Long title: Generalists and specialists: a new view of how MHC class I molecules fight infectious pathogens

Jim Kaufman

University of Cambridge
Department of Pathology, Tennis Court Road, Cambridge, CB2 1QP, UK
Department of Veterinary Medicine, Madingley Road, Cambridge, CB2 0ES, UK

Correspondence: jfk31@cam.ac.uk (J. Kaufman)

Summary:

In comparison to the MHC of typical mammals, the chicken MHC is simple and compact, with a single dominantly-expressed class I molecule that can determine the immune response. In addition to providing useful information for the poultry industry and allowing insights into the evolution of the adaptive immune system, the simplicity of the chicken MHC has allowed discovery of phenomena that are more difficult to discern in the more complicated mammalian systems. This review discusses the new concept that poorly-expressed promiscuous class I alleles act as generalists to protect from a wide variety of infectious pathogens, while highly-expressed fastidious class I alleles can act as specialists to protect against new and dangerous pathogens.

Insights from studying a simpler system

An enormous body of knowledge about the major histocompatibility complex (MHC) and MHC molecules has been amassed over the last 50 years, mostly due to work on humans and important biomedical model species like mice [1]. This information is extremely detailed, complex but well-integrated, and crucially important, both for basic scientific understanding of immune and autoimmune responses, and for practical medical applications, including transplantation [2,3]. What is the point of trying to understand the MHC in non-mammalian vertebrates, when there is such rich and relevant knowledge for placental mammals?

Besides the obvious importance to disease resistance and vaccination in poultry [4,5], research into the chicken MHC has led to novel insights about the evolution of the adaptive immune system [6-9]. This short review highlights a third advantage: how the simplicity (at least in some senses) of the chicken MHC has permitted discovery and/or study of phenomena that have been more difficult to discern in complex MHC biology in humans and other typical mammals.

Resistance to infectious disease

It is generally accepted that the high level of allelic polymorphism of MHC class I and class II genes is driven by a molecular arms race with pathogens [10,11]. An expectation from this relationship is that particular MHC alleles would confer resistance or susceptibility to particular infectious pathogens. The human MHC

49 does have many strong genetic associations with autoimmune disease, but the
50 reported associations with infectious disease are much weaker [2,12]. In
51 essence, it has taken the best immunologists, epidemiologists and geneticists
52 decades to provide convincing evidence for such genetic associations. The best-
53 studied example is the slow progression of human immunodeficiency virus (HIV)
54 infection to acquired immunodeficiency syndrome (AIDS) conferred by the
55 presence of certain HLA-B alleles as well as cell surface expression levels of HLA-
56 C alleles [13,14].
57
58 In contrast, already decades ago the poultry immunologists were stumbling over
59 extremely strong associations between the B blood group and resistance to a
60 variety of economically-important infectious diseases [15]. The MHC encoding
61 classical class I and class II molecules is one region (the so-called BF-BL region)
62 within this B locus [16]; nearby are regions with CD1 genes, TRIM genes and the
63 mysterious BG genes that have some similarities to butyrophilins [4,5]. Initially,
64 these associations were with responses to oncogenic viral diseases such as
65 Marek's disease and Rous sarcoma, with the B locus determining life or death of
66 the individual chickens. Now there is a long list of viruses, bacteria and even
67 parasites that have significant associations with the BF-BL region [4,5,17,18].
68
69 A minimal essential MHC with a single dominantly-expressed class I molecule
70
71 Compared to the MHC of typical mammals, the BF-BL region of chickens (also
72 sometimes called the "classical MHC" or the "core MHC") is compact, simple and
73 arranged differently (Fig. 1), with two class II B (so-called BLB) genes flanking

74 the tapasin gene located near to the DM genes, followed by a pair of class I (so-
75 called BF) genes that flank the TAP genes, and finally the class III region genes
76 [16]. Moreover, there has been no recombination within the BF-BL region
77 observed in experiments [19-22], although comparison of haplotypes shows that
78 there has been some recombination over unknown spans of time [23-25]. Also,
79 the genes involved in peptide loading (tapasin, TAP and DM) are all highly
80 polymorphic with each BF-BL haplotype generally having a unique set of alleles
81 [24,26-28]. The monomorphic DR-like class II A gene (BLA) is located some 5 cM
82 away [29], the monomorphic β_2-microglobulin (β_2m) gene is on a different
83 chromosome [30,31], and inducible proteasome (LMP/PSMB) genes have not
84 been found in the genome [32]. Thus, the polymorphic classical class I and class
85 II B genes are in strong linkage disequilibrium with polymorphic peptide-loading
86 genes, leading to relatively stable MHC haplotypes of polymorphic co-evolving
87 genes [33,34].
88
89 This co-evolution is clearly seen in the chicken class I system, in which the
90 specificity of peptide translocation by the TAP alleles correlates with the peptide
91 motif of the class I molecule encoded by the BF2 (but not the BF1) gene
92 [27,34,35]. Thus, the BF2 class I molecule receives lots of peptides whereas the
93 BF1 molecule gets far fewer peptides and might be expected to have become
94 much less important for antigen presentation. In fact, the BF1 gene has suffered
95 deletions and insertions leading to far less expression at the RNA and protein
96 levels than the BF2 gene [36]. Most importantly, the peptides presented by the
97 dominantly-expressed BF2 molecule can explain the immune response to certain
98 economically-important viruses and vaccines [37-39].

Such a system of co-evolving alleles is not found in most placental mammals. In humans and other typical mammals, the antigen processing and peptide-loading genes are located in the class II and extended class II regions, far away from the class I genes that they serve [40]. Thus, alleles of antigen processing and peptide-loading genes that were advantageous for any particular class I allele would relatively rapidly be switched by recombination [41], and any advantage lost. In fact, there are few sequence alleles of TAP, tapasin and inducible proteasome components, and these appear to be functionally monomorphic [42-44], working as average best fits to provide peptides for all class I molecules, regardless of locus or allele. This situation allows for a multigene family of class I genes, all of which are (or can be, for HLA-C) relatively well-expressed. Just to be clear, there are mammals (like rats) for which the classical class I genes have moved close to the antigen processing and peptide-loading genes, with the result that one of the TAP genes is oligomorphic and co-evolves with the class I molecule(s) [45].

The difference in the number of class I loci that encode well-expressed class I molecules provides at least part of the explanation for the difference between human and chicken MHC in genetic association with infectious disease [4,33]. In humans, if one class I molecule doesn't bind a protective peptide, it is likely that another one will, so that overall, most MHC haplotypes confer more-or-less resistance to most pathogens, which reads out as low genetic associations. In chickens, the single dominantly-expressed class I molecule either finds a protective peptide from a particular pathogen or it doesn't, and this life-and-death difference between haplotypes reads out as strong genetic associations

(Fig. 2). Thus, the simplicity of the chicken MHC has allowed greater appreciation of this phenomenon of resistance to infectious disease.

Evolution of the MHC

Why do chickens and typical mammals differ in the genomic organization of the MHC, if the end result can be so dire for an individual chicken? The salient features of the chicken MHC class I system can be found in many if not most non-mammalian vertebrates [6,10,46]. For example, ducks have polymorphic TAP genes next to five class I genes, only one of which is expressed at a high level [47,48]. *Xenopus* frogs have a single classical class I gene along with the TAP (apparently at least oligomorphic) and tapasin genes located together, with this class I region in between the class II region and the class III region [49]. Atlantic salmon have a single classical class I gene close to the TAP2 gene, with this region having a strong genetic association with resistance to at least one economically-important virus [50,51].

The organization originally discovered for chickens is likely to be the ancestral one. The genes for antigen processing, peptide loading and antigen presentation are not closely-related, so they did not evolve by gene duplication and acquisition of new functions. Instead, unrelated genes co-evolved to work together as a pathway, and such co-evolution is favored by close linkage. In other words, the genes of the class I system and by extension the class II system, T cell receptors and natural killer (NK) cell receptors are likely to have emerged in one region, a primordial MHC, which has been falling apart ever since [6,9]. In

149 support of this notion, genes found in various locations around the genome of
150 mammals are found in or near the MHC of non-mammalian vertebrates. For
151 example, the genes for an NK cell receptor and putative ligand (BNK and Blec,
152 like NKR-P1 and LLT1-clr) are found in the chicken MHC, rather than in the
153 region syntenic to the natural killer complex (NKC) as in mammals [16,52].
154
155 Thus it is the mammalian MHC that is novel, and indeed the MHC of at least one
156 marsupial is organized like chickens [53], so that the change happened in the
157 lineage leading to placental mammals [6,9,27]. A potential mechanistic
158 explanation for this change would be an inversion (Fig. 3) that brought the class
159 III region into the center of the MHC and swung the class I gene(s) to the outside,
160 with the breakpoint such that the antigen processing and peptide loading genes
161 were left behind and eventually became part of the class II region. As discussed
162 above, sufficient levels of recombination meant that advantageous combinations
163 of genes could not stay together, and the TAP, tapasin and inducible proteasome
164 genes become average best fits for whatever class I allele appeared by
165 recombination. Once many alleles could be serviced, a multigene family became
166 possible.
167
168 Low-expressing promiscuous and high-expressing fastidious chicken class I
169 alleles
170
171 Some associations with the chicken MHC could be easily explained by the BF2
172 class I allele from a resistant (but not a susceptible) chicken finding a protective
173 peptide [37,39], but the very strong associations with Marek's disease were

more challenging to explain. Marek's disease virus (MDV), an oncogenic herpes virus with a complex life cycle and significant evolution of virulence in historic times, has been an enormous economic problem [54]. It wasn't clear how the MHC might confer susceptibility, since any class I molecule would be expected to bind a protective peptide from at least one of the 100 MDV genes. However, MHC haplotypes like B19 were strongly associated with susceptibility while B2 and B21 were strongly associated with resistance [55].

