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Antibody Response to Sheep Red Blood Cells in a Major Histocompatibility (B) Complex Aneuploid Line of Chickens

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ABSTRACT An integral part of the immune response is the production of antibodies specific for different antigenic challenges. Genes of the MHC encode products that regulate immunity. This study utilized the FCT-15 line of chickens, which is aneuploid for the chromosome containing the ribosomal RNA genes (rDNA) and the MHC or B complex to determine whether an antibody response to SRBC would vary as a function of B complex gene dose. Mating of trisomic parents (B15B15B15) animals produced progeny having either a disomic (B15B15), trisomic (B15B15B15), or tetrasomic (B15B15B15B15) B complex dosage. The number of B/rDNA chromosomes, and thus the B complex dosage, was determined by feather pulp nucleolar typing of chicks at hatch. A 5% SRBC antigenic challenge, which induces a T cell-dependent antibody response, was injected at 6 wk of age. Samples taken prior to SRBC injection as well as 5, 8, and 12 d postinjection were assayed for total and mercaptoethanol-resistant antibody. Peak antibody titers (log2), day of peak titer and rate of titer decline were calculated using a quadratic equation for each bird. Differences among the three B complex dosages were evaluated by analysis of variance. Antibody titers rose from 5 to 8 d postinjection and declined thereafter without significant differences among the three B complex doses. Calculations from the quadratic equations showed that B complex dose affected neither peak antibody titer nor day of peak titer. However, trisomic and tetrasomic animals had significantly more rapid rates of decline from the maximum titer. In aneuploid chickens, changes in antigen processing, antigen presentation, or persistence of processed antigen may maintain levels of antibody production found in disomic chickens and explain the more rapid decline of titer.

(Key words: aneuploidy, B complex, antigen, immune response, major histocompatibility complex)

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INTRODUCTION

Pathways leading to an antibody response require complex, cellular interactions. Genes of the MHC encode products that expedite these essential interactions in a competent immune response. In the case of particulate antigens, a particle is first engulfed by phagocytic cells, usually macrophages or possibly antigen-specific B cells. Upon intracellular degradation of the antigen, a portion of processed peptide is expressed on the extracellular surface in conjunction with self antigen encoded by the MHC. The complex of MHC molecules and coexpressed processed peptides allows effective interaction with T helper (T_h) cells that release lymphokines enhancing B cell proliferation and differentiation into antibody secreting plasma cells (Kuby, 1991).

In chickens, the MHC or B complex is found on a single chromosome approximately 16th in size that also contains the ribosomal RNA genes or nucleolar organizer (Bloom and Bacon, 1985; Bloom et al., 1987). The FCT-15 line consists of chickens that are aneuploid for the B complex-bearing chromosome and it represents the only animal model of viable trisomy and tetrasomy (Bloom and Bacon, 1985). This model has facilitated studies of B complex dosage effects on immune development.

Alterations of B complex dosage influence primary lymphoid organ development (Hemendinger et al., 1992), bursal and thymic lymphocyte counts (Hemendinger et al., 1992), B lymphocyte subpopulation profiles (Delany et al., 1992), and expression of B complex antigen on B lymphocytes (Delany et al., 1988). Macrophages also expressed B complex class II molecules in relation to their B complex chromosome dose (Qureshi et al., 1989; Lin et al., 1991; 1993). Functional analysis revealed aneuploidy affected SRBC phagocytosis (Qureshi et al., 1989), anti-tumor activity (Lin et al., 1993), production of nitrite (Lin et al., 1993), and in vitro antigen presentation (Lin et al., 1991).

The influence of B complex chromosome dosage on in vivo immune responses is unknown. Aneuploid effects

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were postulated based upon the perturbations found previously in macrophages, T cells, and B cells. The current investigation was undertaken to determine whether B complex dosage would affect T cell-dependent antibody production to an erythrocyte antigen, SRBC.

**MATERIALS AND METHODS**

**Stock**

Animals utilized in this investigation were derived from the FCT-15 trisomic line (B15B15B15) developed at Cornell University (Bloom and Bacon, 1985; Bloom et al., 1987). These birds, hereafter referred to as the trisomic line, exhibit an aneuploid condition for the 16th chromosome, which bears the MHC (B) complex. This aneuploid condition is stably inherited in a Mendelian fashion such that matings between trisomic parents produce a 1:2:1 ratio of disomic, trisomic, and tetrasomic progeny. Fertile eggs were incubated and hatched at the University of New Hampshire Poultry Research Farm. Chicks were banded for identification and were brooded in electrically heated brooding batteries. Chicks were fed a commercially prepared chick starter consisting of 20.0% crude protein and 2,860 kcal ME/kg, which met the essential nutrient requirement (NRC, 1984), throughout the experiment.

**Determination of Chromosome Dosage**

Pin feathers were harvested from chicks at hatch. Samples were prepared as previously described (Bloom and Bacon, 1985; Muscarella et al., 1985). Phase contrast examination was accomplished by the method of Delany et al. (1992). Chromosome dosages from phase contrast examination were confirmed using an acridine orange staining technique (Bloom and Bacon, 1985).

