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Genetic Variation in the Recruitment and Activation of Chicken Peritoneal Macrophages (42293)

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Abstract. Genetic variation in the ability to recruit and activate peritoneal macrophages was examined in seven partially developed $15I_5$-*B* congenic White Leghorn chicken lines. While the ability to generate peritoneal exudate cells (PECs) was similar in all lines, major differences were observed in the numbers, composition, and functional activity of harvestable peritoneal adherent cell populations. In response to a general stimulant, Sephadex, lines .7-*2* and .6-*2* produced the greatest numbers of adherent peritoneal cells while lines .C-*12* and .15I-*5* were among the poorest responders. Macrophage percentage of adherent PECs varied between lines. $15I_5$ chickens produced a consistently high percentage of adherent macrophages while .6-*2* birds exhibited the lowest macrophage percentage at all ages examined. Phagocytosis was used as one measure of the level of macrophage activation and similar results were obtained using both opsonized and unopsonized sheep erythrocytes; adherent peritoneal cells from lines .6-*2*, .7-*2*, and .P-*13* exhibited the highest activity and .C-*12*, .15I-*5*, and background $15I_5$ (B^{15}) lines produced cells with the lowest phagocytic activity. In a second functional assay, the killing of *Salmonella typhimurium*, macrophage-rich cells from line .P-*13* exhibited the lowest activity which was significantly lower than that obtained with cells from lines .6-*2* and .15I-*5*. Antigen-specific stimulation of peritoneal adherent cells by ferritin also showed that .C-*12* was a low responder in contrast with other lines. The results indicate that these genetic lines differ in peritoneal macrophage function and suggest that the chicken major histocompatibility complex may influence certain properties of chicken macrophage function.

Macrophages play a dual role in immune response. First, the phagocytic ability of macrophages is an important component of nonspecific immunity. Second, effective cellular responses involved in specific immune response are dependent on antigen processing and presentation by macrophages and other accessory cells to appropriate lymphocytes. Through a variety of studies, it has been shown that mammalian reticuloendothelial cell functions are subject to genetic influences both from within the major histocompatibility complex (MHC) as well as from non-MHC loci. The genetic influence on RES cells such as macrophages may be manifested at the level of ingestion, degradation, or presentation of antigens.

Biozzi *et al.* (1) demonstrated a polygenic control of the humoral response that was not antigen specific and which operated at the level of macrophage antigen processing and presentation to B-lymphocytes. Both MHC-linked and nonlinked genes were found to influence this response. Defects in phagocytosis of opsonized sheep erythrocytes by macrophages have been shown to be associated with certain HLA haplotypes (2). Inbred strains of mice were used to demonstrate the existence of genetic control of macrophage phagocytosis (3) and of activated macrophage cytotoxicity (4).

Most studies have been performed in mammals with only limited information available concerning genetic variation of macrophage function in other vertebrates. The sparsity of macrophage research on avian species is based on several factors. In contrast with the mouse, Glick *et al.* (5) demonstrated that few, if any, resident macrophage cells could be obtained from the chicken peritoneal cavity. Usable preparations of peritoneal macrophages could only be obtained following the recruitment of these cells into the peritoneal cavity by the introduction of an irritant (6–9). In addition, the availability of appropriate avian genetic material has been a limitation until recently. Following the development of inbred lines of chickens, Powell *et al.* (6) demonstrated that blood monocytes derived from two lines carrying the same MHC haplotype possessed dif-

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ferent phagocytic characteristics. Presumably, these differences resulted from non-MHC genetic influences. Congenic lines have been instrumental in the analyses of genetic control of immune response. With recent progress on the development of lines of chickens congenic for the MHC (10, 11), new opportunities are now available to examine genetic variation in avian macrophage function.

This paper describes a comparison of both general and antigen-specific peritoneal macrophage activation in several partially developed $15I_5$-*B*-congenic lines of chickens. These lines have previously served as the basis of comparisons involving hematological, immunological, and growth parameters (11). Results of these present macrophage comparisons are discussed in light of previous studies based on inbred and congenic lines.

