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Immune-Related Gene Expression in Two B-Complex Disparate Genetically Inbred Fayoumi Chicken Lines Following *Eimeria maxima* Infection

Abstract

To investigate the influence of genetic differences in the MHC on susceptibility to avian coccidiosis, M5.1 and M15.2 B-haplotype-disparate Fayoumi chickens were orally infected with live *Eimeria maxima* oocysts, and BW gain, fecal oocyst production, and expression of 14 immune-related genes were determined as parameters of protective immunity. Weight loss was reduced and fecal parasite numbers were lower in birds of the M5.1 line compared with M15.2 line birds. Intestinal intraepithelial lymphocytes from M5.1 chickens expressed greater levels of transcripts encoding interferon- γ (IFN- γ), interleukin-1 β (IL-1 β), IL-6, IL-8, IL-12, IL-15, IL-17A, inducible nitric oxide synthase, and lipopolysaccharide-induced tumor necrosis factor- α factor and lower levels of mRNA for IFN- α , IL-10, IL-17D, NK-lysin, and tumor necrosis factor superfamily 15 compared with the M15.2 line. In the spleen, *E. maxima* infection was associated with greater expression levels of IFN- γ , IL-15, and IL-8 and lower levels of IL-6, IL-17D, and IL-12 in M5.1 vs. M15.2 birds. These results suggest that genetic determinants within the chicken MHC influence resistance to *E. maxima* infection by controlling the local and systemic expression of immune-related cytokine and chemokine genes.

Keywords

major histocompatibility complex, Fayoumi, *Eimeria maxima*, immune-related, disease resistance

Disciplines

Agriculture | Animal Sciences | Genetics and Genomics | Poultry or Avian Science

Comments

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Immune-Related Gene Expression in Two *B*-Complex Disparate Genetically Inbred Fayoumi Chicken Lines Following *Eimeria maxima* Infection

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ABSTRACT To investigate the influence of genetic differences in the MHC on susceptibility to avian coccidiosis, M5.1 and M15.2 *B*-haplotype-disparate Fayoumi chickens were orally infected with live *Eimeria maxima* oocysts, and BW gain, fecal oocyst production, and expression of 14 immune-related genes were determined as parameters of protective immunity. Weight loss was reduced and fecal parasite numbers were lower in birds of the M5.1 line compared with M15.2 line birds. Intestinal intraepithelial lymphocytes from M5.1 chickens expressed greater levels of transcripts encoding interferon- γ (IFN- γ), interleukin-1 β (IL-1 β), IL-6, IL-8, IL-12, IL-15, IL-17A, inducible nitric

oxide synthase, and lipopolysaccharide-induced tumor necrosis factor- α factor and lower levels of mRNA for IFN- α , IL-10, IL-17D, NK-lysin, and tumor necrosis factor superfamily 15 compared with the M15.2 line. In the spleen, *E. maxima* infection was associated with greater expression levels of IFN- γ , IL-15, and IL-8 and lower levels of IL-6, IL-17D, and IL-12 in M5.1 vs. M15.2 birds. These results suggest that genetic determinants within the chicken MHC influence resistance to *E. maxima* infection by controlling the local and systemic expression of immune-related cytokine and chemokine genes.

Key words: major histocompatibility complex, Fayoumi, *Eimeria maxima*, immune-related, disease resistance

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INTRODUCTION

Coccidiosis, the major parasitic disease of poultry, is caused by protozoa belonging to the genus *Eimeria*. Avian coccidiosis causes significant economic losses to the world poultry industry because of the reduction in production efficiency as a result of mortality, nutrient malabsorption, retarded growth rate, and decreased egg production (Lillehoj and Li, 2004; Lillehoj et al., 2007). Conventional disease control strategies have relied on prophylactic chemotherapy and vaccination, but neither is without serious drawbacks (Dalloul and Lillehoj, 2005). Field selection of drug-resistant parasites and consumer apprehension concerning chemical residues in food hamper the use of coccidiostats while the risk of clinical disease induced by live parasite vaccination is increasing. Accordingly, alternative methods to control avian coccidiosis need to be identified. Genetic selection for disease resistance in commercial broiler chickens may be one of the best ways to achieve this goal and may ultimately lead to the elimination of drugs in commercial poultry production.

Most investigations of genetic resistance to avian diseases have been conducted on viruses, including Newcastle disease, Marek's disease, and avian leukosis viruses (Hassan et al., 2002; Zekarias et al., 2002; Parghi et al., 2004), and on bacteria (e.g., *Salmonella*; Malek et al., 2004). A limited number of studies have reported on resistance to intestinal parasites in genetically different chicken lines. For example, the comparative resistance to *Ascaridia galli* infection in commercial layer lines and indigenous chicken breeds, and the immune responses and resistance to *Eimeria* species in inbred chicken lines have been described (Bumstead et al., 1995; Caron et al., 1997; Pinard-Van Der Laan et al., 1998; Parmentier et al., 2001; Permin and Ranvig, 2001; Schou et al., 2003; Lillehoj and Li, 2004).

The Fayoumi breed, which originated in Egypt, was imported to the United States in 1954, primarily because of its reported resistance to avian leukosis. Subsequently, it was shown to develop a robust protective response against *Eimeria tenella* (Pinard-Van Der Laan et al., 1998). Derived from the original Fayoumi breed, the M5.1 and M15.2 congenic pair of lines share an identical genetic background and differ only in the microchromosome bearing the MHC. Lines M5.1 and M15.2 are highly inbred, each with an inbreeding coefficient of 0.99, and are genetically distant from the broiler and Leghorn lines (Zhou and Lamont, 1999). The present study was con-

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Table 1. Sequences of the oligonucleotide primers used in the quantitative reverse transcription PCR