**Sheep Red Blood Cell Challenge**

The SRBC, collected in Alsever's solution, were washed three times in normal saline. After the final wash, the packed cells were brought to a 5% vol/vol solution in the 0.9% NaCl solution. Two hatches using a total of 98 chicks were used at 6 wk of age. A blood sample was drawn from each chick prior to injection (Day 0). Each experimental subject was then injected i.v. in the wing vein with 1 mL of the 5% SRBC solution to induce a T cell-dependent antibody response. Additional blood samples were drawn at 5, 8, and 12 d postinjection intervals. Serum was recovered from clotted blood by centrifugation and was stored at -0°C until tested.

**Hemagglutination Assay**

Total SRBC antibody titers for each bird were measured using the microtiter procedure of Wegman and Smithies (1966). Mercaptoethanol (ME)-resistant antibody (IgG) was measured as described by Yamamoto and Glick (1982). The titers, both total and IgG, were expressed as the log2 of the reciprocal of the highest dilution giving visible agglutination.

**Statistical Analysis**

The titers (log2) were evaluated by analysis of variance with hatch, B complex dose and time as main effects. In addition, titer values for each bird were used to calculate a quadratic equation:

\[ Y = a + bX - cX^2, \]

after the method of Siegel et al. (1984). The first derivative of the equation gives the day of maximum titer. Substituting for X in the above equation, allows the determination of the maximum titer. The value of the c coefficient estimates the decline from the maximum titer. Student-Newman-Keuls test was used to separate significant means.

**RESULTS**

Antibody production profiles of FCT-15 chickens were significantly affected by time but not by hatch or B complex dosage (Table 1). Data were then pooled across hatches and gene doses. The highest titer occurred 8 d postinjection. Titers rose from a low of 4.8 ± 0.3 at 5 d to a peak of 6.5 ± 0.2 at 8 d. By 12 d, the titers declined to 4.5 ± 0.2. A similar titer response profile was also evident in the individual gene dosages. The ME-resistant antibody did not differ significantly among the three B complex dosages for the sample periods (data not shown).

Using the quadratic equation analysis (Figure 1), disomic birds had the highest calculated peak titer (6.41) but this value was not significantly different from either the trisomic (6.26) or tetrasomic birds (6.34) (Table 2). Likewise, the day of peak titer was not statistically different among the three gene dosage types. Disomic individuals had a slightly later (8.44) day of peak titer compared to trisomic and tetrasomic individuals. Although the peak antibody titers and day of peak titer were not significantly different as a function of gene dose, the rate of decline from the maximum titer defined by the c coefficient of the quadratic equation was more rapid in the trisomic and tetrasomic animals (Table 2).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatch</td>
<td>1</td>
<td>0.686</td>
</tr>
<tr>
<td>Dose (D)</td>
<td>2</td>
<td>0.876</td>
</tr>
<tr>
<td>Time (T)</td>
<td>2</td>
<td>0.001</td>
</tr>
<tr>
<td>D x T</td>
<td>4</td>
<td>0.505</td>
</tr>
</tbody>
</table>

**TABLE 1. Summary of probability values for main effects and interaction from ANOVA of antibody titers to SRBC in FCT-15 chickens at 6 wk of age**
FIGURE 1. Mean total antibody titers (± SE) to SRBC as a function of B complex dose in FCT-15 chickens inoculated at 6 wk of age. Data were analyzed by a quadratic equation $Y = a + bX - cX^2$ (Siegel et al., 1984). The peak day is calculated from the first derivative of the equation. Peak titer is calculated by substituting for X in the original equation. The c coefficient estimates the decline from the maximum titer. Values having no common letter differ significantly ($P < 0.05$).

Disomic birds had a c coefficient of 0.088 compared to 0.098 and 0.097 in the trisomic and tetrasomic animals, respectively.

DISCUSSION

The results demonstrate that antibody to SRBC challenge rose to a peak and then declined but the response was not disparate among chickens having different B complex chromosome dosage. Neither the peak antibody titer nor the day of peak titer as determined from the quadratic equation were statistically different as a function of B complex gene dosage. The ME-resistant antibody was similar in all three gene doses. Aneuploid chickens did have a more rapid decline from peak antibody titer compared to disomic chickens.

Prior studies using this model found that trisomic or tetrasomic chickens had retarded growth as well as reduced bursal and thymic sizes at 3 and 6 wk posthatch (Hemendinger et al., 1992). Bursal lymphocyte counts averaged 50% lower and bursal follicular histology was altered in tetrasomic chickens compared to disomic chickens. Delany et al. (1992) showed that small bursal cells and cell surface B complex class II Ia antigen expression were increased in aneuploid chickens compared to disomic chickens. The B cell proliferation decreased as gene dosage increased. On the other hand, aneuploid chickens had lower thymic lymphocyte counts without significant alterations in thymic or splenic histology.

