Chickens as a simple system for scientific discovery

Chickens as a simple system for scientific discovery: the example of the MHC

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Abstract

Chickens have played many roles in human societies over thousands of years, most recently as an important model species for scientific discovery, particularly for embryology, virology and immunology. In the last few decades, biomedical models like mice have become the most important model organism for understanding the mechanisms of disease, but for the study of outbred populations, they have many limitations. Research on humans directly addresses many questions about disease, but frank experiments into mechanisms are limited by practicality and ethics. For research into all levels of disease simultaneously, chickens combine many of the advantages of humans and of mice, and could provide an independent, integrated and overarching system to validate and/or challenge the dogmas that have arisen from current biomedical research. Moreover, some important systems are simpler in chickens than in typical mammals. An example is the major histocompatibility complex (MHC) that encodes the classical MHC molecules, which play crucial roles in the innate and adaptive immune systems. Compared to the large and complex MHCs of typical mammals, the chicken MHC is compact and simple, with single dominantly-expressed MHC molecules that can determine the response to infectious pathogens. As a result, some fundamental principles have been easier to discover in chickens, with the importance of generalist and specialist MHC alleles being the latest example.

Keywords:

B locus, BF-BL region, BF molecules, HLA, disease association, infectious pathogen

Introduction

The chicken has been an iconic domesticated animal for many thousands of years, for sport and gambling, for meat and eggs, for ritual and collecting, and most recently for scientific discovery and understanding (Smith 2000, Lawler 2016). Chickens have played pivotal roles in various scientific fields. One major area has been embryology, based on the large and flat chick embryo that is easily accessible in the egg (Stern 2005). Other important areas derive from studies of infectious disease in chickens (Samal 2019, Kaspers et al 2021), including seminal contributions to virology, bacteriology, parasitology, cancer biology, immunology, vaccinology and genetics over 150 years. A few important examples are described below.

Louis Pasteur investigated the nature of fowl paralysis in the 1870s, discovering the pathogenic bacterium *Pasteurella multocida* as part of his contributions to the germ theory of disease. Some authorities consider that he also started the scientific fields of immunology and vaccinology by noticing that chickens inoculated with old cultures survived challenge with virulent bacteria, naming the process "vaccination" in honor of Edward Jenner (Berche 2012).

Peyton Rous first showed in the 1910s that a pathogen could cause tumors by discovering the first retrovirus, Rous sarcoma virus (RSV), as a pathogenic agent that would pass through filters for bacteria and parasites (Weiss and Vogt 2011). Decades of research culminating in the studies by Vogt, Duesberg, Bishop, Varmus, Erikson, Hunter and many others showed that RSV had acquired and modified a cellular gene to cause cancer, the first tyrosine kinase discovered (Bister 2015). Moreover, the action of reverse transcriptase encoded by RSV was discovered by Temin and Baltimore, upsetting the central dogma of the time (Coffin and Fan 2016).

Many other viruses were first described as a result of poultry diseases, including the first coronavirus, found as the pathogen responsible for infectious bronchitis that is still a major economically-important disease (Reagan and Brueckner 1952, Kenney et al 2020). Avian influenza virus has caused various zoonotic outbreaks, potentially as the origin of the Spanish flu epidemic of 1918, and more recently for so-called bird flu (Monto et al 2020). In attempting to understand interference with influenza infection, Issacs and Lindenmann discovered the first cytokine, interferon, using techniques from avian virus biology such as growing virus on chorioallantoic membranes of chicken eggs (Gale 2015).

The first cancer vaccine was developed in response to the decimation of commercial chicken flocks upon the intensification of poultry farming in the early 1960s (Schat 2016). Studies by Biggs, Witter and many others disentangled the cause, finding two tumour viruses: avian leukosis virus (a chronic retrovirus related to RSV) and Marek's disease virus (MDV) that was known as the causative agent of fowl paralysis discovered 60 years before. Subsequently, live attenuated MDV vaccines were developed as the first anti-cancer vaccines. Unfortunately, these vaccines controlled the disease but not viral transmission,

leading to the concept of leaky vaccines that drive virulence (Witter 1997, Read et al 2015).

A major contribution to immunology by Glick was the discovery of B lymphocytes whose development was dependent on the bursa of Fabricius, leading to the concept of B and T cell dichotomy by Cooper and colleagues that was foundational for understanding adaptive immunity in humans and all other jawed vertebrates (Taylor and McCorckel 2009). The discovery of the B blood group as the major histocompatibility complex (MHC) by Briles, Gilmour, Simonsen and others was only the second MHC to be described, and has been particularly important both scientifically and practically, based on the strong genetic associations with resistance to a variety of infectious diseases (Miller and Taylor 2016, da Silvo and Gallardo 2020).

