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Abstract

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What chickens might tell us about the MHC class II system

Parker and Kaufman (submitted January 2017)

Declaration of Interest

The authors are not aware of any conflicts of interest.

What chickens might tell us about the MHC class II system

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Abstract

Almost all knowledge about the structure and function of MHC class II molecules outside of mammals comes from work with chickens. Most of the genes implicated in the class II system are present in chickens, so it is likely that the machinery of antigen processing and peptide-loading is similar to mammals. However, there is only one isotype (lineage) of classical class II genes, with one monomorphic DR-like BLA gene and two polymorphic BLB genes, located near one DMA and two DMB genes. The DMB2 and BLB2 genes are widely expressed at high levels, whereas the DMB1 and BLB1 genes are only expressed at highest levels in spleen and intestine, suggesting the possibility of two class II systems in chickens.

Introduction

Outside of mammals (1-4), most of what we know about antigen processing and peptide loading comes from work with chickens (5-7), for which there have been decades of intense research in the service of the global poultry industry. As a result, there is much known about pathogens and disease, disease resistance, vaccine responses, innate and adaptive immunity, immunological assays, genetics and genomics (8). Most striking, in contrast to humans and those placental mammals examined, are the many and often strong genetic associations of the B locus (which contains the major histocompatibility complex, MHC) with resistance and susceptibility to infectious disease, with pathogens ranging from viruses to bacteria and parasites (7,9).

The chicken BF-BL region (which corresponds functionally with the MHC) is much smaller and simpler than the MHC of typical mammals, and is organised differently (10)(Fig. 1). In particular, the chicken class I system has been particularly well-studied, giving clear lessons about the structure, function and evolution of the class I system, and the MHC more generally (5-7). In contrast, much less is known about the chicken class II system, and what it might tell us about evolution. Here, we describe what has been learned from studies of the chicken class I system, review the class II system of mammals and chickens, and then describe some new discoveries in chickens and consider what they might mean.

Co-evolution with polymorphic peptide loading genes leads to a single dominantly-expressed class I gene that determines the immune response

In chickens, the two class I heavy chain genes BF1 and BF2 flank polymorphic TAP1 and TAP2 genes, with the polymorphic tapasin gene nearby (Fig. 1)(10). Recombination across the BF-BL region is rare, not having been observed in thousands of experimental matings although the results of historic recombination and apparent gene conversion events are evident (11-17), suggesting that chicken MHC exists as more-or-less stable haplotypes. This lack of recombination allows co-evolution between the interacting MHC genes, with particular alleles of tapasin, TAP1 and TAP2 working together with one of the class I genes, the BF2 gene (18-20). The BF2 gene is far better expressed at the level of RNA, protein and antigenic peptide, and has more alleles than the BF1 gene, with the latter having mutations and deletions in the promoter and a splicing site, as well as disruptive insertions (16,22-24).

The dominantly-expressed BF2 molecule determines class I-dependent responses in chickens, explaining (at least in part) the strong genetic associations with infectious disease (22,25,26). Unexpectedly, the alleles encoded by this class I locus vary from relatively poorly-expressed molecules that bind a wide variety of peptides (i. e., promiscuous) and therefore confer resistance to many different pathogens, to highly-expressed molecules that bind more narrow variety of peptides (i. e., fastidious) that in general confer susceptibility (19,21,27). A similar hierarchy has been found for human class I alleles, with some fastidious molecules associated with slow progression from HIV infection to AIDS, leading to a new concept of generalists and specialists (27-29).

The presence of only one well-expressed class I gene tightly-linked to polymorphic antigen processing and peptide loading genes is likely the ancestral organisation. At

least some of the salient features of the chicken class I system are found in various vertebrates outside of placental mammals, from marsupials like the American opossum, to frogs like *Xenopus* and bony fish like salmon (30,31). We have proposed that a genomic inversion within the ancestral organisation swung the class III region into the middle of the MHC and the class I gene(s) to the outside, but leaving behind the tapasin, TAP and inducible proteasome components to become part of the class II region (18,31). This separation broke the co-evolutionary relationships, leading to monomorphic antigen processing and peptide loading genes that could work with which ever class I alleles appeared by recombination, thus allowing the appearance of a multigene family of well-expressed class I genes in mammals. This same mechanism of co-evolution between closely-linked genes is postulated to have been crucial for setting up the antigen processing, presentation and recognition pathways in the primordial MHC (18,31).

Many genes expected from the class II system in mammals are found in chickens

In placental mammals, there are typically several distinct isotypes (or lineages) of highly polymorphic class II molecules, each with characteristic α and β chains encoded by A and B genes usually paired in opposite transcriptional orientation. These isotypes are easily recognised throughout mammals; for instance, human DR is like mouse E and human DQ is like mouse A (4). However, there are elaborations on this theme, leading to complications that frustrate broad generalisations. For instance, human DQA and DPA (and mouse Aα) genes are highly polymorphic but DRA (and mouse Eα) are virtually monomorphic (32), most human class II haplotypes have two functional DRB genes that have survived from a larger multigene family (33), humans have DP genes which are missing or pseudogenes in mice and rats (34), mole rats have DP but not DR (34), some ruminants have another isotype DY (35,36), and there are genes not expressed widely, well or at all, such as human DQA2/DQB2 and DPA2/DPB2, and mouse Eβ2 (37-39).