Materials and Methods. The origin and histocompatibility status of the Ea-*B* (*B*) congenic chickens used in these experiments have been previously described (11, 12). The RPRL-$15I_5$ inbred line of White Leghorn chickens served as the background line for the preparation of the congenics (13). This line ($15I_5$) including inbred lines RPRL-6_1, -7_2, -$15I_4$, and Reaseheath C contributed the B^{15}, B^2, B^2, B^5, and B^{12} haplotypes, respectively, used in the construction of the *B*-congenics (13). B^{13} and B^{19} haplotypes were derived from line JM-P (14). All *B*-congenic lines were developed and are maintained at the Regional Poultry Research Laboratory, East Lansing. Serology, skin grafting, and mixed lymphocyte reaction tests have certified both the *B* homozygosity within each line and the line differences for the histocompatibility region of the *B* complex (12). The nomenclature utilized in the paper is based on that of Lyon (15) developed for the mouse system and has been previously utilized in publications of these *B*-congenic lines. Since the congenic lines are still in development, the total number of crosses made for each line is shown in the Table I footnotes.

Cornell Line II Japanese quail (16) were used to produce antibodies against both sheep red blood cells (SRBCs) and *Salmonella typhimurium.* Pedigreed sheep obtained from the Cornell Animal Science Department were maintained as a source of fresh SRBCs throughout the study.

B-Congenic chickens were hatched from two separate shipments of fertilized eggs, sexed by vent identification and administered a bivalent Marek's disease vaccine generously provided by Dr. B. W. Calnek and Dr. K. A. Schat, Cornell University. All chickens were

TABLE I. SEPHADEX STIMULATION OF PERITONEAL EXUDATE CELLS

Congenic line[a]	Haplotype	6 Weeks (age) Mean adherent cells[b]	6 Weeks (age) % Macrophages[c]	8 Weeks (age) % Macrophages[c]	12 Weeks (age) Mean adherent cells[b]	12 Weeks (age) % Macrophages[c]
$15I_5$	B^{15}	166.0 (027.5)^{d}	89.0 (01.1)^{f}	93.7 (1.7)^{f}	234.0 (19.2)^{f,g}	94.9 (1.8)^{e,f,g}
15.6-B^2	B^2	286.0 (115.0)^{d,e,f}	52.2 (10.1)^{d}	83.3 (0.5)^{d}	233.0 (11.0)^{g}	88.3 (1.7)^{d}
15.7-B^2	B^2	359.7 (034.0)^{e}	70.3 (10.0)^{e}	93.6 (2.6)^{e,f}	265.0 (20.7)^{g}	97.8 (0.3)^{h}
15.15I-B^5	B^5	276.7 (034.0)^{f}	71.3 (06.9)^{e}	91.6 (0.6)^{e,f}	173.3 (17.6)^{d}	97.4 (1.3)^{g,h}
15.C-B^{12}	B^{12}	—	—	—	186.3 (16.5)^{d,e}	95.0 (2.0)^{e,f,g,h}
15.P-B^{13}	B^{13}	242.0 (062.0)^{d,f}	70.4 (05.2)^{e}	88.4 (1.7)^{e}	257.7 (28.7)^{g}	94.0 (0.3)^{f}
15.P-B^{19}	B^{19}	—	—	—	204.3 (04.9)^{e,f}	90.5 (2.1)^{d,e}

[a] The base line is followed by period, then the donating strain, a hyphen, and the introduced *B*-haplotype. All lines are homozygous for the indicated haplotype. The total number of crosses for the lines are indicated in parentheses as follows: .6-*2* (5), .7-*2* (5), .15I-*5* (4), .C-*12* (2), .P-*13* (4), .P-*19* (5).

[b] Represents the number of adherent cells counted in four randomly selected fields per coverslip. Each sample consisted of a pool from one male and one female. The number indicated is the mean of four pooled samples. The standard deviations are enclosed by parentheses.

[c] Represents mean of three to four pools. Each pool consisted of two chickens (one male and one female). Standard deviations for the means are enclosed by parentheses.

