Effects of Increased Major Histocompatibility Complex Dosage on Chicken Monocyte-Macrophage Function (42850)

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Abstract. **The influence of major histocompatibility complex (*B* complex) dosage on monocyte-macrophage function was examined using 4- to 6-week-old trisomic strain chickens. Di- ($B^{15}B^{15}$), tri- ($B^{15}B^{15}B^{15}$), and tetrasomic ($B^{15}B^{15}B^{15}B^{15}$) progeny were produced from trisomic × trisomic crosses. Although mononuclear leukocytes from tetrasomics exhibited enhanced chemotactic activity in response to both f-met-leu-phe and *Enterobacter cloacae* culture supernatant as compared with that of cells from other groups, the ability to generate peritoneal exudate cells in response to intraperitoneal Sephadex stimulation was similar in all groups. Among peritoneal exudate cells, tetrasomic birds produced a significantly lower percentage of adherent macrophages with a higher proportion of Fc receptor-positive and CMTD-2-reactive macrophages than either disomic or trisomic chickens. Both tetrasomic and trisomic peritoneal macrophages exhibited a reduced phagocytic activity for unopsonized but not opsonized SRBC than was found with disomic macrophages. Thus, the number of major histocompatibility complex copies present in cells appears to influence monocyte-macrophage function.**

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The major histocompatibility complex (MHC) is a chromosomal region which includes families of genes denoted as class I, class II, and class III. Because class I and II genes encode cell surface molecules that allow the cells of the immune system to communicate and interact in immune processes, the MHC governs the overall responsiveness of an individual to a variety of antigens including bacterial or viral pathogens. MHC genetic regions have been defined in a variety of species including mouse (1), human (2), and chicken (3). The chicken MHC, or *B* complex, is linked to the ribosomal RNA gene cluster on a microchromosome containing less than 1% of cell DNA (4, 5). The major classes of *B* complex genes have been cloned and partially sequenced (6–8). *B* haplotypes from a large variety of genetic strains have been characterized for their class II and class IV restriction fragment length polymorphisms (9, 10). There is a high degree of homology (~65%) between human and chicken MHC genes (6).

Through a variety of studies, it has been shown that mammalian macrophage function is subject to genetic influences both from within the MHC as well as from non-MHC loci (11–13). Most studies have been performed in mammals with only limited information available concerning genetic variation of macrophage function in other vertebrates. In contrast with the mouse, chickens have few harvestable resident peritoneal exudate cells (PEC) (14); therefore, stimulation of the chicken peritoneal cavity with irritants is necessary to obtain significant numbers of macrophages (15, 16).

Previous studies using congenic chicken lines have shown that certain chicken macrophage functions may be influenced by allelic differences for *B* congenic genes (17). We have further explored MHC-based influences on macrophages in the present work by examining MHC dosage effects for a particular haplotype on chicken macrophage differentiation and function. We suspected this might be the case since previous work with animals having extra MHC copies in cells showed

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altered immune development at least in the bursal cell compartment (18).

The trisomic chicken line represents an animal model that permits the study of the effects of increased MHC dosage on immune development and functions (4, 19). Trisomic line birds can be used to produce chickens with two, three, or four copies of the microchromosome that encode both the MHC and rRNA gene clusters (the nucleolar organizer region, NOR) (4). By combining cytochemical and immunologic techniques, Bloom *et al.* (20) demonstrated that chickens with two NOR (disomic) expressed a maximum of two *B* haplotypes, chickens with three NOR (trisomic) expressed three distinct *B* haplotypes, and tetrasomic chickens expressed four different haplotypes on erythrocytes. Later studies revealed that erythrocyte glycoproteins and lymphocyte class II antigens were expressed in a dosage-dependent fashion in aneuploid chickens (18, 21) whereas rDNA products are regulated to maintain diploid levels of 18S + 28S rRNA (5). Thus, the only known (and suspected) gene complex on the microchromosome for dosage-dependent expression is the *B* complex (22). The present study was undertaken to examine the hypothesis that quantitative differences in MHC expression influence monocyte-macrophage differentiation and functions. The results are discussed in light of previous studies concerning the MHC and macrophage function and the influence of *B* complex dosages on chicken lymphoid development.

