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Coevolution of MHC genes (LMP/TAP/class Ia; NKT-class Ib; NKp30-B7H6): Lessons from cold-blooded vertebrates

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Summary

Comparative immunology provides the long view of what is conserved across all vertebrate taxa versus what is specific to particular organisms or group of organisms. Regarding the major histocompatibility complex (MHC) and coevolution, three striking cases have been revealed in cold-blooded vertebrates: lineages of class Ia antigen-processing and -presenting genes, evolutionary conservation of NKT-class Ib recognition, and the ancient emergence of the natural cytotoxicity receptor NKp30 and its ligand B7H6. While coevolution of transporter associated with antigen processing (TAP) and class Ia has been documented in endothermic birds and two mammals, lineages of LMP7 are restricted to ectotherms. The unambiguous discovery of natural killer T (NKT) cells in *Xenopus* demonstrated that NKT cells are not restricted to mammals and are likely to have emerged at the same time in evolution as classical α/β and γ/δ T cells. NK cell receptors evolve at a rapid rate, and orthologues are nearly impossible to identify in different vertebrate classes. By contrast, we have detected NKp30 in all gnathostomes, except in species where it was lost. The recently discovered ligand of NKp30, B7H6, shows strong signs of coevolution with NKp30 throughout evolution, i.e. coincident loss or expansion of both genes in some species. NKp30 also offers an attractive IgSF candidate for the invasion of the RAG transposon, which is believed to have initiated T-cell receptor/immunoglobulin adaptive immunity. Besides reviewing these intriguing features of MHC evolution and coevolution, we offer suggestions for future studies and propose a model for the primordial or proto MHC.

Keywords

TAP; immunoproteasome; coevolution; NKT cells; MHC organization; NCR; NKp30; B7H6; Proto MHC

Introduction

The major histocompatibility complex (MHC), complete with class I, class II, antigen-processing genes, and genes involved in the development and signaling in the immune system, arose early in gnathostome evolution (1–3). The oldest living jawed vertebrates, the

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cartilaginous fish, clearly have such features, in addition to preservation of the ancient MHC-linkage of β 2-microglobulin (4). Unfortunately, cartilaginous fish genes and intergenic regions are large, so linkage studies to date have been performed piecemeal (5, 6). The recent elephant shark genome project did not provide new information on MHC linkage or association, for unknown technical reasons (7). Nevertheless, sharks and most nonmammalian vertebrates have what we have called the ‘adaptive MHC’, with close linkage of class Ia and the genes involved in class I antigen presentation, LMP and TAP (as well as class II in almost all groups but bony fish) (6, 8, 9). Class III genes such as C4 and factor B (Bf) and other genes involved in innate immunity, and immune development and signaling, are also in the MHC, and they likely formed its core before the emergence of adaptive immunity (10–13). Furthermore, in addition to constitutive proteasome subunits, immunoglobulin superfamily (IgSF) members that are now part of the antigen receptor, NK receptors, class I/II, and B7 costimulatory families also were present in this primordial MHC, which we discuss herein.

This review is focused on three non-overlapping aspects of MHC coevolution: lineages of class I processing and presenting genes, the emergence of NKT cell-class Ib interaction in vertebrates, and evolutionary conservation of an NKR-B7 ligand pair. In all cases such coevolution is ancient, and in one instance it may have predated adaptive immunity.

Coevolution of TAP, Imp7, and class I in ectothermic vertebrates

The class I processing/presentation system

Several seminal papers in the early 1990s demonstrated MHC linkage of the genes involved in the production (proteasome, Imp) and transport [transporter associated with antigen processing (TAP)] of peptides that bind to classical class I (class Ia) molecules (reviewed in 14). However, in most mammals, the class Ia processing genes were found to be closely linked to class II genes rather than the class I genes (Fig. 1). In contrast, almost all other vertebrates examined (sharks, bony fish, amphibians, birds) were shown to have a primordial, ‘true’ class I region with close linkage of the class Ia processing and presenting genes (8, 9). Most mammalian species have a permissive TAP that provides peptides of all sorts to class Ia alleles, likely due to the translocation of class I genes relatively far from the class Ia processing genes and loss of TAP/class Ia coevolution (3). The rat is one of a few mammalian species in which coevolution of TAP and class Ia is found, and this was likely made possible because of a translocation of the class Ia gene to close proximity of TAP (15, 16). In chickens, certain TAP alleles are closely linked to particular class Ia alleles, and definitive studies have shown that peptides transported by certain TAP allelic products bind to the linked class I molecules (17;18). Thus, birds, like all cold-blooded gnathostomes, display the primordial state of class Ia genes closely linked to the transport genes, promoting coevolution of the evolutionarily distinct gene families.