It has become clear that the BF2 molecules from susceptible haplotypes have peptide motifs much like typical mammalian class I molecules, with several pockets in the binding groove, each one of which binds only one or a few similar amino acids [37,56]. Such class I molecules might be called fastidious, with stringent peptide motifs and narrow peptide repertoires. In contrast, the BF2 molecules from resistant haplotypes can bind an exceedingly large variety of peptides and might be called promiscuous, with relaxed peptide motifs and wide peptide repertoires [38,57]. For instance, the molecule BF2*021:01 remodels the binding site to accommodate three anchor residues, at peptide positions P_2, P_{c-2} and P_c, with nearly every single amino acid found at P_2 and P_{c-2} [38,57]. Other chicken class I molecules like BF2*02:01 and BF2*14:01 use broad binding pockets capable of accommodating many amino acids with hydrophobic side chains, which are particularly common in most proteins [57].

The correlation of peptide repertoire with resistance to Marek's disease was unexpected. One hypothesis to explain this correlation [57] is that the few MDV peptides presented by fastidious molecules activate too few T cell clones to be

199 effective (as seems to be the case based on one study [58]), while the
200 promiscuous class I molecules provide a wide-ranging response involving many
201 T cell clones. Alternatively, the truly protective peptides might be so few in
202 number that the promiscuous BF2 molecules have a greater chance of presenting
203 such peptides.
204
205 Intriguingly, the fastidious class I molecules are found on the cell surface at a
206 high level, whereas the promiscuous molecules are expressed on the cell surface
207 at a lower level. This cell surface expression level is not dependent on the level of
208 transcription or translation, or on kinetics of translocation to the cell surface or
209 degradation. Overall, the population of highly-expressed fastidious molecules
210 shows greater thermal stability than the poorly-expressed promiscuous
211 molecules, although very stable complexes with particular peptides are found for
212 both kinds of molecules [37,38,56,57].
213
214 This inverse correlation of peptide repertoire with cell surface expression was
215 also unexpected. It seems most likely that the number of molecules arriving on
216 the cell surface is determined by the interaction of the particular TAP, tapasin
217 and BF2 alleles in the peptide-loading complex. The underlying reason for this
218 mechanism might just be the biochemistry of peptide loading, but alternatively
219 there could be evolutionary selection for the inverse correlation. One hypothesis
220 is that promiscuous BF2 molecules present so many self-peptides that negative
221 selection would deplete too many T cell clones in the thymus, and that reducing
222 the cell surface expression would reduce the extent of negative selection, with a
223 balance of peptide repertoire and cell surface expression resulting in an optimal

T cell repertoire [57]. Several other hypotheses can be imagined, including a balance between the responses to pathogens for protection versus the recognition of self that could lead to autoimmunity, or the balance between antigen presentation for T cell recognition and a role as a ligand for NK cells.

An inverse correlation of cell surface expression and peptide repertoire for human classical class I alleles

To what extents do these observations about class I molecules extend beyond chickens? Obviously, the potential contributions of polymorphism in TAP and tapasin cannot extend to mammals which have monomorphic antigen processing and peptide loading genes. However, the linkages of peptide repertoire, cell surface expression, translocation to the cell surface, stability and resistance to a viral disease are found for human class I molecules. Striking differences were reported in the predicted peptide repertoire of four HLA-B alleles that correlated with the speed of progression from HIV infection to AIDS, with the fastidious HLA-B*057:01 and HLA-B*027:05 alleles associated with long-term non-progression compared to the promiscuous HLA-B*07:02 and HLA-B*035:01 associated with rapid progression [59]. Subsequently, the cell surface expression levels of these four HLA-B alleles were shown to vary inversely with peptide repertoire, mirroring the findings in chickens [57]. Measurements of direct peptide binding for 27 HLA-A and HLA-B alleles showed a wide range of peptide repertoires [60]. An early immunoprecipitation study reported that one HLA-A and six HLA-B alleles were mostly in a peptide-bound conformation, while seven HLA-A and three HLA-B alleles were mostly bound to TAP molecules, suggesting

249 a range of peptide-loading efficiencies [61]. Assays with transfected cDNA clones
250 for 27 HLA-B alleles show that some alleles have a strong tapasin-dependence on
251 cell surface expression (tapasin-independent alleles generally being correlated
252 with faster HIV progression) [62], implicating dependence on translocation to
253 the cell surface as in chickens [35]. Although the data is not strictly comparable
254 between all these reports, wider peptide repertoire, lower cell surface
255 expression level, longer TAP binding, tapasin-independence of translocation and
256 faster HIV progression for HLA-A and HLA-B alleles seem to be broadly (but not
257 perfectly) correlated [63], with many (but not all) HLA-A molecules being more
258 promiscuous and many (but not all) HLA-B molecules being more fastidious.
259 Overall, the similarities between chickens and humans suggest that these are
260 fundamental properties of classical class I molecules.
261
262 However, there are clearly differences between the human and chicken class I
263 systems. The range of peptide binding for human class I alleles appears to be less
264 than that of chickens. For instance, HLA-A*02 molecules are the most
265 promiscuous human class I molecules, accommodating hydrophobic amino acids
266 that are very common in proteins, but only two or three different amino acids in
267 each pocket as opposed to the six different amino acids accommodated by the
268 highly promiscuous chicken BF2*002:01 [57,64,65]. Similarly, the very fastidious
269 HLA-B*57:01 only specifies amino acids for two pockets, one of which requires
270 the rare amino acid tryptophan, whereas the other pocket allows the very
271 common amino acids Ala, Ser and Thr. In contrast, the highly fastidious chicken
272 class I molecule BF2*004:01 requires binding of acidic amino acids in each of
273 three pockets [37,56,66]. Perhaps the presence of a multigene family of human

274 class I molecules means that the selective pressure for extremely promiscuous
275 and fastidious molecules is lower than in chickens.
276
277 A second difference might be that cell surface expression has been correlated
278 with tapasin-dependence in humans but thus far only with TAP specificity in
279 chickens; the effect of chicken tapasin has not been examined [35,62]. In any
280 case, human tapasin and TAP genes are functionally monomorphic, so any effect
281 in the peptide-loading complex would depend on the polymorphic positions in
282 the class I allele [42-44]. In contrast, chicken tapasin and TAPs genes are all
283 polymorphic, and appear to co-evolve with the dominantly-expressed class I BF2
284 gene [26,27], so the interactions could be more complex.
285
286 HLA-C presents a special challenge, perhaps because the relative importance of
287 various sequence features remains controversial. HLA-C molecules are found
288 expressed on the surface of most cells at a much lower level than HLA-A and
289 HLA-B (perhaps commensurate with a greater role for HLA-C as ligand for NK
290 cells rather than as an antigen presentation molecule for T cells) [67]. In
291 addition, HLA-C alleles vary in their cell surface expression (with higher
292 expression correlated with slower HIV progression, perhaps due to T cell
293 recognition) [68-70]. Various features of HLA-C have been reported to contribute
294 to these two kinds of differences, including promoter sequence and
295 transcription; miRNA sites in the 3'UTR sequences and RNA stability; β_2m
296 association, peptide motif and peptide repertoire; TAP residency and
297 translocation to the surface [67, 71-75]. In early studies [61, 71], certain HLA-C
298 alleles were found to be present inside the cell at the same level as HLA-A and

HLA-B molecules but remained bound to TAP and not translocated to the cell surface, similar to promiscuous chicken class I alleles. Indeed, the available data from predicted or actual peptide motifs is often interpreted to show a limited number of peptides that can bind HLA-C molecules compared to typical HLA-A and HLA-B molecules [67, 74-76]. A more recent report [76] compares two HLA-C alleles, confirming that several features of the HLA-C gene contribute in a complex way to cell surface expression, but finding that the peptide-binding domains of one HLA-C allele which binds a greater diversity of peptides are better expressed at the cell surface (at least when fused to another class I molecule), the opposite as found for chicken class I molecules. How all these observations fit together is at the moment unknown.

