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The simple chicken major histocompatibility complex: life and death in the face of pathogens and vaccines

Jim Kaufman

Institute for Animal Health, Compton, Berkshire RG20 7NN, UK (jim.kaufman@bbsrc.ac.uk)

In contrast to the major histocompatibility complex (MHC) of well-studied mammals such as humans and mice, the particular haplotype of the B-F/B-L region of the chicken B locus determines life and death in response to certain infectious pathogens as well as to certain vaccines. We found that the B-F/B-L region is much smaller and simpler than the typical mammalian MHC, with an important difference being the expression of a single class I gene at a high level of RNA and protein. The peptide-binding specificity of this dominantly expressed class I molecule in different haplotypes correlates with resistance to tumours caused by Rous sarcoma virus, while the cell-surface expression level correlates with susceptibility to tumours caused by Marek's disease virus. A similar story is developing with class II β genes and response to killed viral vaccines. This apparently suicidal strategy of single dominantly expressed class I and class II molecules may be due to coevolution between genes within the compact chicken MHC.

Keywords: chicken; major histocompatibility complex; pathogen; vaccine

1. INTRODUCTION

It perhaps should be no surprise, given the enormous and concentrated enquiry into host–pathogen relationships in human disease, that the dynamics of virus infections in humans and in animals that serve as biomedical models can be studied with precision and elegance. There are at least three levels of such pathogen–host interactions. A great deal is known about the course of certain viral infections in individuals, with respect to both the pathogen and the host response, to the point that mathematical modelling has become a useful tool, not just for depicting what is already known but for predicting what may not be obvious, as exemplified in the accompanying reports (see other papers in this issue). A certain amount is also known about how and why these viruses evolve in relation to the immune response in populations of hosts. Much less is clear about how and why both viruses and hosts choose particular strategies—for instance, when different host species are considered.

Given the sophistication of the analysis in well-studied biomedical model species, why examine these questions in any other animal? We believe that there are some real advantages in using the humble domestic chicken to study all three levels of host–pathogen interaction.

First, there are many chickens around. Some 34 billion chickens are alive, however briefly, each year, and the health and welfare of these animals is of serious concern to the poultry industry. In comparison to other non-mammalian vertebrates, avian genetics and immunology are very well studied, in part because of this economic importance. In contrast to mice, a great deal of field data are continually gathered on a variety of natural infectious diseases, and compared with humans, laboratory experiments can easily be performed.

Second, chickens are beset with a large variety of natural pathogens. Many of these pathogens are well studied in the laboratory, well monitored in the field, and known to be locked in a continuing and lethal molecular arms race with their hosts. Indeed, the shift to intensive rearing practices may have accentuated the rise in virulence, partly through an increase in population density, but probably also through husbandry practices and vaccination strategies.

Third, there are many interesting differences between avians and mammals. Particularly relevant to this discussion are the observations that the genetic loci encoding certain immune system molecules are smaller and simpler in chickens than in humans and mice, and that this simplicity can have important functional implications.

In well-characterized mammals such as humans and mice, the major histocompatibility complex (MHC) is a large, complicated and redundant genetic region, with many highly expressed classical class I and class II genes (Aguado *et al.* 1999; Trowsdale 1995). Moreover, despite the fact that the high polymorphism of mammalian MHC genes is thought to be driven by pathogen variation, different haplotypes all confer roughly equal protection against most infectious pathogens. In fact, the strong associations with the human MHC are with autoimmune diseases or biochemical defects. The best examples of associations with infectious diseases are slight and the level of selection on individual alleles on mammalian MHC genes has been calculated to be low (Hill 1998; Satta *et al.* 1994; Tiwari & Terasaki 1985).

