Animal Science Journal (2006) **77,** 285–289 doi: 10.1111/j.1740-0929.2006.00350.x

ORIGINAL ARTICLE

Influences of major histocompatibility complex class I haplotypes on avian influenza virus disease traits in Thai indigenous chickens

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ABSTRACT

Natural infections with influenza viruses have been reported in a variety of animal species including humans, pigs, horses, sea mammals, mustelids and birds. Occasionally, devastating pandemics occur in domestic chickens (broiler and layers) and in humans. From November 2003 to March 2004 in many countries in Asia, there were outbreaks of H5N1 avian influenza virus, causing death of infected patients, and devastating the poultry industry. Some groups of Thai indigenous chickens survived and were therefore classified as resistant. These traits were related to immunogenetics, in particular, the major histocompatibility complex (MHC) class I and class II molecules. The chicken *MHC class I* were investigated as candidate genes for avian influenza virus disease resistance. Seven hundred and thirty Thai indigenous chickens from smallholder farms in the rural area of avian influenza virus disease outbreaks in the central part of Thailand were used in the present study. They were separated into two groups, 340 surviving chickens and 390 dead chickens (resistant and susceptible). Genomic DNA were precipitated from blood samples and feathers. The DNA were used to amplify the *MHC class I* gene. Data were analyzed using χ^2 analysis to test significant differences of influences of *MHC class I* haplotypes on avian influenza virus disease traits. The results represented nine *MHC class I* haplotypes: *A1*, *B12*, *B13*, *B15*, *B19*, *B21*, *B2*, *B6*, and *BA12*, and included 10 of their heterozygotes. The homozygous *B21* from these collected samples had a 100% survival rate and they were the major survival group. In addition, the heterozygous *B21* also had a high survival rate because of co-dominant expression of these genes. In contrast, the homozygous *B13* had a 100% mortality rate and they were the major mortality group. These results confirmed that *MHC class I* haplotypes influence avian influenza virus disease-resistant traits in Thai indigenous chicken. The *MHC* genes can be used as genetic markers to improve disease-resistant traits in chicken.

Key words: *avian influenza virus, major histocompatibility complex, Thai indigenous chicken.*

INTRODUCTION

In rural areas of Thailand, farmers keep indigenous chickens for food, as a hobby, as fighting chickens, and as an alarm clock, therefore indigenous chickens have genetic diversity. The flavor of Thai indigenous chicken meat is delicious and appropriate to Thai food cooking. Because of these reasons, farmers can use indigenous chickens for home consumption and home income. The poultry industry is facing increasing problems due to the emergence of progressively more virulent forms of pathogenic viruses that require increasingly effective vaccines to prevent disease. However, vaccination is now proving useless

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Received 30 April 2005; accepted for publication 16 November 2005.

against very virulent strains and those that could affect humans. In comparision, indigenous chickens are resistant to diseases. Thai indigenous chickens are can be used to improve genetics for disease-resistant traits.

Chickens suffer from a number of diseases, some of which are fatal, and all of which affect the growth, production and welfare of the birds. From November 2003 to March 2004 in many countries in Asia, there were outbreaks of H5N1 avian influenza virus, causing death of infected patients and devastating the poultry industry. Avian influenza outbreaks can be difficult to control and often have a major economic impact on poultry farmers in affected countries because mortality rates are high and infected fowl generally must be destroyed in order to prevent the spread of the disease. As a result of ongoing outbreak in Asia, the Food and Agriculture Organization estimated that approximately 20–25 million birds have been destroyed. For this reason, the local economies of both commercial poultry operations and smallholders were devastated, especially in Thailand, where the industry is heavily reliant on trade. In 2003, poultry exports from Thailand accounted for nearly 7% of the global poultry meat trade, with an export value of approximately US$1bn. The effects of avian influenza virus disease outbreak severely decreased frozen poultry meat exports. Of greatest importance of this outbreak was the death of infected patients in Thailand and Vietnam. Thus, disease resistance is an important economic consideration in the poultry industry.

