Major Histocompatibility Complex-Linked Immune Response of Young Chickens Vaccinated with an Attenuated Live Infectious Bursal Disease Virus Vaccine Followed by an Infection


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ABSTRACT The influence of the MHC on infectious bursal disease virus (IBDV) vaccine response in chickens was investigated in three different chicken lines containing four different MHC haplotypes. Two MHC haplotypes were present in all three lines with one haplotype (B19) shared between the lines. Line 1 further contains the BW1 haplotype isolated from a Red Jungle Fowl. Line 131 further contains the B131 haplotype isolated from a meat-type chicken. Finally, Line 21 further contains the international B21 haplotype. The chickens were vaccinated with live attenuated commercial IBDV vaccine at 3 wk of age, followed by a challenge with virulent IBDV at 6 wk of age. In this study, we found a notable MHC haplotype effect on the specific antibody response against IBDV, as measured by ELISA. The BW1 haplotype was found to have a significantly higher serum antibody titer against IBDV (7,872) than haplotypes B19 (mean 5,243), B21 (5,570), and B131 (5,333) at 8 d postinfection. However, a virus-neutralizing antibody test did not reflect this result. Nevertheless, the MHC haplotype-associated protective immunity was further supported by the bursa of Fabricius (bursa) recovery from the disease, as measured by histological scorings of the bursa. Chickens carrying the BW1 haplotype had a significantly lower bursa lesion score (1.7) than the haplotypes B19 (mean 3.8), B21 (3.6), and B131 (4.3) 8 d postinfection. Furthermore, multiple line effects were found in other variables when comparing Day 6 with Day 8. Body weight, relative weights of the bursa and the spleen, percentage and relative number of MHC II molecules on MHC II-positive lymphocytes, percentage and relative number of CD4 molecules on CD4-positive lymphocytes, and the specific antibody response all differed significantly among lines. Line 1, with Red Jungle Fowl genes, was clearly differentiated from the other two investigated lines. These results suggest an MHC II restricted T-cell dependent secondary antibody response against IBDV.

(Key words: chicken, major histocompatibility complex, disease resistance, vaccination, infectious bursal disease virus)

INTRODUCTION Infectious bursal disease (IBD) in chicken flocks is caused by a birnavirus that induces inflammation and necrosis in the bursa of Fabricius (bursa). Two serotypes have been recognized, of which serotype 1 may cause disease in chickens, whereas serotype 2 is avirulent (Saif, 1998). The virus infection leads to the destruction of B-lymphocytes in the bursa and, to a lesser degree, in other lymphoid organs such as cecal tonsils, spleen, thymus, and gland of Harder (Lukert and Saif, 1991; Saif, 1998).

The major changes in the bursa begin 3 d postinfection (PI) where the size of the bursa increases, reaching up to twice its normal size by 4 d PI due to hyperemia and infiltration of heterophils and T-lymphocytes in the bursa. Then, atrophy follows and the bursa diminished to one third of its original weight by 8 d PI (Tanimura and Sharma, 1997; Saif, 1998; Kim et al., 1999). When chickens

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Abbreviation Key: CE = chicken embryo; IBDV = infectious bursal disease virus; PI = postinfection.
of 3 to 6 wk of age are infected with the serotype 1 strain, they typically develop clinical symptoms with increased mortality within 3 to 5 d (Fadly and Nazarian, 1983; Tanimura and Sharma, 1998). Younger chickens more often develop a subclinical infection and subsequent immunosuppression that manifests itself as a complete lack of IgG and the presence of only monomeric IgM (Ivanyi and Morris, 1976) with a subsequent poor response to routinely used vaccines (Faragher et al., 1974).

The disease caused by infectious bursal disease virus (IBDV) is economically very important because of its immunodepressive effects, as well as its ability to induce morbidity and mortality (Lukert and Saif, 1991). The use of vaccines generated by attenuating the virus has been very successful in controlling the disease. However, IBDV continues to cause problems in the field.