There are also non-classical class II genes, with minimal polymorphism and roles in peptide loading (40). The DM genes (human DMA and DMB, and mouse Ma, Mb1 and Mb2) are distantly-related to classical class II genes, and encode heterodimers acting as class II-specific chaperones and peptide editors. The DO genes (human DOA and DOB, and mouse Oa and Ob) encode heterodimers that are most closely-related to DR molecules, and act as competitive inhibitors of DM action in certain cell types like B cells. Unlike the many non-classical class I genes that vary in their locations in the genome, functional roles and levels of polymorphism (41,42), all class II genes are located in the MHC of typical placental mammals (34).

There are many structurally-unrelated molecules involved in the class II system, some of which are apparently class II-specific and others with much wider roles (1-4). The most specific include the transcription factor called class II transactivator (CIITA) responsible for class II expression in some cells and/or increased expression in others, invariant chain (Ii or CD74) that directs class II molecules to the specialised MHC class II peptide loading compartment (MIIC) as well as preventing peptide-binding until the MIIC is reached, CD4 that is generally expressed in cells that recognise class II molecules to aid T cell receptor binding and signaling, and the CD4 homologue lymphocyte activating gene 3 (LAG-3 or CD223). More general molecules include various cathepsin proteases and legumain (LGMN), as well as γ-interferon-inducible lysosomal thiol reductase

(GILT/IFI30), that together turn antigenic proteins into suitable peptides for loading. Certain cathepsins (like CTSS and CTSL2) and the signal sequence peptidase-like 2a (SSPL2A) are important for trimming and degrading Ii. Key roles are played by factors involved in membrane protein trafficking, and there are likely many others whose roles are only beginning to be understood (1).

In chickens, many of the genes expected from the mammalian class II system are found (Table 1). There are two regions on chromosome 16 with recognisable class II genes (10,43,44). The classical class II genes as well as DM genes are found in the BF-BL region of the B locus (Fig. 1), responsible for rapid allograft rejection, mixed lymphocyte reaction (MLR), T-B cell collaboration and many associations with infectious disease (7,9). In addition, a small number of non-polymorphic and poorly-expressed class II B genes (closely-related to BLB genes and sometimes referred to as YLB genes) are located in the poorly-characterised Rfp-Y region, which is separated from the B locus by a region of recombinogenic repeats (7,44-46).

In chickens, there is only one isotype of highly polymorphic classical class II molecules, encoded by a virtually monomorphic BLA gene located some 5 cM outside of the BF-BL region (47), and two closely-related and highly polymorphic BLB genes that flank the tapasin gene in the BF-BL region (Fig. 1). The BLB2 gene is much better expressed in spleen RNA than the BLB1 gene (48,49). The BLB genes do not closely-resemble any particular mammalian class II isotype, but BLA is clearly a DR homologue, with many residues in the peptide-binding domain identical between mammals and chicken (47). There are also three polymorphic DM genes located in a cluster very near to the BLB genes: DMA, DMB1 and DMB2 (10,50,51). There are no obvious counterparts to DO genes, unless the non-polymorphic YLB molecules play the same role. Other class II-specific genes including CIITA, Ii and CD4 are found in chickens, located in the same genomic regions as (syntenic with) their mammalian counterparts, while the cathepsin, legumain, GILT and SSPL2a genes are scattered throughout the genome (Table 1).

Differential tissue-specific expression rather than dominant expression of chicken class II B genes

There are superficial similarities between the class I and class II systems in chickens. Similar to the arrangement of chicken β2m, BF1 and BF2, the single monomorphic class II A gene (BLA) is located outside of the BF-BL region, there are two closely-linked class II B genes in opposite transcriptional orientation, and one of the class II B genes (BLB2) is far better expressed at the RNA level in spleen than the other (BLB1). Moreover, just like chicken tapasin, there are polymorphic DM genes closely-linked to BLB2 and BLB1, with unique alleles in each MHC haplotype. Thus, a model of co-evolution between polymorphic peptide-loading genes and the gene for a single dominantly-expressed class II molecule might seem obvious.