Finally, perhaps the most striking difference is that poorly-expressed promiscuous alleles confer protection from Marek's disease in chickens, while well-expressed fastidious alleles are responsible for slow progression to AIDs in humans. Any pretense to an overarching model must explain this difference.

Promiscuous generalists and fastidious specialists

What could be the evolutionary basis for having well-expressed fastidious and poorly-expressed promiscuous class I alleles? Looking through the literature, it appears that the promiscuous BF2 alleles protect chickens against a range of common infectious diseases in addition to Marek's disease [77-80]. For instance, typing chickens in rural Thailand after an outbreak of avian influenza found that all B21 homozygotes survived, that all chickens homozygous for the B12, B13

and B15 haplotypes with fastidious BF2 molecules died, and that in all but one combination, heterozygotes with one promiscuous class I allele survived [79] (Fig. 4). It appears that promiscuous BF2 molecules, wrapping up the specificities of several fastidious molecules into one molecule, generally confer protection to most pathogens (Fig. 2), much like a mammalian MHC haplotype with multiple mammalian class I molecules. In contrast, the fastidious human class I alleles HLA-B*057:01 and HLA-B*027:05 confer protection from the very dangerous zoonotic pathogen HIV, which they do by binding a particular protective peptide that the virus cannot change without a drastic loss of fitness [13,59,81,82]. Another dangerous and possibly new pathogen, hepatitis C virus (HCV) is also controlled by HLA-B*027:05 [83,84].

Putting these two ideas together, one evolutionary hypothesis would be that low-expressing promiscuous class I alleles function as generalists while the high-expressing fastidious alleles act as specialists [57,63]. Most of the time, the generalists deal well with common pathogens, but may not always be able to cope with the appearance of a new and virulent pathogen. In this case, a particular fastidious molecule may present a special peptide from the new pathogen, conferring protection and leading to an increase in gene frequency for that allele (Fig. 5).

How does this new view stack up against the current concepts and available data? The many class I alleles found in most human populations have long been interpreted to mean that high levels of polymorphism are important for survival. However, a population with a few generalist alleles may provide enough

protection under normal circumstances. If true, then the current view that MHC typing can identify the risk of extinction for endangered species [85] may need to be re-considered, to also take into account the peptide repertoire of MHC molecules present in the remaining population. It is also easy to imagine that the original class I alleles were promiscuous, which seems superficially similar to class II molecules from which they may have arisen [6,9,86]. The major change in structure-function relationships caused by rearrangement of the MHC in the lineage leading to placental mammals discussed above [6,9,27] might have been facilitated by a promiscuous class I allele closely-linked to promiscuous TAP genes.

Fastidious alleles might arise by a few mutations from promiscuous alleles and remain at a low gene frequency unless selected by a pathogen challenge, and might diminish in frequency once that challenge is relaxed. However, it is also possible that a selective sweep ensures near fixation of fastidious alleles. Chimpanzees, which are thought to have been strongly selected during a retroviral catastrophe, have two kinds of class I molecules [87-89]: those with fastidious peptide motifs very similar to HLA-B*57:01 and HLA-B*27:05, and those with promiscuous motifs very similar to BF2*02:01. Finally, it is important to note the classification of generalist and specialist alleles may be more useful for the explanation of a population response to a given pathogen than for individual predictions: a promiscuous molecule may be able to protect from a new and virulent pathogen (as does HLA-B*35:01 for HIV clade C but not B viruses [13]), while most fastidious molecules will be unlikely to recognize a protective peptide for any one particular pathogen. Having said that, a potential

advantage might arise from bundling together alleles that are promiscuous or fastidious, allowing greater statistical power in genetic association studies.

Concluding remarks

The observations and hypotheses described in this review still require much additional work for support and testing (see Outstanding Questions). First, careful and quantitative measurement of peptide repertoire breadth, cell surface expression levels and translocation to the surface for class I alleles in homozygotes is required. If the broad correlations discussed here are confirmed, then a comprehensive re-assessment of the extent to which (low-expressing) promiscuous and (high-expressing) fastidious class I alleles confer protection to various kinds of pathogens (as well as correlating with other biological and medical phenomena) would be valuable.

A much deeper understanding of the mechanisms underlying the phenomena is clearly required. If it is a fundamental property that leads to these correlations for classical class I molecules, it is natural to ask whether the same phenomena may be true for classical class II molecules. In fact, the concept of generalist and specialist has already been used for class II genes in relation to nematode burden of striped mice in Africa [90]. Perhaps the same idea of promiscuous and fastidious recognition might be true for other (particularly innate) immune receptors.

Finally, much can be learned from evolutionary biology approaches, including observation and simulation, typically with wild outbred populations. For detailed disease associations including autoimmunity and for mechanistic studies, humans and mice are obviously much better suited for rapid progress than chickens. However, at the least, the chicken MHC again has provided a simple model to discover phenomena that have been difficult to discern, both in the more complicated MHC of typical mammals and the less well-characterized MHC of wild species.

406 Acknowledgements. I thank Ellen Palmer, Becky Martin, Tom (C. H. T.) Yip and
407 Dan Wise for critical reading, and acknowledge a Wellcome Trust programme
408 grant (089305/Z/09/Z) that funded much of the initial research leading to the
409 concept of generalists and specialists, and a Wellcome Trust Investigator Award
410 (110106/Z/15/Z) for current support.

411 Figure legends

412

413 Figure 1. The chicken MHC (BF-BL region) is much smaller and simpler than the
414 human MHC (HLA locus), with a single dominantly-expressed class I molecule
415 due to co-evolution with peptide-loading genes. Colored vertical lines or boxes
416 indicate genes, with names above; very thin vertical lines indicate regions, with
417 names above or below; location is roughly to scale, with the length of
418 approximately 100 kB indicated. Thickness of arrows pointing up indicate level
419 of expression, co-evolution between the TAP genes and the BF2 class I gene
420 indicated by a curved arrow beneath the genes. Red are genes from the class I
421 system; blue are genes from the class II system; green are genes from the class III
422 or other regions; solid colors indicate classical genes while striped colors
423 indicate genes involved in peptide loading. Figure modified from references 16
424 and 91.

425

426 Figure 2. In comparison to mammals, the MHC of chickens has strong genetic
427 associations with resistance and susceptibility to infectious diseases. Left panel.
428 A multigene family in the human MHC can encode multiple fastidious class I
429 molecules, each of which has a chance to find a protective peptide. Altogether the
430 typical human MHC haplotype confers more-or-less resistance to most
431 pathogens, a situation that reads out as a weak genetic association (since there is
432 not much difference between haplotypes). Middle panel. In contrast, the single
433 dominantly-expressed class I molecule encoded by the chicken MHC can have a
434 fastidious peptide motif that may or may not find a protective peptide from any
435 given pathogen, a situation that reads out as strong genetic associations (since

436 there can be enormous differences between haplotypes). Right panel. However,
437 the single dominantly-expressed class I molecule encoded by the chicken MHC
438 can have a promiscuous peptide motif, capable of binding a wide variety of
439 peptides (much like the multigene family of human class I molecules acting
440 together). Comparing two promiscuous alleles may read out as a weak genetic
441 association (since there is not much difference between them), but comparison
442 of a fastidious allele with a promiscuous allele in chickens may give strong
443 genetic associations. Figure modified extensively from reference 33.
444
445 Figure 3. The presence of a multigene family of well-expressed classical class I
446 molecules in typical placental mammals can be explained by a genomic inversion
447 that disrupted the co-evolutionary relationships between the closely-linked
448 genes of the class I system found in many other vertebrates. Top panel. The
449 genomic organization of an ancestral MHC haplotype, based on data from the
450 chicken and throughout the non-mammalian vertebrates, has class II genes in a
451 class II region, class I genes and the genes encoding antigen processing and
452 peptide-loading components in a class I region, and the class III region genes on
453 the outside. The close linkage within the class I region leads to a single
454 dominantly-expressed class I gene (red), whose peptide motif reflects the
455 specificities of the polymorphic antigen processing and peptide-loading genes
456 (all red) with which it co-evolves. Middle panel. A genomic inversion can lead to
457 the class III region moving in between the single dominantly-expressed class I
458 gene and the rest of the MHC, marooning the particular alleles of the antigen
459 processing and peptide-loading genes near the class II genes and far from the
460 class I allele that they serve. Bottom panel. The antigen processing and peptide-