Genetic disease resistance is known to influence several diseases in poultry. In many cases, this resistance has been mapped to the MHC region. In chickens, the MHC is called the B-system. The B-system is considered to be the strong transplantation locus and contains at least two B-FA genes that are homologous to mammalian MHC class I and at least two B-LB genes that are homologous to mammalian MHC class IIB genes. In addition, the B-system contains genes encoding a third group of highly polymorphic molecules, the B-G genes, which have been found in birds only (Guillemot et al., 1989; Kaufman et al., 1989). One of the most significant associations between MHC and disease resistance is that observed for Marek’s disease (Lamont, 1998), but resistance to other virus-induced diseases such as Rous sarcoma virus (Schierman and Collins, 1987) and avian leukemia (Yoo and Sheldon, 1992) have been linked to the MHC region as well. In addition to viral diseases, other diseases have been shown to be influenced by the MHC. These diseases include fowl cholera (Lamont et al., 1987), coccidiosis (Lillehoj et al., 1989), and salmonella infections (Cotter et al., 1998). However, previous research on the association of MHC and resistance to IBDV has produced conflicting data (Fadly and Bacon, 1992; Nielsen et al., 1998).

In addition to genetic differences in immune response to virus infections, there is also evidence that chickens vary in their response to attenuated viruses currently used as vaccines (Bumstead et al., 1993). However, no influence of the MHC region on antibody response to IBDV vaccination has yet been found (Fadly and Bacon, 1992). The aim of this study was to investigate the association between the MHC region and the protection induced by vaccination against Infectious Bursal disease.

**MATERIALS AND METHODS**

**Birds**

The parents of the experimental chickens were reared in a filtered-air, positive-pressure house. The parent flock was tested serologically for antibodies against IBDV and was found to be negative. Breeding pairs were set up to yield MHC homozygous offspring. The experimental chickens were hatched in the filtered-air, positive-pressure house and transferred at 1 d of age to special isolators. The chicks were kept there during the experiment under positive pressure with sterile-filtered air. The chickens were fed diets that met or exceeded NRC requirements. Feed and water were provided ad libitum. Line 1 originates from meat type chickens and consists of 50% White Cornish and 50% Scandinavian White Leghorn. Line 21 is 100% international White Leghorn (Hedemand et al., 1993; Juul-Madsen et al., 2000).

**Vaccination**

Vaccination was performed with the attenuated live IBDV Vaccine Nobilis Gumboro based on the PBG98 virus strain from Intervet.2 When the birds were 3 wk old, the vaccine was added to the drinking water, in accordance with the instructions of the manufacturer.

**Virus**

Experimental infection was performed with homogenized bursa taken from SPF chickens 3 d PI with the Faragher 52/70 IBDV strain. Each chicken was given 10^{4.5} 50% egg lethal dose (ELD_{50}) of IBDV orally and by eyedrop at 6 wk of age.

**Termination of the Experiment**

The birds were killed 6 or 8 d PI by cervical dislocation; sex, body, spleen, and bursa weight were recorded. One milliliter of EDTA-stabilized blood for flow cytometric analysis and 1 mL of unstabilized blood for preparation of serum were collected. The bursa was fixed in 10% (vol/ vol) formalin in neutral buffered saline. The relative weights of the bursa and spleen were calculated as weight of organ (g) × 100/body weight (g).

**MHC Serological Typing**

Serological MHC typing was carried out by hemagglutination on open glass plates with specific MHC alloantisera (B-F and B-G) according to the procedure of Juul-Madsen et al. (1993). All the experimental birds were typed on the day of killing for verification of the MHC haplotypes.

**Histology**

The degree of depletion or necrosis of the lymphocytes in the bursa was evaluated on formalin-fixed, paraffin wax-embedded, and hematoxylin and eosin-stained equatorial sections, cut perpendicularly to the long axis

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2Intervet, Cambridge, CB4 4FP UK.
of the plicae. The total area of the section was evaluated and recorded based on lesion scores, i.e., 0 = 0 to 5%, 1 = 6 to 32%, 2 = 33 to 65%, 3 = 66 to 94%, 4 = 95 to 100% depletion or necrosis, and 5 = loss of follicular architecture with fibroplasia (modified after Muskett et al., 1997).