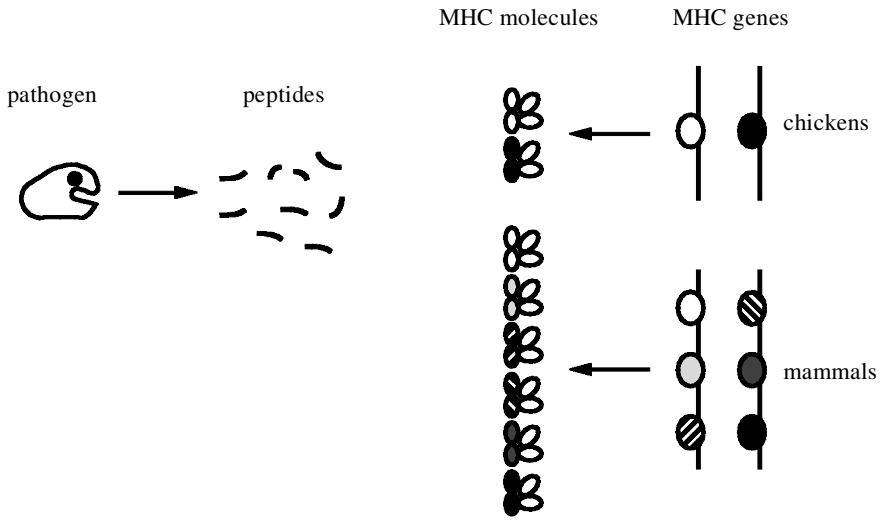

Figure 1. The effect of multiple well-expressed class I genes compared to a single dominantly expressed class I gene. Pathogen proteins are proteolysed in host cells and the resulting peptides are bound by MHC molecules and presented to T lymphocytes of the immune system. In typical mammals, a multigene family of MHC genes (each with two alleles in MHC heterozygotes) encodes multiple MHC molecules on the cell surface. Each molecule has a different peptide-binding specificity, so overall there are many chances to find a peptide that binds a class I molecule. With the single dominantly expressed MHC gene in chickens, there are far fewer chances to bind a peptide to protect the individual (even in MHC heterozygotes).

In contrast, the chicken MHC is simple and compact, with single dominantly expressed class I and class II genes in common MHC haplotypes (Kaufman *et al.* 1995, 1999*a*,*b*). Moreover, the chicken MHC determines life and death in response to certain infectious pathogens, both relatively small and simple pathogens as well as at least one large and complicated virus, the herpesvirus that causes Marek's disease (Calnek 1985; Dietert *et al.* 1990; Kaufman & Lamont 1996; Plachy *et al.* 1992; Schat 1987). We have developed a simple model, the 'minimal essential MHC of the chicken', to relate the structural simplicity of the chicken MHC with the striking functional associations, in comparison with the well-characterized mammalian models (Kaufman 1999; Kaufman *et al.* 1995, 1999*a*,*b*; Kaufman & Salomonsen 1997; Kaufman & Venugopal 1998; Kaufman & Wallny 1996).

In this review, we would like to consider the three points leading to the hypothesis of the 'minimal essential MHC'—that in contrast to the MHCs of humans and mice, the chicken MHC (i) determines resistance and susceptibility to small pathogens such as Rous sarcoma virus (RSV); (ii) determines resistance and susceptibility to a large pathogen, Marek's disease virus (MDV); and (iii) is small and simple. For each point, we will summarize a few of our published and unpublished data, relate those data to our model of the 'minimal essential MHC' of the chicken, and then describe a potential application of mathematical modelling that could have a beneficial impact on such work.

2. A BIRD'S EYE VIEW OF LIFE AND DEATH IN THE FACE OF (SMALL) PATHOGENS

The hypothesis of the 'minimal essential MHC of the chicken' attempts to provide a molecular basis for the striking disease associations of the chicken MHC, in comparison to what is known for well-characterized mammalian models (figure 1). Consider a pathogen that is proteolysed into peptides by the systems of antigen processing, with the peptides bound by MHC molecules and presented to the T lymphocytes of the immune system. Mammals have multigene families of well-expressed MHC molecules, for instance the human class I molecules HLA-A, HLA-B and HLA-C, each of which has a different peptide-binding specificity. Individual humans heterozygous for the MHC have six possibilities for finding an appropriate peptide to bind and present to T cells, in order to make an effective response. In contrast, chickens have single dominantly expressed MHC genes, so a heterozygous individual would have just two chances to find a protective peptide. We believe that this may be the explanation for the strong chicken MHC associations with disease caused by certain small infectious pathogens—those individuals that find a peptide survive, while those that do not die.

As the first step in examining this hypothesis, we have shown that there is a single dominantly expressed class I molecule in common chicken MHC haplotypes, and we have determined the peptide-binding specificities of some of these dominantly expressed class I molecules, the first, to our knowledge, peptide-binding motifs identified in any non-mammalian vertebrate (Kaufman *et al.* 1995).

We then chose to examine a natural disease with a very strong MHC association: progression and regression of RSV-induced tumours. RSV, one of the first retroviruses described, is the classic replication-competent transforming retrovirus with four genes (*gag*, *env*, *pol* and *src*) flanked by long terminal repeats (LTRs). The proteins encoded by the *gag*, *env* and *pol* genes are involved in replication of the virus, whereas the *v-src* gene encodes a tyrosine kinase that appears to have been transduced from the normal chicken gene *c-src*. Infected chickens rapidly develop tumours transformed by the *v-src* gene,

which progress in some individuals and regress in others. In many studies of both inbred lines and chicken populations, the chicken MHC (the B-F/B-L region of the B locus) is the major determinant of this regression and progression, with regression being genetically dominant. More detailed work in certain lines has shown that the regression depends on a functioning immune system, is associated with CD8-bearing cells, and is targeted to the *v-src* gene (Kaufman & Venugopal 1998; Plachy *et al*. 1992). All of these attributes are consistent with the hypothesis that the regression is due to recognition by cytolytic T lymphocytes of v-src peptides bound to class I molecules located on the surface of tumour cells.