461 loading genes are selected to support any class I allele that might appear due to
462 recombination (rainbow color), which would then allow duplication within an
463 MHC haplotype to give a multigene family encoding class I molecules with
464 different peptide motifs (red, green, blue), as is found in typical mammals.
465 Regions separated by thin vertical lines; genes indicated by thicker vertical lines;
466 TAP, transporter associated with antigen presentation; LMP, inducible
467 proteasome component, originally known as low molecular weight protein; C2,
468 complement component 2; C4, complement component 4; fB, factor B. Figure
469 modified from reference 9.
470
471 Figure 4. Chicken MHC haplotypes encoding promiscuous class I molecules
472 (blue) can confer protection from a variety of viral infections under experimental
473 and field conditions, whereas MHC haplotypes encoding fastidious class I
474 molecules (red) generally confer susceptibility. Percentage of MHC genotypes in
475 a flock before and after experimental infection with Marek's disease virus (MDV),
476 with the B2 and B21 haplotypes conferring protection (a). Percentage of Rous
477 sarcoma virus (RSV) strains that progress to give lethal tumors after
478 experimental infection, with the B6 haplotype conferring survival (b).
479 Percentage survival after natural infection with avian influenza virus (AIV)
480 under field conditions in rural Thailand, with presence of a single promiscuous
481 haplotype conferring protection, except in one combination (B2/B13) for
482 reasons that are not understood (c). Percentage of chickens ill from infectious
483 bronchitis virus (IBV) on day 10 after experimental infection, with the B2
484 haplotype conferring protection (d). Data from references 77-80.
485

Fig. 5. A model illustrates the shift in gene frequencies from a few predominant generalist MHC alleles upon selection by new and/or particularly virulent pathogens. The diameter of each circle indicates the frequency of a particular MHC allele in a population before and after selection by a pathogen. The rainbow colors indicate promiscuous molecules that act as generalists, conferring protection to most pathogens including those regularly found in the environment. The single colors indicate fastidious molecules encoded by genes that arise by mutation and are present at low frequency, but with the possibility of presenting a protective peptide from a particular pathogen. Scenarios after three different pathogens are shown: one of the generalist molecules confers protection to the first pathogen (top), one of the specialist molecules confers protection to the second pathogen (middle), and another of the specialist molecules confers protection to the third pathogen (bottom). Figure modified extensively from reference 63.

500 References

501

502 1. Klein J. 1986. *The natural history of the major histocompatibility complex*.
503 New York: Wiley.
504 2. Trowsdale, J. and Knight, J.C. (2013) Major histocompatibility complex
505 genomics and human disease. Annu. Rev. Genomics. Hum. Genet. 14, 301-323.
506 3. Tiercy, J.M. and Claas, F. (2013) Impact of HLA diversity on donor
507 selection in organ and stem cell transplantation. Hum. Hered 76, 178-186.
508 4. Kaufman, J. (2014) The Avian MHC. In *Avian Immunology, second edition*.
509 (Schat, K.A., Kaiser, P. and Kaspers, B., eds) pp. 149-167, Elsevier, Ltd.
510 5. Miller, M.M. and Taylor, R.L. (2016) Brief review of the chicken Major
511 Histocompatibility Complex: the genes, their distribution on chromosome 16,
512 and their contributions to disease resistance. Poult. Sci. 95, 375-392.
513 6. Kaufman, J. (2011) The evolutionary origins of the adaptive immune
514 system of jawed vertebrates (Chapter 3). In *The Immune Response to Infection*
515 (Kaufmann, S.H.E., Rouse, B.T. and Sachs, D.L., eds.) pp. 41-54, American Society
516 of Microbiology Press.
517 7. Kaufman, J. (2013) Antigen processing and presentation: evolution from a
518 bird's eye view. Molec. Immunol. 55,159-161.
519 8. Kaufman, J. (2015) What chickens would tell you about the evolution of
520 antigen processing and presentation. Curr. Opin. Immunol. 34, 35-42.
521 9. Kaufman, J. (2018) Unfinished business: the evolution of the MHC and the
522 adaptive immune system of jawed vertebrates. Annu. Rev. Immunol., in press.

523 10. Bernatchez, L. and Landry, C. (2003) MHC studies in nonmodel
524 vertebrates: what have we learned about natural selection in 15 years? J. Evol.
525 Biol. 16, 363-377.
526 11. Spurgin, L.G. and Richardson, D.S. (2010) How pathogens drive genetic
527 diversity: MHC, mechanisms and misunderstandings. Proc. Biol. Sci. 277, 979-
528 988.
529 12. Hill, A.V. (1998) The immunogenetics of human infectious diseases. Annu.
530 Rev. Immunol. 16, 593-617.
531 13. Goulder, P.J. and Walker, B.D. (2012) HIV and HLA class I: an evolving
532 relationship. Immunity 37, 426-40.
533 14. McLaren, P.J. et al. (2015) Polymorphisms of large effect explain the
534 majority of the host genetic contribution to variation of HIV-1 virus load. Proc.
535 Natl. Acad. Sci. USA 112, 14658-14663.
536 15. Briles, W.E. et al. (1983) Resistance to a malignant lymphoma in chickens
537 is mapped to subregion of major histocompatibility (B) complex. Science 219,
538 977-979.
539 16. Kaufman, J. et al. (1999) The chicken B locus is a minimal essential major
540 histocompatibility complex. Nature 401, 923-925.
541 17. Psifidi, A. et al. (2016) Genome-wide association studies of immune,
542 disease and production traits in indigenous chicken ecotypes. Genet. Sel. Evol.
543 48, 74.
544 18. Psifidi, A. et al. (2016) The genomic architecture of resistance to
545 Campylobacter jejuni intestinal colonisation in chickens. BMC Genomics. 17, 293.
546 19. Hala, K. et al. (1979) A new recombinant allele in the B complex of the
547 chicken. Folia Biol. (Praha) 25, 323-324.

548 20. Koch, C. et al. (1983) New recombinants within the MHC (B-complex) of
549 the chicken. Tissue Antigens 21, 129-137.
550 21. Skjødt, K. et al (1988) Analysis of chickens for recombination within the
551 MHC (B-complex). Tissue Antigens, 1985, 25: 278-282.
552 22. Hala, K. et al. (1988) Attempt to detect recombination between B-F and B-
553 L genes within the chicken B complex by serological typing, in vitro MLR, and
554 RFLP analyses. Immunogenetics 28, 433-438.
555 23. Simonsen, M. et al. (1982) The MHC haplotypes of the chicken.
556 Immunogenetics 16, 513-532.
557 24. Hosomichi, K. et al. (2008) Contribution of mutation, recombination, and
558 gene conversion to chicken MHC-B haplotype diversity. J. Immunol. 181, 3393-
559 3399.
560 25. Fulton, J.E. et al. (2016) A high-density SNP panel reveals extensive
561 diversity, frequent recombination and multiple recombination hotspots within
562 the chicken major histocompatibility complex B region between BG2 and CD1A1.
563 Genet. Sel. Evol. 48:1.
564 26. van Hateren, A. et al. (2013) A mechanistic basis for the co-evolution of
565 chicken tapasin and major histocompatibility complex class I (MHC I) proteins. J.
566 Biol. Chem. 288, 32797-32808.
567 27. Walker, B.A. et al. (2011) The dominantly expressed class I molecule of
568 the chicken MHC is explained by coevolution with the polymorphic peptide
569 transporter (TAP) genes. Proc. Natl. Acad. Sci. USA 108, 8396-8401.
570 28. Chazara, O. et al. (2011) Organisation and diversity of the class II DM
571 region of the chicken MHC. Mol. Immunol. 48, 1263-1271.

572 29. Salomonsen, J. et al. (2003) The properties of the single chicken MHC
573 classical class II alpha chain (B-LA) gene indicate an ancient origin for the DR/E-
574 like isotype of class II molecules. Immunogenetics 55, 605-614.
575 30. Riegert, P et al. (1996) The chicken beta2-microglobulin gene is located
576 on a non-major histocompatibility complex microchromosome: a small, G+C-rich
577 gene with X and Y boxes in the promoter. Proc. Natl. Acad. Sc.i USA. 93, 1243-
578 1248.
579 31. Jones, C.T. et al. (1997) Gene homologs on human chromosome 15q21-
580 q26 and a chicken microchromosome identify a new conserved segment. Mamm.
581 Genome. 8, 436-440.
582 32. Erath, S. and Groettrup, M. (2015) No evidence for immunoproteasomes
583 in chicken lymphoid organs and activated lymphocytes. Immunogenetics 67, 51-
584 60.
585 33. Kaufman, J. et al. (1995) A "minimal essential Mhc" and an "unrecognized
586 Mhc": two extremes in selection for polymorphism. Immunol. Rev. 143: 63-88.
587 34. Kaufman, J. (2015) Co-evolution with chicken class I genes. Immunol. Rev.
588 267, 56-71.
589 35. Tregaskes, C.A. et al. (2016) Surface expression, peptide repertoire, and
590 thermostability of chicken class I molecules correlate with peptide transporter
591 specificity. Proc. Natl. Acad. Sci. USA 113, 692-697.
592 36. Shaw, I. et al (2007) Different evolutionary histories of the two classical
593 class I genes BF1 and BF2 illustrate drift and selection within the stable MHC
594 haplotypes of chickens. J. Immunol. 178, 5744-5752.