Target gene ¹	Primer sequence		Product size	Accession no.	Reference
	Forward	Reverse			
GAPDH	5'-GGTGGTCTAAGCGTGTTAT-3'	5'-ACCTCTGCATCTCTCCACA-3'	264	K01458	Hong et al. (2006d)
IFN- α	5'-GACATCCCTTCAGCACTCTTCA-3'	5'-AGGCGCTGTAATCGTTGICT-3'	238	AB021154	Hong et al. (2006d)
IFN- γ	5'-AGCTCAGCGTGGACCTATTATT-3'	5'-GGCTTTCGGCTGGATTTC-3'	259	Y07922	Hong et al. (2006d)
IL-1 β	5'-TGGGCATCAAGGCTACA-3'	5'-TCGGGTGGTGGGATG-3'	244	Y15006	Hong et al. (2006d)
IL-6	5'-CAAGGTACCGAGGAGGAC-3'	5'-TGGCGAGGAGGATTTCT-3'	254	AJ309540	Hong et al. (2006d)
IL-8	5'-GGCTGTAGGGAAATGA-3'	5'-AGCTGACTCTGACTAGAAACTGT-3'	200	AJ009800	Hong et al. (2006d)
IL-10	5'-CGGGAGCTAGGGTGAA-3'	5'-GTGAAGAAGCCGTGACAGC-3'	272	AJ621614	Hong et al. (2006d)
IL-12p40	5'-AGACTCCAATGGCAAAATGA-3'	5'-CTCTCGCAATGGACAGT-3'	274	NM_213571	Hong et al. (2006d)
IL-15	5'-TCTGTCTCTGTTCTGAGTATG-3'	5'-AGTGAATTCCTCTCTTTGGTA-3'	243	AF139097	Hong et al. (2006d)
IL-17A	5'-TCCGATCCCTTATCTCCIC-3'	5'-AAGCGGTTTGGTCTCAT-3'	292	AJ493595	Hong et al. (2006d)
iNOS	5'-TCGGTGGAAAGCCGAAATA-3'	5'-GTACCAGCCGTTGAAAGGAC-3'	241	U46504	Hong et al. (2006d)
IL-17D	5'-GCTGCTCATGGGATCTTTGGTG-3'	5'-CGATGACGGCTTTCCTGTTGAC-3'	248	EF570583	Hong et al. (2006c)
LITAF	5'-TGTGTATGTCAGCAACCCGTAGT-3'	5'-GGCATTCGAATTTGGACAGAACT-3'	229	AY765397	Hong et al. (2006d)
TNFSF15	5'-CTGAGTATCCAGCAACGCA-3'	5'-ATCCACGAGTGTGACTAAC-3'	292	AB194710	Takimoto et al. (2005)
NK-lysin	5'-GATGTTTCAGTCCGTGGGATGC-3'	5'-CTGCCGAGCTTCTTCAACA-3'	217	DQ186291	Hong et al. (2006a)

¹GAPDH = glyceraldehyde 3-phosphate dehydrogenase; IFN = interferon; IL = interleukin; iNOS = inducible nitric oxide synthase; LITAF = lipopolysaccharide-induced tumor necrosis factor- α factor; TNFSF15 = tumor necrosis factor superfamily 15.

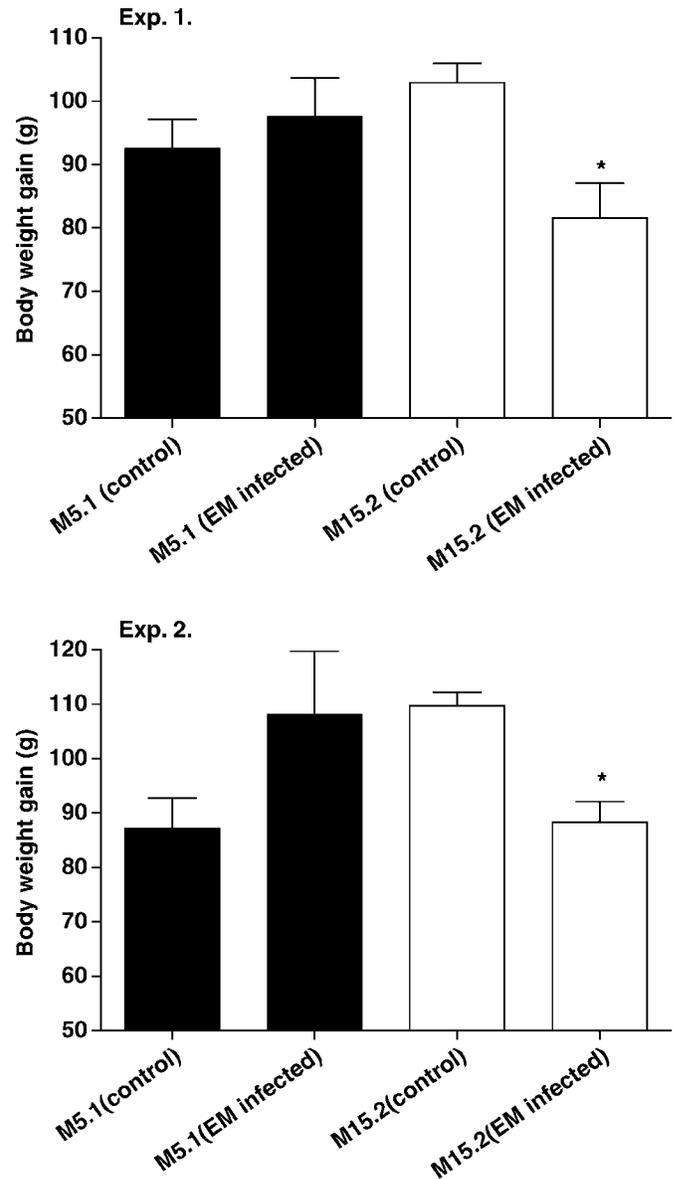


Figure 1. Body weight gain of M5.1 and M15.2 chickens between 0 and 9 d postinfection with *Eimeria maxima* (EM). Each bar represents the mean \pm SD values for each group (n = 5; *P < 0.05).

ducted to compare the expression of immune-related cytokines and chemokines in the M5.1 and M15.2 lines after experimental infection with *Eimeria maxima* and to determine whether the B-complex would influence the development of protective immunity to coccidiosis.

MATERIALS AND METHODS

Birds

Chicks were produced from the Fayoumi lines maintained in the Poultry Genetics Program at Iowa State University (Ames, IA). All matings were carried out by artificial insemination to ensure pedigree accuracy, and B-complex genotypes were confirmed by serological testing.