Coevolution of TAP and class Ia in ectotherms

In the amphibian *Xenopus*, like the rat, there are biallelic lineages of TAP and class Ia (19). However, in *Xenopus* these lineages for both TAP1 and TAP2 are very old, found in both the and *X. tropicalis* and the common *X. laevis*, separated by at least 30 million years, and

apparently in all other *Xenopus* species as well. Furthermore, the amino acid divergence is very high between the *Xenopus* alleles in comparison to other species having TAP allelic diversity. The data support a model, again like in rats, in which one TAP is 'permissive' and the other 'restrictive' of the types of peptides transported. The class Ia molecules also fall into two lineages, most noticeable in *X. laevis* by their transmembrane and cytoplasmic regions (20). Analysis of the peptide-binding regions (PBR) in alleles comprising the two frog class Ia lineages suggested that the F pocket is biallelic and thus might accommodate peptides with different C-termini (21). Such a scenario would be consistent with studies in endotherms. Unfortunately, to date neither the peptides binding to the class Ia molecules nor the peptides transported by TAP have been elucidated in any ectotherm. Finally, while TAP biallelic lineages clearly exist in frogs, we have not yet determined whether there might be minor polymorphisms among MHC haplotypes, like in birds (18), which may further promote coevolution between TAP and class Ia.

Consistent biallelism of Imp7 in ectotherms

Unlike the situation in all endotherms including birds (interestingly, birds have lost *all* of the immunoproteasome and thymic proteasome genes ($\beta 5t$ or *psmb11*), unusual creatures that they are, considering the loss of many other immune genes as well (22–24)(see below), all cold-blooded vertebrates studied to date have biallelic lineages of Imp7 (*psmb8* or $\beta 5i$). Lineages are clearly restricted to Imp7, not the other immunoproteasome members Imp2 (*psmb10* or $\beta 2i$), and *mecl1* (*psmb10* or $\beta 2i$), where there is clearly no allelic variation among MHC haplotypes. In all of the species examined, including cartilaginous fish, bony fish, and amphibians, there are two forms of Imp7, one with an unusual catalytic site (5, 25–27). The catalytic site of one allele is similar to the constitutive form (Impx or *psmb5*), and likely provides peptides to class Ia with hydrophobic C-termini. The other allele has a bulky phenylalanine rather than alanine or valine in this site, which would likely modify the catalysis, perhaps even inhibiting enzymatic function.

In all *Xenopus* species so far studied, there is unfailing close linkage between the *Imp7*, *TAP1*, *TAP2*, and class Ia lineages in all animals tested, indicating a strong selection to maintain the association (19). Interestingly, preliminary analysis suggests that the lineages are in different gene frequencies in *X. laevis* and *X. tropicalis*. *X. laevis* is a tetraploid, and there is differential silencing of genes within the MHC (28, 29). Functional *Imp2* is expressed on the chromosome with silenced class Ia and class II, but *Imp7* has maintained its close linkage to the functional TAP 1/2 and class Ia genes. *Mecl1*, like in bony fish, is linked to the MHC in *Xenopus*, and it is actually present on both the functional and 'silenced' MHC chromosomes in the tetraploid (Ohta, Du Pasquier, and Flajnik, unpublished data). Furthermore, *mecl1* is found in the class III region in the *Xenopus* MHC, which seems to be the ancestral condition despite its close linkage to class Ia in teleosts (30). Additionally, in mammals, *mecl1* has been translocated out of the MHC, supporting the idea that coevolution with class Ia is not crucial for this subunit. Taken together, these findings suggest that Imp7 is a lynchpin in the formation/function of the immunoproteasome, consistent with studies in mammals (31).

The *Imp7* lineages are found in both teleost and cartilaginous fish as well, with biallelic lineages having a similar type of change in the catalytic site (5). Nonaka has argued that this same or similar change arose via convergent evolution, but more work must be done to corroborate this hypothesis, considering the appearance of the two forms as alleles or pseudoalleles in all ectotherms tested (32). In cartilaginous fish, we have identified two categories of MHC haplotypes, a complex one having two *Imp7* genes (one pseudogene) and a simpler one only with *Imp7*; nevertheless, the biallelic forms are found in different haplotypes. To date, we have not determined whether there are divergent TAP alleles in sharks, but Southern blotting has suggested that (at least) *TAP2* is found in lineages (5). *TAP2* polymorphisms have been detected in one bony fish, but *TAP1* has lost its association with the teleost MHC (33;34).