Chickens that are vulnerable to disease also might destroy Thai domestic supplies as well as exports of this important food commodity. An interesting alternative to standard disease control methods would be selective breeding to increase disease resistance in livestock. Genetic resistance to disease involves many facets of the body's defense system and interactions, and is extremely complex. Production traits had negative correlation to disease resistant traits. In addition, disease resistant traits had low heritability, which made it difficult to use conventional breeding to improve the disease resistant traits. New opportunities to improve our understanding of the genetic nature of disease resistance have been produced through recent advances in molecular biology, gene mapping, and immunology which makes selection for disease resistance possible in the future. However, testing and selection for disease resistance and improved immune responsiveness require knowledge of the genetic correlations between disease resistance and immune responsiveness, and production traits.

Some groups of Thai indigenous chicken have survived H5N1 outbreaks, and are considered to be resistant. These traits are related to immunogenetics, in particular, the major histocompatibility complex (MHC) class I and class II molecules. The chicken *MHC* lies on chromosome 16, which is a microchromosome. It includes various linked multigene families of highly polymorphic genes (Li *et al*. 1997). It was discovered to contain three loci, *BF*, *BL*, and *BG* (Pink *et al*. 1977), producing class I, II, and IV antigens, which are expressed on the surface of all nucleated cells, immunological cells, and erythrocytes, respectively (Ewert *et al*. 1980; Nordskog *et al*. 1987). MHC worked as an antigen presenting to T lymphocytes (Abbas *et al*. 2000). It was first identified by the ability of leukocytes to produce a strong graft rejection. There is some evidence for different *MHC* haplotypes having different competence in graft versus host responses (GVHR) as a mechanism for resistance to Marek's disease; that is, less proliferation would decrease the chance of lymphoma development, but may also lead to a general decrease in T-cell responses (Pazderka *et al*. 1975). However, there is no research on the association of MHC and avian influenza virus disease. It is very important to find candidate genes relating to avian influenza virus disease as genetic markers for disease-resistant trait improvement. The objective of the present study was to investigate influences of *MHC class I* gene polymorphisms on avian influenza virus disease resistance in Thai indigenous chickens, to facilitate the improvement of disease-resistant traits, animal welfare, safe production, and safe food.

MATERIALS AND METHODS

Chickens

Seven hundred and thirty Thai indigenous chickens (Leung-Hahng-Kow chickens (LHK); Pradoo-Hahng-Dam (PHD)) were used in the present study. They were sourced from rural areas of the central part of Thailand in the area of avian influenza virus outbreak, such as Ayuthaya, Kanchanaburi, Nakhon Pathom, Singburi, and Suphanburi province, and consisted of 390 dead and 340 surviving chickens. The chickens were collected from smallholder farms and each farm was randomized to collect chicken blood for titer testing.

Chicken DNA extraction

Blood and feather samples were collected from two groups of chickens (surviving and dead) in the outbreak area. They were used to extract genomic DNA.

Polymerase chain reaction amplification

Genomic DNA from chickens were used to identify the *MHC* haplotype by polymerase chain reaction (PCR) of *MHC class I αI* and *αIV* (BF) exon 2 (*BFαI* primer: forward primer, 5′-GTGGACGGGGAACTCTTC-3′; reverse primer, 5′TCTGGTTGTAGCGCCG-3′; *BFαIV* primer: forward primer, 5′GTGGACGGGGAACTCTTC-3′; reverse primer, 5′-ACCGCCGGTCTGGTTGTA-3′; Goto *et al*. 2002). Partial sequencing of *BFα1* and *BFα4* genes was carried out to standardize and identify *MHC* haplotype.

Major histocompatibility complex class I haplotypes: Identification by single strand conformation polymorphism

A total of 1–3 μL of PCR reaction product was denatured at 94°C in 3–9 μL formamide dye (30 mL formamide, 0.05 g each of xylene cyanol and bromophenol blue, and NaOH 10 mmol/L) for 10 min and immediately chilled on ice to prevent reannealing. The denatured PCR products were electrophoresed for 2–2.5 h at 200 V (equivalent to 400–500 volt-h) in 12% polyacrylamide, 1× TBE (45 mmol/L Tris, 45 mmol/L boric acid, 1 mmol/L ethylenediamine tetra-acetic acid) gels in a Hoefer miniVe electrophoresis and electrotransfer unit. Runs were routinely performed in a refrigerator (4–5°C; Goto *et al.* 2002). Silver nitrate staining gels were scanned and images were produced with Adobe Photoshop.