However, there are also differences between the class I and class II systems in chickens. Unlike BF genes, there are unique alleles of BLB1 and BLB2 in every (non-recombinant) MHC haplotype investigated, leading to similar numbers of alleles (16,49). Moreover, there is little variation in the proximal promoters of BLB1 and BLB2, and the two proximal promoters drive a reporter gene to roughly the same extent (J. Jacob and J. Kaufman, unpublished). Finally, instead of a single polymorphic tapasin molecule to act

as a chaperone and peptide editor, there are potentially two DM molecules, with the α chain from the DMA gene expected to associate with either of two β chains, encoded by DMB1 and DMB2. Unlike tapasin, the sequence diversity between DM alleles is low, and phylogenetic analysis gives no indication that DM alleles group in the same topology as the alleles of the highly-expressed BLB2 gene (50). Initially, these discrepancies left us in the dark about both how and why there might be dominant expression of BLB2.

One ray of light from work to amplify transcripts from cDNA was the discovery that DMB2 is better expressed in spleen and expressed sequence tags (ESTs) compared to DMB1 (50,51). Moreover, inspection of the genomic sequence from the B12 haplotype showed that the promoter of DMB2 had the appropriate elements expected for class II genes, including S/W-X/X2-Y box motifs, whereas the region upstream of DMB1 (in between the DMA and DMB1 genes) was extremely short, with much less obvious promoter motifs (51) [Aimée Parker, PhD thesis, University of Cambridge, 2012]. Finally, mammalian DMB sequences are more similar to chicken DMB2 than chicken DMB1 (50). Taken together, the data and interpretations could support a model in which DMB2 co-evolves with the dominantly-expressed BLB2 but not the minor BLB1, much like the class I system.

From these considerations, it wasn't clear to what extent DMB1 is functional. Therefore, a reverse transcriptase-polymerase chain reaction (RT-PCR) screen of many tissues from many MHC haplotypes was undertaken, which showed that DMB2 is well-expressed compared to DMB1 in a variety of tissues, but that DMB1 is relatively well-expressed in tissues of the intestine [Aimée Parker, PhD thesis, University of Cambridge, 2012]. A preliminary RT-PCR analysis of BLB2 versus BLB1 supports the same story (Fig. 2). These findings led to the possibility that there are two class II systems in chickens: a general BLB2/DMB2 system in many tissues, and a more specialised BLB1/DMB1 system expressed predominantly in the intestine.

How could a specialised class II system work?

The presence of a BLB1/DMB1 system in the intestine might have a developmental basis (tissue- or even cell-specific), or be stimulated by signals in the gut (for instance, from the microbiome). Among the questions to consider are: what might be the function, how might it work mechanistically, and could it be more general than just chickens?

Class II molecules lie at the heart of adaptive immune responses to pathogens, binding peptides that generally arise from intracellular vesicles or the extracellular space, and then stimulating antigen-specific Th1, Th2 and Th17 populations that function both as direct effectors and through helping other cells, primarily by secreting specific cytokines (1-4). A specialised class II molecule conceivably could be involved in the earliest responses in the gut, perhaps being recognised by specialised T cells with αβ or γδ T cell receptors, or even innate immune lymphocytes (52). Class II molecules are also crucial for down-regulating the immune response, primarily through stimulating regulatory T (Treg) cells (53). Given that the gut responds to pathogenic organisms among many commensal organisms in the microbiome, a specialised class II molecule could be involved in tolerance to those organisms that are not dangerous. Finally, class II genes play a central role in autoimmunity, with most autoimmune diseases having strong associations with particular alleles of class II genes (4). A specialised class II molecule

might explain why it has been difficult to determine the peptides responsible for initiation of autoimmunity, before epitope spreading to conventional class II molecules.

There are also several possibilities for the interaction of DM and class II molecules in chickens (Fig. 3). The simplest model would be that DMB2 co-evolves with BLB2, while DMB1 co-evolves with BLB1, resulting in different repertoires of peptides presented by the class II molecules containing BLB1 and BLB2 (which we could call BL1 and BL2). However, another possibility is that the class II molecules containing DMB1 and DMB2 (DM1 and DM2) are both capable of interacting appropriately with BL1 and BL2. If so, they might act similarly, in which case the repertoires of BL1 and BL2 would be the same no matter which DMB was present. Alternatively, each DMB might result in a characteristic repertoire, in which case if they were all present at reasonable levels in the same cell, there would be different repertoires of BL1 after interaction with DM1 versus DM2, and the same with BL2. Even if this were the case, there might be only DMB1/BLB1 and DMB2/BLB2 repertoires if DMB2 and BLB2 are expressed in one set of cells (for instance, haemopoietic cells), while DMB1 and BLB1 are expressed in another set of cells (for instance, epithelial cells). Finally, perhaps DM1 doesn't interact at all with either BL1 or BL2, but has some entirely different function.

Are such specialised class II molecules a feature only in chickens, or could they be in mammals as well? In fact, there are some orphan class II genes in humans and mice. The human gene pairs DQA2/DQB2 and DPA2/DPB2 were long considered to be pseudogenes based on lack of expression and polymorphism, but DQA2/DQB2 is expressed in Langerhans cells (37). The mouse Eb2 gene is expressed at low levels in cell lines (38,39). There are also two DMB genes in mouse, which are reported to have different tissue distributions (54,55). So, like the presence of fastidious and promiscuous class I molecules discovered in chickens and then found in humans, it is possible that the class II molecules of chickens may have something to teach us about the class II molecules of mammals.