We used our peptide-binding motifs to predict the potential binding peptides encoded by the four genes of RSV Prague strain C (the first completely sequenced RSV; Schwartz *et al*. 1983; Takeya & Hanafusa 1983). The number of predicted peptides that fit the motif of the B12 molecule from the resistant CB strain was far greater than the number that fit the motif of the B4 molecule from the susceptible CC strain (Kaufman *et al*. 1995). We then synthesized the predicted peptides for the *v-src* gene and tested their ability to bind the appropriate class I molecules. A number of the peptides predicted to bind the class I molecule from the resistant CB line did in fact bind, while none of the peptides predicted to bind the class I molecule from the susceptible CC line bound significantly. We then used some of the peptides to vaccinate CB chickens against RSV infection, the first application of such peptide vaccination to a non-mammalian vertebrate. We found that one peptide, the peptide with the strongest activity in the binding assay, protected the CB chickens from RSV-induced tumours (A. Hofmann, K. Hala and J. Kaufman, unpublished observation), an observation consistent with our hypothesis.

Most interesting is the position of this protective peptide in the structure of the protein encoded by the *v-src* gene (figure 2). Both c-src and v-src proteins have three domains called src-homology regions (SH) 1, 2 and 3, which are followed by a C-terminal tail with no obvious secondary structure. A major mechanism for regulating the c-src kinase involves a tyrosine residue in the tail, which, when phosphorylated, binds to the SH2 domain, preventing the SH1 domain from acting as a kinase. The sequences of c-src and v-src are nearly identical in the SH1, 2 and 3 domains, but the C-terminal tails are completely different. In particular, the regulatory tyrosine is absent from the tail of v-src, so the viral kinase is not well regulated by the cell (leading to transformation). The peptide, which, upon vaccination, confers protection from tumours, is located in the C-terminal tail. This is consistent with the fact that peptides derived elsewhere in the v-src protein are likely to be the same as the self-peptides from the c-src protein, and so will not be recognized because of T-cell tolerance.

The observation that no peptide predicted to bind the dominantly expressed class I molecule of the susceptible CC (B4) chicken actually did bind, is also consistent with our prediction that the susceptible chickens do not present any protective peptide and therefore do not elicit any cytolytic T cells to regress the tumours. Such unresponsiveness is not restricted to the B4 haplotype, since we predicted that the class I motif for the B15 haplotype fits

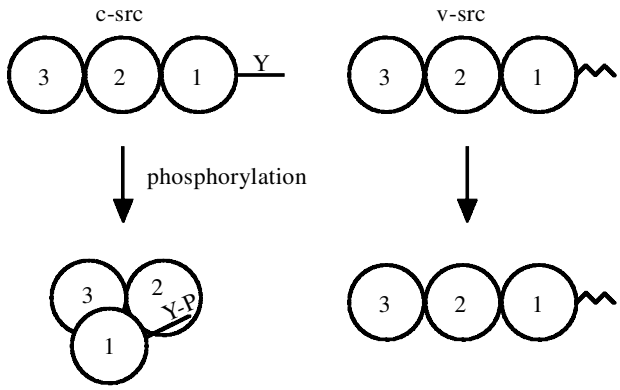

Figure 2. The cellular tyrosine kinase c-src and the viral homologue v-src differ at the C-terminal tail, leading to differences in regulation. The three src-homology domains (SH1, SH2 and SH3) are depicted as circles and the C-terminal tail as a straight line (c-src) or a jagged line (v-src). The C-terminal tail of c-src bears a tyrosine (Y), which, when phosphorylated (Y-P), binds to the SH2 domain inhibiting the kinase activity of the SH1 domain. In contrast, the C-terminal tail of v-src bears no tyrosine and thus is not regulated in this fashion.

few peptides from RSV, and data in the literature show that the B15 haplotype confers susceptibility to a number of strains of RSV (Brown *et al*. 1984; Cutting *et al*. 1981; Kaufman *et al*. 1995).

While there are other experiments to be done, we feel confident that the reason why some chickens die on infection with certain small pathogens is because no effective peptide derived from the pathogen is presented by the class I molecules to T cells. We have evidence to support the same explanation for the response to vaccines that elicit a class I or class II MHC-restricted response. In mammals, such phenomena have been extensively examined as 'immune response (Ir) gene effects', but were only discernible when inbred mouse and hamster strains were immunized with molecules bearing very limited epitopes (for instance, repetitive synthetic peptides) (Kantor *et al*. 1963; McDevitt & Chinitz 1969). In contrast, we find that chicken strains can show striking differences in response to complicated commercial vaccines.