595 37. Wallny, H.J. et al. (2006) Peptide motifs of the single dominantly
596 expressed class I molecule explain the striking MHC-determined response to
597 Rous sarcoma virus in chickens. Proc. Natl. Acad. Sci. USA 103, 1434-1439.
598 38. Koch, M. et al. (2007) Structures of an MHC class I molecule from B21
599 chickens illustrate promiscuous peptide binding. Immunity 27, 885-899.
600 39. Butter, C. et al. (2013) The peptide motif of the single dominantly
601 expressed class I molecule of the chicken MHC can explain the response to a
602 molecular defined vaccine of infectious bursal disease virus (IBDV).
603 Immunogenetics 65, 609-618.
604 40. MHC Sequencing Consortium (1999) Complete sequence and gene map of
605 a human major histocompatibility complex. Nature 401, 921–923.
606 41. Carrington, M. (1999) Recombination within the human MHC. Immunol
607 Rev 167, 245–256.
608 42. Momburg, F. et al. (1994) Selectivity of MHC-encoded peptide
609 transporters from human, mouse and rat. Nature 367,648–651.
610 43. Obst, R. et al. (1995) TAP polymorphism does not influence transport of
611 peptide variants in mice and humans. Eur. J. Immunol. 25, 2170–2176.
612 44. Williams, A.P. et al (2000) Identification of novel Tapasin polymorphisms
613 and linkage disequilibrium to MHC class I alleles. Immunogenetics 52, 9-11.
614 45. Joly, E. et al. (1998) Co-evolution of rat TAP transporters and MHC class I
615 RT1-A molecules. Curr. Biol. 8, 169-172.
616 46. Kaufman, J. (1999) Co-evolving genes in MHC haplotypes: the "rule" for
617 nonmammalian vertebrates? Immunogenetics 50, 228-236.
618 47. Moon, D.A. et al. (2005) The MHC of the duck (Anas platyrhynchos)
619 contains five differentially expressed class I genes. J. Immunol.175, 6702-6712.

620 48. Mesa, C.M. et al. (2004) The dominant MHC class I gene is adjacent to the
621 polymorphic TAP2 gene in the duck, Anas platyrhynchos. Immunogenetics 56,
622 192-203.
623 49. Ohta, Y. et al. (2006) Ancestral organization of the MHC revealed in the
624 amphibian Xenopus. J. Immunol. 176, 3674-3685.
625 50. Lukacs, M.F. et al. (2007) Genomic organization of duplicated major
626 histocompatibility complex class I regions in Atlantic salmon (Salmo salar). BMC
627 Genomics 8, 251.
628 51. Grimholt, U. et al. (2003) MHC polymorphism and disease resistance in
629 Atlantic salmon (Salmo salar); facing pathogens with single expressed major
630 histocompatibility class I and class II loci. Immunogenetics 55, 210-219.
631 52. Rogers, S.L. et al. (2005) Characterization of the chicken C-type lectin-like
632 receptors B-NK and B-lec suggests that the NK complex and the MHC share a
633 common ancestral region. J. Immunol. 174, 3475-3483.
634 53. Belov, K. et al. (2006) Reconstructing an ancestral mammalian immune
635 supercomplex from a marsupial major histocompatibility complex. PLoS Biol. 4,
636 e46
637 54. Osterrieder, N. et al. (2006) Marek's disease virus: from miasma to model.
638 Nat. Rev. Microbiol. 4, 283-294.
639 55. Plachy, J. et al. (1992) Biology of the chicken MHC (B complex). Crit. Rev.
640 Immunol. 12, 47-79.
641 56. Zhang, J. et al. (2012) Narrow groove and restricted anchors of MHC class
642 I molecule BF2*0401 plus peptide transporter restriction can explain disease
643 susceptibility of B4 chickens. J. Immunol. 189, 4478-4487.

644 57. Chappell, P. et al. (2015) Expression levels of MHC class I molecules are
645 inversely correlated with promiscuity of peptide binding. eLIFE 4, 05345.
646 58. Mwangi, W.N. et al. (2011). Clonal structure of rapid-onset MDV-driven
647 CD4+ lymphomas and responding CD8+ T cells. PLoS Pathog. 7, e1001337.
648 59. Kosmrlj, A. et al. (2010) Effects of thymic selection of the T-cell repertoire
649 on HLA class I-associated control of HIV infection. Nature 465: 350-354.
650 60. Paul, S. et al. (2013) HLA class I alleles are associated with peptide-
651 binding repertoires of different size, affinity, and immunogenicity. J. Immunol.
652 191, 5831-5839.
653 61. Neisig, A. et al. (1996) Allele-specific differences in the interaction of MHC
654 class I molecules with transporters associated with antigen processing. J.
655 Immunol. 156, 3196-206.
656 62. Rizvi, S.M. et al. (2014) Distinct assembly profiles of HLA-B molecules. J.
657 Immunol. 192, 4967-4976.
658 63. Kaufman, J. (2017) A New View of How MHC Class I Molecules Fight
659 Disease: Generalists and Specialists. In: Evolutionary Biology: Self/Nonself
660 Evolution, Species and Complex Traits Evolution, Methods and Concepts. Pierre
661 Pontarotti, editor. Springer, Switzerland.
662 64. Madden, D.R. et al. (1993) The antigenic identity of peptide-MHC complexes:
663 a comparison of the conformations of five viral peptides presented by HLA-A2.
664 Cell 75, 693-708.
665 65. Chen, K.Y. et al. (2012) Structural and functional distinctiveness of HLA-A2
666 allelic variants. Immunol. Res. 53, 182-190.
667 66. Illing, P.T. et al. (2012) Immune self-reactivity triggered by drug-modified
668 HLA-peptide repertoire. Nature 486, 554-558.

669 67. Blais, M.E. et al. (2011) HLA-C as a mediator of natural killer and T-cell
670 activation: spectator or key player? Immunology 133, 1-7.
671 68. Thomas, R. et al. (2009) HLA-C cell surface expression and control of
672 HIV/AIDS correlate with a variant upstream of HLA-C. Nat. Genet. 41, 1290-1294.
673 69. Apps, R. et al. (2013) Influence of HLA-C expression level on HIV control.
674 Science 340, 87-91.
675 70. McLaren, P.J. et al. (2015) Polymorphisms of large effect explain the majority
676 of the host genetic contribution to variation of HIV-1 virus load. Proc. Natl. Acad.
677 Sci. U S A. 112, 14658-14663.
678 71. Neisig, A. et al. (1998) Reduced cell surface expression of HLA-C molecules
679 correlates with restricted peptide binding and stable TAP interaction. J.
680 Immunol. 160, 171-9.
681 72. Kulkarni, S. et al. (2011) Differential microRNA regulation of HLA-C
682 expression and its association with HIV control. Nature 472, 495-498.
683 73. Vince, N. et al. (2016) HLA-C level is regulated by a polymorphic Oct1 binding
684 site in the HLA-C promoter region. Am. J. Hum. Genet. 99, 1353-1358.
685 74. Falk, K. et al. (1993) Allele-specific peptide ligand motifs of HLA-C molecules.
686 Proc. Natl. Acad. Sci. U S A. 90, 12005-9.
687 75. Rasmussen, M. et al. (2014) Uncovering the peptide-binding specificities
688 of HLA-C: a general strategy to determine the specificity of any MHC class I
689 molecule. J. Immunol. 193, 4790-4802.
690 76. Kaur, G. et al. 2017. Structural and regulatory diversity shape HLA-C
691 protein expression levels. Nat. Commun. 8, 15924.