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Figure Legends.

Fig. 1. The chicken MHC (BF-BL region of the B locus) contains classical class I and class II B genes, along with genes involved in peptide-loading, among other genes. The polymorphic TAP1, TAP2 and tapasin genes co-evolve only with the polymorphic class I locus called BF2, leading to high expression of a single class I gene. All the DM and class II B genes are polymorphic, but are expressed at different levels depending on the tissue. DMB2 and BLB2 are well-expressed in a wide variety of tissues and cell lines, whereas DMB1 and BLB1 are poorly expressed except in the intestine. Potential co-evolution of these genes is as yet unknown. Boxes indicate genes, with names above; thickness of arrows pointing up indicate level of expression in blood and spleen, (potential) co-evolution indicated by arrows underneath the genes. Red and pink are genes from the class I system; dark blue, light blue and black are genes from the class II system; solid colours indicate classical genes while striped colours indicate genes involved in peptide loading.

Fig. 2. BLB2 is well-expressed in a variety of tissues, whereas BLB1 is expressed most strongly in some intestinal tissues (ileum, duodenum, caecal tonsil) and spleen, as assessed by end-point reverse transcriptase-polymerase chain reaction (RT-PCR) from RNA. Tissues are from a P2a chicken (B19 MHC haplotype), and the B cell line (IS19, derived from P2a spleen cells by transformation with reticuloendotheliosis virus, REV) is a positive control, used to normalize signal in the two RT-PCR reactions. Data from [Aimée Parker, PhD thesis, University of Cambridge, 2012].

Fig. 3. Cartoons illustrate that the two DM molecules could interact with the two BL molecules and edit peptides in a variety of different ways. A. DM2 (the DM molecule with DMA and DMB2) interacts only with BL2 (the BL molecule with BLA and BLB2), while DM1 interacts only with BL1, leading to different peptide repertoires due to the BL peptide-binding specificity (but perhaps also to the particular DM molecule). B. Both DM molecules interact with both BL molecules, but have the same effects, so that the peptide repertoires depend only on the BL peptide-binding specificity. C. Both DM molecules interact with both BL molecules, but have differing effects on the peptides loaded, so that the peptide repertoire for any given BL molecule depends on both the BL peptide-binding specificity and the particular DM molecule that interacted with the BL molecule. D. Both DM molecules could interact with both BL molecules and with differing effects on the peptides loaded, but DM1 and BL1 are expressed in one cell type and DM2 and BL2 are expressed in a different cell type. E. Both BL molecules interact with DM2, and DM1 has function that is not peptide editing. BL1, solid light blue; BL2 solid dark blue; DM1, light blue falling stripes; DM2, dark blue rising stripes; peptides determined by DM1, light blue; peptides determined by DM2, dark blue; peptides same whether DM1 or DM2, pink or red.

Table 1. Components of the chicken classical class II system.

Component	ensemble identifier	location (chromosome, nucleotides)
Class II: BLB1	ENSGALG00000000141	Chromosome 16: 116,646-118,450
Class II: BLB2	ENSGALG00000030940	Chromosome 16: 109,677-111,212
Class II: YLB	ENSGALG00000034319	Chromosome 16: 584,101-584,786
Class II: YLB	ENSGALG00000044502	Chromosome 16: 515,889-516,618
Class II: YLB (next to YF)	ENSGALG00000030368	Scaffold AADN04000838.1: 9,984-13,692
DMA	ENSGALG00000000158	Chromosome 16: 96,322-99,142
DMB1	ENSGALG00000000162	Chromosome 16: 93,895-96,168
DMB2	ENSGALG00000038393	Chromosome 16: 90,602-93,633
Ii (CD74)	ENSGALG00000004594	Chromosome 13: 13,146,841-13,150,694
CIITA	ENSGALG00000007171	Chromosome 14: 9,120,605-9,138,407
CD4	ENSGALG00000014477	Chromosome 1: 77,208,503-77,219,970
*LAG3 (CD223)	ENSGALG00000044449	Chromosome 1: 77,197,103-77,198,831
Cathepsin A (CTSA)	ENSGALG00000006876	Chromosome 20: 10,729,767-10,733,859
Cathepsin B (CTSB)	ENSGALG00000030016	Chromosome 3: 107,829,340-107,838,487
Cathepsin C (CTSC)	ENSGALG00000017239	Chromosome 1: 188,325,996-188,327,046
Cathepsin D (CTSD)	ENSGALG00000006613	Chromosome 5: 14,123,783-14,134,951
Cathepsin E (CTSE)	ENSGALG00000010018	Chromosome 1: 35,522,127-35,529,831
Cathepsin G	ENSGALG00000044661	Chromosome 28: 1,271,207-1,272,845
Cathepsin H (CTSH)	ENSGALG00000033557	Chromosome 10: 19,950,725-19,956,592
Cathepsin K (CTSK)	ENSGALG00000028147	Chromosome 25: 2,586,099-2,589,028
Cathepsin L1	ENSGALG00000004252	Chromosome 28: 4,944,768-4,948,239
Cathepsin L2	ENSGALG00000012610	Chromosome Z: 41,408,607-41,414,029
Cathepsin O (CTSO)	ENSGALG00000009373	Chromosome 4: 21,053,776-21,061,372
Cathepsin S (CTSS)	ENSGALG00000000775	Chromosome 25: 2,580,940-2,585,553