From the point of view of mathematical modelling, chickens represent an opportunity to understand the effects of real pathogens on populations with defined genetics but on a scale almost unthinkable for biomedical model species, both in laboratory experiments and in the field. One interesting challenge would be to examine the impact of the single dominantly expressed class I and class II loci found in chickens on the epidemiology of small infectious pathogens and simple vaccines, using modelling to guide the understanding of the evolutionary dynamics of viruses and their variants in host populations. In this sense, the minimal essential MHC of chickens may be useful as a simple model system for biomedical and evolutionary studies.

3. A LARGE PATHOGEN UNCOVERS A NOVEL MECHANISM OF RESISTANCE?

As described above, small pathogens encode few proteins, so MHC-dependent resistance and susceptibility

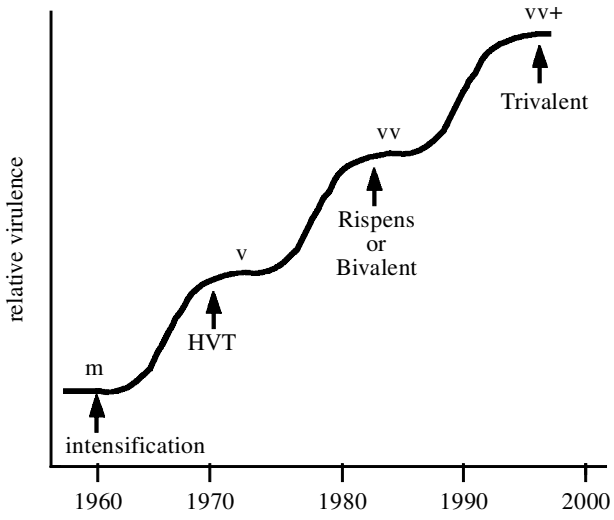

Figure 3. An idealized depiction of the evolution of virulence in MDV field strains over the past 30 years (after Witter 1996). MDV strains have been classified moderate (m), virulent (v), very virulent (vv) and very virulent plus (vv+), based on a variety of criteria. Vaccines used include herpesvirus of turkeys (HVT), SB and HVT (Bivalent), Rispens, and Rispens and Bivalent (also called Trivalent).

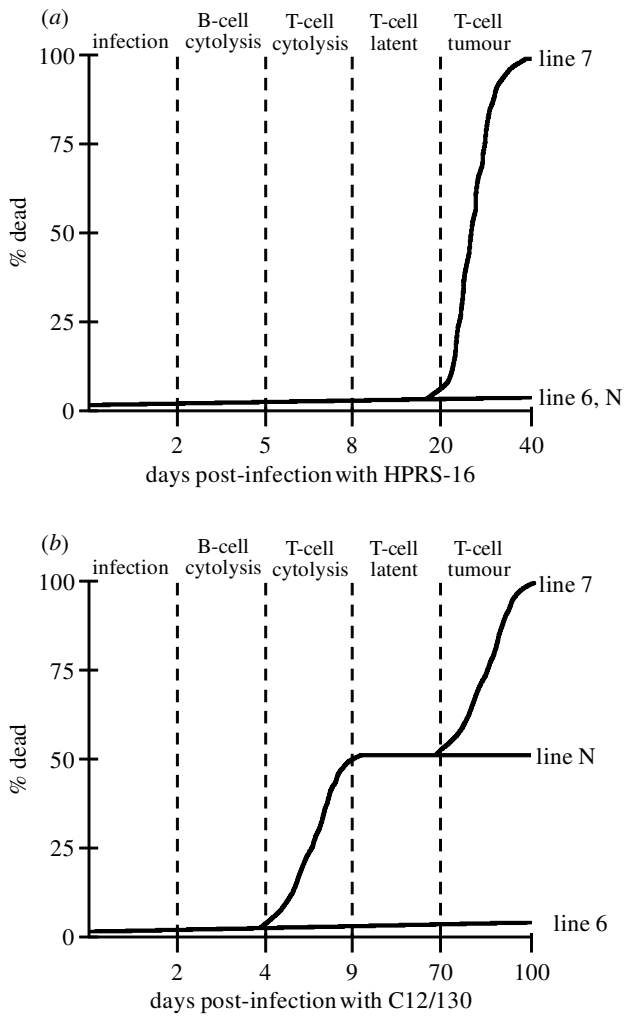

Figure 4. An idealized depiction of mortality after infection with two MDV strains ((*a*) HPRS-16 and (*b*) C12/130) in three experimental chicken lines (lines 6, 7 and N). The differences in mortality at various stages of the disease between strains of MDV are presumably due to differences in pathogen virulence genes, while the differences in mortality between lines of chickens are presumably due to different host resistance genes.

in chickens may depend simply on the peptide-binding specificity of the dominantly expressed class I molecule. In larger pathogens that encode many proteins, appropriate peptides will exist for even the most fastidious class I molecule, making differential resistance based on peptide-binding specificity of a single MHC molecule unlikely. However, the strongest association in any species (to our knowledge) between an MHC and a disease, autoimmune or infectious, is the resistance of the chicken MHC haplotype B21 to tumours caused by classic MDV, a herpesvirus encoding at least 80 proteins (Calnek 1985; Dietert *et al.* 1990; Kaufman & Lamont 1996; Plachy *et al.* 1992; Schat 1987).