[d–h] Numerical values within columns not sharing a common superscript letter are significantly different by Student's *t* test at $P < 0.05$.

coded until the study was completed. Chicks were reared intermingled under identical environmental conditions. They were housed in thermostatically controlled battery brooders with raised wire floors. Feed and water were provided *ad libitum*. The diet consisted of Cornell B ration (17). Chicks were raised with a 15-hr day length. Regular veterinary screening of populations was performed.

Isolation of adherent peritoneal exudate cells (PECs). Adherent peritoneal exudate cells used in SRBC phagocytosis, bacterial killing, determination of adherent cell numbers, and differential cell counts were harvested using a Sephadex stimulation method modified from Sabet *et al.* (8) as previously described (9). A single injection of 3% Sephadex G-40 superfine (Sigma) solution in saline was injected into the peritoneal cavity of each bird at the concentration of 1 ml per 100 g of body weight. On the third day (approximately 42 hr after injection), the birds were sacrificed and the peritoneal cavity was flushed with sterile heparin (0.5 U/ml) in saline. For most assays, cell pellets from a pool of two birds were then resuspended in RPMI 1640 growth medium (GIBCO) with antibiotics (100 U/ml penicillin and 50 μg/ml streptomycin); however, for PECs used in the bacterial killing assay, RPMI 1640 supplemented with 5% fetal bovine serum without antibiotics was used as the culture medium.

After adherent cell collection on coverslips (9), the coverslip cultures were fixed in methanol for 10 min, stained with May–Grünwald–Giemsa (18), mounted on clean glass slides, and 500 adherent cells per coverslip were classified by morphological criteria (18). The adherent cell number of four randomly selected fields was scored for each coverslip at 1000× and the mean from four pooled samples was determined.

Adherent PECs bactericidal assay. The bacterial killing assay was performed as previously described for bovine macrophages by Desiderio and Campbell (19). A bovine strain of *S. typhimurium* (serotype 0-1, 4, 5, 12) was kindly donated by Dr. J. V. Desiderio of the Department of Veterinary Microbiology, College of Veterinary Science, Cornell University for use in the killing assay. Briefly, *S. typhimurium* at log phase growth was coated with antibody for 30 min at 37°C using a subagglutinating concentration of specific antiserum prepared in Line II Japanese quail. Following washing in PBS, the bacterial concentration was adjusted to 2.5×10^7/ml in RPMI 1640 with 5% fetal bovine serum. This concentration was also confirmed by plate counts. After collection of the macrophage-rich peritoneal adherent cells from Sephadex-stimulated 8-week-old chickens, the opsonized bacteria were fed to cell monolayers at a ratio of approximately 25:1 bacteria to macrophage. Cells were harvested at intervals from 0 to 75 min and analyzed as previously described (19). Duplicate experiments were performed.

Ferritin PEC stimulation. At 13 weeks of age, two chickens (one male and one female) in each of three replicate pools per haplotype group were sensitized by injecting 0.2 mg (low dose) or 0.4 mg (high dose) of horse spleen ferritin (Sigma) in Freund's complete adjuvant (Difco) subcutaneously. The low dose was identical to that previously used in chicken cell-mediated assays by Palladino *et al.* (20). One week later, a single injection of 3.2 mg ferritin in sterile 0.75% saline per 300 g of body weight was given in the peritoneal cavity. After 42 hr, PECs were harvested and the same analyses previously described for Sephadex stimulation were performed.

Sheep red blood cell phagocytosis. Freshly collected sheep red blood cells (SRBCs) were washed three times in sterile PBS and a 5% solution coated with a subagglutinating concentration of specific antiserum made in Line II Japanese quail. Following washing in PBS, the cells were adjusted to a 5% concentration in RPMI 1640 medium. A 5% solution of unopsonized SRBCs was separately made in RPMI 1640 medium.

Adherent PEC monolayers rich in macrophages were prepared on the coverslips as previously described. The culture medium from sets of three coverslip cultures was removed and 1.0 ml of 5% opsonized or unopsonized SRBC suspension was dispensed into each petri dish. The cultures were incubated at 37°C in humidified atmosphere of 5% CO_2. After 45 min, cultures were washed with 0.75% saline solution, fixed in methanol for 10 min, and stained with May–Grünwald–Giemsa as before. The percentage of phagocytic macro-

phages and average number of interiorized SRBCs per phagocytic macrophage were scored.