Materials and Methods

Animals. Normal (disomic) and aneuploid (trisomic and tetrasomic) chickens were produced from matings of trisomic sires ($B^{15}B^{15}B^{15}$) × trisomic dams ($B^{15}B^{15}B^{15}$). Only full and half sib chicks were used in the present experiments to minimize potential background gene effects. At hatching, progeny of this cross were classified for the number of NOR (and hence the number of MHC-bearing microchromosomes) in feather-pulp cells using a previously described procedure (4). Groups of chickens categorized as disomic, trisomic, and tetrasomics were coded until the study was completed. The birds were reared in raised wire pens with thermostatically controlled identical environments and a 15-hr day length. Feed and water were provided *ad libitum* with the diet consisting of the Cornell B ration (23).

Chemotaxis of Blood Mononuclear Cells. *Enterobacter cloacae* culture supernatant and the synthetic polypeptide f-met-leu-phe (Sigma) were used as two different chemotactic factors for mononuclear cells. The chemotactic factor from *E. cloacae* (chicken isolate) was produced as previously described (24). The polypeptide f-met-leu-phe was dissolved in RPMI 1640 growth medium (GIBCO) without antibiotics and used at a 10^{-5} M concentration as the chemotactic agent.

For mononuclear cells, a pooled blood sample from 4-week-old chickens (four males and four females) of different groups was collected in siliconized glass tubes. The mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation (Pharmacia and Sigma), washed three times in RPMI 1640 medium, and the final concentration adjusted to 3.0×10^6 cells/ml.

The chemotaxis assay was performed in blind well chambers as previously described (25). Control medium (Brain Heart Infusion broth or RPMI 1640 medium) or the chemoattractant was pipetted into the lower wells of the chambers with cells added to the upper wells. Polycarbonate filters (5 μm) were used as membrane barriers. Following a 1-hr incubation for f-met-leu-phe and 2 hr for bacterial supernatant at 37°C and 5% CO_2, the filters were fixed in methanol, stained with Mayer's hemotoxylin, and scored as previously described (25). Each pooled sample was assayed in triplicate and results from three independent experiments were obtained on separate hatches.

Isolation of Adherent Peritoneal Exudate Cells. PEC from 5- and 6-week-old disomic, trisomic, and tetrasomic chickens were harvested using a Sephadex G-50 stimulation method modified from Sabet *et al.* (15) as previously described (16). Briefly, a single injection of 3% Sephadex G-50 superfine (Sigma) solution in sterile 0.75% saline was given ip at an amount of 1 ml/100 g body wt. Approximately 42 hr after injection, the birds were killed and the peritoneal cavity flushed with sterile heparin (0.5 units/ml) in 0.75% saline. The peritoneal lavage was allowed to sit on ice for 20 min to remove unabsorbed Sephadex or any tissue debris. The supernatant was centrifuged (Fisher Centrific centrifuge) at 1200 rpm for 20 min and the PEC pellets from individual bird were resuspended into 2 ml of RPMI 1640 growth medium with antibiotics (100 units/ml penicillin and 50 μg/ml streptomycin) and antimycotics (0.25 μg/ml) (GIBCO). Total nonerythroid PEC from each bird were counted on a hemacytometer (Brightline, American Optical). To collect the adherent exudate cells, the PEC suspension from each bird was loaded onto the glass coverslips and incubated for 30 min at 37°C in a humidified incubator with 5% CO_2. Cultures were then washed with sterile 0.75% saline solution, fixed in methanol, stained with May-Grünwald-Giemsa, mounted on clean glass slides, and 500 adherent cells per coverslip were classified by morphologic criteria (26). The adherent cell number of four randomly selected fields were scored for each coverslip at 1000× and the mean from four pooled samples was determined.

Sheep Red Blood Cell Phagocytosis Assay. The phagocytic activity of macrophages from PEC of chickens belonging to different groups was determined using an *in vitro* SRBC phagocytosis assay as previously described (17). Briefly, freshly collected SRBC were washed three times in sterile phosphate-buffered saline (PBS, pH 7.2) and a 5% solution coated with a subagglutinating concentration of specific hyperimmune antiserum (previously heat inactivated at 56°C for 45 min), made in Line II Japanese quail (27). After washing in PBS, the cells were adjusted to a 5% concentration in RPMI 1640 growth medium. This opsonized SRBC suspension was also used in the rosette assay where rosetting should be based primarily on the presence of Fc receptors (FcR). A 5% solution of unopsonized SRBC was separately made in RPMI 1640 growth medium.