**Flow Cytometric Analysis**

Flow cytometric analysis was performed on white blood cells of all birds as described by Juul-Madsen et al. (2000). The primary antibody supernatants were as follows: anti-chicken β2-microglobulin (F21-21), anti-chicken B-LB (2G-11), anti-chicken CD4 (2-6), and anti-chicken CD8 (11-39). The secondary antibody was a fluorescein isothiocyanate (FITC)-labeled goat F(ab′)2 fragment anti-mouse IgG (H + L).3 Lymphoprep isolated mononuclear cells were analyzed on a flow cytometer (Coulter Epics)3 with excitation at 488 nm from an argon laser. Analytic gates were chosen based on forward and side scatter to include lymphocytes (including contaminating thrombocytes) and to exclude debris, dead cells, and erythrocytes. Flow cytometer alignment verification was performed with Flow-Check fluorospheres,2 and day-to-day standardization of the flow cytometer was performed with uniform dyed microspheres (0.96 µm).4

**Serum Antibody Titers Against IBDV**

A ProFLOK infectious bursal disease ELISA test kit5 was used to measure serum antibody titers against IBDV. The ELISA assay was performed according to the kit manual. Briefly, 96-well microtiter plates coated with IBDV antigen were incubated with 5 µL serum samples and positive and negative controls included in the kit, followed by incubation with a horseradish peroxidase-conjugated affinity-purified antibody from a pool of serum from goats immunized with chicken IgG (H + L).2,2′-Azinodio (3-ethyl benzthiazoline sulfonic acid) was used as chromogen; 5% SDS was the stop solution. The result was monitored as optical density (OD) at 405 nm, and the antibody titer was calculated as SP = (sample absorbance) – (average normal control absorbance)/corrected positive control absorbance.

**Virus-Neutralizing Antibody Titer Determination**

A serum-neutralizing antibody test was performed in chicken embryo (CE) cell cultures in 96-well microtiter plates. A tissue culture infectious dose of 102.2 of the IBDV vaccine strain Poultv Bursine 2* Vet6 was used in each culture of CE cells. The inverse of the highest dilution of each serum that was able to inhibit the growth of IBDV was designated as the neutralization titer of the chicken serum. In each test, dilutions of a positive and a negative control serum against IBDV were included in the range of 1:20 to 1:1,280. Furthermore, controls of CE cells were incubated with plain medium and serum from each chicken (lowest dilution), and the IBDV inoculum was titrated on the CE cells to confirm the virus neutralization titer. All samples and controls were run as duplicates.

**Statistical Analyses**

The model used was as follows: Y = µ + Gi + Si + G × Sj + e, where µ = overall mean, G = fixed effect of the ith genetic group, S = fixed effect of the jth sampling day, and e = residual—not explained. The analysis of variance was performed by the GLM procedure of SAS software (1994).

**RESULTS**

To estimate the vaccination response resulting from the MHC, a system in which three genetically different lines containing four different B haplotypes was developed and used in the experiment (Table 1; Materials and Methods). The BW1 haplotype was present exclusively in Line 1. The B131 haplotype was present exclusively in Line 131. The B21 haplotype was present exclusively in Line 21. However, the B19 haplotype was present in all three lines. The BW1 haplotype isolated from a Red Jungle Fowl and the B131 haplotype isolated from a meat-type chicken were characterized as B21-like haplotypes (Hedemand et al., 1993; Juul-Madsen et al., 2000). This experimental design made it possible to estimate the contribution of the background genes by fixing the MHC-locus (comparison of the three genetic groups containing the B19 haplotype), and further, if there were no background genes involved in a measured variable, to study the contribution of the different B haplotypes on the vaccine response.

Thirty-two birds from each genetic group were first vaccinated with an attenuated live IBDV vaccine at 3 wk of age, followed by a challenge with IBDV at 6 wk of age. None of the chickens died during the experiment. Half of the birds of each group were killed 6 d PI, and the rest of the birds were killed 8 d PI.

Due to the disease, the relative bursa weight decreased significantly from Days 6 to 8 in all genetic groups (P < 0.05) except for L1-BW1 (P = 0.14) and L131-B19 (P = 0.49) in which the decrease was not significant (data not shown). This result was expected as we have shown that

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3Beckman Coulter, Fullerton, CA 92834.
4Bangs Laboratories, Fishers, IN 46038.
5Kirkegaard and Perry Laboratories, Gaithersburg, MD 20878.
6Fort Dodge Animal Health, Overland Park, KS.
Figure 1. The specific antibody titer against infectious bursal disease virus (IBDV) measured by the ELISA test on Days 6 and 8 postinfection in six different genetic groups vaccinated at 3 wk of age and later infected with IBDV at 6 wk of age. The genetic groups and their MHC haplotypes are described in Table 1. Means without a common letter are statistically different \( (P < 0.05) \).

