Poultry Immunogenetics: Which Way Do We Go?[1]

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ABSTRACT A major goal in poultry immunogenetics is the enhancement of innate immunoresponsiveness and resistance to disease. This may be pursued by studying either single genes or polygenic traits. The MHC is perhaps the best-characterized family of host genes that modulates response to a variety of antigens and pathogenic challenges. The association of different MHC alleles with disease resistance has been known for decades. But only recently has analysis at the DNA level opened new avenues of understanding and new opportunities for application of genetic variation in the MHC with immunoresponsiveness. An alternate approach to molecular analysis is selection for a desired phenotype controlled by polygenes. Several studies have illustrated the successful alteration of immunoresponsiveness by genetic selection for antibody production. Recently, a selection program based upon multiple traits of immune response was conducted. Results of this project demonstrated that selection on multiple immune-response traits altered immunophysiology, MHC allelic frequencies, and disease resistance. Several areas for future pursuits in poultry immunogenetics research are proposed.
(*Key words*: major histocompatibility complex, immune response, genetics, selection, disease)

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INTRODUCTION

Efficient production of poultry is dependent upon a properly functioning immune system to defend individuals against disease. Modern management practices, however, which strive to protect animals by isolation from disease agents or by induction of vaccination immunity, may be antagonistic to natural genetic selection for disease resistance and may allow genetic susceptibility to increase in a breeding population. Genetic selection for increased immune responsiveness and disease resistance can make permanent improvements in fitness and also enhance vaccine effectiveness.

Improving disease resistance via genetic selection is a desirable approach for many reasons. Although the progress per generation may be small, it is heritable and therefore cumulative over generations. Improved immunoresponsiveness conserves natural resources by decreasing the losses in production efficiency due to disease presence. Animal well-being is enhanced by greater resistance to disease. If suitable markers are validated, selection can be by marker-assisted selection, rather than by direct challenge with disease agents. A reduction of microbial contamination of poultry products will improve food safety and consumer perception of poultry products as a wholesome food source.

Three different strategies can be used to identify markers associated with traits of immune response and disease resistance. First, after generation of a saturated map of the poultry genome, quantitative trait loci (QTL) can be added to the map. Although rapid progress is being made in gene mapping (Bumstead and Palyga, 1992; Crittenden *et al.*, 1993; Levin *et al.*, 1994), the chicken genome map is not yet

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at the level of resolution to allow efficient use of this approach. Second, populations that differ in quantitative traits (i.e., disease or immune response) can be screened for differences that may be associated with the divergent trait (Bacon, 1992). These genetic or physiologic differences can then be examined for linkage with the traits of interest and for potential use as selection markers. Third, candidate genes that have a high probability of involvement in immune function, based upon either previous studies in poultry or function of homologous genes in other species, can be examined directly. The MHC is an excellent example of a set of candidate genes for immune function (Lamont, 1989, 1991, 1993).

MAJOR HISTOCOMPATIBILITY COMPLEX

The location of genes encoding the erythrocyte antigen B (Ea-B) within the chicken MHC (Schierman and Nordskog, 1961) has long allowed the use of hemagglutination as a means to identify genetic variation in the chicken MHC (Briles *et al.*, 1950). This use of products of a linked gene to analyze or effect changes associated with the chicken MHC is one of the earliest examples of marker-assisted selection in any agricultural species. Today, research techniques are available that allow molecular analysis of the genes and gene products involved in immune response (Lamont *et al.*, 1987; Lamont and Dietert, 1990).

Numerous studies confirm that gene products encoded by the chicken MHC affect resistance to disease, including viral, bacterial, parasitic, and autoimmune disease (Bacon, 1987; Dietert *et al.*, 1991). Recent discoveries about the crucial role of MHC antigens on antigen processing and presentation help to explain the MHC's association with immune responsiveness and the mechanism of MHC restriction of immune cell activity. As detailed in a companion paper (Schat, 1994) of this symposium, Class I and Class II molecules are involved in two distinct pathways of antigen presentation. The MHC Class I proteins bind endogenously synthesized peptides and present them to cytotoxic T lymphocytes. The Class II proteins bind short peptides (about nine amino acids) that were derived from exogenous proteins that were endocytosed and degraded. Because the binding site of MHC proteins for antigens is so small, genetic variation in the MHC can readily alter antigen-binding capacity and, therefore, immune responsiveness.

Recent years have witnessed a great increase in knowledge regarding the molecular genetic structure of the chicken MHC, including genomic organization, numbers of genes, expression of genes, genetic regulatory elements, and allelic polymorphisms. In contrast to mammalian MHC, the chicken MHC has much smaller introns, and the Class I and II genes are interspersed (Guillemot *et al.*, 1988). At least six Class I (Kaufman *et al.*, 1992) and six Class II genes (Zoorob *et al.*, 1993) have been cloned. The expressed Class II β genes seem to be clustered into a single isotopic family (Pharr *et al.*, 1993a; Sung *et al.*, 1993). Allelic polymorphisms in the putative antigen-binding site of chicken Class II have been identified (Pharr *et al.*, 1993b). Differences in capacity to bind antigenic peptides for presentation to effector T cells may be one source of MHC-linked variation in immune response and disease resistance.