As with other herpesviruses, the disease course after classic MDV infection is long and complicated, with an initial cytolytic infection of B cells and later T cells, followed by a latent infection of $CD4^+$ T cells, with lethal T-cell tumours arising thereafter, dependent on many factors including age, sex and genetic background. Under the pressures of intensive husbandry practices and vaccination, the field strains of MDV have changed in various ways, including the tissue location of tumours, the stage of disease at which animals die and the ability to cause disease after vaccination (figures 3 and 4) (Witter 1996).

The chicken MHC is one important resistance locus, but there are others. The only other resistance locus whose genetic location is known has recently been shown to be syntenic with the natural killer complex (NKC), a genetic region in mice and humans that encodes lectin-like NK cell receptors and determines resistance to herpesviruses (Bumstead 1998; Scalzo *et al.* 1995). However, there is no evidence yet for the mechanism of action determined by any of the resistance loci, nor is it clear at which stage of the disease any of the resistance loci act.

An interesting feature of the MHC association is the rank order of resistance of MHC haplotypes, which appears to be roughly the same in many studies over many years, in which different experimental and commercial chicken flocks were infected with different field and laboratory MDV strains. Classically (with background genes segregating freely), B21 haplotypes are the most resistant to MDV, the haplotypes B2, B6 and B14 are moderately resistant, and other haplotypes are much less resistant, with B19 being the most susceptible (Calnek 1985; Plachy *et al.* 1992). This pattern is difficult to reconcile with a simple recognition of peptide(s) by cytolytic T cells, since escape variants of MDV would be expected to have mutated precisely the peptide(s) recognized, so that the rank order of resistance would change. The pattern is also unlikely to be due to resistance unrelated to the immune system (such as sickle cell haemoglobin-mediated resistance to malaria), since the genes that determine such resistance in mammals have few alleles.

In fact, what correlates with the rank order of the MHC-determined resistance and susceptibility to MDV reported in the literature is the relative level of class I molecules found on the surface of cells (Kaufman *et al.* 1995; Kaufman & Salomonsen 1997). In mammals, the level of cell-surface class I expression is remarkably consistent between MHC haplotypes (although it varies considerably between cell types). In chickens, the level of class I molecules expressed on the surface of cells varies depending on the MHC haplotype of the chicken, differing by as much as tenfold on certain cell types. Remarkably, the MDV-resistant B21 haplotype has the fewest class I molecules on the cell surface, the MDV-susceptible B19 haplotype has the most and the others are ranged in between, reflecting their susceptibility to classic MDV. We have found that the difference in cell-surface expression is not due to transcription, translation, association with β_2-microglobulin, acquisition of peptides or stability, but to some aspect of transport to the cell surface. We currently know nothing about the molecular and cellular mechanisms responsible for this disease association in chickens, although we favour the idea that NK cell recognition of the class I cell-surface expression level is an important factor (Kaufman *et al.* 1995, 1999*a*,*b*; Kaufman & Salomonsen 1997).

There is no precedent among mammals for a relationship between cell-surface class I levels and resistance to a disease. Indeed, variation in total class I levels on the cell surface between mammalian MHC haplotypes has not been reported. However, there is strong evidence for differences in cell-surface expression between alleles at particular mammalian class I loci (Hansen *et al.* 2000; Neefjes & Ploegh 1988; Neisig *et al.* 1996). Thus, it may be that the cell-surface class I expression in mammals appears constant because it is an average of many loci, whereas in chickens, the transport of a single dominantly expressed class I molecule to the cell surface determines the cell-surface expression level and so differences are easy to see. If so, it may be that the same disease-resistance mechanism is operative in mammals, just waiting to be discovered. In this case, the minimal essential MHC of chickens may serve as a simple model system for the more complicated systems in humans and other mammals.

From the point of view of mathematical modelling, the interaction of host resistance and pathogen virulence loci during the complicated course of Marek's disease presents an interesting opportunity, given the variation in both host and pathogen. It seems clear that MDV is evolving in response to various control measures (primarily vaccination), with new 'vaccine breakthrough variants' appearing sometime after the introduction of each new vaccine (figure 3). Indeed, the less attenuated vaccines used now can cause some disease in susceptible chickens, so one challenge at present is to develop a 'sustainable' disease control strategy. Also, the polymorphism in the important disease-resistance loci suggests that the chickens have also been evolving in response to MDV. By following the disease after infection of genetically defined lines of chickens with different strains of MDV, these host–pathogen interactions can be examined (figure 4), first at the level of immunopathology, and then at the levels of cells and molecules. Such studies have both academic and economic interest.