From the initial discovery as a genetic locus responsible for allograft rejection, an enormous amount has been learned about the MHC and the highly polymorphic MHC molecules responsible for transplantation phenomena, the classical class I and class II molecules (Klein 1986, Trowsdale and Knight 2013, Kaufman 2016). Almost all this vast amount of knowledge has been learned by research with humans and other mammals, much of which has hardened into dogma and swiftly passed into textbooks. However, the genes encoding MHC molecules along with many other genes located in the human MHC are found in all jawed vertebrates, and comparative genomics has highlighted many differences between mammals and other vertebrates, and between different non-mammalian taxa (Kaufman 2018a). Outside of mammals, almost all that is known about the MHC between the genome and population genetics comes from the work on chickens (Kaufman 2013, Kaufman 2015, Parker and Kaufman 2017, Kaufman 2021).

This brief article will review how the simplicity of the chicken MHC has led to discoveries about the interplay of structure, function and evolution of the adaptive immune system, in particular how such discoveries allowed appreciation of concepts that were more difficult to discern from the complex MHC of humans and other typical mammals. The article will end with a more general discussion of the advantages of chickens as a system to integrate understanding of disease at many levels, combining the advantages of humans and of biomedical models such as mice.

The complex and the simple in the MHC

The classical MHC molecules that evoke transplant rejection are now known (Djaoud and Parham 2020) to present peptides to thymus-derived (T) lymphocytes of the adaptive immune system, class I molecules presenting peptides derived mainly from proteins in the cytoplasm and nucleus to CD8 T cells, and class II molecules presenting peptides derived mainly from the lumen of intracellular vesicles and the extracellular space to CD4 T cells. In addition, class I molecules can be detected by natural killer (NK) cells, a lymphocyte subset of the innate immune system. These MHC molecules play crucial roles in

the immune responses to infectious pathogens, and the molecular arms race between the pathogens and hosts leads to the high polymorphism of classical MHC molecules, with different alleles binding different sets of peptides.

The classical MHC molecules require other proteins to acquire the peptides, and some of these antigen processing and presentation (APP) molecules are encoded in the MHC (Blum et al 2013, Trowsdale and Knight 2013, Kaufman 2016). For example, the digestion of cytoplasmic proteins by the proteasome is aided by inducible proteasome components. The resulting peptides are pumped into the lumen of the endoplasmic reticulum by the heterodimeric transporter for antigen processing (TAP), which is part of the peptide-loading complex (PLC) that includes a class I-specific chaperone called tapasin (also known as TAP binding protein, TAPBP) that optimizes the peptides bound. These class I APP molecules are important for some but not all of the so-called non-classical class I molecules. The non-classical MHC molecules have the same gene structure as classical molecules, but lack one or more of the key features: widespread and high expression, high polymorphism and/or peptide presentation. The APP genes for class II molecules include the non-classical DM genes.

The MHC of typical mammals (Klein 1986, Trowsdale and Knight 2013, Kaufman 2016) is large and complex, with multigene families of classical class I and class II molecules peppered among many other genes. As the iconic example (Fig. 1), the human MHC is around four megabase pairs (Mbp) and 2-4 centimorgans (cM), with hundreds of genes. Three classical class I genes, HLA-A, -B and -C, along with several different non-classical class I genes and many other kinds of genes, are found in the class I region. The class II region typically encodes 3-4 classical class II heterodimers, including HLA-DR, -DQ and -DP, along with non-classical class II genes, a serine/threonine kinase and, most importantly, the tapasin, TAP and some inducible proteasome genes required for class I presentation. The class III region is located in between the class I and class II regions, but contains many genes that are unrelated to antigen processing and presentation systems.