692 77. Simonsen, M. (1987) The MHC of the chicken, genomic structure, gene
693 products, and resistance to oncogenic DNA and RNA viruses. Vet. Immunol.
694 Immunopathol. 17, 243-53.
695 78. McBride, R.A. et al. (1981) MHC gene control of growth of avian sarcoma
696 virus-induced tumours in chickens: a study on the role of virus strains. J.
697 Immunogenet. 8, 207-214.
698 79. Boonyanuwat, K. et al. (2006) Influences of major histocompatibility complex
699 class I haplotypes on avian influenza virus disease traits in Thai indigenous
700 chickens. Anim. Sci. J. 77, 285–289
701 80. Banat, G.R. et al. (2013) Association of the chicken MHC B haplotypes with
702 resistance to avian coronavirus. Dev. Comp. Immunol. 39, 430-437.
703 81. Brennan, C.A. et al. (2012) Early HLA-B*57-restricted CD8+ T lymphocyte
704 responses predict HIV-1 disease progression. J. Virol. 86, 10505-10516.
705 82. Schneidewind, A. et al. (2007) Escape from the dominant HLA-B27-restricted
706 cytotoxic T-lymphocyte response in Gag is associated with a dramatic reduction
707 in human immunodeficiency virus type 1 replication. J. Virol. 81, 12382-12393.
708 83. Pybus, O.G. and Thézé, J. (2016) Hepacivirus cross-species transmission and
709 the origins of the hepatitis C virus. Curr. Opin. Virol. 16, 1-7.
710 84. Dazert, E. et al. (2009) Loss of viral fitness and cross-recognition by CD8+ T
711 cells limit HCV escape from a protective HLA-B27-restricted human immune
712 response. J. Clin. Invest. 119, 376-386.
713 85. Sommer, S. (2005) The importance of immune gene variability (MHC) in
714 evolutionary ecology and conservation. Frontiers in Zoology 2, 16.
715 86. Kaufman, J.F. et al. (1984) The class II molecules of the human and murine
716 major histocompatibility complex. Cell 36, 1-13.

717 87. de Groot, N.G. et al. (2002) Evidence for an ancient selective sweep in the
718 MHC class I gene repertoire of chimpanzees. Proc. Natl. Acad. Sci. U S A. 99,
719 11748-11753.
720 88. Sidney, J. et al. (2006) Detailed characterization of the peptide binding
721 specificity of five common Patr class I MHC molecules. Immunogenetics 58, 559–
722 570.
723 89. de Groot, N.G. et al. (2010) AIDS-protective HLA-B*27/B*57 and chimpanzee
724 MHC class I molecules target analogous conserved areas of HIV-1/SIVcpz. Proc
725 Natl Acad Sci U S A. 107, 15175–15180.
726 90. Froeschke, G. and Sommer, S. (2012) Insights into the complex associations
727 between MHC class II DRB polymorphism and multiple gastrointestinal parasite
728 infestations in the striped mouse. PLoS ONE 7, e31820.
729 91. Kaufman, J. (2016) "Genetics and genomic organisation of the major
730 histocompatibility complex (MHC) with infectious disease". In: *Encyclopedia of*
731 *Immunobiology, vol. 2* (Ratcliffe, M.J.H., editor-in-chief) pp. 166-173, Academic
732 Press, Oxford.

Trends Box (900 characters and spaces, 3-5 bullet points on recent developments)

- A broad overview of classical MHC I expression and bound peptides reveals an inverse correlation between repertoire breadth and cell surface expression in some chicken and human alleles

- Several chicken class I alleles with wide peptide binding repertoire (promiscuity) are associated with resistance to a variety of common diseases

- Conversely, narrow peptide binding repertoire (fastidiousness) in some human HLA-B alleles is associated with resistance to HIV progression

- Cell surface expression of some classical class I alleles depends on the regulation of translocation to the cell surface rather than of translation. MHC translocation is influenced by peptide-translocation in chickens and by tapasin interaction in humans

Comment [DJ(1]: Our style is generally to write these as full sentences, please review the changes below.

Outstanding questions (2000 characters and spaces in bullet points)

Comment [DJ(1]: The questions were reworded and shortened to fit within the character limit. Please review this version. Thank you.

- To what extent is the inverse correlation between cell surface expression and peptide repertoire found for all classical class I molecules in chickens and humans? Is it a true hierarchy or just two groups? If this is not the case for human HLA-C, why?

  What is the mechanism for the inverse correlation between cell surface expression and peptide repertoire found for classical class I molecules? Is it due to intrinsic differences in folding of the class I molecule, efficiency of interactions the peptide loading complex, quality control steps (like TAPBPR/UGT), or other important steps of translocation? In addition to these biochemical mechanisms, what are the selective pressures for the inverse correlation between cell surface expression and peptide repertoire found for classical class I molecules (e.g. optimization of T cell repertoire, avoidance of auto-immunity)?

- To what extent does the low expression level/peptide promiscuity really correlate with resistance to common pathogens? How is this correlation influenced by the type of pathogen involved (e.g. virus with a small or large genome, for which the number of potential protective peptides is different), and what is the underlying mechanism for protection (number of T cells activated, higher probability to bind a given efficacious peptide)?

- Similarly, what are the mechanisms underlying protection against a given zoonosis by high expression level/fastidious peptide binding MHCs? Is the binding of special protective peptides that are critical for viral fitness always involved?

- To what extent is this new view true for a wide variety of species? These studies should be easier in mice and primates where significant literature is available, but are largely relevant across the animal kingdom (e.g. farm and sport animals, farmed fish).

- To what extent are these features found for classical class II molecules?

Figure 1

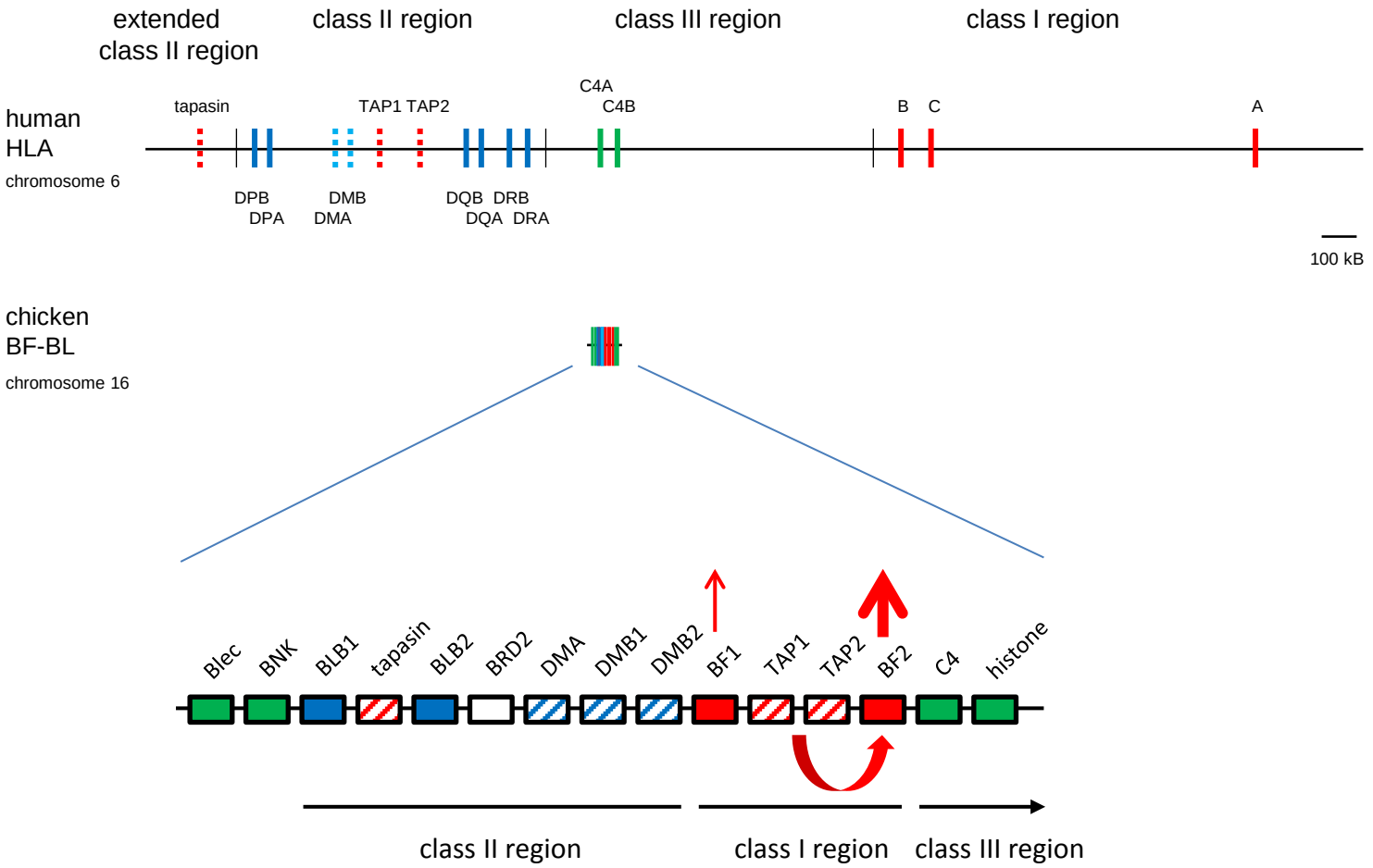

extended
class II region
class II region
class III region
class I region
human
HLA
chromosome 6
tapasin
TAP1 TAP2
C4A
C4B
B
C
A
DPB
DPA
DMB
DMA
DQB DRB
DQA DRA
100 kB
chicken
BF-BL
chromosome 16
Blec
BNK
BLB1
tapasin
BLB2
BRD2
DMA
DMB1
DMB2
BF1
TAP1
TAP2
BF2
C4
histone
class II region
class I region
class III region