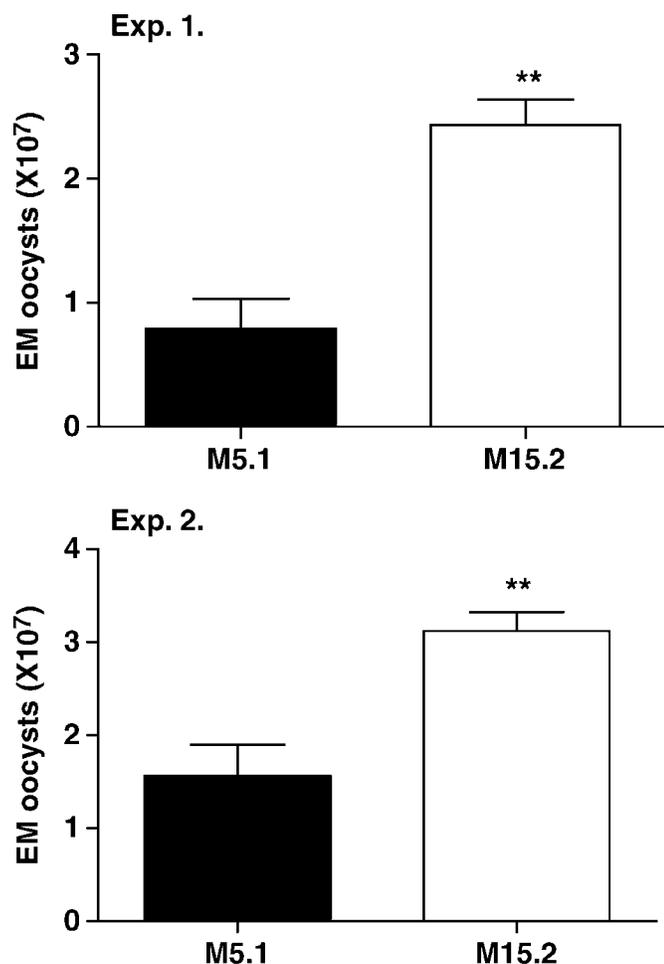


Figure 2. Fecal oocyst numbers of *Eimeria maxima* (EM)-infected M5.1 and M15.2 chickens. Each bar represents the mean \pm SD values for each group ($n = 5$; $**P < 0.01$).

Experimental *Eimeria* infection

At 4 wk of age, 10 birds were transferred to cages (2 birds per cage) and either remained uninfected or were orally infected with 1.0×10^4 sporulated oocysts of *E. maxima*. Birds were weighed at 0 and 9 d postinfection (dpi) and differences in BW were calculated (Lee et al., 2007). Fecal materials were collected at 9 dpi, the number of oocysts was determined, and oocyst shedding per bird was calculated as described previously (Dalloul et al., 2003). Each experiment was performed twice.

RNA Extraction and cDNA Synthesis

After euthanizing the birds, intestines and spleens were taken from 5 birds in each group at 0, 3, 4, and 5 dpi. Intestines were cut longitudinally and washed 3 times with ice-cold Hanks' balanced salt solution containing 100 U/mL of penicillin and 100 mg/mL of streptomycin (Sigma, St. Louis, MO). The mucosal layer was carefully scraped with a surgical scalpel and intraepithelial lymphocytes (IEL) were isolated from the jejunum as described previously (Lee et al., 2007). Spleen samples were prepared by passage through a wire mesh screen, erythro-

cytes were lysed, and the spleen cells were washed with Hanks' balanced salt solution. Total RNA was isolated from a constant number of IEL (5.0×10^7) or splenocytes (1.0×10^8) by using Trizol (Invitrogen, Carlsbad, CA). In preliminary experiments, we determined that equal cell numbers and equal amounts of total RNA were obtained per unit volume of gut mucosa or spleen (data not shown). For each tissue analyzed, 5 μ g of RNA was reverse-transcribed by using the StrataScript first strand synthesis system (Stratagene, La Jolla, CA) according to the manufacturer's protocol. All experiments were approved by the Animal and Natural Resources Institute (Institutional Animal Care and Use Committee, Beltsville, MD).

Quantitative Reverse Transcription PCR

Polymerase chain reaction oligonucleotide primers for chicken cytokines, chemokines, and other immune-related genes are listed in Table 1. Amplification and detection were carried out with equivalent amounts of total RNA by using the Mx3000P system and Brilliant SYBR Green QPCR master mix (Stratagene) as described previously (Hong et al., 2006b). Standard curves were generated by using \log_{10} -diluted standard RNA, and levels of individual transcripts were normalized to those of glyceraldehyde 3-phosphate dehydrogenase analyzed by the Q-gene program (Muller et al., 2002). Each analysis was performed in triplicate. To normalize RNA levels between samples within an experiment, the mean threshold cycle (C_t) values for the amplification products were calculated by pooling values from all samples in that experiment.

Statistical Analysis

Mean \pm SD values for each group were calculated, and differences between groups were analyzed by Bonferroni's multiple comparison test with Prism 4 software (Graphpad, San Diego, CA). Differences were considered significant at $P < 0.05$.

RESULTS

Assessment of Disease Parameters

To discern the difference in immunity, if any, between the Fayoumi M5.1 and M15.2 *B*-haplotype congenic lines, BW gain and fecal oocyst shedding were measured after oral infection with *E. maxima* oocysts. As shown in Figure 1, in 2 separate experiments, *Eimeria*-infected M15.2 birds exhibited approximately 20% decreased BW gain between 0 and 9 dpi compared with uninfected controls. By contrast, infected M5.1 birds displayed a tendency toward increased BW gain compared with uninfected controls, although this difference was not statistically significant. When compared between infected chickens, M5.1 weight gains were significantly increased compared with the M15.2 line. Similarly, M5.1 birds had significantly reduced numbers of fecal *E. maxima* oocysts compared with M15.2 in the 2 experiments (Figure 2).