Studies have recently appeared that map functional promotor elements for the chicken Class I (Zoller *et al.*, 1992) and Class II genes (Chen *et al.*, 1993). The MHC allelic diversity in breeding populations should be surveyed and analyzed for associations with disease resistance so that alterations in allelic frequencies might be used to improve resistance. Information on antigen-binding specificity is crucial to design of effective poultry vaccines (Schat, 1994; Witter and Hunt, 1994), and knowledge of specific MHC types in the flock to be vaccinated could allow specific choices to optimize the vaccine-host genetic combination.

The picture of the chicken MHC is complicated by the existence of sequences homologous but unlinked to the MHC (Briles *et al.*, 1993) as well as genes within the MHC, which are not classical MHC genes. Two examples of the latter are C8.4, which has characteristics of the im-

munoglobulin super family, and C12.3, which is homologous to guanine nucleotide-binding proteins (Guillemot *et al.*, 1989). These genes may potentially contribute to the MHC-linked control of disease resistance, particularly viral transformation.

It seems that chicken MHC Class II clones can also be used to define turkey Class II genotypes (Emara *et al.*, 1992). This is based upon correspondence of restriction fragment length polymorphism (RFLP) patterns with histocompatibility and tissue-specific expression.

The Major Histocompatibility Complex: Which Way Do We Go

Tremendous progress has been made in understanding the relationship of the chicken MHC and disease resistance by using traditional serological identification of MHC diversity. Now that the avenue has opened for exploration of the avian MHC at the DNA level, what should future research address to bring knowledge and techniques into practical usage? Development of appropriate economic technologies for screening large sample numbers will be needed. Initial work has begun to address this need (Shuman *et al.*, 1993). Definition of variation in structural genes, evaluated by using RFLP analysis and gene sequencing, is advancing well but understanding of the genetic regulatory elements of the MHC is minimal. As allelic diversity of each class of genes is defined, the relationship with disease resistance must be defined. And after identifying MHC-disease relationships, but before applying such knowledge in commercial practice, the effects of that MHC variation on production traits must be defined. Additionally, the MHC-linked, non-Class I, non-Class II genes need to be further characterized to determine whether they play a significant role in host defense.

GENETIC SELECTION ON IMMUNOPHYSIOLOGIC TRAITS

An alternative approach to selection on a single gene family, such as the MHC, is selection on one or more traits, on the assumption that these traits are correlated with disease resistance. One successful selection system that has been utilized is selection based upon antibody response to SRBC (Martin *et al.*, 1990; Pinard *et al.*, 1992). The underlying assumption is that response to this complex, nonpathogenic T cell-dependent antigen should be a broad indicator of general immunocompetence. Gross *et al.* (1980) found positive associations between anti-SRBC antibody and resistance to viral and parasitic disease, but negative associations with bacterial diseases. Pinard *et al.* (1993) found divergent Marek's disease responses in high and low anti-SRBC antibody lines. In an effort to improve early immune competence in broilers, chicks were selected for early response to *Escherichia coli* vaccination (Leitner *et al.*, 1992). This resulted in correlated changes in antibody response to other antigens (SRBC, Newcastle disease virus), phagocytic activity, and mitogenic response (Heller *et al.*, 1992).

In view of the delicate balance among the various components of the immune system, a selection program aimed at developing an enhanced, yet balanced, immune system was initiated (Cheng *et al.*, 1991). After several generations of replicated divergent selection for multiple traits of immune response, the high immune-response lines differed from the low immune-response lines in mean breeding values and some individual immune response traits (Kean *et al.*, 1994b). In recent challenge studies with Marek's disease virus, the low immune-response lines were more resistant to Marek's disease than the high immune-response lines (Nelson and Lamont, unpublished data).

A common feature of the aforementioned selection experiments is that, as a correlated response to selection for one or more immune-response traits, there was a significant difference between high and low response lines for MHC allelic frequencies (Martin *et al.*, 1990; Pinard *et al.*, 1993; Uni *et al.*, 1993; Kean *et al.*, 1994a). Thus, it appears that marker-assisted selection based upon the MHC would be a viable approach to accomplish similar genetic and physiologic changes to what were done in these selection experiments. In many instances, however, it is more expeditious to begin a selection with

simple immune system assays than to define the MHC genotypes extant in the population.

Immune Traits: Which Way Do We Go

Although there is a great deal of interest in identifying a marker trait that can be used for selection for disease resistance (Gavora and Spencer, 1983), it is clear that additional research is needed to define efficacious selection criteria. Additional assays, besides antibodies, should be characterized for evaluation of cell-mediated immunity and phagocytic activity. The relationship with disease resistance must be defined. Identification of molecular genetic markers could speed selection by allowing early detection of genotype. And, just as with the MHC, associations of any selection traits or markers with production traits must be evaluated before large-scale application can be recommended.

SUMMARY

Knowledge of the genetic bases for immune response differences allows the opportunity to select for improved disease resistance. Specific genes, such as the MHC, or immune assays may be used as indirect markers to modulate genetic disease resistance in a population.

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