Figure 2

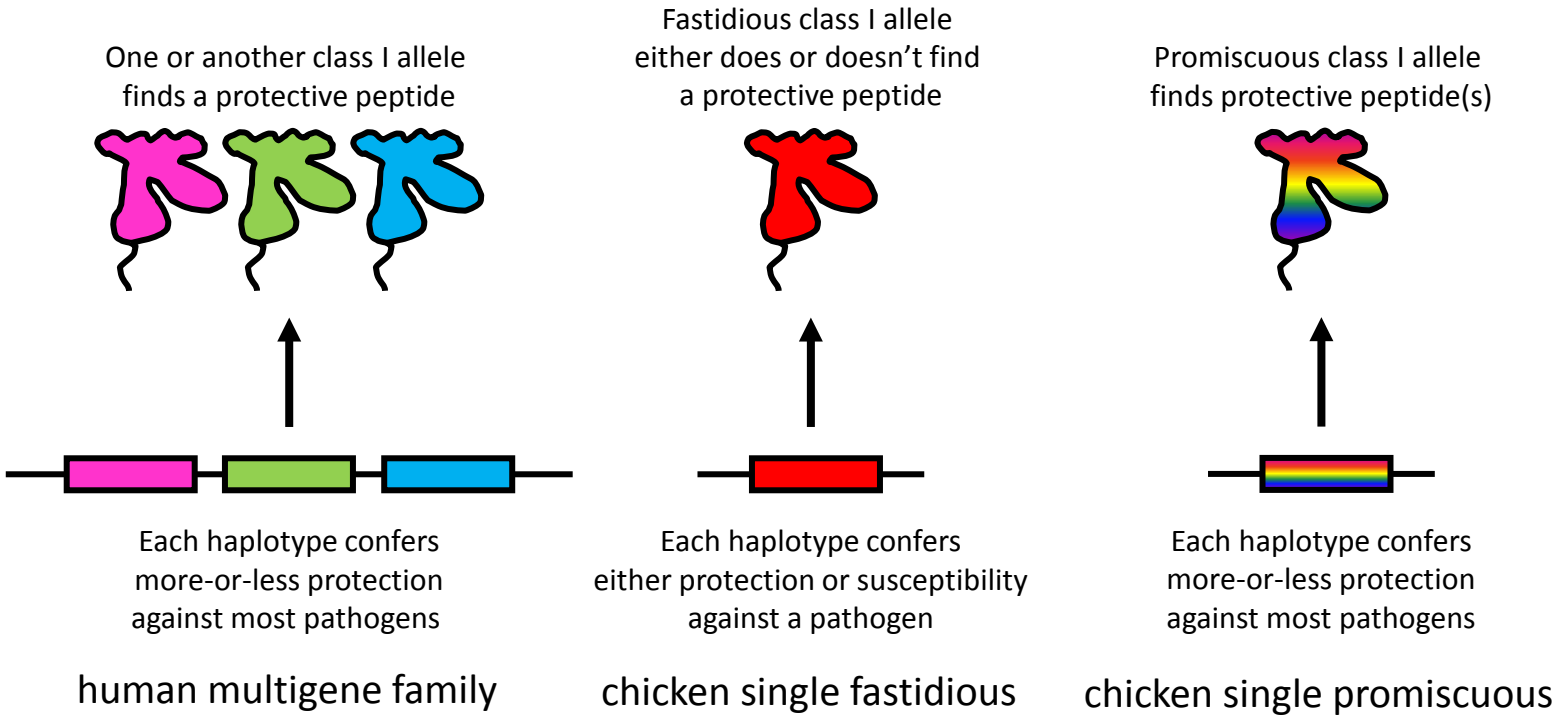

One or another class I allele finds a protective peptide
Each haplotype confers more-or-less protection against most pathogens
human multigene family
Fastidious class I allele either does or doesn't find a protective peptide
Each haplotype confers either protection or susceptibility against a pathogen
chicken single fastidious
Promiscuous class I allele finds protective peptide(s)
Each haplotype confers more-or-less protection against most pathogens
chicken single promiscuous

Figure 3

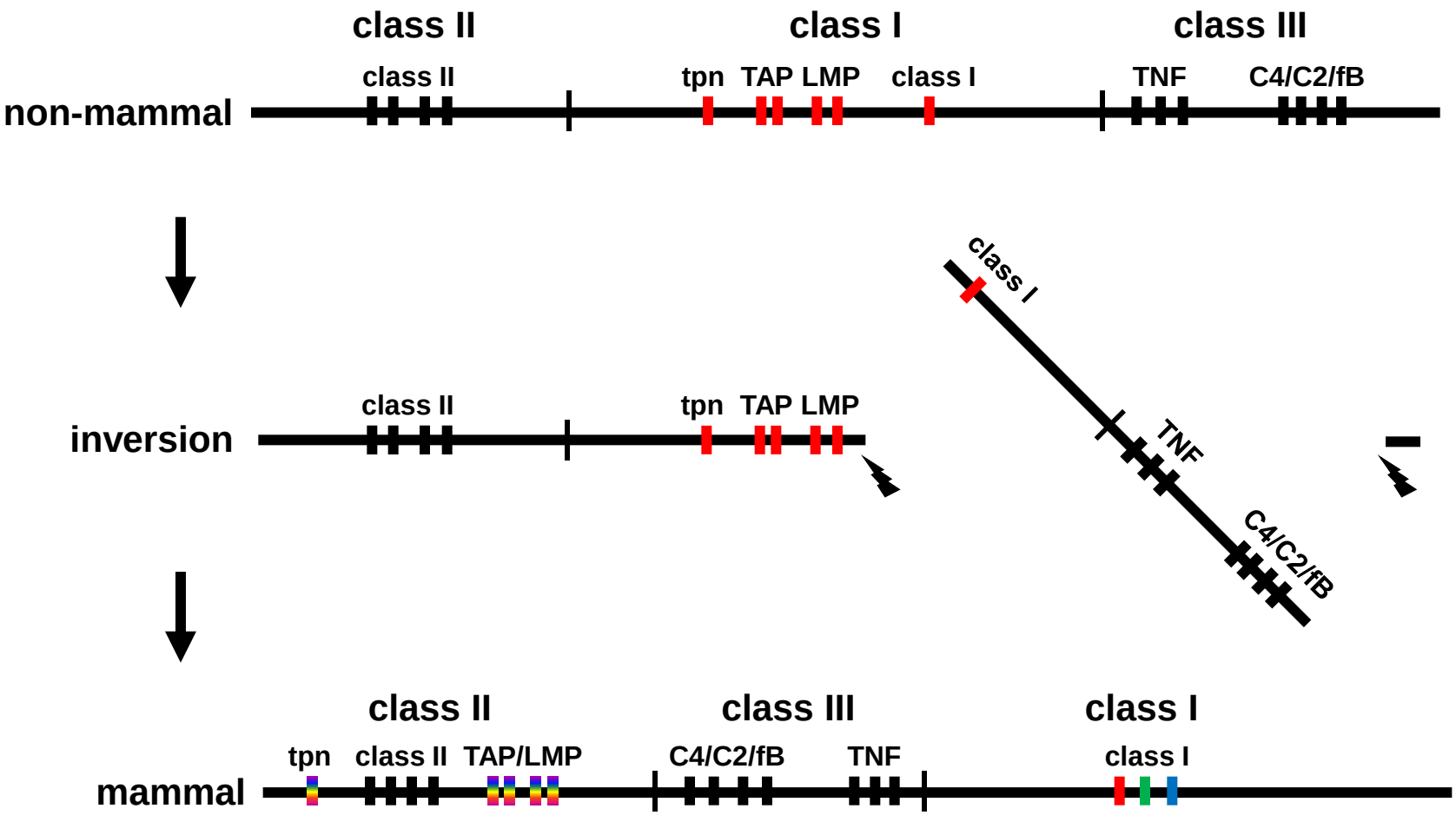

class II
class I
class III
non-mammal
class II
tpn
TAP
LMP
class I
TNF
C4/C2/fB
inversion
class II
tpn
TAP
LMP
class I
TNF
C4/C2/fB
class II
class III
class I
mammal
tpn
class II
TAP/LMP
C4/C2/fB
TNF
class I