Cathepsin Z (CTSZ)	ENSGALG00000007462	Chromosome 20: 11,097,473-11,102,231
legumain (LGMN)	ENSGALG00000010811	Chromosome 5: 44,854,617-44,879,390
SPPL2A	ENSGALG00000031348	Chromosome 10: 10,733,680-10,761,449
GILT (IFI30)	ENSGALG00000033694	Chromosome 28: 4,012,345-4,014,330

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*The LAG3 gene may be wrongly identified, as further search identifies the gene as an interleukin 1 receptor precursor. The GenBank entry XM416510 appears to be correct.

References (* and ** indicate especially interesting reviews and papers in the last two years)

1. Neefjes J, Jongsma ML, Paul P, Bakke O: Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol 2011, 11: 823-836.

2. Blum JS, Wearsch PA, Cresswell P: Pathways of antigen processing. Annu Rev Immunol 2013, 31: 443-473.

3.** Rock KL, Reits E, Neefjes J: Present Yourself! By MHC Class I and MHC Class II Molecules. Trends Immunol 2016, 37: 724-737.

4.** Unanue ER, Turk V, Neefjes J: Variations in MHC Class II Antigen Processing and Presentation in Health and Disease. Annu Rev Immunol 2016, 34: 265-297.

**Clear views of current thoughts about antigen processing as discovered in humans and mice

5. Kaufman J: Antigen processing and presentation: evolution from a bird's eye view. Molec Immunol 2013, 55:159-

6.** Kaufman J. What chickens would tell you about the evolution of antigen processing and presentation. Curr Opin Immunol 2015, 34: 35-42.

**Quick summary of differences between the birds and mammals in terms of structure, function and evolution of the MHC class I system

7.** Kaufman J: The Avian MHC. In Avian Immunology, second edition. Edited by Schat KA, Kaiser P, Kaspers B. Elsevier, Ltd; 2014: 149-167.

**Comprehensive description of the chicken MHC and models arising for evolution of the adaptive immune system

8. Schat AK, Kaspers B, Kaiser P (eds): Avian Immunology second edition. Academic Press; 2014.

9.** Miller MM, Taylor RL Jr: Brief review of the chicken Major Histocompatibility Complex: the genes, their distribution on chromosome 16, and their contributions to disease resistance. Poult Sci 2016, 95: 375-392.

**Up-to-date description of the genes on the chicken MHC chromosome and the known associations with disease

10. Kaufman J, Milne S, Göbel TW, Walker BA, Jacob JP, Auffray C, Zoorob R, Beck S: The chicken B locus is a minimal essential major histocompatibility complex. Nature 1999, 401: 923-925.

11. Hala K, Vilhelmova M, Schulmannova J, Plachy J: A new recombinant allele in the B complex of the chicken. Folia Biol (Praha) 1979, 25: 323-324.

12. Koch C, Skjødt K, Toivanen A, Toivanen P: New recombinants within the MHC (B-complex) of the chicken. Tissue Antigens 1983, 21: 129-137.

13. Skjødt K, Koch C, Crone M, Simonsen M: Analysis of chickens for recombination within the MHC (B-complex). Tissue Antigens, 1985, 25: 278-282.

14. Hala K, Chausse AM, Bourlet Y, Lassila O, Hasler V, Auffray C: Attempt to detect recombination between B-F and B-L genes within the chicken B complex by serological typing, in vitro MLR, and RFLP analyses. Immunogenetics 1988, 28: 433-438.

15. Simonsen M, Crone M, Koch C, Hala K: The MHC haplotypes of the chicken. Immunogenetics 1982, 16: 513-532.

16. Hosomichi K, Miller MM, Goto RM, Wang Y, Suzuki S, Kulski JK, Nishibori M, Inoko H, Hanzawa K, Shiina T: Contribution of mutation, recombination, and gene conversion to chicken MHC-B haplotype diversity. J Immunol 2008, 181: 3393-3399.

17.* Fulton JE, McCarron AM, Lund AR, Pinegar KN, Wolc A, Chazara O, Bed'Hom B, Berres M, Miller MM: A high-density SNP panel reveals extensive diversity, frequent recombination and multiple recombination hotspots within the chicken major histocompatibility complex B region between BG2 and CD1A1. Genet Sel Evol 2016, 48:1.