The PEC suspensions from each group of chickens were pooled and a 20-μl aliquot from each group was mixed with an equal volume of 0.4% trypan blue in PBS to determine the viable cell count (28). The macrophage concentration was adjusted to 1×10^6 viable cells/ml. The adherent PEC monolayers rich in macrophages were prepared in triplicate on the coverslips as previously described (17). 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 a humidified atmosphere and 5% CO_2. After 30 min, cultures were washed with saline, fixed in methanol for 10 min, and stained with May-Grünwald-Giemsa stain as before. The percentages of phagocytic macrophages and average number of interiorized SRBC per phagocytic macrophage were scored. A total of 200 cells were counted from each coverslip.

SRBC-Rosette Assay. Macrophage monolayers made from PEC isolated from different groups of chickens were incubated in triplicate with 5% opsonized SRBC at 22°C in a humidified atmosphere with 5% CO_2. After 15 min, cultures were washed, fixed in methanol and stained with May-Grünwald-Giemsa, and evaluated microscopically. Macrophages with five or more attached SRBC were scored as positive for the opsonin receptor (primarily the FcR) (29). A total of 500 cells were scored from each of the three to four coverslips per petri dish. Rosette-forming cells were expressed as a percentage of the total number of macrophages scored from each group.

Flow Cytometry Analysis for CMTD-2 Monoclonal Antibody (MCA)-Reactive Macrophages. The incidence of Sephadex-stimulated macrophages reactive with CMTD-2, a mouse-MCA reactive with a subpopulation of chicken inflammatory macrophages (30), was determined for each group by flow cytometry as previously described (30). This antibody is known to detect a cytoplasmic epitope. Briefly, 1×10^6/ml of macrophage-rich PEC from each group of chickens were fixed in methanol for 20 min to permit reaction of the monoclonal antibody with the cytoplasmic epitope. The cells were washed three times with cold PBS and the pellets resuspended in 1 ml of PBS. To this, 0.5 ml of CMTD-2 or PAI (myeloma cell line) control supernatant was added. After a 30-min incubation at room temperature, the cells were again washed three times with cold PBS and pelleted. To the pellets were added 200 μl of 1/16 dilution of affinity purified FITC-conjugated goat anti-mouse IgG and IgM (H&L) (The Jackson Laboratory). The suspension was incubated at 4°C for 30 min in the dark and rinsed three times with cold PBS. Finally, pellets were resuspended in 1 ml of PBS for flow cytometry analysis using a Becton-Dickinson II fluorescence-activated cell sorter (FACS). A minimum of 10,000 cells were analyzed per sample. The relative cell numbers reactive with CMTD-2 were determined for disomic, trisomic, and tetrasomic chickens.

Data Analysis. Statistical analysis among groups was performed using a one-way analysis of variance to determine the overall significance. Means were then compared using a two-sided Student's *t* test. To determine if strains × treatment interactions existed, a two-way factorial analysis of variance was done on chemotaxis data. When significant interactions were found, the variables could not be treated as independent and thus were separately analyzed using a one-way analysis of variance or a two-sided Student's *t* test with $\alpha = 0.05$.

Results

When blood mononuclear leukocytes from 4-week-old disomic, trisomic, and tetrasomic birds were tested for their *in vitro* chemotactic ability, cells from tetrasomic birds consistently exhibited a significantly higher chemotactic response to both chemoattractants as compared with that of cells from disomic and trisomic chickens (Table I).

When peritoneal exudate cells and the adherent PEC populations were isolated from 5- to 6-week-old chickens 42 hr following Sephadex stimulation (*in vivo* chemotaxis), mean PEC counts per bird did not differ among the three groups in any of the three separate experiments with values ranging from 2.49, 2.32, and 2.37 ($\times 10^7$) total cells for disomic, trisomic, and tetrasomic groups of chickens, respectively. Likewise, in three of four experiments, PEC from the three genetic groups did not differ in their glass adherence ability (data not shown). In contrast, tetrasomic birds had a significantly lower incidence of macrophages and a resulting higher incidence of heterophils among the adherent PEC as compared with both disomic and trisomic chickens in four of five separate experiments (Table II). For the parameters of peritoneal macrophage production, the trisomic group behaved similarly with the disomic groups but distinct from that of the tetrasomics. Table III illustrates the phagocytic activity of adherent PEC from 5-week-old chickens, for opsonized and unopsonized SRBC. Although no consistent trend