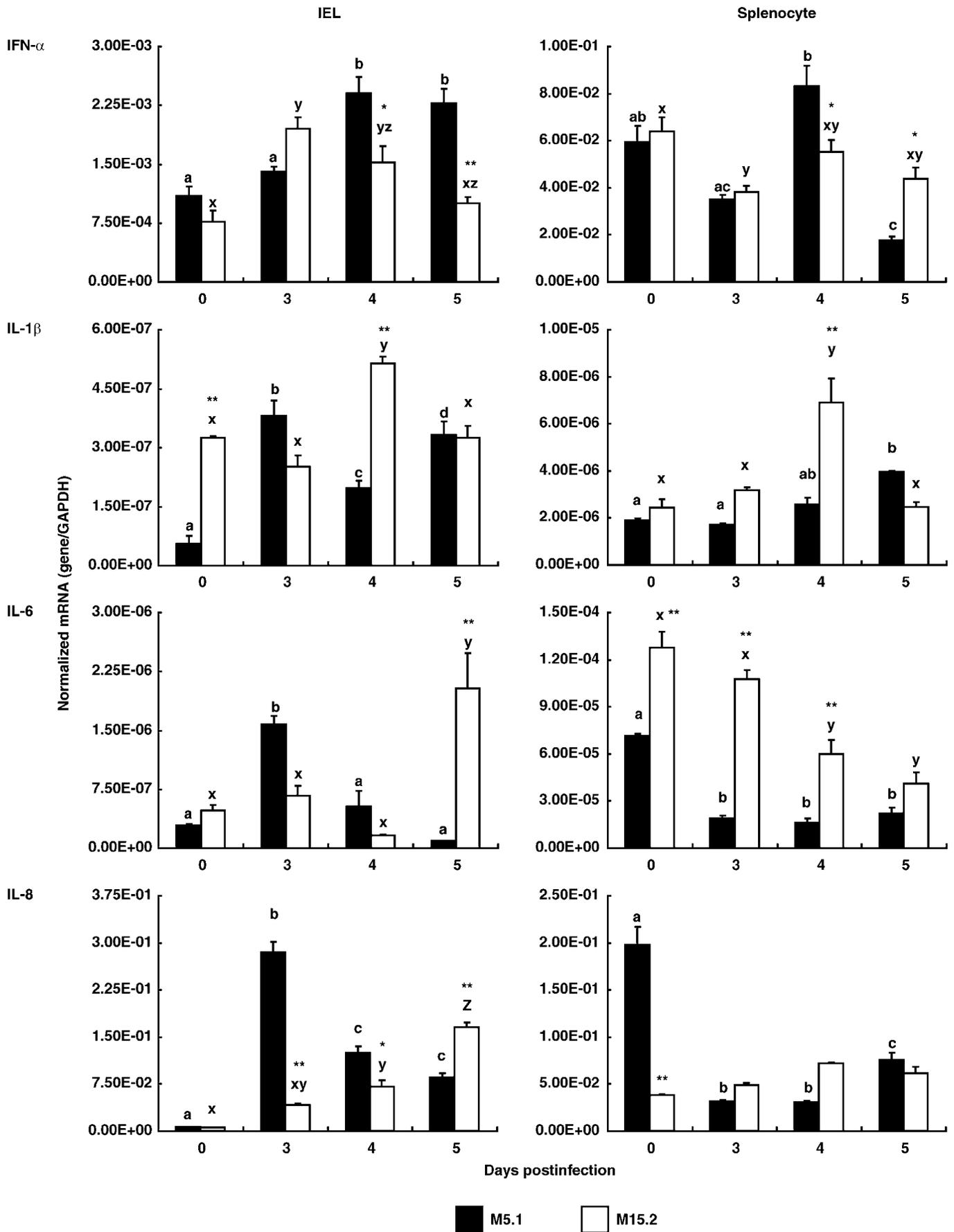


Figure 3. (Continued on next page).