4. CHICKENS HAVE A SMALL AND SIMPLE MHC IN COMPARISON TO MAMMALS

As outlined above, chickens appear unable to protect themselves from certain pathogens that would never bother a human, and also appear unable to benefit from vaccines that would be adequate for a human. Our model of the 'minimal essential MHC' proposes that these functional differences are due to molecular differences between the MHC of chickens and typical mammals.

The recently completed sequence (Aguado *et al.* 1999) shows that the human MHC is at least 4 MB in size (and 4 cM by recombinational distance) and contains at least 280 genes, located in three large regions (figure 5). The class II region contains class IIα- and β-chain genes as well as some genes involved in antigen processing for the class I pathway (TAPs, LMPs and tapasin) and a mysterious nuclear kinase (RING3). The class I region contains the classical class I genes for HLA-A, HLA-B and HLA-C molecules, as well as non-classical class I genes and certain other genes. The class II and class I regions flank the class III region, which encodes many different kinds of genes, including the complement components C4, C2 and factor B. There are many pseudogenes, repeats and repetitive elements in all three regions. The two most important points for the discussion that follows are (i) the fact that there are multiple classical class I genes, each gene locus having a large number of common alleles (that is, they are highly polymorphic), and each allele having a different peptide-binding specificity; and (ii) that the genes (TAPs, LMPs and tapasin), the products of which provide the peptides for these class I molecules, are non-polymorphic and are located far away in the class II region.

To lay the foundation for understanding the disease associations of the chicken MHC on a molecular level, we sequenced the B-F/B-L region of the B locus from the CB chicken strain (B12 haplotype) (figure 6). This region has all of the signal attributes of the MHC of well-studied mammals: it contains the classical class I and class II β-chain genes, and determines serological alloantigens, rapid allograft rejection, strong mixed lymphocyte reaction and cellular cooperation in the immune response. There are many interesting differences between the MHCs of typical mammals and the chicken MHC, but as this work was recently published and reviewed in detail (Kaufman *et al.* 1999*a*,*b*; Kaufman 1999), we will simply summarize the four main points. (i) The B-F/B-L region is simple and compact, with only 11 genes identified in the 44 kB of the central region spanning the class II β-chain and class I genes. In particular, we have shown that there are two classical class I genes, but because of differences in the promoters, only one is both transcribed and present as RNA at a high level. Thus, there is effectively a single dominantly expressed class I molecule in many common chicken MHC haplotypes. (ii) Some of the genes present in the MHC of typical mammals are found in the sequenced region (such as class I, class II β-chain, TAP, DM, RING3 and C4 genes) but many are absent (including class II α-chain, LMP, DO, C2/factor B and other class III region genes). (iii) There are genes present in the sequenced region that would not be expected based on the MHC of typical mammals, including B-G genes