Table I. Chemotaxis of Blood Mononuclear Leukocytes

Experiment	Disomic mean (SD)^a	Trisomic mean (SD)^a	Tetrasomic mean (SD)^a
f-met-leu-phe			
1	6.60 (1.85)^b	6.90 (4.17)^b	17.42 (5.81)^c
2	18.70 (4.00)^c	9.97 (2.54)^b	28.30 (3.52)^d
3	8.98 (6.86)^b	9.63 (8.27)^b	18.28 (9.30)^c
E. cloacae supernatant			
1	13.25 (0.92)^c	6.05 (1.77)^b	21.60 (1.05)^d
2	9.50 (0.10)^b	12.27 (1.08)^c	21.83 (4.55)^d
3	13.00 (0.66)^b	16.07 (2.63)^b	32.97 (4.70)^c

[a] Represents the mean number of cells migrated per microscopic field (1000×). Ten fields per filter were scored. Each experiment included a pool of four to eight chickens per group and was run in triplicate. The control values were previously subtracted from the listed means. Standard deviations from the means are enclosed by parentheses.

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

Table II. Incidence of Macrophages in Sephadex-Elicited Adherent Peritoneal Exudate Cells

Experiment	Disomic mean (SD)^a	Trisomic mean (SD)^a	Tetrasomic mean (SD)^a
1	83.88 (3.45)^b	83.36 (0.56)^b	61.89 (6.61)^c
2	89.92 (2.70)^d	83.86 (2.81)^b	76.36 (2.49)^c
3	84.58 (2.82)^b	77.72 (1.74)^c	81.60 (7.70)^{b,c}
4	90.13 (2.75)^b	87.61 (2.71)^b	59.30 (6.64)^c
5	90.39 (3.38)^b	91.13 (1.02)^b	76.92 (3.83)^c

[a] Represents the mean percentage of macrophages scored from four coverslip cultures per bird. Each group consisted of four birds per experiment. Standard deviations are enclosed in parentheses.

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

Table III. Sheep Red Blood Cell Phagocytosis by Peritoneal Macrophages

Experiment	Disomic mean (SD)^a	Trisomic mean (SD)^a	Tetrasomic mean (SD)^a
Opsonized			
1	89.99 (2.37)^b	70.06 (4.71)^c	82.34 (5.88)^b
2	90.01 (1.59)^c	90.55 (2.38)^c	86.98 (3.39)^c
3	89.43 (2.29)^b	73.99 (3.94)^c	75.55 (2.36)^c
Unopsonized			
1	55.72 (5.01)^d	27.68 (4.58)^c	37.79 (6.23)^b
2	60.06 (2.35)^b	45.68 (1.77)^c	46.00 (2.68)^c
3	71.94 (2.93)^b	50.78 (1.75)^c	54.41 (9.93)^c

[a] Represents the mean percentage of phagocytic macrophages from three separate culture dishes for each group. Each group consisted of a pool of four chickens.

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

was observed for phagocytosis of the opsonized SRBC by macrophages from different groups, macrophages from both the trisomic and tetrasomic groups of chickens were significantly depressed in their ability to phagocytize the unopsonized SRBC. However, the relative activity of individual phagocytic macrophages to engulf opsonized and unopsonized SRBC was similar in all three genetic groups. The means from three experiments ranged from 8.3 to 8.8 for opsonized and 3.4 to 4.0 for unopsonized SRBC per phagocytic macrophage within these groups of chickens.

The incidence of opsonin-receptor-bearing macrophages in the adherent PEC of 6-week-old disomic, trisomic, and tetrasomic birds was examined using a SRBC-rosette assay. Because heat-inactivated antiserum was used in opsonization, receptor binding should involve primarily the FcR. Macrophages from tetrasomic chickens exhibited the highest incidence of FcR$^+$ cells in three of four separate experiments (Table IV). Also, tetrasomic peritoneal exudate macrophages possessed a significantly higher proportion of macrophages reactive with the CMTD-2 MCA than the disomic cells as determined by the flow cytometry analysis (Table V and Fig. 1).