In comparison, the chicken MHC (defined as the region with the classical class I and class II genes, known as the BF-BL region) is compact and simple (Fig. 1), with only 19 genes in 95 kilobase pairs (kbp) (Kaufman et al 1999a, Kaufman et al 1999b), and undergoes recombination very infrequently (Simonsen et al 1982, Skjødt et al 1985, Hala et al 1988, Fulton et al 2016), leading to relatively stable haplotypes. There are only two classical polymorphic class I genes, with only BF2 being expressed at a high level; the gene for the other chain of class I molecules, β_2-microglobulin, is monomorphic and located on another chromosome (as in most other jawed vertebrates) (Riegert et al 1996, Wallny et al 2006, Shaw et al 2007). There are only two classical polymorphic class II B genes (that encode the β chain of the class II heterodimer), of which only BLB2 is expressed widely; the BLA gene that encodes the non-polymorphic α chain is located outside the BF-BL region (Jacob et al 2000, Salomonsen et al 2003). Unlike mammals, the chicken APP genes located in the MHC are all polymorphic: tapasin, TAP1 and TAP2 for the class I system and DMA, DMB1 and DMB2 for the class II system (Atkinson et al 2001, Hosomichi et al 2008, Walker et al 2011, van Hateren et al 2013). No

inducible proteasome or thymoproteasome components have been found in the chicken MHC, genome or proteome (Kaufman et al 1999a, Erath and Groettrup 2015). One functional consequence is that chicken class I molecules can bind peptides with acidic residues at the C-terminus, unlike mammals (Wallny et al 2006).

The genomic organization of the chicken MHC is quite different from that of mammals, and has striking consequences for function (Kaufman et al 1999a, Kaufman et al 1999b, Hosomichi et al 2008, Kaufman 2018b). Most importantly, the two chicken classical class I genes flank the polymorphic TAP genes and the polymorphic tapasin gene is located nearby. In any case the whole BF-BL region has very limited recombination so that unique TAP and tapasin alleles co-evolve with the BF2 gene (Walker et al 2011, van Hateren et al 2013) (Fig. 1), leading to a dominantly-expressed and highly polymorphic class I gene that can determine the CD8 T cell response. In comparison, the BF1 gene is poorly expressed with far fewer alleles, the majority of which have very similar peptide binding sites; in some haplotypes the BF1 gene is missing altogether (Wallny et al 2006, Shaw et al 2007). The available evidence is that the BF1 gene is primarily an NK cell target (Kim et al 2018), perhaps resembling HLA-C in humans. A somewhat similar situation is envisaged for the class II system, with one class II molecule expressed widely and the other expressed only in some locations or upon stimulation, presumably a consequence of co-evolution with the polymorphic DM genes (Parker and Kaufman 2017), although much work remains to test this hypothesis. Overall, the consequence of these systems is strong genetic associations with the response to infectious pathogens, with each haplotype leading to greater or lesser responses to particular pathogens, with life and death at the extremes (Fig. 2). That is precisely what has been found for many pathogens, including many that are of economic importance.

In contrast, the genomic organization of the human MHC (and those of other typical mammals) leads to different functional consequences (Kaufman et al 1995, Kaufman et al 1999b, Kaufman 2015, Kaufman 2018a, Kaufman 2018b). The classical class I genes in the class I region are located far away from the TAP, tapasin and inducible proteasome components located in the class II region so that co-evolution between polymorphic class I genes and APP genes would be disrupted by relatively frequent recombination. As a result, the APP genes evolved to a monomorphic “average best-fit” for any class I alleles that might appear by recombination, and thus could provide peptides for a multigene family of classical class I genes. Such a multigene family would confer more-or-less protection from most infectious pathogens, reading out as weak genetic associations (Fig. 2). Indeed, the strongest genetic associations of the human MHC are with autoimmune diseases, whereas the associations with infectious pathogens are relatively weak, with the strongest found for viruses with small genomes, such as human immunodeficiency virus (HIV). Even for such viruses, the associations with the human MHC may not depend on peptide presentation to T cells (but at least in part to NK cell responses), and may only be one of several genetic loci (which may or may not be located in the MHC).

In the human MHC, the classical class I and class II genes are peppered among hundreds of other genes, and many more genes are located in the extended MHC regions on either side. In contrast, the classical class I and class II genes cluster together in the chicken genome, with only a few other genes that are mostly essential for antigen presentation within this classical region. The simplicity is so striking that the BF-BL region was originally characterized as a "minimal essential MHC" (Kaufman et al 1995, Kaufman et al 1999a). One of the reasons why the chicken MHC is so small is that the class III region, containing a complement C4 gene among others, is outside of the class I and class II regions, an organization suggested to be ancestral.