Figure 4

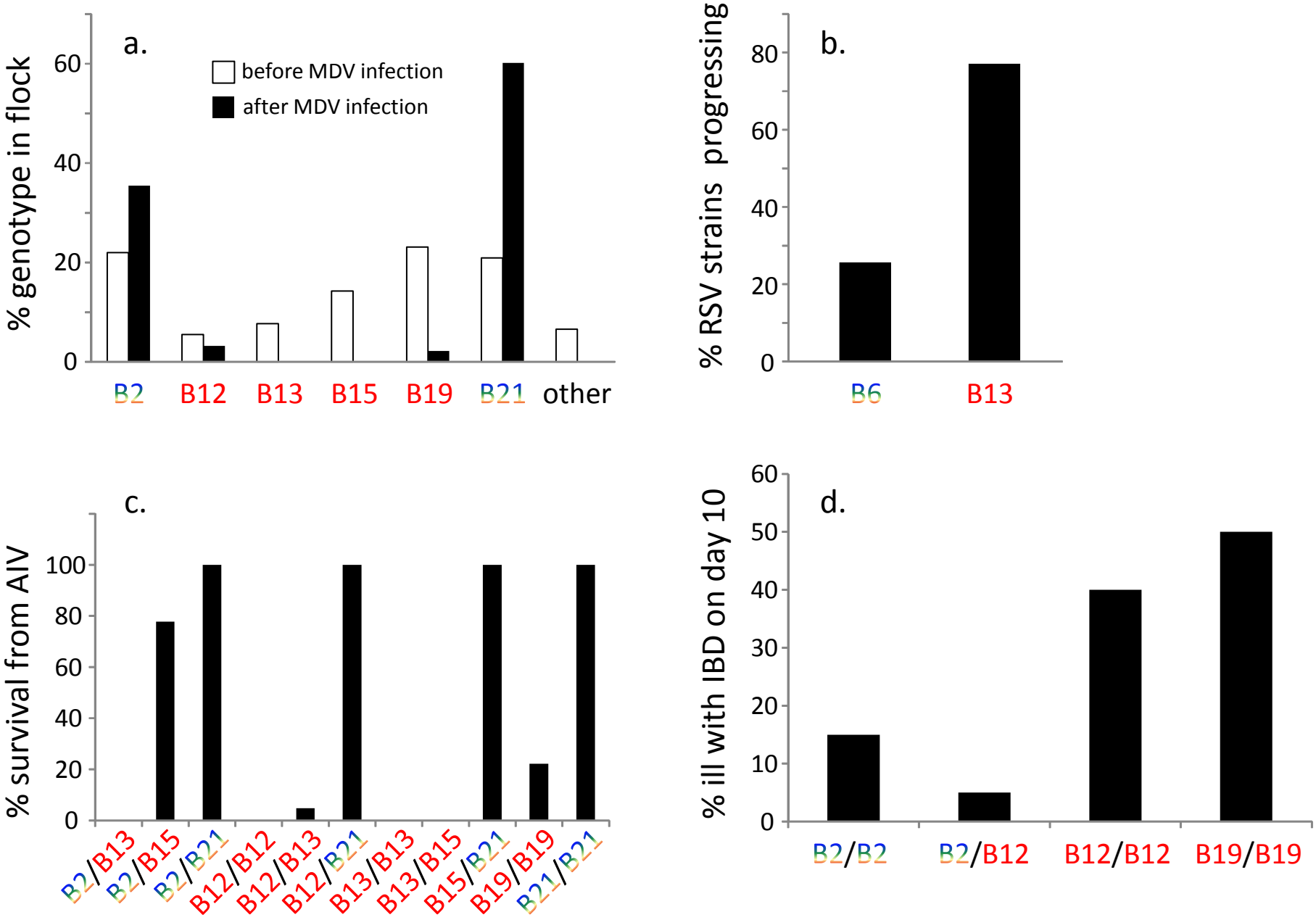
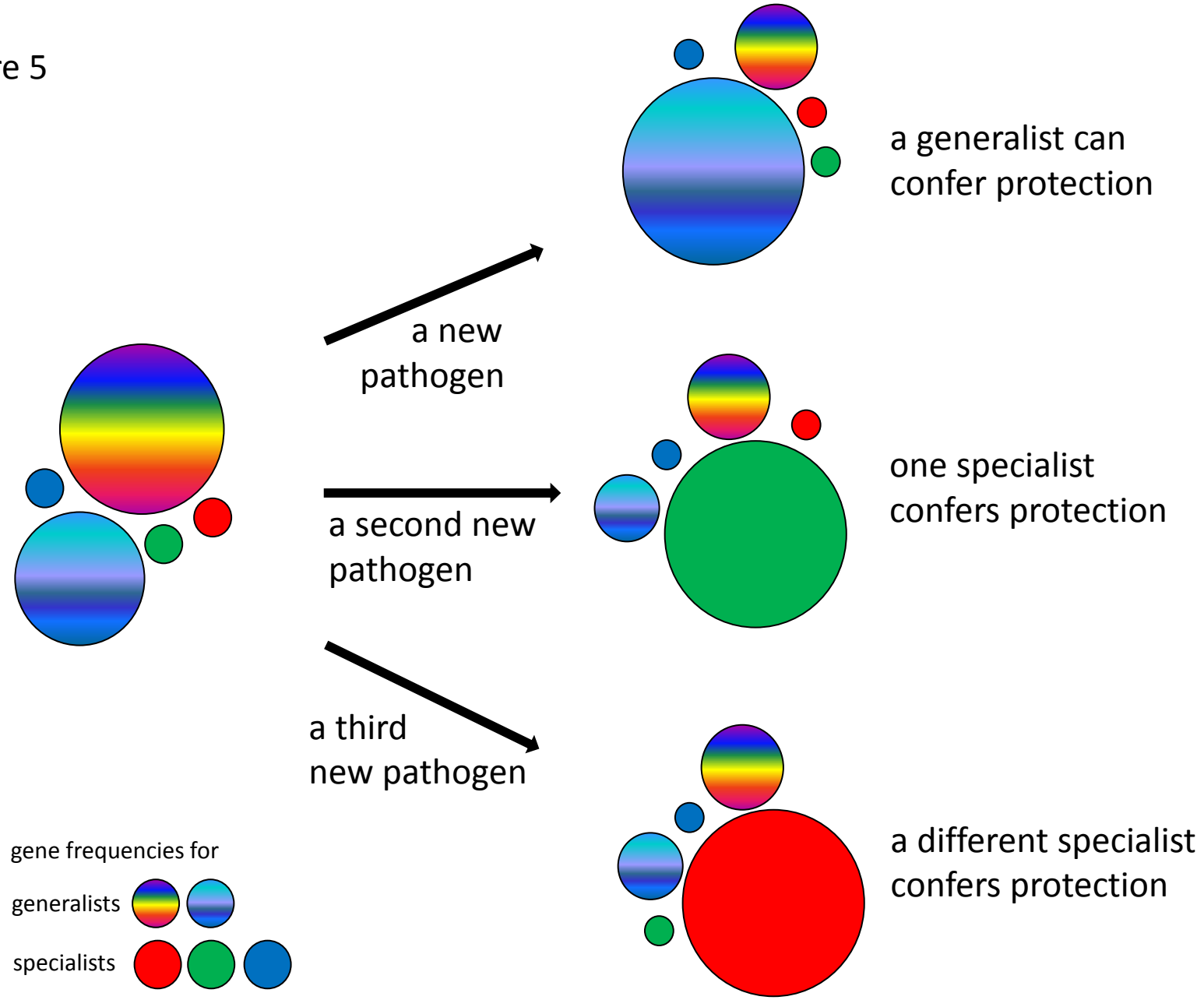

a.
before MDV infection
after MDV infection
% genotype in flock
0
20
40
60
B2
B12
B13
B15
B19
B21
other
b.
% RSV strains progressing
0
20
40
60
80
B6
B13
c.
% survival from AIV
0
20
40
60
80
100
B2/B13
B2/B15
B2/B21
B12/B12
B12/B13
B12/B21
B13/B13
B13/B15
B15/B21
B19/B19
B21/B21
d.
% ill with IBD on day 10
0
10
20
30
40
50
60
B2/B2
B2/B12
B12/B12
B19/B19

Figure 5


a new pathogen
a second new pathogen
a third new pathogen
a generalist can confer protection
one specialist confers protection
a different specialist confers protection
gene frequencies for
generalists
specialists