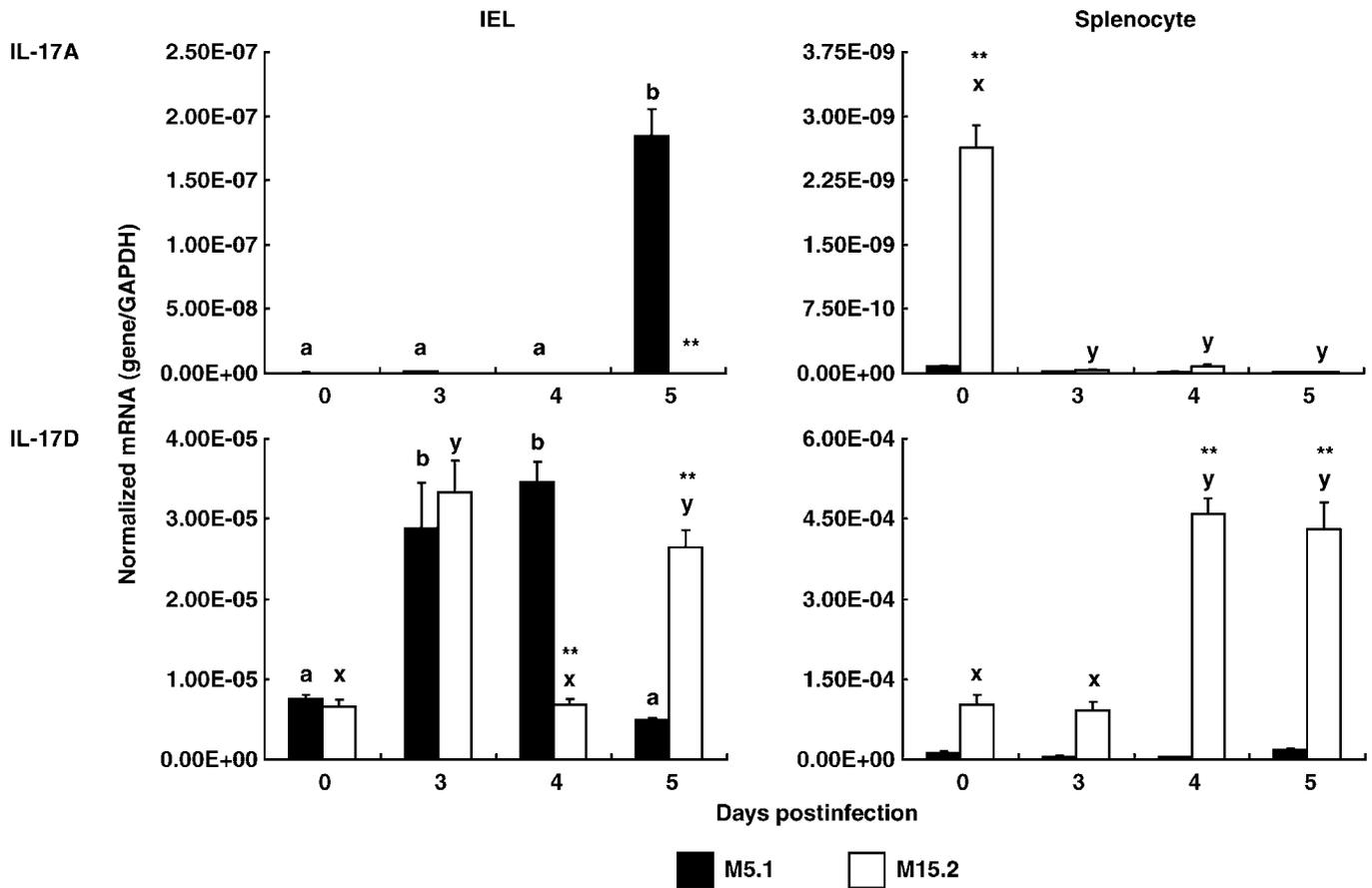


Figure 3. Quantitative analysis of mRNA levels of proinflammatory cytokines and chemokines in jejunum intraepithelial lymphocytes (IEL) and spleen cells of *Eimeria maxima*-infected M5.1 and M15.2 chickens. Each bar represents the mean \pm SD values for each group ($n = 3$). The letters a, b, and c indicate statistically significant differences in transcript levels in the M5.1 line at the indicated days postinfection compared with uninfected controls ($P < 0.05$). The letters x, y, and z indicate statistically significant differences in transcript levels in the M15.2 line at the indicated days postinfection compared with uninfected controls ($P < 0.05$). Asterisks indicate statistically significant differences between transcript levels in the M5.1 and M15.2 lines at the same day postinfection (* $P < 0.05$, ** $P < 0.01$). IFN- α = interferon- α ; IL = interleukin.

Immune-Related Gene Expression

We determined the expression levels of a panel of genes encoding proinflammatory and T helper cell type 1 (Th1)/T helper cell type 2 cytokines, chemokines, and other immune response-related molecules. In general, the proinflammatory cytokines IFN- α , IL-1 β , IL-6, IL-17A, and IL-17D were up-regulated in jejunum IEL at 3, 4, or 5 dpi after *E. maxima* infection of M5.1 or M15.2 chickens, or both (Figure 3). Transcripts encoding IFN- α and IL-17D were expressed at greater levels in M5.1 compared with M15.2 chickens at 4 dpi, whereas those for IL-1 β and IL-6 were expressed at greater levels in M15.2 chickens at 4 and 5 dpi, respectively. Interestingly, although IL-17A levels were undetectable at 0, 3, and 4 dpi in both chicken lines, expression of that cytokine was markedly increased by 576-fold at 5 dpi, but only in the M5.1 line. In spleen cells, IL-6 and IL-17A were down-regulated after *E. maxima* infection, and M15.2 had generally greater levels of IL-6, IL-17A, and IL-17D mRNA during the period examined. Finally, the proinflammatory CXC chemokine IL-8 was down-regulated after *E. maxima* infection in M5.1 birds but was up-regulated in M15.2 birds compared with uninfected controls (0 dpi).

The Th1-related molecules IFN- γ , IL-10, and iNOS were generally up-regulated in jejunum IEL of both M5.1 and M15.2 chickens at 3 to 5 dpi compared with birds at 0 dpi (Figures 4 and 5). In the spleen, expression levels of IFN- γ , IL-10, IL-12, and IL-15 were down-regulated, particularly at 3 dpi in the M15.2 line, whereas the levels of these transcripts were relatively constant in the M5.1 line.

The levels of lipopolysaccharide-induced tumor necrosis factor- α factor (LITAF) transcripts were relatively unchanged in IEL of M5.1 and M15.2 chickens but were significantly decreased in M15.2 spleen cells at 3 to 5 dpi compared with 0 dpi (Figure 5). Similarly, NK-lysin expression was down-regulated in both chicken lines in spleen cells after *E. maxima* infection. By contrast, mRNA encoding tumor necrosis factor superfamily 15 (TNFSF15) were up-regulated in both lines in IEL and spleen cells.

DISCUSSION

In this study, BW gain during experimental *E. maxima* infection was more prominent and fecal parasite numbers were lower in M5.1 birds than in M15.2 birds. The degree