However, there are aspects that are complex, and another view of the chicken MHC, based more on genomic analysis than on function, that emphasizes the various complexities (Kaufman et al 1999b, Miller and Taylor 2016). The NK receptor and ligand gene pair (BNK and Blec) is located at one end of the BF-BL region (Kaufman et al 1999a, Rogers et al 2005, Rogers and Kaufman 2008), and two monomorphic non-classical class I genes (CD1.1 and CD1.2) are located at the other end beyond the class III region (Salomonsen et al 2005, Miller et al 2005, Maruoka et al 2005); both of these systems are located on other chromosomes in mammals. The location of these genes in the chicken MHC has been considered evidence that receptor, ligand and APP genes were located together in a primordial MHC so that the unrelated genes could co-evolve into pathways to give rise to the relevant innate and adaptive systems, before falling apart once the pathways were established (Rogers et al 2005, Walker et al 2011, Kaufman 2018a). Genes of the butyrophilin family (including a complex multigene family of BG genes in the BG region) are located at the end and near the BF-BL region, with various other genes in a TRIM region, altogether being part of the B locus (Shiina et al 2007, Salomonsen et al 2014). Moreover, an Rfp-Y region with non-classical class I and class II B genes is located on the same chromosome as the BF-BL region (Briles et al 1993, Miller et al 1994). One view is that all these genes of the B locus, Rfp-Y region and further afield should be considered the MHC, whereas the view espoused in this review is that all these genes are extremely interesting, but that based on function the classical MHC genes are the core of the MHC, around which other genes come and go as evolutionary history unfolds (Kaufman 2018a).

Thinking beyond the MHC molecules as ligands, the receptors for classical MHC molecules include the αβ (and perhaps the γδ) T cell receptors (TCRs) and the NK receptors. The chicken TCR β locus is famously simple, with around ten V genes, which has been taken to suggest that the classical class I genes and αβ TCRs are co-evolving, despite being on different chromosomes (Smith and Göbel 2013, Kaufman et al 2015). However, the homologs of the killer immunoglobulin receptors (KIRs) in humans are members of the chicken immunoglobulin-like receptor (ChIR) multigene family, found in the equivalent of the leukocyte receptor complex (LRC), and appear at least as complex as in mammals (Straub et al 2013). In contrast, the two lectin-like receptor genes found in the equivalent of the NK complex (NKC) of the chicken do not appear to be NK receptors (Chiang et al 2007, Neulen and Göbel 2012), while the equivalents of the NK receptor NKR-P1/NK1.1/KLRB1 and the ligand LLT1/clr found in the human and

mouse NKC are BNK and Blec found in the BF-BL region (with other Blec-like genes in the BG region) (Kaufman et al 1999a, Rogers et al 2005, Rogers and Kaufman 2008, Salomonsen et al 2014).

Generalists and specialists: discovered in chickens

Although the fact of peptide binding to MHC molecules was first discovered for human class II molecules, the specificity of binding by different classical MHC alleles was first established for human class I molecules (Babbitt et al 1985, Falk et al 1991). In part, this discovery of anchor residues was due to the fact that typically most peptides bound to a class I molecule have a range of similar lengths (8-10 amino acids being common), with only a few (usually two) peptide positions that determine specificity and with those positions limited to one or a few amino acids of very similar chemical characteristics. By comparison, class II molecules bind peptides that have a variety of lengths, with a core typically of nine amino acids, within which four peptide positions determine specificity, each of which is based on several amino acids (typically more than four) that can have a variety of chemical characteristics (Rammensee et al 1993, Engelhard 1994). As a result, it was easy to apply the idea of a class I peptide motif as the property that would determine peptide binding, which in turn would be responsible for the immune responses (for example, Kast and Melief 1991, Kast et al 1991), including resistance to infectious pathogens. The concept was also extended to class II molecules, although it was much harder to discern clear differences for infectious pathogens.

For some chicken class I molecules, the determination of peptide motifs was easy, with clear peptide motifs that were more stringent than typical mammalian molecules: often a single peptide length with three peptide positions each of which with only one or two closely-related amino acids (Kaufman et al 1995, Wallny et al 2006). As an oft-quoted example, the dominantly-expressed class I molecule of the B4 haplotype (BF2*004:01) is almost entirely octamers (8mers), requiring a negatively-charged amino acid at positions 2 and 5, and a glutamic acid almost exclusively at position 8. This peptide motif is explained both by the crystal structure of the binding site of the class I molecule and by the peptide translocation specificity of the B4 cells (Zhang et al 2012, Walker et al 2011).