Discussion

Previous studies using congenic chicken lines for the *B* complex suggested that the MHC influences production and activation of harvestable peritoneal adherent cell populations in chickens. Major line differences were seen in Sephadex-elicited adherent cell production and the incidence of macrophages in the adherent PEC. The apparent MHC influence extended to the functional ability of both monocytes and macrophages. Blood monocytes from 15I_5-*B* congenic lines differed in chemotactic activity to f-met-leu-phe (24) and macrophages from these lines possessed different phagocytic and bactericidal activities (17). More recently, blood monocytes from these MHC congenic lines were found to exhibit different functional capabilities using direct *in vitro* activation with lipopolysaccharide (Puzzi *et al.*, unpublished observation). These findings suggest that MHC differences in the chicken can influence the response capacities of monocytes to inflammatory signals.

The present study was conducted to investigate the possible effects of increased dosage of the chicken MHC

Table IV. Incidence of Opsonin Receptor-Positive Macrophages in Sephadex-Elicited Peritoneal Macrophages

Experiment	Disomic mean (SD)[a]	Trisomic mean (SD)[a]	Tetrasomic mean (SD)[a]
1	22.53 (6.56)[b]	25.27 (4.77)[b]	44.33 (6.05)[c]
2	42.39 (6.90)[c]	34.89 (6.34)[b]	43.56 (4.56)[c]
3	42.91 (7.39)[b]	39.83 (3.47)[b]	53.82 (3.92)[c]
4	33.21 (5.11)[b]	39.62 (3.62)[c]	56.70 (5.72)[d]

[a] Represents the mean percentage of rosette-positive cells scored from three separate coverslip culture dishes. Rosetteing should be mediated via the FcR. Each group consisted of a pool of four birds.
[b,c,d] Numerical values within rows not sharing a common superscript letter are significantly different by Student's t test at $P < 0.05$.

Table V. Incidence of Peritoneal Exudate Macrophages Reactive with CMTD-2 Monoclonal Antibody[a]

Experiment	Disomic	Trisomic	Tetrasomic
1	12.29	10.90	17.20
2	11.90	21.60	17.70
Mean (SD)	12.09 (0.27)[b]	16.25 (7.57)[b,c]	17.45 (0.35)[c]

[a] Sephadex-stimulated PEC from 4- to 5-week-old trisomic strain chickens were fixed in methanol and then allowed to react with primary (CMTD-2) and secondary (goat anti-mouse Ig fluorescein isothiocyanate) antibodies. Samples were run through FACS along with PEC treated with myeloma supernatant as controls.
[b,c] Represents the mean of the percentage of positive PEC as determined by FACS analysis. The PAI control values were already substracted from the test values. Numerical values within a row not sharing a common superscript letter are significantly different by Student's t test at $P < 0.05$.

(*B* complex) on recruitment, incidence, and functional abilities of peritoneal macrophages and blood monocytes. This investigation extended parallel results from the trisomic line involving the same laboratories that demonstrated the association of enhanced *B* complex class II expression (B-L products) with altered B lymphocyte differentiation (18, 21). This trisomic strain of White Leghorn chickens is unique since the birds carry extra copies of the microchromosome encoding the MHC. MHC-encoding genes on each of the microchromosomes in aneuploid cells are transcriptionally active and increased levels of product per cell has been demonstrated for both B-G/B-F- and B-L-encoded glycoproteins (22).

The functional parameter examined for monocytes was that of directed migration of chemotaxis. When trisomic line chickens were tested for their ability to respond to a chemoattractant, tetrasomic monocytes responded significantly higher than disomic and trisomic cells in response to both *E. cloacae* supernatant and f-met-leu-phe. These two chemotactic agents are related in that f-met-leu-phe shares a structural similarity with a peptide released by bacteria, e.g., *Escherichia coli* (31).