The relative bursa weight in IBDV-infected birds from Line 1 and Line 131 was still low on Day 17 PI (Nielsen et al., 1998). No differences were observed among the six groups when comparing results exclusively from Day 6 or 8.

The serum antibody titers against IBDV increased significantly from Days 6 to 8 PI in two of the six genetic groups (L1-B19 and L1-BW1) (Figure 1). A comparison of L1-B19, L21-B19, and L131-B19 on Day 6 or 8 showed no significant differences, indicating that no major effect of the background genes was involved in the antibody response against IBDV. However, the extent to which L1-BW1 increased the serum antibody titer from Days 6 to 8 resulted in a significantly higher titer in this genetic group than in the other five genetic groups on Day 8 \( (P < 0.001) \).

Furthermore, the depletion or necrosis of the lymphocytes in the bursa was scored on Days 6 and 8 (Figure 2). A comparison of the L1-B19, L21-B19, and L131-B19 genetic groups with identical B haplotypes revealed no differences on Day 6 or 8, indicating that no background genes were involved in the degree of depletion of the bursa. However, a major MHC haplotype effect was observed when the four B haplotypes were compared. On Day 6, the lesion scores of the bursa were similar in all genetic groups. However, on Day 8 no change in the lesion scores of the bursa in birds with the BW1 haplotype was observed, whereas chickens with the other B haplotypes continued to suffer from the IBDV infection with a score higher than 3.

Finally, the serum samples collected on Day 8 PI were assessed for their ability to neutralize IBDV; the results for each genetic group are shown in Figure 3. Significant differences were found among the three lines examined; however, no B haplotype effect was observed when comparing the two haplotypes within each line.

In addition, several other immunological components were measured, and multiple line effects among lines were found. For comparison, only genetic groups containing identical B haplotypes were included (L1-B19, L21-B19, L131-B19). The data have been summarized in Table 2. Line 1 only increased the body weight significantly from Days 6 to 8, a fact indicating less morbidity. Lines 1 and 21 decreased in bursa weight, whereas Line 131 did not. Line 1 decreased the relative weight of the spleen from Days 6 to 8, whereas Lines 21 and 131 increased the relative weight of the spleen, probably due to hyperplasia. The relative numbers of MHC I molecules increased in Lines 1 and 131 but not in Line 21. No change was observed in the relative number and percentage of CD8-positive lymphocytes. A notably low percentage of MHC II-positive lymphocytes were found in Line 1 and the relative numbers of MHC II molecules per cell was increasing in Line 1 only. For CD4-positive cells, there was a remarkable increase in the relative number of molecules per cell and the percentages of CD4-positive cells in Line 1 compared to Lines 21 and 131, in which no change or a decrease occurred. The change in bursa scores was highest in Lines 21 and 131. For the antibody titer, the increase from Days 6 to 8 was significant only in Line 1. Finally, the neutralizing antibody titer was found to be significantly higher in Line 1 compared to the other two lines.

**DISCUSSION**

In this study, the protective immunity against IBDV was shown to be associated with the MHC region of the
MHC-LINKED VACCINE PROTECTION

FIGURE 2. The degree of depletion or necrosis of lymphocytes, recorded from zero to five based on lesion scores (see Materials and Methods), in the bursa on Days 6 and 8 postinfection in six different genetic groups vaccinated and later infected with IBDV. The genetic groups and their MHC haplotypes are described in Table 1. Means without a common letter are statistically different (P < 0.05).

Chicken. A notable MHC effect was found on the specific antibody response against IBDV in chickens vaccinated with a live, attenuated vaccine strain of IBDV at 3 wk of age followed by an infection with live IBDV at 6 wk of age (Figure 1). On Day 6 PI, no difference in the specific antibody response was found between the six genetic groups with the exception of L21-B21, which was slightly different from the other five genetic groups (P < 0.04). However, on day 8 PI, the genetic group containing the BW1 haplotype clearly differentiates from the rest of the genetic groups (P < 0.001). There seems to be a relationship between the highest IBDV-specific antibody response found in L1-BW1 birds (Figure 1) and the depletion or necrosis of the lymphocytes in the bursa caused by the disease (Figure 2) because all birds except the L1-BW1 had high bursa scores on Day 8 PI, indicating that sera from the L1-BW1 contain more protective antibodies.