The simple and stringent peptide motifs of B4, B12 and B15 molecules were used to predict the peptides from Rous sarcoma virus (RSV Prague strain C) that might bind, with many more peptides predicted for the resistant B12 haplotype than for the susceptible B4 and B15 haplotypes (Wallny et al 2006). Peptide binding studies showed that only three B12 peptides from the RSV *src* gene actually did bind *in vitro*, while none of the predicted peptides for B4 and B15 haplotypes bound. Vaccination of B12 birds showed that the strongest binding peptide reduced the eventual tumour burden from RSV infection, in vaccination schedules down to zero (Hofmann et al 2003). Thus, peptide motifs of chicken class I molecules determined resistance to an oncogenic virus, and these motifs were used to determine peptides for avian influenza virus, for infectious bronchitis virus and for a molecular-defined vaccine to infectious bursal disease

virus (Reemers et al 2012, Butter et al 2013, Tan et al 2016). However, these chicken class I molecules with simple peptide motifs were not encoded by those MHC haplotypes widely recognised to confer resistance to economically-important infectious diseases in chickens (Simonsen 1987, Plachy et al 1992).

The path to understanding the protective chicken MHC haplotypes was long and convoluted, starting with an unexpected observation that the level of class I molecules from different MHC haplotypes expressed on the surface of chicken erythrocytes varied over a ten-fold range, and that this hierarchy was correlated with the susceptibility to Marek's disease (MD) caused by an oncogenic herpesvirus (Kaufman et al 1995). The BF-BL regions of the low-expressing haplotypes such as B2 and B21 were most clearly involved with resistance to MD. With time, it became clear that the cell surface expression level was part of a suite of correlated properties, including ease of refolding *in vitro*, thermal stability and size of peptide repertoire for chicken class I molecules, as well as specificity of peptide translocation (Koch et al 2007, Chappell et al 2015, Tregaskes et al 2016, Kaufman 2017b, Kaufman 2018b). In essence, the "fastidious" alleles had high cell surface expression, narrow peptide repertoires and high stability but did not refold well *in vitro* (which was interpreted as a measure of tapasin-dependence), while the "promiscuous" alleles had lower cell surface expression, much wider peptide repertoires and lower stability but refolded relatively easily *in vitro* (which was interpreted as a measure of tapasin-independence).

There are many questions that arose from this new idea (Chappell et al 2015, Kaufman 2018b), all subject to ongoing investigation. One question is how the promiscuous molecules bind such a great variety of peptides (there are at least two mechanisms reported thus far, and a third even more startling one that is not yet published). Another question is whether the promiscuous class I molecules protect due to presentation of a greater number of peptides to a wider variety of T cells, or to a greater chance of presenting a single protective peptide to a particular group of T cells. A third question is whether the inverse correlation of peptide repertoire with cell surface expression level could be due to the requirements of developing an optimal TCR repertoire after selection of T cells in the thymus. A fourth question is whether the peptide repertoire and expression level can affect NK cell responses. A fifth question is whether the hierarchy of class I alleles has effects outside of infectious diseases, for instance, cancer, autoimmunity, reproduction or brain development. Finally, perhaps the most immediately important question is the nature of the selection for this suite of properties.

A review of the historic literature found that promiscuous molecules generally protected from a range of economically-important viral diseases in deliberate experiments, with most examples pitting a haplotype now known to have a promiscuous class I allele against a haplotype with a fastidious allele (Kaufman et al 2018b), including infectious bronchitis virus (IBV), the first discovered coronavirus (Banat et al 2013). Even more informative are field studies with natural infections, such as was carried out during an outbreak of avian influenza in northern Thailand, with class I typing of dead and surviving birds performed

by sequencing (Boonyanuwat et al 2006). Those birds with B21 haplotypes nearly all survived, while those birds with B4 haplotypes mostly died (Table 1). A subsequent experimental test failed to replicate the result (Hunt et al 2010); although there are several potential explanations, an unbiased field study would seem to be most telling. More generally in the field study, for those haplotypes for which the class I alleles are known in detail, those birds with only fastidious class I molecules mostly died whereas birds with one or more promiscuous haplotypes (with one exception) survived (Table 1) (Kaufman 2018b). Overall, these observations suggest that promiscuous haplotypes generally protect from infectious pathogens, that is to say the promiscuous class I molecules are generalists.

There is a flip side to this idea of generalists. Even for the case of RSV Prague strain C (noted above), the resistant B12 haplotype has a dominantly-expressed class I molecule that is more promiscuous than the susceptible haplotypes B4 and B15 (Wallny et al 2006). A historic analysis of MHC haplotypes showed that the B4 haplotype conferred susceptibility for some strains of RSV, but conferred protection for others (McBride et al 1981) (Table 2). This latter result might be understood in the context of specialists, an idea that owes more to data for HIV progression in humans.