Summary of part 1

Functional studies of immunoproteasome and TAP specificity are required in the sharks, bony fish, and amphibians. Mutagenesis of the mammalian *Imp7* subunit to the residues in the catalytic site that differ between the lineages would provide the first test of peptides produced by the allelic forms. The *Xenopus* class I alleles have been studied for many years and it would be possible to isolate peptides from purified molecules. Lastly, conditions for generating transgenic and knockout lines exist in several ectotherms now for study of the class I region lineages.

The coevolution of *Imp7*, *TAP*, and class Ia is clearly the primordial condition, and the gene sets likely work as a team in antigen presentation. In most mammals, the movement of class I away from the *Imp* and *TAP* genes apparently allowed for a more permissive system of peptide supply and the original coevolution was lost. Birds have seemingly taken the primordial coevolution system to extremes, with many TAP alleles, loss of immunoproteasome and the thymic-specific proteasome $\beta 5t$, and with some class Ia alleles that are more permissive in their peptide-binding (3). Since $\beta 5t$ has such an important role in positive selection for CD8⁺ T cells in mammals, and is found in all gnathostomes so far studied except birds (22), it will be interesting to compare TCR repertoires (and T-cell function in general) of chickens to all other gnathostomes with these differences in mind.

NKT cell-nonclassical class I evolution

In mammals, nearly 30 years ago a subset of T cells were discovered to have semi-invariant TCR that was later shown to be restricted by the non-classical class I molecule CD1, and were thus named natural killer T cells (NKT) (35–37). Subsequently, other lineages of NKTs were uncovered, for example MAIT cells, which use the non-classical class I molecule MR1 as their ligand to present vitamin metabolites produced by pathogens (38). Despite this recent fascinating discovery for MAITs, it has been difficult to determine the physiological role of NKT cells in mammals, and thus it has been suspected that this cell type might have been a recent addition to the immune system, perhaps a feature such as germinal centers that are only found in endotherms (39).

Evolution of NKT cells

The first evidence that suggested that NKT might not be limited to mammals was the finding that birds [and now lizards (40)] have *bona fide* CD1 (41, 42). Recent work in amphibians by Jacques Robert and colleagues (43) has shed definitive light on this subject. In 1993, our laboratory found that, in addition to the single class Ia gene encoded in the MHC, there is a large number of class Ib genes encoded at the telomere of the MHC chromosome that we called the *Xenopus* nonclassical class I genes, or *XNC* genes (44, 45). Our first sets of experiments showed that several of *XNC* genes were expressed in a tissue-specific fashion, suggesting a division of labor among them (46). Robert began studying these genes in more detail, mainly in the context of tumor immunology. One gene that he identified, *XNC10*, was expressed in tumors and shown to induce specific immunity (47). Furthermore, unlike what we showed previously for many *XNC* genes, *XNC10* was found to be expressed during tadpole life (see below). Expression in the thymus was found on cortical thymocytes, similar to CD1 and TL in mammals, suggesting that there might be positive selection of NKT cells on these thymocytes as is found in mice (37, 48). *XNC10* tetramers were made in insect cells and used as reagents to identify potential specific T cells (49). Indeed, a population of cells stained with these reagents had the hallmarks of NKT cells including an invariant TCR alpha chain. These T cells participated in a viral-specific response.

NKT cells and amphibian development

It has been known for a long time that the classical MHC molecules are differentially expressed during development in *Xenopus* (50, 51). Class II molecules are found on T cells, B cells, and APC in adults, but only on B cells and APC in larvae (52). Furthermore, class Ia expression is very low, to non-existent, on most larval tissues (51, 52). It was suggested that larvae might only use class II during larval life and add class Ia-mediated functions during adult life. Now Robert (53) has found that a large proportion of larval T cells bears invariant TCR α chains, suggesting that the larval immune system could make do primarily with NKT cell recognition of class Ib (or other ligands). This makes sense as lymphocyte numbers are much lower in tadpoles as compared with adult frogs, and thus the T and B-cell precursor frequency is quite low as well. For B cells, the smaller repertoire can be accounted for by the lack of N-region addition in Ig rearrangement junctions (54). Now, for T cells the answer may be that larvae use NKT-like TCRs of limited diversity to maintain a useful precursor frequency (53). Interestingly, like class Ia, *Imp7* is expressed at low amounts during larval life (46), suggesting that typical class Ia ligands are not generated, perhaps paving the way for production of novel ligands for class Ib molecules.