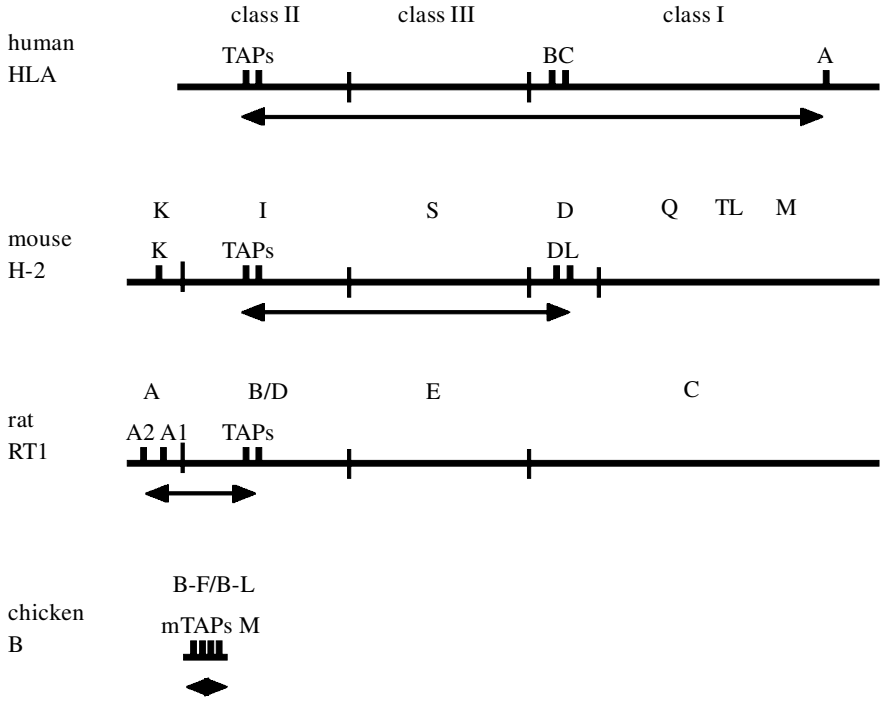

Figure 5. Organization of human, mouse, rat and chicken MHCs, highlighting the locations and relative proximities of TAP and classical class I genes (indicated with double-headed arrow). The HLA complex of humans is divided into three regions, with almost 4 MB (*ca.* 4 cM of recombinational distance) between the TAP genes and the furthest of the classical class I genes (HLA-A). The H-2 complex of mice has an additional region encoding a classical class I gene (the K gene in the K region), but the furthest classical class I gene (D and in some haplotypes L in the D region) is roughly 2 MB away from the TAP genes. The RT1 complex of rats has classical class I genes only in the A region (equivalent to the mouse K region), which is located some 300 kB from the TAP genes. The B locus of the chicken only has classical class I genes in the B-F/B-L region, flanking the TAP genes at a distance of 30 nucleotides.

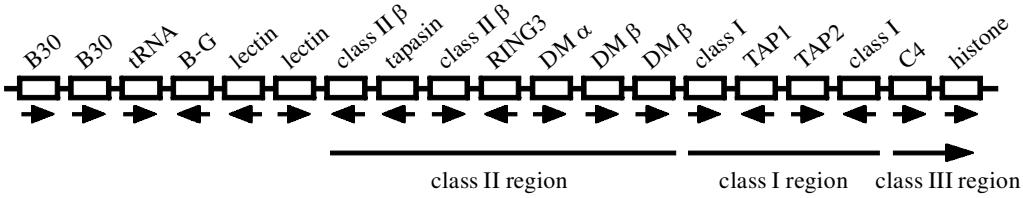

Figure 6. Cartoon of the B-F/B-L region from the B12 haplotype that was sequenced. Open boxes indicate genes; arrows indicate transcriptional orientation. Solid lines underneath indicate the regions equivalent to the class I, class II and class III regions of the mammalian MHC.

and C-type animal lectin genes. (iv) The chicken genes are organized differently from the mammalian MHC, with the TAP genes flanked by class I genes, the tapasin gene flanked by class II β-chain genes, and class I/TAP genes in between class II β-chain and C4 genes. The two most important points for the discussion that follows are (i) that there is a single dominantly expressed classical class I gene, with many alleles, each of which has a unique peptide-binding specificity, and (ii) that the TAP and tapasin genes are polymorphic and located nearby.

The central 44 kB region is very compact, with an average gene size of 1.3 kB, average intron size of 200 nucleotides and intergenic distances (excluding predicted promoters) of as little as 30 nucleotides. Moreover, there are no obvious repetitive elements, pseudogenes or gene fragments identified in the central region. In the absence of recombinational hot spots, such simplicity and compactness would be expected to result in a very low level of recombination. This is precisely the result found experimentally (Skjødt *et al.* 1985): not a single recombinant was found between the genes determining the serologically detected class I (B-F) and class II (B-L) molecules in over 6000 progeny, giving an upper limit of 0.017 cM across the chicken MHC in these experimental crosses compared with 4 cM across the human MHC, a difference of at least 250-fold.

The important implication from this low level of recombination is that the genes of the chicken MHC can evolve together as an allelic group, giving rise to distinct haplotypes that are relatively stable in evolution. In humans and mice, certain combinations of alleles of MHC genes ('haplotypes') are found together, either somewhat more frequently than expected by chance among outbred human populations or fixed by the founder effect in inbred strains of mice, leading to the idea of stable haplotypes selected for differential disease

resistance (Bodmer 1978). However, in reality most mammalian MHCs in real populations are patchworks because of the relatively high level of recombination, and in comparison, the chicken is the realization of the concept of a haplotype.

As mentioned above, we believe that the large, complicated and redundant nature of the typical mammalian MHC means that most haplotypes confer more or less equal protection against most infectious pathogens (at least at the level of peptide presentation), whereas the small and simple nature of the chicken MHC, particularly the dominant expression of a single class I gene, confers striking differences between individuals with different MHC haplotypes in resistance and susceptibility to certain infectious pathogens. The evidence for this differential resistance was discussed in §§ 2 and 3. If these arguments are accepted, then one of the most important questions is why the chicken would evolve an apparently suicidal strategy, in which some MHC haplotypes lead to death simply because the dominantly expressed class I molecule fails to bind a protective peptide derived from an infectious pathogen. The problem is especially perplexing in light of the fact that most chicken MHC haplotypes express more than one classical class I molecule, so that it would not seem to be such a difficult evolutionary step to upregulate expression of the poorly expressed gene, giving the chicken multiple well-expressed class I molecules like typical mammals.