Generalists and specialists: extended to humans

After the inverse correlation between cell surface expression level and peptide repertoire of chicken class I molecules began to emerge, it was of immediate interest to determine whether the results were specific only for chickens or had wider (biomedical) relevance. It would be more difficult to disentangle such relationships in humans and typical mammals, since the results would be an amalgam of the effects from all members of the multigene families of class I molecules rather than the straightforward results from the single dominantly-expressed class I molecule of chickens.

At the time, only one paper on human class I molecules was found (Kosmrlj et al 2010), comparing the number of peptides from the whole human proteome predicted to bind four HLA-B alleles to the odds ratios of progression from HIV infection to acquired immunodeficiency syndrome (AIDS). The elite controller alleles HLA-B*57:01 and -B*27:01 were predicted to bind fewer self-peptides than the faster progressing alleles HLA-B*07:02 and -B*35:01. Just to be clear, the predictive methods strongly over- and under-predict (Paul et al 2020), and can yield different answers in separate studies, as is clear from a re-evaluation with a more HLA alleles (Arora et al 2019). In any case, *ex vivo* lymphocytes and monocytes from volunteers homozygous for HLA-B alleles (and bearing HLA-A alleles that were not cross-reactive with the antibodies used) were analysed by flow cytometry using three different antibodies, finding a clear inverse correlation between the number of class I molecules on the surface and the predicted peptide repertoire (Chappell et al 2015). This inverse correlation was exactly as found for chickens, but the disease associations were the opposite: promiscuous class I molecules were associated with general resistance in

chickens, but certain fastidious class I molecules were associated with slow progression to AIDS in humans.

The characteristics of elite controlling class I alleles like HLA-B*57:01 and -B*27:01 (and similar alleles in non-human primates, de Groot et al 2018) have been investigated in detail, and one overarching conclusion is that such alleles bind HIV peptides that the virus can mutate to escape the immune response, but only with much decreased viral replication (Schneidewind et al 2007, Miura et al 2009). For these class I alleles, the virus is stuck between immune evasion and viral fitness, which slows down the appearance of escape mutants and thus the progression to AIDS. The peptides responsible are somehow special, which led to the idea of such fastidious class I molecules being "specialists" for binding special peptides from particular pathogens. Putting the data from chicken and human class I molecules together led to the generalist and specialist hypothesis (Chappell et al 2015, Kaufman 2018b), in which promiscuous generalist alleles would provide protection from most common pathogens, but that particular fastidious specialists might protect upon the appearance of new and virulent pathogens for which the promiscuous alleles could not afford protection.

Among the interesting thoughts that follow from this hypothesis, it may be that populations with only a few promiscuous MHC alleles would be well protected under typical circumstances, so that high levels of polymorphism typically found really do reflect the accidents of evolutionary history rather than requirements for survival of contemporary populations. If this view is true, then high MHC polymorphism may not always be a crucial guide for conservation of endangered species. Conversely, it may be that species other than chickens and humans have other strategies for MHC function, for instance having a large number of fastidious alleles and no promiscuous alleles. It is clear that much more work is required to understand the ramifications of the hypothesis of generalists and specialists.

As these ideas were being formulated, another seminal paper on human class I molecules appeared (Paul et al 2013), showing that the number of peptides predicted to bind 27 HLA-A and -B alleles varied from over 10% to less than 1% of total peptides from the Dengue virus proteome, and that the number of predicted peptides inversely correlated to the predicted affinity of binding. Thus, just as in chickens, there is a range of peptide repertoires and the most promiscuous molecules are less stable. The rank order of the human class I alleles in common between this study and the one mentioned above was the same (Kosmrlj et al 2010, Paul et al 2013), but a recent report apparently using the same methodology contradicts both of these earlier studies (Arora et al 2019). It is likely that any discrepancies arise from the fact that these algorithms both over-predict and under-predict, and that the parameters chosen for the predictions are important to the reported results. A comparison shows that the rank order of peptide repertoire determined by predictions of Dengue peptides (Paul et al 2013) does not correlate with peptide motifs of many class I molecules, as illustrated by the A1, A24 and B7 supertypes (Kaufman 2020), so that these two properties may be disentangled experimentally.