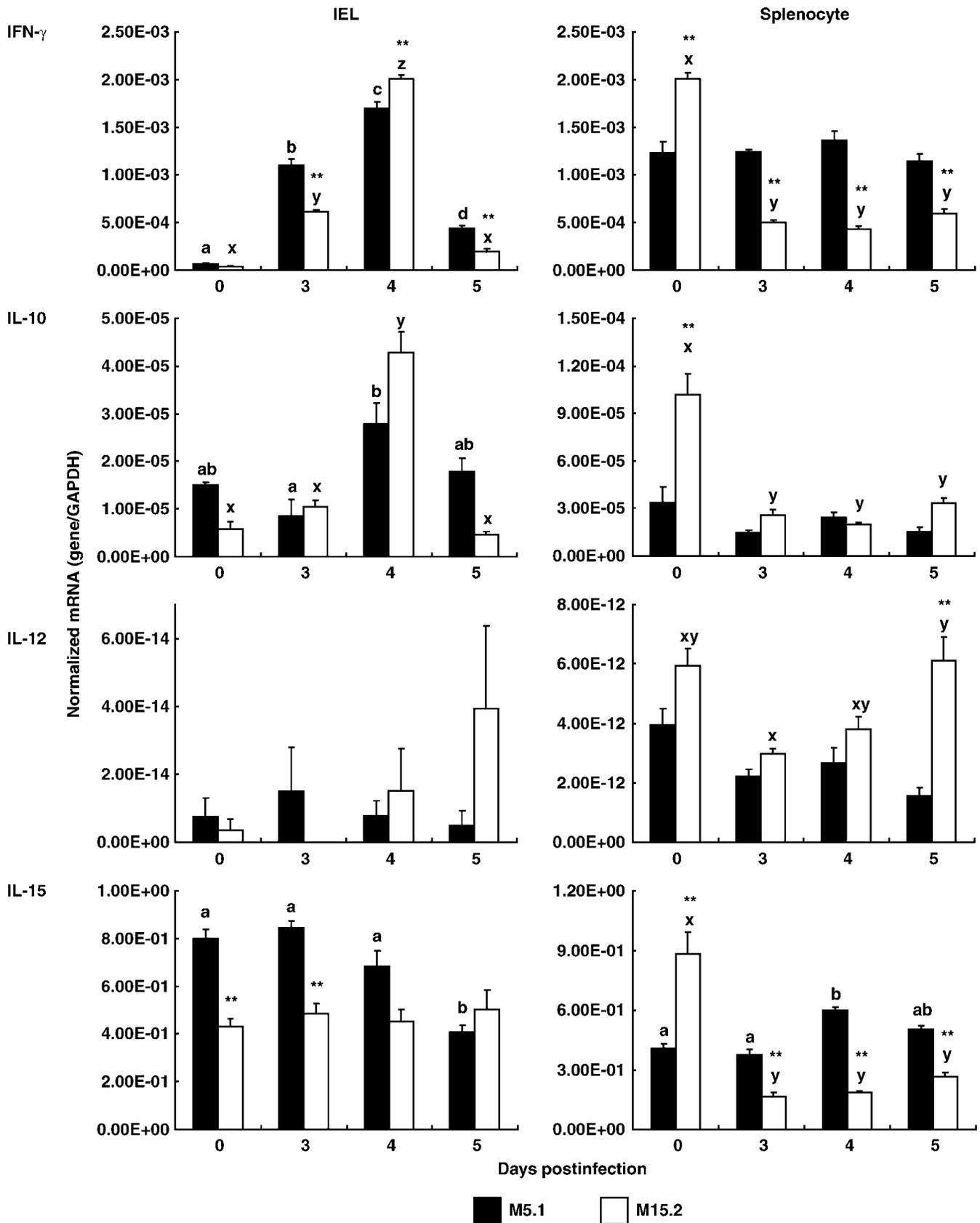


Figure 4. Quantitative analysis of mRNA levels of T helper cell type 1/T helper cell type 2 cytokines in jejunum intraepithelial lymphocytes (IEL) and spleen cells of *Eimeria maxima*-infected M5.1 and M15.2 chickens. Each bar represents the mean \pm SD values for each group ($n = 3$). The letters a, b, c, and d indicate statistically significant differences in transcript levels in the M5.1 line at the indicated days postinfection compared with uninfected controls ($P < 0.05$). The letters x, y, and z indicate statistically significant differences in transcript levels in the M15.2 line at the indicated days postinfection compared with uninfected controls ($P < 0.05$). Asterisks indicate statistically significant differences between transcript levels in the M5.1 and M15.2 lines at the same day postinfection ($**P < 0.01$). IFN- α = interferon- α ; IL = interleukin.