Each single strand conformation polymorphism group was standardized by partial sequencing of *BFαI* and *BFαIV* genes in the exon 2 region to identify *MHC* haplotype.

Estimation of influences of major histocompatibility complex haplotypes on avian influenza virus

Disease

Significant differences in influences of *MHC class I* haplotypes on avian influenza virus disease traits in Thai indigenous chickens were investigated using the χ^2 test.

RESULTS AND DISCUSSION

Situation of avian influenza virus disease outbreaks in Thailand

Avian influenza virus disease was caused by avian influenza virus type A, capsulated RNA virus of the Orthomyxoviridae family. It has two specific surface antigens, hemagglutinin (H, H1-H15) and neuraminidase (N, N1-N9). From November 2003 to March 2004 in many countries in Asia, such as Cambodia, China, Hong Kong, Indonesia, Japan, Korea, Laos, Vietnam, and Thailand, there were outbreaks of avian influenza virus, especially in Vietnam and Thailand, of strain H5N1, causing death of infected patients and devastating the poultry industry. The H5N1 strain had a different genetic sequence and therefore is believed to come from a different source. It is a violent pathogenic avian influenza. In Thailand, there are ongoing outbreaks of avian influenza. It devastated local economies and both commercial poultry operations and smallholders. Chicken prices are down slightly this year in the European Union. While a ban on Thai chicken will leave a deficit in the market for white meat processing, it is likely that Brazil, a competitive producer of a similar type of product, will move to supply the gap. In the Japanese market, domestic poultry supplies are dropping as avian influenza-infected flocks are disposed of and imports are banned from Thailand (Thailand accounts for 30% of Japanese poultry imports). This will push prices of all meats higher. Interestingly, some Thai indigenous chickens survived these outbreaks. This means that these chickens are resistant to avian influenza virus disease, and were therefore suitable for use as a genetic resource for genetic improvement of virulent disease-resistant traits in the poultry industry in Thailand.

Major histocompatibility complex class I haplotypes genotyping

Major histocompatibility complex class I haplotypes were genotyped using single strand conformation polymorphism. The results represent nine *MHC class I* haplotypes, *A1*, *B12*, *B13*, *B15*, *B19*, *B21*, *B2*, *B6*, and *BA12*, and include 10 of their heterozygotes.

Haplotype *A1* of *MHC class I* was found by Li *et al.* (1999) in broiler chickens, to be different from standard haplotypes in White Leghorn chickens. The standard haplotype in White Leghorn chickens is preceded with the letter '*B*' and followed by numerals. Many scientists suggested that nucleotide sequencing of *MHC* haplotypes from different breeds would be different because of the cross-reaction of serological reagents of White Leghorn chickens, used in broiler chickens (Li *et al*. 1999). *B2–B21 MHC class I* haplotypes were identical to standard haplotypes of White Leghorn chickens.

Influences of major histocompatibility complex class I gene polymorphisms on avian influenza virus disease traits

The *B* haplotypes were identified in Thai indigenous chickens from smallholder farms in the rural area of avian influenza virus disease outbreaks in the central part of Thailand, representing 28 groups of homozygous and heterozygous haplotypes (Table 1).

The *B21* haplotype in *MHC class I* was the strongest resistant haplotype and had a 100% survival rate in *class I* (Table 1). This was the largest group of surviving chickens. The *B12* haplotype was found in the dead chickens. The *B12* were classed as the susceptible group. The *B12* haplotype is the same as that used in other studies. The *B12* haplotype was susceptible to disease (not all disease) (Bacon 1987). The heterozygous *B12* haplotype had a higher survival rate than the *B12* homozygous haplotype. The majority of dead chickens had haplotype *B13* and were heterozygous (100% mortality rate for heterozygous *B13* haplotype). In addition, in the survival group, *B21* haplotype and the heterozygote had the highest survival rate (100%); the *B2* and *B6* haplotypes also had a 100% survival rate but these were in the minority.