Evidence for the existence of distinct monocyte subpopulations in the peripheral blood of the chicken capable of chemotactic responsiveness to f-met-leu-phe has been recently presented by Golemboski and Dietert (32). Similarly, Delany *et al.* (18), using the same trisomic line chickens, showed a clear alteration in bursal B lymphocytes in the aneuploid chickens with regard to both their cell compositions and Ia expression. Therefore, the differences observed in the chemotactic ability of blood monocytes between disomic and aneuploid chickens may be due to (i) a shift in number or composition of the responsive subpopulations of monocytes, (ii) an increase in number or affinity of cell surface receptors specific for recognizing the particular chemotactic signal, or (iii) an altered transmembrane signal transduction pathway leading to enhanced migration of aneuploid cells. To evaluate the breadth of the enhanced chemotactic response of tetrasomic cells, it would be useful to examine additional chemotactic agents.

In the present study, no significant group differences were observed in the total number of harvestable PEC elicited by Sephadex. This was similar to the lack of PEC number differences observed in *B* congenic chicken lines (17). In a recent study of murine macrophages functional differences in the A/J and B10 inbred lines were found, but there were no differences in total number of exudate cells (33).

Disomic and trisomic chickens produced high numbers of macrophages whereas tetrasomics had a heterogeneous cellular response with an increased incidence of heterophils in lieu of macrophages. The mixed cellular inflammatory response generated by the tetrasomics might be important in considering the depressed phagocytic activity of tetrasomic macrophages for unopsonized SRBC. However, in a previous study, no correlation was observed between the peritoneal macrophage-heterophil ratio and macrophage phagocytic activity in Sephadex response of chicken *B* congenic lines (17).

When macrophage monolayers from disomic, trisomic, and tetrasomic birds were incubated with antibody-coated SRBC, no differences were observed among the groups in the ability to phagocytize opsonized SRBC. This process of phagocytosis is apparently mediated through the FcR present on the macrophage surface. When macrophages from these groups of chickens were tested for opsonin receptors (likely FcR), tetrasomic birds expressed an increased incidence of macrophages positive for opsonin receptors. The increased FcR$^+$ macrophage population may represent a compensatory mechanism for the lower percentage of macrophages in tetrasomic birds, thereby enabling

