table 8. Ability of 15I₅ Congenic chickens to respond in one-way MLR.¹

Responder Line	B-type	96 hour response ² stimulator strain		120 hour response ² stimulator strain	
		Syngeneic	RLC(B12B12)	Syngeneic	RLC(B12B12)
15I ₅	15/15	1(1.7) ^a	8.5(14.9) ^{bc}	1.2(2.2) ^a	1.6(1.7) ^a
.6-2	2/2	1(2.1) ^{ab}	15.2(33.4) ^{bc}	3.2(5.6) ^{ab}	14.7(22.2) ^b
.7-2	2/2	1(1.6) ^a	22.7(36.5) ^c	2.4(5.2) ^b	25.6(18.5) ^b
.15I-5	5/5	1(2.8) ^{ab}	12.5(35.3) ^c	2.9(8.2) ^b	10.7(17.9) ^b
.C-12	12/12	1(2.4) ^{ab}	1.1(2.6) ^a	2.4(5.6) ^b	14.1(20.6) ^b
.P-13	13/13	1(4.1) ^b	10.3(42.1) ^c	1.0(4.2) ^{ab}	11.2(21.3) ^b
.P-19	19/19	1(1.8) ^{ab}	3.6(6.4) ^{ab}	2.9(5.2) ^b	39.3(31.5) ^b
.N-21	21/21	1(3.6) ^b	8.4(30.7) ^c	1.8(6.4) ^b	11.8(23.4) ^b

¹For each response-time test 4 responders were used for each line in 2 tests to give 8 responders except for the 96 hr. test .15I-5 = 5, and for the 120 hr test lines .15I-5 and .P-19 = 7. Chickens were the same age in each experiment, ie. 13 weeks.

²Stimulator cells were buffy coat peripheral blood lymphocytes from 3 RLC and 3 15I₅ 18 week old chickens pooled prior to mitomycin-C treatment. Syngeneic stimulator cells were prepared from the 4 responder birds within that line.

³Stimulation index followed by counts per minute X 10³ in parenthesis. Counts differ significantly from other values within a column if followed by different letters (P < .05).

Table-9. B-Locus phenotypes detected in selected Israeli broiler strains using RPRL B-alloantisera.

"B" Phenotype	Low Line		High Line	
	Number	%	Number	%
13/13	4	9	6	12
13/-	16	36	19	36
0/-	6	13	21	40
19/-	11	24	0	0
x/-	4	9	0	0
15/-	4	9	0	0
5/-	0	0	6	12
Total	45	100	52	100

FIG. 1

FIG. 2

FIG. 3

FIG. 4

mitogenic response to Con-A in two selected lines.

16 chickens from line L
16 chickens from line H

mitogenic response to PHA in two selected lines.

FIG. 6

16 chickens from line L
16 chickens from line H

mitogenic response to PWM in two selected lines.

16 chickens from line L
16 chickens from line H