Thai indigenous chickens in smallholder farms in the rural areas have been raised according to natural selection. The strong chickens are alive and reproductive. The susceptible chickens have been culled through many generations, and are extinct. Further experiments need to be carried out to study the influences of the combination of the *MHC class I* and *II* genes on avian influenza virus disease. The *MHC class I* haplotypes were highly polymorphic in the region of *BFαI* exon 2, a pathogen binding site region. This region was specific to pathogen, so *MHC* haplotypes were highly polymorphic in disease-resistant traits (Livant *et al*. 2001).

The MHC molecules were the central role of the immunological system, MHC class I presented intracellular antigen to T-cytotoxic after antigen went into host cell by antigen specific receptor, antigens were destroyed. By the same case, reported by Ito (2000), it was reported that virulent avian influenza A viruses are lethal in chickens. Because cell death could be caused by either necrosis or apoptosis, the types of cell death that occur in natural hosts, chickens, infected with virulent avian viruses, were investigated. Using biochemistry it was demonstrated that harmful avian influenza viruses induced apoptosis of vascular endothelial cells in liver, kidney, and brain. Viral antigens were also detected in these organs, suggesting that viral replication induced apoptosis in infected chickens. These results indicated that apoptosis did occur in virulent avian influenza virus infection in a natural host, and might contribute to the lethality of the virus. In this way, *MHC class I* is associated with avian influenza virus disease in chickens.

Table 1 Association of MHC class I gene polymorphisms with avian influenza virus disease-resistant traits

Haplotypes	No. chickens†	% Survival
LHK		
A1A1	10	0.00^{d}
B12B12	33	0.00^{d}
B12B13	21	4.76^{d}
B12B21	18	100.00^{a}
B13B13	44	0.00^{d}
B13B15	19	0.00^{d}
B13B2	8	0.00^{d}
B13BA12	4	0.00^{d}
B15B2	9	77.77^{a}
B15B21	26	100.00^{a}
B15B5	10	30.00^{c}
B19B19	9	22.22^{c}
B19B5	10	50.00^{b}
B21B21	53	100.00^{a}
B21BA12	16	87.50^{a}
B2B21	9	100.00^{a}
PHD		
A1A1	18	0.00^{d}
B12B12	111	2.70^{d}
B12B13	14	7.14^{d}
B12B21	8	100.00^{a}
B13B13	50	0.00^{d}
B13B15	27	0.00^{d}
B13B2	10	0.00^{d}
B13BA12	4	0.00^{d}
B15B2	7	100.00^{a}
B15B21	10	100.00^{a}
B15B5	6	33.33^{c}
B19B19	5	80.00^{a}
B19B5	8	50.00^{b}
B21B21	121	100.00^{a}
B21BA12	16	87.50^{a}
B2B21	7	100.00^{a}

†Data not shown for minor groups of chicken. $^{a–d}$Different letters in the same class of MHC means highly significant difference ($P < 0.01$). LHK, Leung-Hahng-Kow chicken; MHC, major histocompatibility complex; PHD, Pradoo-Hahng-Dam.

After the outbreaks, many chickens (including the Thai indigenous chickens that survived) were destroyed to eradicate avian influenza virus disease. This is suitable for short-term control. In the long term it is better to improve disease-resistant traits in poultry by using candidate genes of disease resistance as genetic markers in conventional breeding. It is difficult for disease-resistant trait improvement to be carried out in conventional breeding because of the negative correlation between production traits and disease-resistant traits. A high selection pressure for production traits compresses disease-resistant traits (Rothschild 1989). Therefore, genetic markers of disease-resistant genes such as *MHC* genes, are suitable to improve disease-resistant traits.

Conclusions

The *MHC class I* genes influenced avian influenza virus disease traits in Thai indigenous chickens. There are polymorphisms of these genes. The *MHC* genes can be used as genetic markers to improve disease-resistant traits in chickens (indigenous chickens, broilers, and layers) in order to prevent disease. Finally, the poultry industry has high standards for raising healthy livestock and providing consumers with safe products.

ACKNOWLEDGMENTS

The authors thank Mrs Sommart Suwanmajo for providing samples, and Center for Agricultural Biotechnology for laboratory support. Thanks also go to Dr Tadayoshi Mitsuhashi for recommendation to journal editor.

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