Data analysis. Statistical analysis among groups was performed using a one-way analysis of variance to determine overall significance. Means were then compared using a two-sided Student's t test with $\alpha = 0.05$.

Results. Peritoneal exudate cells (PECs) and adherent PEC populations were isolated from *B*-congenic chickens following Sephadex stimulation at 6, 8, and 12 weeks of age. The total mean PEC counts from a pool of two birds ranged from 0.7 to 1.7×10^7 among different lines. Due to the large standard deviations, there was no statistical difference between lines in the total number of harvested nonerythrocyte PECs (data not shown). In the 6, 8, and 12 week samples, adherent cells from the peritoneal exudate were collected onto coverslips to determine the relative number of adherent cells and the percentage of cell types present in the adherent cell isolates (Table I). Lines .7-*2* and .6-*2* chickens consistently had the highest number of adherent cells at both 6 and 12 weeks. Adherent cell numbers were not determined for 8 week samples. Lines .C-*12* and .P-*19* were only tested at 12 weeks and were significantly lower in adherent cell production than all lines except .15I-*5*. The percentage of macrophages in the adherent cell population at 6 weeks of age for line $15I_5$ chickens was nearly 90% while the adherent cells of line .6-*2* birds contained only 52% macrophages. Other groups tested at this age had intermediate percentages that were significantly different from the $15I_5$ and .6-*2* values. The percentage of adherent macrophages was also determined from 8 week samples; line .6-*2* chickens still generated the lowest percentage of adherent macrophages. At 12 weeks, the .6-*2* line again produced a significantly lower percentage of macrophages than other lines except .P-*19* while $15I_5$ did not differ from most other lines. Samples with low percentages of macrophages were observed to have a proportionally higher incidence of heterophils, the avian equivalent of mammalian neutrophils. Thus, the number of heterophils in the adherent cell population at 6 weeks was greater in line .6-*2* than in other groups (unpublished observation).

Phagocytic ability of adherent PECs from 12-week-old chickens was determined using opsonized and unopsonized sheep RBCs (Table II). As observed for adherent cell stimulation at 12 weeks, .6-*2*, .7-*2*, and .P-*13* chickens were found to exhibit the greatest overall phagocytic activity. These lines produced a high percentage of phagocytic macrophages for both opsonized and unopsonized SRBCs. In addition, the average number of SRBCs en-

TABLE II. SHEEP RED BLOOD CELL PHAGOCYTOSIS BY PERITONEAL ADHERENT CELLS OF 12-WEEK-OLD CHICKENS

Congenic[a] line	% Phagocytic macrophages[b]		Average SRBCs engulfed per phagocytic macrophage	
	Opsonized SRBCs	Unopsonized SRBCs	Opsonized SRBCs	Unopsonized SRBCs
$15I_5$	62.4 (8.8)^{c,d}	49.8 (6.7)^{c,d,e}	4.2 (0.3)^{c,d}	2.1 (0.2)^{d,e,f}
15.6-B^2	90.3 (2.2)^{f}	82.6 (1.2)^{g}	5.4 (0.3)^{e}	3.6 (0.1)^{g}
15.7-B^2	81.7 (5.5)^{e,f}	76.1 (4.3)^{f,g}	4.7 (0.4)^{c,d,e}	2.8 (0.5)^{f,g}
15.15I-B^5	61.5 (4.2)^{c}	37.0 (6.0)^{c}	4.6 (0.3)^{d}	1.8 (0.2)^{c,e}
15.C-B^{12}	72.0 (0.9)^{d}	50.8 (1.7)^{d}	3.6 (0.5)^{c}	1.5 (0.1)^{e}
15.P-B^{13}	83.9 (3.2)^{e}	69.8 (1.4)^{f}	4.1 (0.4)^{c,d}	2.2 (0.1)^{d,f}
15.P-B^{19}	78.4 (6.8)^{d,e}	61.8 (4.1)^{e}	4.6 (0.4)^{c,d}	1.6 (0.4)^{c,d}

[a] See Table I for lines, haplotypes, and number of crosses.