Again the answer, at least at one level, would appear to be rooted in the simple and compact nature of the chicken MHC. As mentioned above, the low rate of recombination in the chicken MHC means that alleles of the MHC genes can coevolve. While such coevolution may apply to all the genes of the chicken MHC, thus far we have produced only the first pieces of evidence in the relationship between the chicken class I and TAP genes, as proposed in several recent reviews. In essence, we believe that the specificity for peptide translocation by the TAP molecules and the specificity for peptide binding by the class I molecules converge in each haplotype, and that this leads to a single dominantly expressed class I molecule (or the equivalent, several molecules all with very similar peptide-binding specificities).

In every chicken MHC haplotype that we have examined (Kaufman *et al.* 1999*a*), we found two classical class I genes that flank the *TAP1* and *TAP2* genes, of which one gene (the 'minor' gene) was transcribed very poorly compared with the other (the dominantly expressed or 'major' gene). Interestingly, there were many more alleles of the major class I gene than the minor gene. The TAP genes are also highly polymorphic, and some of the sequence variation is consistent with differences in the specificity of peptide translocation. In the most obvious example, we found that the *TAP1* in the B4 haplotype has positively charged residues in three positions where negatively charged residues are found in the other haplotypes examined. The peptides eluted from total class I molecules of the B4 haplotype have three negatively charged residues, and the dominantly expressed class I molecule of the B4 haplotype has complementary positively charged residues in the binding site. It seems very likely that the B4 TAP only pumps peptides that have three negatively charged residues into the lumen of the endoplasmic reticulum where they can bind to class I molecules. The sequence of the minor class I molecule of the B4 haplotype is incompatible with binding peptides with three negatively charged residues, so it will not assemble with the pumped peptides and be transported to the surface. Therefore, even if the minor gene was well expressed at the RNA and protein levels, it would not be involved in much antigen presentation. Thus, we believe that the convergence of the specificity for peptide translocation and peptide binding is the reason for the single dominantly expressed class I molecule in chickens.

In contrast to the proposed situation in chickens, the TAPs of well-studied mammals are not highly polymorphic (Momberg *et al.* 1994; Pamer & Cresswell 1998). Indeed, there appears to be only one specificity for translocation in human TAP genes, one for mouse TAP genes and two for rat TAP genes. In rats, the two specificities are due to differences in the *TAP2* gene, which determine the specificity for the amino acid at the C-terminus of the peptide, which is relatively unrestricted for one *TAP2* allele but must be hydrophobic for the other allele. Interestingly, the class I molecules in particular rat haplotypes nearly always have the same specificity for the C-terminal residue as the linked *TAP2* gene. In humans, the TAP specificity appears relatively unrestricted, whereas in mice the TAP specificity is for hydrophobic C-terminal residues.

We believe that these data can be explained by coevolution (Joly *et al.* 1998; Kaufman *et al.* 1999*a*,*b*; Kaufman 1999), in terms of genetic linkage (figure 5). In essence, the less recombination that occurs between two genetic loci, the greater the probability of coevolution of the genes and the greater the specificity of interaction between the products they encode. For humans and mice, class I and TAP genes are located far apart and are frequently separated by recombination, so that the TAPs could only evolve to a 'best average fit' for all class I specificities. In rats, the classical class I and TAP genes are located much nearer and are separated by recombination less frequently, so that the advantageous combinations of alleles stay together often enough to allow some coevolution. This results in two sets of coevolving alleles, each with a particular specificity for the last position of the antigenic peptide. In chickens, the TAPs are flanked by the class I genes with only tens of nucleotides between them, and are virtually never separated by recombination. This result in many sets of coevolving alleles, each one of which affects a number of peptide positions.

Thus, chickens (and perhaps most other non-mammalian vertebrates; Kaufman 1999) may be susceptible to certain pathogens ultimately because of the genetic organization of their MHC, meaning that genome evolution plays a striking role in the life and death of individuals. Of course, it is a mechanism of evolution that variation at the DNA level leads to phenotypic differences that are acted on by natural selection. There are also examples of groups of genes evolving together in so-called 'concerted evolution', although all examples that we have been able to find (some 380 papers from the Medline database dating back to 1980) involve multigene families or repetitive elements. Indeed, 'concerted evolution' was originally defined as 'the tendency of a family of repeated genes to evolve in unison' (Zimmer *et al.* 1980). A fascinating aspect of the potential coevolution of genes within

the MHC is the fact that the genes are not related in sequence or structure. Such coevolution between structurally unrelated genes must have been an important feature of the evolution of many series of proteins involved in a particular function (for instance, synthesis of a molecule by a metabolic pathway). The evolution of such ancient events is very difficult to study, whereas the coevolution of MHC genes may still be happening. From the point of view of molecular modelling, the exploration of the relationships between the alleles of two genes and their recombinational distance, the probability of coevolution and the stringency of interaction will be most interesting.

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