Surprisingly, the in vitro neutralizing antibody test did not reflect the specific antibody titer measured by ELISA. In all three lines, there was no significant difference in the neutralization titer between the two B haplotypes in each line (Figure 3); however, major differences were observed among the lines. Line 1 had the highest neutralization titer. One explanation could be that the ELISA test recognizes some immunoglobulins not detected by the neutralization test because the ELISA test uses a pool of serum from goats immunized with purified chicken IgG (H + L), which may recognize other immunoglobulin types that have light chains in common with IgG.

Another explanation could be that antibodies determined in the neutralization test are not the protective antibodies, because the L1-B19 genetic group has a high amount of neutralizing antibodies but no protection against bursa depletion (Figure 2). This result has been observed for other viruses as well (Bachmann and Zinkernagel, 1997). However, the high amount of neutralizing antibodies (natural antibodies) in Line 1 could be an advantage for the T-cell dependent antigen-specific antibody response in L1-BW1 by trapping the IBDV antigens to the splenic marginal zone on follicular dendritic cells and thereby influencing the outcome of the T-cell dependent antibody response (Ochsenbein and Zinkernagel, 2000).

A third explanation could be that the viral neutralizing antibody test contains some limitations since pathogenic strains of IBDV, like the one used in this study (Faragher 52/70), do not replicate easily in vitro and hence cannot be used in the assay. A nonpathogenic vaccine strain of IBDV (attenuated IBDV strain Poulvac Bursine 2* Vet) adapted to tissue culture was, therefore, used instead. The potential differences between the strains used in vivo and in vitro are an unavoidable problem and a limitation of the in vitro IBDV neutralization assay. The result may therefore not reflect the true number of neutralizing antibodies against the IBDV strain Faragher 52/70 or neutralizing antibodies against the vaccine strain (PBG98).

The most logical explanation of the significant difference in the IBDV-specific antibody response (Figure 1), with L1-BW1 being superior to the other haplotypes, would be specific peptide-MHC complexes on B-cells triggering antigen-specific helper T-cells to activate antigen-specific B-cells, and memory B-cells to make membrane-bound and secreted molecules, which may be part of the explanation. However, we have sequenced the antigen-binding domains of both MHC IA and MHC IIB molecules in BW1, B21, and B131 (Juul-Madsen et al., 2000; J. Salomonsen, 2001, Royal Veterinary and Agricultural University, Copenhagen, Denmark, personal communication) and found that these three haplotypes are identical in the analyzed domains. Therefore, the MHC effect on
### TABLE 2. Alteration in the variables investigated from Days 6 to 8 (least squares means)

<table>
<thead>
<tr>
<th>Variables</th>
<th>L1-B19†</th>
<th>L21-B19†</th>
<th>L131-B19†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>666 ↑ 760*</td>
<td>597 → 624</td>
<td>872 → 853</td>
</tr>
<tr>
<td>Relative bursa weight (ratio)²</td>
<td>0.15 ↓ 0.08*</td>
<td>0.19 ↓ 0.11*</td>
<td>0.16 → 0.14</td>
</tr>
<tr>
<td>Relative spleen weight (ratio)²</td>
<td>0.21 ↓↓ 0.14**</td>
<td>0.16 ↑↑ 0.20**</td>
<td>0.14 ↑↑ 0.26**</td>
</tr>
<tr>
<td>Relative number of MHC I molecules (log mean fluorescence)</td>
<td>27.6 ↑ 31.3*</td>
<td>26.7 → 26.7</td>
<td>31.0 ↑ 33.4*</td>
</tr>
<tr>
<td>Relative number of CD8 molecules (log mean fluorescence)</td>
<td>7.6 → 7.2</td>
<td>9.5 → 9.6</td>
<td>11.6 → 11.3</td>
</tr>
<tr>
<td>Percentage of CD8+ cells</td>
<td>9.7 → 11.8</td>
<td>10.1 → 9.7</td>
<td>10.7 → 13.2</td>
</tr>
<tr>
<td>Relative number of MHC II molecules (log mean fluorescence)</td>
<td>41 ↑↑ 47.5*</td>
<td>47.4 → 44.4</td>
<td>52 ↓ 44.7*</td>
</tr>
<tr>
<td>Percentage of MHC II+ cells</td>
<td>2.5 → 2.6</td>
<td>9.1 ↓ 6.6*</td>
<td>4.5 ↑↑ 9.1**</td>
</tr>
<tr>
<td>Relative number of CD4 molecules (log mean fluorescence)</td>
<td>8 ↑↑ 9.9**</td>
<td>9.6 → 10.3</td>
<td>10.4 → 10</td>
</tr>
<tr>
<td>Percentage of CD4+ cells</td>
<td>23.1 ↑↑ 38.9**</td>
<td>35.6 ↓ 21.9**</td>
<td>36.9 → 32.4</td>
</tr>
<tr>
<td>Anti IBDV antibodies (titer)</td>
<td>3,877 ↑ 5,655*</td>
<td>4,170 → 5,353</td>
<td>4,546 → 5,333</td>
</tr>
<tr>
<td>Bursa depletion or necrosis (scoring)</td>
<td>2 ↑ 3.3*</td>
<td>1.6 ↑↑ 4.2**</td>
<td>1.5 ↑↑ 4.3**</td>
</tr>
<tr>
<td>Neutralizing antibodies (titer)</td>
<td>7,500*</td>
<td>805*</td>
<td>3,033*</td>
</tr>
</tbody>
</table>