[b] Represents mean of three pools. Each pool consisted of two chickens (one male and one female). Standard deviations are included in parentheses.

[c–g] Numerical values within columns not sharing a common superscript letter are significantly different by Student's t test at $P < 0.05$.

gulfed by each phagocytic macrophage was greater in line .6-*2* than in other lines except .7-*2* while line .C-*12* phagocytes were found to have the lowest activity (Table II). An example of the phagocytosis of SRBCs by .6-*2* adherent cells is illustrated in Fig. 1.

The ability of adherent cells from 13 to 14-week-old chickens to infiltrate the peritoneal cavity in response to ferritin was studied using two sensitizing doses (0.2 and 0.4 mg) of ferritin (Table III). As with Sephadex stimulation, no significant differences between lines were observed in the numbers of ferritin-stimulated harvested PECs at either dose (data not shown). In the low dose experiment, lines .P-*19*, .P-*13*, and $15I_5$ had the highest number of adherent cells, while lines .6-*2*, .7-*2*, the highest responders to Sephadex, and line .C-*12* were among the lowest ferritin responders (Table III). At the higher dose, line $15I_5$ produced the highest number of adherent cells and .C-*12* chickens achieved only 50% of this level. .C-*12* chickens also exhibited the lowest percentage of macrophages among the groups receiving low dose of ferritin while $15I_5$ chickens had the highest percentage of macrophages (Table III). The total numbers of adherent cells were generally higher in the low dose experiment, while the percentages of macrophages present in the adherent cell population were higher in the high dose ferritin experiment. At the high ferritin dose, all groups exhibited >90% macrophages among adherent cells.

Adherent Sephadex-elicited PECs from different congenic lines at 8 weeks of age were used to compare their bactericidal ability against opsonized *S. typhimurium*. The isolated monolayers were rich in macrophages ranging from 83.3 to 93.7% macrophages among the different lines utilized in the assay (Table I). Figure 2 illustrates the phagocytosis of opsonized bacteria by adherent cell monolayers. The maximum killing by cells of line .P-*13* chickens was found to be significantly lower than that obtained with cells from lines .6-*2* and .15I-*5* (Fig. 3). This result is unlikely