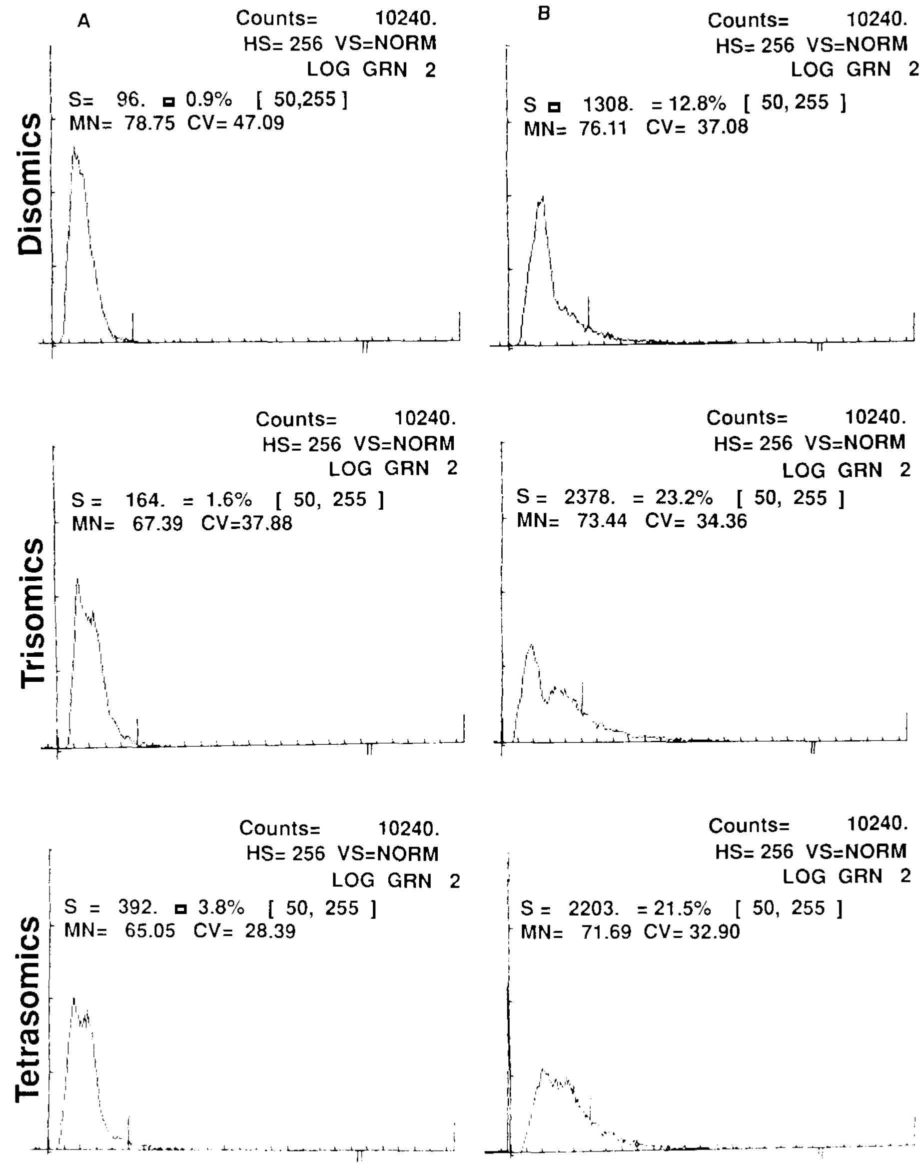

Figure 1. Flow cytometry analysis of peritoneal exudate cells from disomic, trisomic, and tetrasomic birds. Percentage of positive cells to control supernatant (panel A) and CMTD-2 MCA (panel B) are shown.

tetrasomic macrophages to express Fc-mediated SRBC phagocytosis activity similar to the one expressed by macrophages from other groups. It has been shown that during the inflammatory response to Sephadex, macrophages capable of Fc-mediated phagocytosis appear earlier than cells with the receptor for unopsonized SRBC uptake (34).

It has been shown in mice that the intraperitoneal injection of an irritant like Sephadex (35) or an antigen like *Corynebacterium parvum* (36) results in the recruitment of macrophages into the cavity. The macrophages thus recruited are not only highly activated but are recruited in different steps or stages. They vary in size, e.g., small and medium macrophages which are derived from newly arrived monocytes or large tumoricidal macrophages which are relatively more differentiated. Therefore, the presence of high numbers of FcR$^+$ macrophages in the peritoneum of tetrasomic birds might result from differences in the incidence of two different subpopulations of macrophages and/or differences in the degree of differentiation of these macrophages. Similar changes in the incidence of FcR$^+$ cells have been observed in the mouse following immunization with SRBC (33). Whether this difference in tetrasomic chickens results from an endogenous genetic-based shift in the differentiation pattern of macrophages or in the response patterns of these cells to Sephadex stimulation is as yet unclear. Delany *et al.* (18) found that the aneuploid birds show an increased percentage of small bursacytes and altered Ia-positive bursacyte subpopulations compared with disomics. An analogous situation might be true for monocyte-macrophages which may express an altered differentiation pattern in aneuploid chickens.

The mouse MCA, CMTD-2, was produced against Sephadex-stimulated chicken peritoneal macrophages and was shown to react with a subpopulation of inflammatory macrophages (30) that is also detected during spontaneous autoimmune thyroiditis (37). In the present study, peritoneal exudate macrophages from tetrasomic birds contained a significantly higher incidence of CMTD-2-reactive macrophages than did disomic preparations. Although the nature and functional properties of CMTD-2-reactive macrophages is not known, this result is consistent with the hypothesis that there is a shift in peritoneal macrophage populations obtained in the peritoneal cavity of tetrasomic chickens.

This study has demonstrated that the dosage of a specific *B* haplotype of the MHC is associated with differences in chicken monocyte-macrophage functions. The differences appear to be based on altered monocyte-macrophage subpopulations within aneuploid chickens perhaps due to altered differentiation. The overall responsiveness of monocyte-macrophages from aneuploids varied significantly from normal chickens both in macrophage production and other functional parameters tested. When combined with previous analysis on *B* congenic lines of chickens, the results suggest that MHC gene products influence chicken macrophage differentiation and functions. Although the trisomic strain of chickens represents an excellent gene dosage model for the *B* complex, the existence of possible influences from non-MHC genes on this microchromosome in the chicken cannot be completely excluded. As yet there is no evidence that genes other than MHC and rDNA are present in this microchromosome containing less than 1% of cell DNA. Non-MHC genetic differences have been described for chicken monocyte-macrophage parameters (38–41). Whether non-MHC genetic differences could play a role in the present results requires further investigation.

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