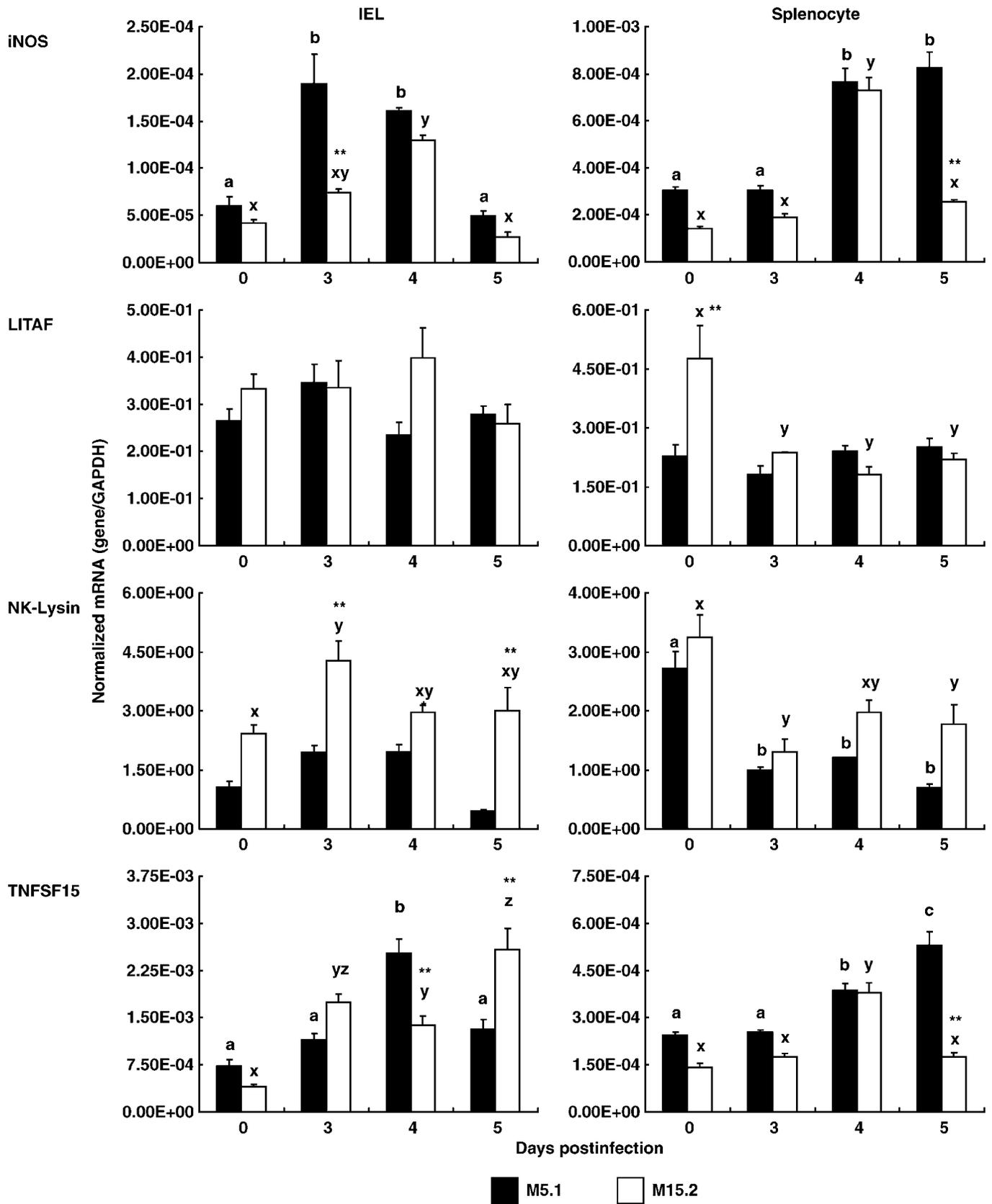


Figure 5. Quantitative analysis of mRNA levels of inducible nitric oxide synthase (iNOS), lipopolysaccharide-induced tumor necrosis factor- α factor (LITAF), NK-lysin, and tumor necrosis factor superfamily 15 (TNFSF15) in jejunum intraepithelial lymphocytes (IEL) and spleen cells of *Eimeria maxima*-infected M5.1 and M15.2 chickens. The letters a, b, and c indicate statistically significant differences in transcript levels in the M5.1 line at the indicated days postinfection compared with uninfected controls ($P < 0.05$). The letters x, y, and z indicate statistically significant differences in transcript levels in the M15.2 line at the indicated days postinfection compared with uninfected controls ($P < 0.05$). Asterisks indicate statistically significant differences between transcript levels in the M5.1 and M15.2 lines at the same day postinfection (** $P < 0.01$).

of diminished weight gain in M15.2 chickens was consistent with that seen in other chicken breeds orally inoculated with *E. maxima* (Jang et al., 2007). However, the weight gain in M5.1 birds was slightly increased, although the difference from uninfected controls was not statistically significant. Because weight gain and oocyst production are commonly regarded as the best indicators of susceptibility to avian coccidiosis (Lillehoj and Lillehoj, 2000), these results suggest that M5.1 chickens are more disease resistant than M15.2 chickens. Analysis of various immune-related molecules in the gut and spleen indicated that the enhanced resistance of M5.1 over M15.2 birds may be explained, in part, by their differential patterns of expression, particularly during the early phase of parasite infection.

The Fayoumi line of chickens has been reported to be relatively resistant to development of infectious diseases. For example, this breed was the first genetic stock in which resistance to Rous Sarcoma virus in the embryonic chorioallantoic membrane was reported (Prince, 1958). Compared with Leghorn chickens, the Fayoumi lines were more resistant to the development of tumors induced by Marek's disease virus (Lakshmanan et al., 1996). When compared with the White Leghorn, Rhode Island Red, and Mandarah lines for resistance to *E. tenella* infection, the Fayoumi lines were reported to exhibit the most resistance to development of coccidiosis (Pinard-Van Der Laan et al., 1998).

Genetic effects on resistance to avian coccidiosis have been studied in prior investigations, both in outbred and inbred *B*-complex congenic lines. For example, Pinard-Van Der Laan et al. (1998) compared 5 outbred lines and noted large differences in resistance to *E. tenella*. In that study, the Fayoumi line appeared as the most resistant, showing no mortality, less severe lesions than other lines, and a 30% reduction in growth compared with control birds. Major histocompatibility complex effects on immunity to coccidiosis have been investigated by using *B*-complex congenic chicken lines other than those used in this study. The resistance to *E. tenella* (Clare et al., 1985, 1989; Lillehoj et al., 1989; Brake et al., 1997; Caron et al., 1997) and *E. maxima* (Bumstead et al., 1995) among those congenic lines indicated a cause-effect relationship between the MHC haplotypes and protective immunity.

The Fayoumi M5.1 and M15.2 lines do not differ in the percentage of cells from normal adult birds expressing the Bu-1, CD3, CD4, CD8, and CD14 cell surface antigens (Cheeseman et al., 2004). Thus, line differences in susceptibility to coccidiosis likely arise from immunological mechanisms that do not influence the subpopulation distribution of these cells, or possibly from momentary changes in cell subpopulations only after *Eimeria* infection. Rather, the results presented in this study suggested that the observed differences in *Eimeria* infection may be related to altered cytokine or chemokine gene expression. Our previous studies revealed that *Eimeria* infection of chickens stimulated the production of a plethora of cytokines and chemokines in the gut (Hong et al., 2006b,d). How this response of immune mediators is related to

resistance to coccidiosis is still not well established and remains the focus of many research laboratories worldwide.

Chicken IFN- α was initially recognized for its antiviral activity (Sekellick et al., 1994; Sick et al., 1996). The effect of IFN- α on *Eimeria* infection was subsequently investigated by in vitro treatment of chicken cells with the recombinant cytokine, and in vivo administration of its cDNA in conjunction with an *Eimeria* DNA vaccine before infection (Heriveau et al., 2000; Min et al., 2001). In both experiments, however, parasite viability was not affected by treatment. Similarly, in our previous study, *E. maxima* infection was not affected in the level of IFN- α expression (Hong et al., 2006d), although increased levels of IFN- α gene expression were noted after *Eimeria acervulina* or *E. tenella* infection (Hong et al., 2006b). The observed increase in IFN- α expression seen in the Fayoumi chickens may reflect a real difference in the response to *Eimeria* by this strain or a response to a secondary infection associated with coccidiosis; however, the latter is not likely in the short time frame of the current study.

The IL-17 family of cytokines constitutes a group of immune mediators associated with inflammation and immunity (Aggarwal and Gurney, 2002). Originally, we reported that IL-17 expression was stimulated during experimental coccidiosis (Hong et al., 2006b,d). Subsequently, we developed PCR primers to distinguish IL-17A from IL-17D, which allowed us to determine in the current report that IL-17A transcripts in jejunum IEL were dramatically increased at 5 dpi in M5.1 birds but were completely down-regulated in the spleen of M15.2 birds. By contrast, IL-17D expression in the M15.2 line was up-regulated after *E. maxima* infection.