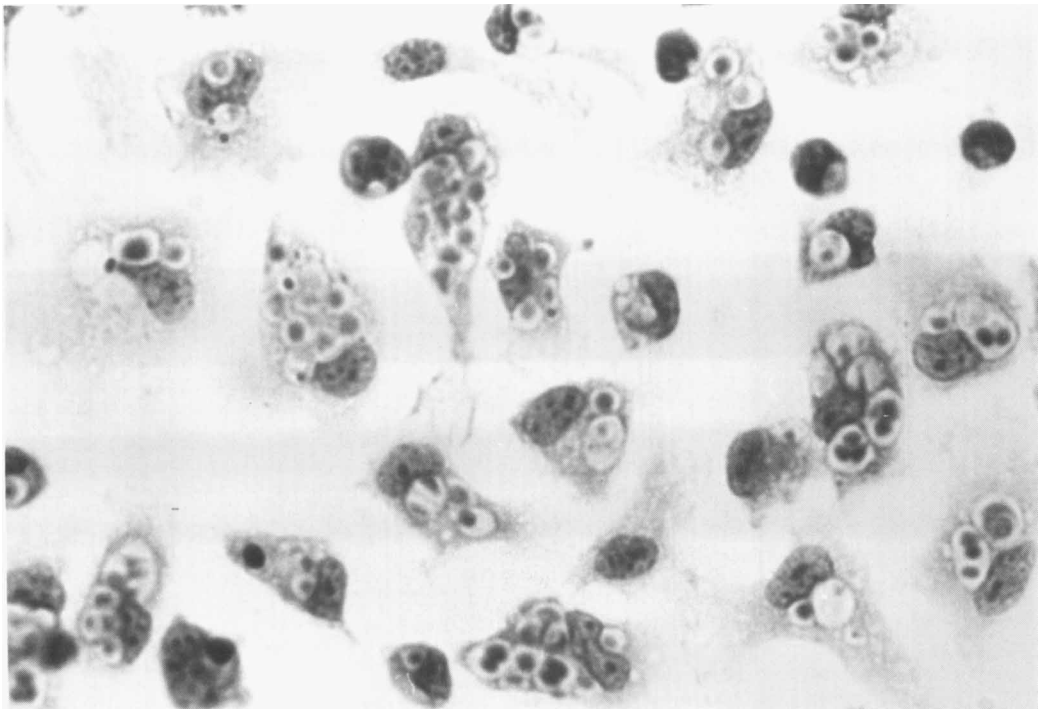

FIG. 1. The phagocytosis of SRBCs by macrophage-rich peritoneal adherent cell monolayers derived from Sephadex-stimulated .6-*2* line chickens is illustrated. The magnification is 850×.

TABLE III. FERRITIN STIMULATION OF PERITONEAL EXUDATE CELLS

Congenic[a] line	Low dose (0.2 mg) Mean adherent cells[b]	Low dose (0.2 mg) % Macrophages[c]	High dose (0.4 mg) Mean adherent cells[b]	High dose (0.4 mg) % Macrophages[c]
$15I_5$	292.3 (34.6)^{e,f}	90.7 (2.1)^{f}	245.3 (30.9)^{f}	97.3 (2.8)^{d,e}
15.6-B^2	207.3 (04.0)^{d}	86.0 (5.7)^{e,f}	205.0 (26.8)^{d,f}	94.5 (4.0)^{d,e}
15.7-B^2	226.3 (27.2)^{d}	88.7 (2.7)^{f}	202.0 (23.3)^{e,f}	97.4 (0.6)^{d}
15.15I-B^5	261.3 (03.5)^{d,e}	85.7 (2.7)^{e,f}	212.5 (13.4)^{e,f}	96.6 (0.9)^{d,e}
15.C-B^{12}	227.3 (19.9)^{d,f}	60.9 (9.4)^{d}	123.0 (05.7)^{d}	98.0 (0.6)^{d}
15.P-B^{13}	283.0 (42.9)^{e,f,g}	79.5 (2.9)^{e}	147.1 (47.4)^{d,e}	95.5 (0.4)^{e}
15.P-B^{19}	314.0 (24.64)^{g}	74.7 (4.6)^{d,e}	166.0 (21.2)^{d,f}	95.3 (0.5)^{e}

[a] See Table I for lines, haplotypes, and number of crosses.

[b] Represents the number of adherent cells counted in four randomly selected fields per coverslip. Each sample consisted of a pool from one male and one female. The number indicated is the mean of three to four pooled samples. Standard deviations are included in parentheses.

[c] Represents mean of three pools. Each pool consisted of two chickens (one male and one female). Standard deviations are included in parentheses.

[d-g] Numerical values within columns not sharing a common superscript letter are significantly different by Student's t test at $P < 0.05$.

to be a result of the cellular composition on the coverslips since .P-*13* isolates exhibited a percentage of macrophages intermediate between that of .6-*2* and .15I-*5*. Possible line differences in the kinetic profiles of killing warrant further examination.