*Up-to-date analysis showing the low levels of recombination within the chicken MHC (BF/BL region) compared to a neighbouring (TRIM) region

18. Walker BA, Hunt LG, Sowa AK, Skjødt K, Göbel TW, Lehner PJ, Kaufman J: The dominantly expressed class I molecule of the chicken MHC is explained by coevolution with the polymorphic peptide transporter (TAP) genes. Proc Natl Acad Sci U S A 2011, 108: 8396-8401.

19.* Tregaskes CA, Harrison M, Sowa AK, van Hateren A, Hunt LG, Vainio O, Kaufman J: Surface expression, peptide repertoire, and thermostability of chicken class I molecules correlate with peptide transporter specificity. Proc Natl Acad Sci U S A 2016, 113 :692-697.

*Data comparing the properties of alleles of the dominantly-expressed class I molecule BF2

20. van Hateren A, Carter R, Bailey A, Kontouli N, Williams AP, Kaufman J, Elliott T: A mechanistic basis for the co-evolution of chicken tapasin and major histocompatibility complex class I (MHC I) proteins. J Biol Chem 2013, 288: 32797-32808.

21.* Kaufman J. Co-evolution with chicken class I genes. Immunol Rev 2015, 267: 56-71.

*A clear description of co-evolution within and outside of the chicken MHC

22. Wallny HJ, Avila D, Hunt LG, Powell TJ, Riegert P, Salomonsen J, Skjødt K, Vainio O, Vilbois F, Wiles MV, Kaufman J: Peptide motifs of the single dominantly expressed class I molecule explain the striking MHC-determined response to Rous sarcoma virus in chickens. Proc Natl Acad Sci U S A 2006, 103: 1434-1439.

23. Shaw I, Powell TJ, Marston DA, Baker K, van Hateren A, Riegert P, Wiles MV, Milne S, Beck S, Kaufman J: Different evolutionary histories of the two classical class I genes BF1 and BF2 illustrate drift and selection within the stable MHC haplotypes of chickens. J Immunol 2007, 178: 5744-5752.

24. O'Neill AM, Livant EJ, Ewald SJ: The chicken BF1 (classical MHC class I) gene shows evidence of selection for diversity in expression and in promoter and signal peptide regions. Immunogenetics 2009, 61: 289-302.

25. Butter C, Staines K, van Hateren A, Davison TF, Kaufman J: The peptide motif of the single dominantly expressed class I molecule of the chicken MHC can explain the response to a

molecular defined vaccine of infectious bursal disease virus (IBDV). Immunogenetics 2013, 65: 609-618.

26. Koch M, Camp S, Collen T, Avila D, Salomonsen J, Wallny HJ, van Hateren A, Hunt L, Jacob JP, Johnston F, Marston DA, et al: Structures of an MHC class I molecule from B21 chickens illustrate promiscuous peptide binding. Immunity 2007, 27: 885-899.

27.** Chappell P, Meziane el K, Harrison M, Magiera Ł, Hermann C, Mears L, Wrobel AG, Durant C, Nielsen LL, Buus S, Ternette N, Mwangi W, Butter C, Nair V, Ahyee T, Duggleby R, Madrigal A, Roversi P, Lea SM, Kaufman J: Expression levels of MHC class I molecules are inversely correlated with promiscuity of peptide binding. eLIFE 2015, 4: 05345.

**Introduction of the model for promiscuous generalist and fastidious specialist class I molecules found in chickens and humans

28. Paul S, Weiskopf D, Angelo MA, Sidney J, Peters B, Sette A: HLA class I alleles are associated with peptide-binding repertoires of different size, affinity, and immunogenicity. J Immunol 2013, 191: 5831-5839.

29. Kosmrlj A, Read EL, Qi Y, Allen TM, Altfeld M, Deeks SG, Pereyra F, Carrington M, Walker BD, Chakraborty AK: Effects of thymic selection of the T-cell repertoire on HLA class I-associated control of HIV infection. Nature 2010, 465: 350-354.

30. Kaufman J: Co-evolving genes in MHC haplotypes: the "rule" for nonmammalian vertebrates? Immunogenetics 1999, 50: 228-236.

31. Kaufman J: The evolutionary origins of the adaptive immune system of jawed vertebrates (Chapter 3). In The Immune Response to Infection. Edited by Kaufmann SHE, Rouse BT, Sachs DL. American Society of Microbiology Press, 2011: 41-54.

32. Germain RN, Bentley DM, Quill H: Influence of allelic polymorphism on the assembly and surface expression of class II MHC (Ia) molecules. Cell 1985, 43: 233-242.