Other important papers (Rizvi et al 2014, Raghaven et al 2015) focused on the relative tapasin-dependence of human class I alleles compared to their role in controlling HIV progression. The initial studies used transfection of 50 HLA-B alleles into tapasin-deficient and wild-type cells, finding a hierarchy based on tapasin-dependence for cell surface expression. Moreover, the efficiency of refolding class I alleles *in vitro* correlated with the tapasin-independence. Comparing those human alleles in common with the studies mentioned above, a strong correlation was noted for the number of predicted peptides and tapasin-independence, finding that fastidious class I alleles required tapasin for cell surface expression, while promiscuous alleles did not (Kaufman 2015, Kaufman 2017, Kaufman 2018b). This analysis allowed the human and chicken alleles to be compared, finding that promiscuous alleles in both humans and chickens refold better *in vitro*.

One major concern with the generalist and specialist hypothesis was the fact that promiscuous chicken alleles protected from many common pathogens but fastidious human alleles protected from AIDS progression. This discrepancy was resolved by a very recent study (Bashirova et al 2020) that extended both the analysis of tapasin-dependence to 96 HLA-A, -B and -C alleles and the comparison to HIV progression. Clear hierarchies of tapasin-dependence were shown for the class I molecules from all three class I loci, and evidence provided that the predicted peptide repertoires were inversely correlated with tapasin-dependence (as previously discussed, Kaufman 2015, Kaufman 2017, Kaufman 2018b). In addition, tapasin-dependence (and thus peptide repertoire) was compared to HIV progression for all the alleles with the exclusion of known elite controllers, and led to the conclusion that, in the absence of elite controlling alleles, the number of promiscuous HLA-B alleles present in an individual correlated with protection. Thus, promiscuous human alleles confer protection to HIV as generalists while some fastidious human alleles can confer protection to HIV as specialists.

Although the peptide motifs for class II molecules are very promiscuous compared to class I molecules, an analysis of the numbers of peptides predicted to bind to human class II molecules was interpreted to define a hierarchy of alleles based on peptide repertoires. Moreover, it was proposed that promiscuous alleles arise independently and repeatedly from fastidious alleles in geographical locations with higher numbers of pathogens (Manczinger et al 2019), not exactly the same hypothesis as generalists and specialists. Although little is known about the peptide repertoires of chicken class II molecules, one report notes differences in the cell surface expression levels of class II molecules from different chicken MHC haplotypes (Larsen et al 2019).

Chickens as a system to understand disease at all levels

Almost everything known about the MHC comes from research with humans and biomedical models like mice, so why should anyone consider working with chickens? As discussed above, there are many differences between typical mammals and chickens, so it is not obvious that all textbook information based

on mammals can be extended to other jawed vertebrates. In fact, chickens provide an outstanding model to challenge and/or validate the generality of results provided by biomedicine.

There are huge numbers of outbred humans subject to natural infections by a wide range of natural pathogens. Moreover, people are closely monitored for health and many other biological traits by extensive medical and public health systems, with many tools and resources developed over decades. However, it has been extremely challenging, both practically and ethically, to undertake frank experiments using humans.

Biomedical model organisms have been the choice for detailed hypothesis-driven research for many decades. In particular, mice have become important for general enquiries, due to their relatively small size, rapid generation time and genetic homogeneity. The tools and resources for working with mice are impressive, including genetic manipulation that has led to such rapid progress in understanding detailed mechanisms *in vivo*. However, most research is carried out with a few highly inbred mouse strains, using unnatural pathogens in unnatural environments. Although research using outbred, wild and "dirty" mice is increasing (Ilmonen et al 2008, Nelson et al 2013, Poltorak et al 2018, Hamilton et al 2020), few studies look at natural mice in natural environments beset by natural pathogens.

In some senses, chickens provide a system that combines the best of humans and mice. There is wide overall diversity starting with wild jungle fowl through many different kinds throughout the world, living in a wide range of environments (Groeneveld et al 2010, Tixier-Boichard et al 2011, Lawal et al 2020). There are huge numbers of commercial flocks that are constantly monitored for health, both by the commercial organisations and by government agencies. Much is known about the genetics and other properties of these commercial chickens, but there are also well-characterised inbred lines of chickens for deliberate experimentation (Bacon et al 2000, Zhang et al 2020, Kaspers et al 2021). There are many immunological reagents and assays, ongoing genetic and genomic analyses, and increasingly sophisticated and simple methods for genomic modification (Sid et al 2018, Davey et al 2018, Guzman and Montoya 2018, Kaspers et al 2021). There is detailed and wide-ranging information on the wide range of natural pathogens that continually threaten chicken flocks around the world (Samal 2019, Rouger et al 2017, Mitra et al 2018, Fatoba and Adeleke 2018). Finally, it is relatively easy to bring new strains of pathogens and chickens from the field into the lab to do careful experiments, and then return with the results for practical intervention in the field.