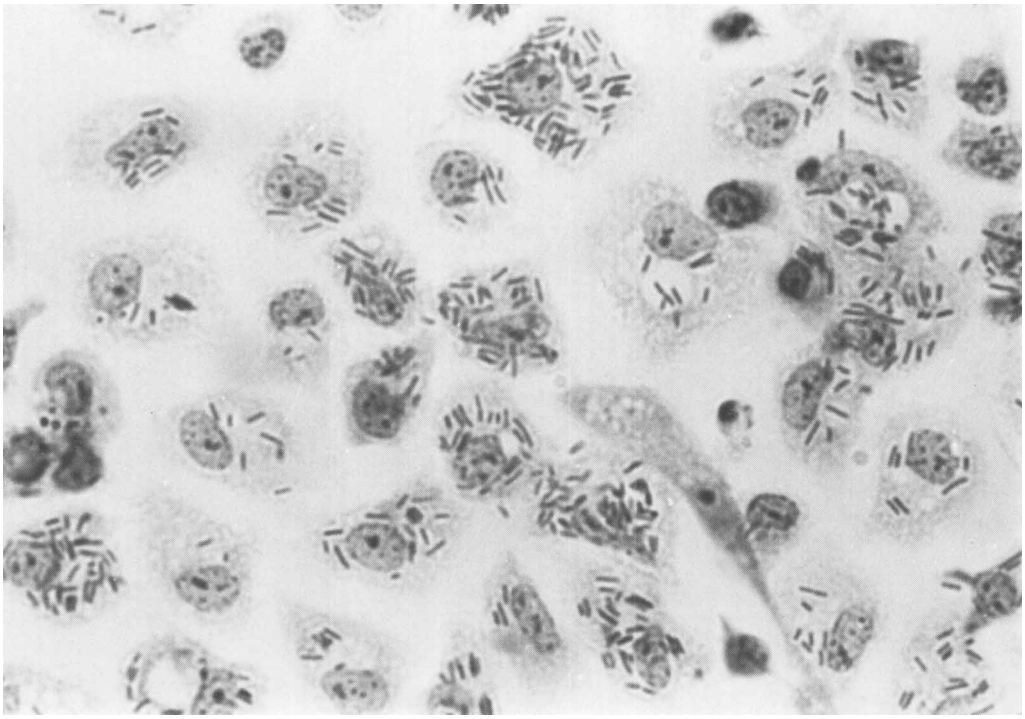

FIG. 2. The phagocytosis of antibody-coated *S. typhimurium* by .$15I_5$-*5* line chicken peritoneal adherent-cells is shown. The magnification is 850×.

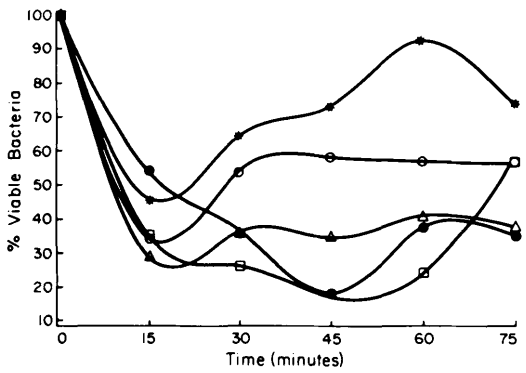

FIG. 3. Kinetics of intracellular killing of opsonized *S. typhimurium* by adherent peritoneal exudate cells from $15I_5$ (△), 15.6-*2* (●), 15.7-*2* (○), 15.15I-*5* (□), and 15.P-*13* (*) congenic chickens. Analysis of maximum killing using a pooled Student's *t*-test indicated that line .P-*13* killing was significantly lower than that of lines .6-*2* and .15I-*5* at $P < 0.05$.

Discussion. Genetic influences on chicken monocyte–macrophage function were first suggested in studies utilizing the East Lansing inbred chicken lines 6 and 7. Gilmour and Fredericksen (21) reported that these sublines of 6 and 7 differed in peripheral blood mitogen responsiveness and that the difference involved adherent cell populations (Schaefer *et al.*, 1985, unpublished observation). Powell *et al.* (6) demonstrated inherent line differences in the phagocytic activity of line 6 and 7 peripheral blood leukocytes. These lines have also been shown to differ in specific disease resistance (22), and responsiveness to ferritin (20), but are known to carry the same B^2 MHC haplotype (23). Therefore, these differences in monocyte–macrophage function are presumably due to non-MHC genetic differences between the lines (24). In a similar observation, Kline and Sanders (25) found a mitogen-specific suppressor cell population among splenic RES cells of hereditary muscular dystrophic (MD) chickens that was absent in MHC-matched control chickens. Dietert and Sanders demonstrated that adult MD chickens possessed abnormal heterophil vs macrophage activity (26).