33. Doxiadis GG, Hoof I, de Groot N, Bontrop RE: Evolution of HLA-DRB genes. Mol Biol Evol 2012, 29: 3843-3853.

34. Kumánovics A, Takada T, Lindahl KF: Genomic organization of the mammalian MHC. Annu Rev Immunol 2003, 21: 629-657.

35. Nizetić D, Figueroa F, Dembić Z, Nevo E, Klein J: Major histocompatibility complex gene organization in the mole rat Spalax ehrenbergi: evidence for transfer of function between class II genes. Proc Natl Acad Sci U S A. 1987, 84: 5828-5832.

35. Andersson L, Lundén A, Sigurdardottir S, Davies CJ, Rask L: Linkage relationships in the bovine MHC region. High recombination frequency between class II subregions. Immunogenetics 1988, 27: 273-280.

36. Ballingall KT, Ellis SA, MacHugh ND, Archibald SD, McKeever DJ: The DY genes of the cattle MHC: expression and comparative analysis of an unusual class II MHC gene pair. Immunogenetics 2004, 55: 748-755.

37. Lenormand C, Bausinger H, Gross F, Signorino-Gelo F, Koch S, Peressin M, Fricker D, Cazenave JP, Bieber T, Hanau D, de la Salle H, Tourne S: HLA-DQA2 and HLA-DQB2 genes are specifically expressed in human Langerhans cells and encode a new HLA class II molecule. J Immunol 2012, 188: 3903-3911.

38. Braunstein NS, Germain RN: The mouse E beta 2 gene: a class II MHC beta gene with limited intraspecies polymorphism and an unusual pattern of transcription. EMBO J. 1986, 5: 2469-2476.

39.* Monzón-Casanova E, Rudolf R, Starick L, Müller I, Söllner C, Müller N, Westphal N, Miyoshi-Akiyama T, Uchiyama T, Berberich I, Walter L, Herrmann T: The Forgotten: Identification and Functional Characterization of MHC Class II Molecules H2-Eb2 and RT1-Db2. J Immunol 2016, 196: 988-999.

*The latest information on the orphan mouse class II B gene Eb

40.** Mellins ED, Stern LJ: HLA-DM and HLA-DO, key regulators of MHC-II processing and presentation. Curr Opin Immunol 2014, 26: 115-122.

**A clear description of the molecular basis for DM and DO action

41. Rodgers JR, Cook RG: MHC class Ib molecules bridge innate and acquired immunity. Nat Rev Immunol 2005, 5: 459-471.

42. Adams EJ, Luoma AM: The adaptable major histocompatibility complex (MHC) fold: structure and function of nonclassical and MHC class I-like molecules. Annu Rev Immunol 2013, 31: 529-561.

43. Guillemot F, Billault A, Pourquié O, Béhar G, Chaussé AM, Zoorob R, Kreibich G, Auffray C: A molecular map of the chicken major histocompatibility complex: the class II beta genes are closely linked to the class I genes and the nucleolar organizer. EMBO J 1988, 7: 2775-2785.

44. Miller MM, Goto RM, Taylor RL Jr, Zoorob R, Auffray C, Briles RW, Briles WE, Bloom SE: Assignment of Rfp-Y to the chicken major histocompatibility complex/NOR microchromosome and evidence for high-frequency recombination associated with the nucleolar organizer region. Proc Natl Acad Sci U S A 1996, 93: 3958-3962.

45. Zoorob R, Béhar G, Kroemer G, Auffray C: Organization of a functional chicken class II B gene. Immunogenetics 1990, 31: 179-187.

46. Zoorob R, Bernot A, Renoir D, Choukri F, Auffray C: Chicken major histocompatibility complex class II B genes: analysis of interallelic and interlocus sequence variance. Eur J Immunol 1993, 23: 1139-1145.

47. Salomonsen J, Marston D, Avila D, Bumstead N, Johansson B, Juul-Madsen H, Olesen GD, Riegert P, Skjødt K, Vainio O, Wiles MV, Kaufman J: The properties of the single chicken MHC classical class II alpha chain (B-LA) gene indicate an ancient origin for the DR/E-like isotype of class II molecules. Immunogenetics 2003, 55: 605-614.

48. Pharr GT, Dodgson JB, Hunt HD, Bacon LD: Class II MHC cDNAs in 15I5 B-congenic chickens. Immunogenetics 1998, 47: 350-354.

49. Jacob JP, Milne S, Beck S, Kaufman J: The major and a minor class II beta-chain (B-LB) gene flank the Tapasin gene in the B-F /B-L region of the chicken major histocompatibility complex. Immunogenetics 2000, 51: 138-147.

50. Atkinson D, Shaw I, Jacob J, Kaufman J: DM gene polymorphisms: co-evolution or coincidence? In Proceedings of the Avian Immunology Research Group, 7-10 October 2000, Ithaca NY. Edited by KA Schat; 2001, 163-165.