Overall, chickens can be used to understand infectious disease at all levels, from evolution and epidemiology to molecular mechanisms, providing a system that is both underutilized and underappreciated. The closer relationship to humans may be considered an advantage of mammalian biomedical model species, although many studies show detailed differences between the immune responses of humans and mice. As described in this brief review, chickens do have the advantage of a simpler immune response, at least as far and the MHC

and αβ T cell system are concerned, leading to discoveries that have been harder to make with the more complicated adaptive immune system of mammals.

5795 words

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Figure legends

Fig. 1. The BF-BL region (the chicken MHC) is smaller and simpler than HLA (the human MHC), with co-evolution with APP genes leading to the single dominantly-expressed BF2 (class I) gene. Colored boxes indicate genes with names above; thin vertical lines indicate region boundaries with names above or below; location is roughly to scale, with a bar indicating approximately 100 kB. Level of gene expression indicated by thickness of arrows pointing up, co-evolution between the TAP genes and the BF2 class I gene indicated by a curved arrow. Genes from the class I system, red; the class II system, blue; the class III or other regions, green; solid colors indicate classical genes while striped colors indicate APP genes. Figure from Kaufman 2018b.

Fig. 2. The chicken MHC can have strong genetic associations with resistance to infectious diseases, compared to the MHC of typical mammals. Left panel. A multigene family in the human MHC encodes several fastidious class I molecules, each with a chance of presenting a protective peptide, so the typical human MHC haplotype can confer more-or-less resistance to most pathogens, reading out as weak genetic associations (because there is little difference between haplotypes). Middle panel. In comparison, the chicken MHC encodes a single dominantly-expressed class I molecule, which can have a fastidious peptide motif that may or may not present a protective peptide from a pathogen, reading out as strong genetic associations (because there can be large differences between haplotypes). Right panel. The single dominantly-expressed class I molecule encoded by the chicken MHC may have a promiscuous peptide motif, binding a wide range of peptides (like a multigene family acting together). Comparing promiscuous alleles may read out as weak genetic associations (since there is not much difference between haplotypes), but comparing fastidious alleles with promiscuous alleles may give strong genetic associations. Figure from Kaufman 2018b.

Fig. 3. The predictive peptide repertoires for 27 common HLA class I alleles (bars in graph) compared to the supertypes of these alleles (in circles below) show that the peptide motifs for some supertypes do not correlate well with peptide repertoire. Figure from Kaufman 2020.

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Genotype	number of chickens		percent survival	
	LHK	PHD	LHK	PHD
A1/A1	10	18	0	0
B2/B15	9	7	78	100
B5/B15	10	6	30	33
B5/B19	10	8	50	50
B12/B12	33	111	0	3
B19/B19	9	5	22	80
B2/B13	8	10	0	0
B12/B13	21	14	5	7
B13/B13	44	50	0	0
B13/B15	19	27	0	0
B13/BA12	4	4	0	0
B2/B21	9	7	100	100
B12/B21	18	8	100	100
B15/B21	26	10	100	100
B21/B21	53	121	100	100
B21/BA12	16	16	88	88

Table 1. Avian influenza epidemic among Leung-Hahng-Kow (LHK) and Pradoo-Hahng-Dam (PHD) indigenous chickens from small holder farms in central Thailand shows that MHC haplotypes with promiscuous class I molecules confer survival (rounded to nearest integer, data from Boonyanuwat et al 2006 Anim Sci J). NB: promiscuous haplotypes include B2 and B21; fastidious haplotypes include B12, B13 (equivalent to B4 in the BF-BL region), B15 and B19; B5, A1 and BA12 are not known at present.

Virus subtype	Virus strain	GB1 chicken B13 haplotype	GB2 chicken B6 haplotype
B	Harris	P	R
B	Bryan High titre	R	R
C	Prague	P	R
C	Bratislava 77	R	P

Table 2. Challenge experiments show that the same MHC haplotype can confer regression or progression, depending on the strain of Rous sarcoma virus (data from McBride et al 1981 J Immunogenet). NB: GB1 and GB2 are congenic lines, bred to be the same everywhere but the B locus, which includes the MHC. B13 (equivalent to B4 in the BF-BL region) has a fastidious class I molecule, while B6 has a relatively promiscuous class I molecule. R, tumour regressor (resistant); P, tumour progressor (susceptible).