The present investigation employed some of the first developing *B*-congenic chicken lines to examine the genetic variation in chicken peritoneal macrophage function. The same lines were recently characterized for a variety of hematological and immunological functions (11) and are being examined for resistance to avian leukosis virus (27) and coccidiosis (28). Of all the congenic lines examined, .6-*2* and .7-*2* exhibited the most similar RES profiles; they were similar in both general and ferritin-specific *in vivo* chemotaxis and in phagocytic parameters and differed only in the composition of Sephadex-stimulated peritoneal cells. The two congenic lines possess B^2 haplotypes donated by lines 6 and 7.

The series of experiments provided a comparison between the genetic lines in both general and antigen-specific macrophage recruitment and activation. Since chickens have few, if any, resident peritoneal adherent cells (5), recruitment of these cells into the cavity in response to Sephadex vs ferritin was used as a measure of general vs antigen-specific *in vivo* chemotaxis (9). Several *B*-congenic lines exhibited significant differences in the response of peritoneal cells to each of these stimuli and different levels of response in the general vs antigen-specific comparison. Lines .6-*2* and .7-*2* were among the best adherent cell responders to Sephadex regardless of age; while the adherent cell production of these lines was low with low dose ferritin, lines .6-*2* and .7-*2* were comparable to other lines in the production of adherent cells with high dose ferritin and produced a high percentage of macrophages at both ferritin doses. Line .C-*12* possessed the poorest overall response with low adherent cell production to Sephadex and both doses of ferritin and poor phagocytic activity of Sephadex-activated peritoneal macrophages. Both the percentage of phagocytic macrophages and the relative activity of each phagocyte were depressed in this line compared with most other *B*-congenic lines. The .C-*12* line was recently shown to exhibit a greater susceptibility to *Eimeria tenella* coccidiosis compared with most other $15I_5$-*B* congenic lines (28). Resistance to coccidiosis is known to be dependent on effective cell-mediated immune response (29).

While line $15I_5$ consistently exhibited high responses and line .C-*12* low responses to ferritin, other lines showed varied responses dependent upon the antigen sensitizing dose. The basis of this dose effect observed in some but not all of the lines is not known. How-

ever, from the PEC counts and percentage of peritoneal macrophages produced, the higher sensitizing dose of ferritin appeared to elicit a more homogeneous but lower level of cellular response than was obtained with the low dose regime. Relative differences in the ferritin response of these genetic lines between the low dose vs high dose immunization protocol could reflect the known influence of antigen dose on MHC-controlled antigen response (30).

Results from *in vivo* chemotaxis and *in vitro* phagocytosis assays were obtained using assay times selected to maximize macrophage activity. Observed line differences in response to given stimuli likely reflect inherent line differences in macrophage activation. However, major differences in the kinetics of the responses between the lines could also produce the similar results. This is supported by our observation that the initial peritoneal macrophage population isolated 6 hr after Sephadex stimulation from K-strain chickens is virtually nonphagocytic and lacks the Fc receptor; later in the response the majority of the macrophages are phagocytic and carry the Fc receptor (Yi and Dietert, unpublished observation). Sabet *et al.* (8) found that Sephadex stimulation could generate highly phagocytic chicken macrophages. In addition, Sephadex was recently shown to activate mouse macrophages for tumoricidal activity (31).

The kinetics of intracellular killing of opsonized bacteria by macrophage-rich adherent cells suggested a possible low activity in line .P-*13* chickens. Factors known to influence the interaction between bacteria and phagocytes include the virulence of the bacterial strain (32), the state of macrophage activation (33), and the genetic susceptibility of the host to the bacteria (34).

The present study has demonstrated the existence of genetic-based influences on chicken peritoneal macrophage function. Significant differences were observed in several macrophage parameters in partially-developed *B*-congenic lines of chickens. Relative high and low peritoneal macrophage responses in the lines depended upon the nature of the antigen. The comparisons between *B*-congenic lines suggest that some of the observed differences in peritoneal macrophage function may be influenced by the MHC; however, the possibility of non-MHC influences cannot be excluded due to the developmental status of the lines. Future studies should facilitate the distinction between MHC vs non-MHC influences on chicken macrophage function.

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