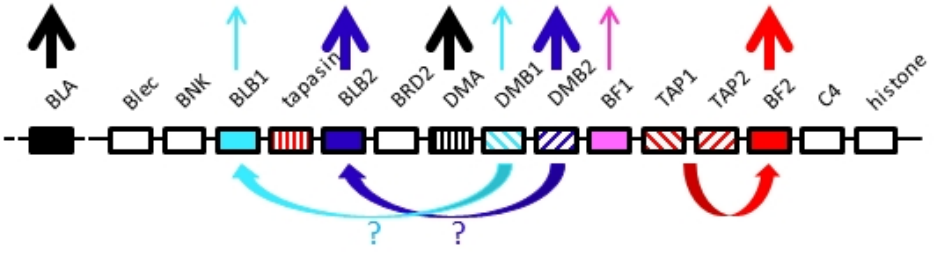
51. Chazara O, Tixier-Boichard M, Morin V, Zoorob R, Bed'hom B: Organisation and diversity of the class II DM region of the chicken MHC. Mol Immunol 2011, 48: 1263-1271.

52. Robinette ML, Colonna M: Innate lymphoid cells and the MHC. HLA 2016, 87: 5-11.

53. Weissler KA, Caton AJ: The role of T-cell receptor recognition of peptide:MHC complexes in the formation and activity of Foxp3^{+} regulatory T cells. Immunol Rev 2014, 259: 11-22.

54. Walter W, Loos M, Maeurer MJ: Differential expression of alternative H2-M isoforms in B cells, dendritic cells and macrophages by proinflammatory cytokines. Mol Immunol 1999, 36: 733-43.

55. Walter W, Scheuer C, Loos M, Reichert TE, Maeurer MJ: H2-Mbeta 1 and H2-Mbeta 2 heterodimers equally promote clip removal in I-A(q) molecules from autoimmune-prone DBA/1 mice. J Biol Chem 2001, 276: 11086-11091.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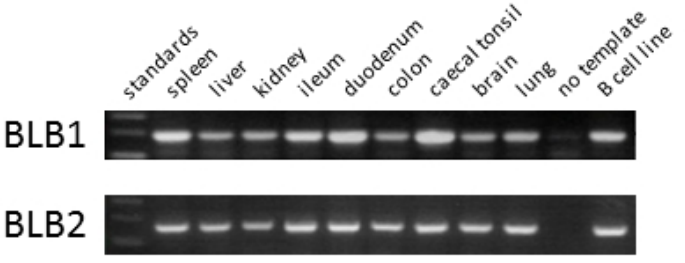
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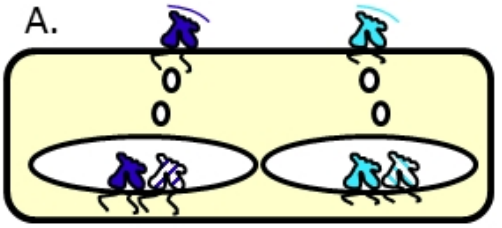

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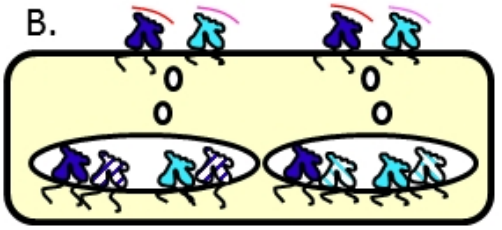

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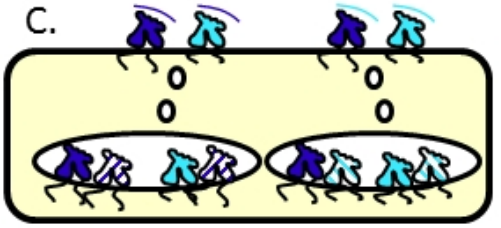

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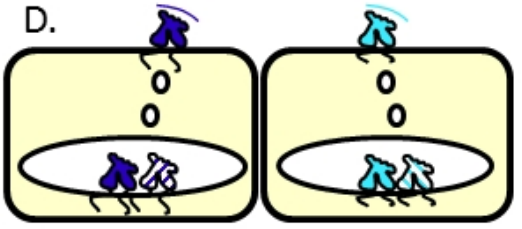

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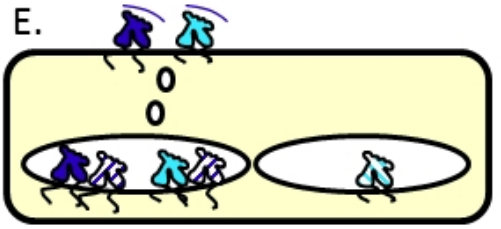

What chickens might tell us about the MHC class II system

Parker and Kaufman (submitted January 2017)

Highlights

- Chickens provide most knowledge of antigen presentation outside of mammals
- Chickens have most of the genes expected for the class II system from mammals
- Chickens have two class II B genes and two DMB genes
- One class II B/DMB gene pair is widely expressed
- A second class II B/DMB gene pair is expressed most strongly in spleen and intestine