1Arrows ↑ and ↓ indicate significant differences up or down, whereas → indicates no difference.

2Ratio = weight of organ (g) × 100/body weight (g).

*P < 0.05.

**P < 0.001.

the humoral immune response (Figure 1) and the recovery from the disease measured by bursa depletion (Figure 2) may not only come from MHC genes presenting different sets of peptides to the T cells. Part of the MHC effect may derive from other polymorphic molecules located in the B region. From unpublished data, we know that the BW1 haplotype is distinguishable from the other two haplotypes (B21 and B131) in a micro-satellite (LEI0258) that has been found in the B-system juxtaposed to the B-LB genes (McConnell et al., 1999).

An interesting molecule to contemplate in that connection would be the polymorphic 8.5 B-G gene that is located in the B-F/B-L region close to the micro-satellite (Guillemot et al., 1989; Kaufman et al., 1991). The B-G molecules are polymorphic cell surface proteins that are encoded by the B system and have sequence and structural similarities to the T-cell costimulatory gene family B7 (Linsley et al., 1994; Henry et al., 1999). MHC molecules on antigen-presenting cells are not sufficient to initiate an immune response. Co-stimulatory interactions between additional elements on the cell surface are required for optimal immune response. The 8.5 B-G molecule that has been found on B-cells only (Salomensen et al., 1991; Kaufman and Salomonsen, 1992) may be involved in costimulatory T-cell interactions other than the B7 ligand-CD28 receptor interaction that has already been described in chickens.
Another possibility of B-G functions would be B to B-cell stimulation or regulation because CD28 molecules have been found on activated B-cells also (Kozbor et al., 1987; Zhang et al., 1998).

Finally, we found remarkable line effects on other immunological parameters in the line containing 6.25% Red Jungle Fowl genes (Table 2). In most cases, Line 1 differs from the other two lines containing White Leghorn or meat-type genes. The relative number of MHC II molecules on MHC II-positive cells increases significantly from Days 6 to 8 in Line 1, whereas it decreased significantly in Line 131. The percentage of MHC II was extremely low in Line 1 compared to Lines 21 and 131 and did not change from Days 6 to 8, opposite the other two lines. The percentage and the relative numbers of CD4 molecules on CD4-positive cells increases significantly in Line 1 only, whereas the other two lines have no change or decreases significantly. The specific antibody titer increases significantly in Line 1 only. The bursa lesion score rate is lowest in Line 1 and finally the neutralization titer is significantly higher in Line 1 compared to the other two lines on Day 8. Furthermore, body weight increased significantly in Line 1 only, and the relative spleen weight decreased significantly in Line 1 only, whereas in Lines 21 and 131, it decreased significantly from Days 6 to 8. The superiority of Line 1, at least when compared to the B19 haplotype, may contribute in a positive way to the superiority of birds having the BW1 haplotype when it comes to recovering from the disease.

In conclusion, these results suggest an MHC II-restricted T-cell-dependent secondary antibody response as the only immunological parameters that vary between Days 6 and 8 PI are MHC II and CD4 and the amount of IBDV antibodies.

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