Several laboratories have reported that IL-17 up-regulates numerous other cytokines and chemokines, such as IL-1 β , IL-6, IL-8, IL-10, and IL-12 (Fossiez et al., 1996; Jovanovic et al., 1998; Min and Lillehoj, 2002). Both IL-1 β and IL-6 are proinflammatory cytokines that play multiple roles in the regulation of protective immunity, and the expression of both mRNA was increased after experimental infection of chickens with *E. tenella*, *E. maxima*, or *E. acervulina* (Laurent et al., 2001; Min et al., 2001; Hong et al., 2006b,d). In the current study, IL-1 β expression was increased 7-fold in the jejunum IEL of M5.1 chickens at 3 dpi, whereas it was decreased in M15.2 chickens. Similarly, IL-6 transcripts were significantly up-regulated only in M5.1 birds. Thus, increased IL-17A in M5.1 birds may induce up-regulation of IL-1 β and IL-6 during *Eimeria* infection. In view of the proinflammatory properties of IL-1 β and IL-6, it is tempting to speculate that differential expression of these cytokines in M5.1 vs. M15.2 birds may underlie, to a large degree, their difference in susceptibility to *E. maxima* infection.

In the case of IL-8, an inverse relationship was observed in the expression of this proinflammatory chemokine between M5.1 and M15.2 chickens during the course of *E. maxima* infection. In M5.1, IL-8 transcript levels were greatest at 3 dpi and progressively decreased thereafter, whereas in M15.2 the IL-8 response was delayed, reaching

the greatest levels at 5 dpi. The early robust IL-8 response in M5.1 may confer the ability of this strain to limit *Eimeria* infection in the gut, whereas the belated response in M15.2 may be too late to counteract parasite dissemination and intestinal destruction.

The Th1 cytokines (IFN- γ , IL-10, IL-12, and IL-15) promote cell-mediated immune responses mediated by cytotoxic T cells and macrophages. Interferon- γ levels have been correlated with the resistance to several avian diseases, including Marek's disease, infectious bronchitis, Newcastle disease, and salmonellosis (Hong and Sevoian, 1971; Sadeyen et al., 2004). Chicken strains that are resistant to coccidiosis are reported to have high levels of IFN- γ expression (Byrnes et al., 1993; Lowenthal et al., 1997; Yun et al., 2000). Our results showed that IEL of M5.1 and M15.2 chickens displayed similar kinetics of IFN- γ expression, with the greatest levels at 4 dpi. In contrast, IFN- γ in the M5.1 spleen cells was relatively unchanged after *E. maxima* infection and was markedly down-regulated in M15.2 spleen cells.

Interleukin-10 exerts inhibitory effects on proinflammatory cytokines, as exemplified by the fact that chicken IFN- γ and IL-12 were down-regulated after expression of IL-10 (Groux and Powrie, 1999; Rothwell et al., 2004). Indeed, IL-12 expression was greatest at 5 dpi in M15.2 spleen cells when IFN- γ expression was concurrently low, although the expression levels of IL-12 were not significantly different. On the other hand, the kinetics of IFN- γ and IL-10 expression were identical in both IEL and spleen cells of *E. maxima*-infected chickens, suggesting that IFN- γ expression was not affected by IL-10. It remains to be determined whether the relationship between IFN- γ and IL-10 is unique to coccidiosis, to these particular chicken lines, or both.

In addition to the cytokines and chemokines discussed above, in this study we analyzed the expression of several additional mediators of innate immunity, iNOS, TNFSF15, NK-lysin, and LITAF. Expression of iNOS was reported to be induced by IFN- γ in chicken macrophages and was greatly up-regulated during experimental *E. tenella* infection (Laurent et al., 2001). Our results showed that the temporal patterns of iNOS and IFN- γ expression were identical in M5.1 and M15.2 IEL, that is, biphasic responses with maximum levels at 3 or 4 dpi. In spleen cells, however, the 2 genes displayed unique patterns of expression. NK-Lysin possesses antimicrobial and tumor cytolytic activities, and its gene expression was shown to increase in jejunum IEL after *E. maxima* infection (Hong et al., 2006a). Our data revealed that although IEL of *E. maxima*-infected birds exhibited increased NK-lysin expression, its levels in the spleen were down-regulated. Furthermore, this pattern was consistent in M5.1 and M15.2 chickens.

Tumor necrosis factor superfamily 15 is the avian homolog of mammalian TL1A and was previously reported to reduce feed intake and increase rectal temperature in vivo, and to augment cytotoxicity in vitro (Takimoto et al., 2005). Tumor necrosis factor superfamily 15 was up-regulated in IEL after infection of chickens with *E. maxima*

or *E. acervulina*, but not *E. tenella* (Park et al., 2007). Our results confirmed these observations in *E. maxima*-infected M5.1 and M15.2 birds and extended them to spleen cells, in which a similar increase in TNFSF15 expression was detected. Expression of chicken LITAF, another member of the tumor necrosis factor superfamily, was up-regulated by *Escherichia coli*, *Salmonella*, or *Eimeria* species in vitro, and recombinant LITAF promoted the cytotoxicity of chicken tumor cell lines (Hong et al., 2006c). In support of these observations, we detected greater levels of LITAF at 4 dpi in the IEL of M15.2 birds but significantly decreased levels in spleen cells.

In conclusion, the current results provide clear evidence that genetically determined resistance to *E. maxima* infection entails a T-cell-mediated immune mechanism involving key molecular mediators of protective immunity. Early and high expression of proinflammatory cytokine and chemokine genes, including IL-1 β , IL-6, and IL-8, may be induced by IL-17D expression. Moreover, these results, combined with lower levels of IL-10, an anti-inflammatory cytokine, were associated with enhanced resistance to *E. maxima* infection in the Fayoumi M5.1 breeding line compared with the M15.2 strain. The Th1-related cytokines (IFN- γ and IL-15) as well as other mediators of innate immunity (iNOS) also were early markers associated with the resistant M5.1 line. Also noted were strain-specific patterns of IFN- α and IL-8 expression in comparison with previous studies, and most of the genes examined exhibited tissue-specific expression between IEL and spleen cells. Future studies based on these results will provide a foundation for the use of cytokines and chemokines, either as recombinant proteins or DNA vaccines, as therapeutic agents against coccidiosis.

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