Activity of another immune cell, the macrophage, varies as a function of gene dose. Expression of Ia and Fc receptors (FcR) on the macrophage cell surface is altered in aneuploid birds (Lin et al., 1991; Qureshi et al., 1989). Such a B complex dependent effect on cell surface molecules is similar to B cell populations dosage patterns described by Delany et al. (1992). Phagocytic activity toward unopsonized SRBC was reduced in aneuploid birds despite increased levels of FcR (Qureshi et al., 1989), whereas phagocytosis of opsonized SRBC was not different among the three B complex doses.

Lin et al. (1991) compared the antigen presenting capacity of macrophages collected from the peritoneal cavity of aneuploid birds either 24 or 42 h after Sephadex injection. All three B complex gene doses were compared in their ability to present BSA to primed disomic peripheral blood lymphocytes. No significant difference was found in 24 h macrophages but trisomic and tetrasomic macrophages collected at 42 h had reduced antigen presentation.

There are two alternative explanations for the results of the current study. First, if aneuploid macrophage presentation of the SRBC antigen is reduced as was found with BSA (Lin et al., 1991), then the activity of antigen-specific B cells or T cells or the two combined may be higher resulting in antibody titers that are similar to those found in disomic birds. Such a mechanism would generate an effective immune response despite reduced antigen presentation. However, aneuploid chickens have lower bursal lymphocyte counts (Hemendinger et al., 1992) as well as lower numbers of bursal B cells in the proliferative phase at 1 wk posthatch (Delany et al., 1992), which would argue against increased proliferation at later ages. Higher activity in T cells would remain plausible because no major differences were found in thymic histology (Hemendinger et al., 1992).

<table>
<thead>
<tr>
<th>Gene dose</th>
<th>n</th>
<th>Peak titer (log$_2$)</th>
<th>Day of peak titer</th>
<th>c Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>16</td>
<td>6.41</td>
<td>8.44</td>
<td>0.068*</td>
</tr>
<tr>
<td>3</td>
<td>49</td>
<td>6.26</td>
<td>7.99</td>
<td>0.086*</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>6.34</td>
<td>8.01</td>
<td>0.097*</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td></td>
<td>0.04</td>
<td>0.15</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*Means within a column with no common superscript differ significantly ($P < 0.05$).
On the other hand, presentation of SRBC determinants may be similar among all three B complex gene doses. Because class II antigen expression on B cells and macrophages is a function of B complex dosage (Delany et al., 1988; Lin et al., 1991), it appears that the antibody response is not merely a function of number of available B complex class II molecule-processed peptide complexes available for T cell receptor interaction. Increased numbers of class II surface molecules on B cells and macrophages may produce steric hindrance allowing only a certain number of class II molecules to interact and thereby serving as a compensatory mechanism that maintains disomic levels of antibody regardless of gene dose. Compensation in response to aneuploid gene dose has already been shown to function in FCT-15 chickens. Muscarella et al. (1985) demonstrated that the level of mature mRNA encoding for ribosomal proteins remains at disomic levels without regard to ribosomal gene dosage.

Aneuploidy for the 16th chromosome does not eliminate the possibility that other genes on this chromosome may affect the response. A polymorphic system, Rfp-Y, which contains at least two MHC class I and two MHC class II genes, assorts independently (Briles et al., 1993; Miller et al., 1994) of the B complex but maps to the same chromosome (M. M. Miller, 1995, Beckman Research Institute, 1450 E. Duarte Rd., Duarte, CA 91010, personal communication). These Rfp-Y genes or other genes, found on the same chromosome may have a role in the observed results.

Macrophages from aneuploid chickens may differ in antigen processing. The data of Lin et al. (1991) may actually reflect the amount of processed antigen on the macrophage surface rather than the macrophage's ability to interact with primed T and B cells. Enzymatic differences in aneuploid macrophages would alter the quantity of processed antigen available on the surface. Barbey et al. (1995) found that organelles isolated from murine B cell lines expressing different MHC haplotypes differed in their in vitro degradation of antigen. The authors propose that such differential processing might be the result of both different enzymatic content and dimorphism at given regions of the MHC. The FCT-15 line is homozygous for the B15 haplotype but this does not exclude enzymatic differences attributable to increased doses of the B complex or other genes on the same chromosome. In fact, superoxide dismutase was reduced in tetrasomic macrophages during the late inflammatory response (Lin et al., 1992) supporting the possibility of other enzyme changes.

The more rapid antibody titer decline from the peak in trisomic and tetrasomic chickens indicated a B complex dosage effect on this facet of the antibody response. Biozzi et al. (1984) demonstrated that persistence of processed antigen on the murine peritoneal macrophages cell surface is a fundamental difference between mouse lines that are known to be high or low antibody responders to a SRBC challenge. Antigen persistence was longer on the surface of macrophages derived from the high titer response lines. Disomic macrophages may have longer antigen persistence that does not elevate the peak antibody titer but does produce a slower decline from peak titer.

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REFERENCES