Summary of part 2

Unambiguous identification of NKT cells in amphibians shows that these cells are not evolutionary latecomers and they probably will be present in all vertebrates. Class Ib molecules are found in all ectotherms, with some old lineages (5, 55–57). Some species, like axolotl (58) and cod (59), have greatly expanded their class Ib gene families, suggesting they might be excellent candidates for recognition by large NKT repertoires; cod has lost its class II system *in toto* and axolotl class II molecules are minimally polymorphic (60), further suggesting that NKT cells might be preeminent in these ‘immunodeficient’ animals.

Generating tetramers with these class Ib molecules *à la* J. Robert should permit detection of NKT cell subsets. Additionally, expression studies to determine whether the class Ib molecules are expressed by cortical thymocytes might also provide an indication of whether they will be found as NKT ligands (most *Xenopus* XNC genes are expressed in the thymus (44;46)). Study of such class Ib molecules may lead to discoveries of other PAMPs, like the vitamin B presentation by MR1 (61). In summary, Robert's recent work has provided one of the most exciting findings in the field of comparative immunology, likely to encourage much future study.

Natural cytotoxicity receptors, B7 molecules, and MHC

Natural killer (NK) cells are involved in elimination of virally infected and transformed cells, and class Ia is used for their surveillance (reviewed in 62). Since class Ia is expressed by almost all the cells of the body, its presence on the cell surface indicates a healthy condition. When cells are stressed, class Ia is often downregulated, and its absence is detected by NK cells as 'missing self,' resulting in the elimination of stressed cells by direct killing or indirectly via cytokine production. Elimination of stressed cells can be also done using a subset of stress-induced class Ib molecules (e.g. MICA, MICB), which are upregulated upon infection or oncogenic transformation. These molecules are detected by different sets of NK receptors (e.g. NKG2D for MICA, MICB binding). Upon engagement, NK cells are stimulated via activating NKR and eliminate the stressed cells. Inhibitory signals are generally dominant over activating signals, but under stressed conditions activating signals can override the negative signals. Generally speaking, NKR are constrained by rapidly evolving pathogens and the highly polymorphic MHC, and therefore are extremely diverse and highly plastic: species-specific expansion and contraction of certain NKR are commonly seen. *Xenopus* KIR-related genes have been previously identified (63) and shown to be upregulated at the cell surface upon co-transfection with FcR γ (64). The function of these KIR-related genes needs to be examined to confirm their relationship to the mammalian genes. Generally (except as detailed below), it is impossible to identify orthologous NKR between different vertebrate taxa (65).

NKp30

Besides the above mentioned conventional NKRs, there are unconventional NKR. Among them are natural cytotoxic receptors (NCRs), NCR1-3, also called NKp46 (66, 67), NKp44 (67), and NKp30 (68), respectively. All NCR genes are activating NKR and expressed by NK cells, subsets of T cells (69), and innate lymphocytes (ILC) (69, 70). NKp44 is induced upon stimulation, while NKp46 and NKp30 are constitutively expressed, although they can be induced by treatment with IL15 and/or IL2 (69). NKp46 has two Ig-like domains of the C2-type, which are commonly found in FcR and other NKR such as KIRs. NKp46 indeed maps to the LRC on human chromosome 19q13, near the KIR genes, and thus shares a common ancestor with KIRs. Both NKp44 and NKp30 have single V-type IgSF domains and map to the MHC on human chromosome 6p21. Our phylogenetic analysis showed that they cluster as a sister group (71). While NKp46 is found only in mammalian species, NKp44 is also present in carp (NILT) in addition to mammals, suggesting NKp44 is evolutionarily older (72). However, NKp44 has not been found so far from other fish

species. In contrast, NKp30 was identified from almost all jawed vertebrate species including cartilaginous fish, making NKp30 the most conserved and evolutionarily the oldest NKR (71). Furthermore, the V domain of NKp30 is unique, the so-called VJ-type, which does not undergo somatic rearrangement like the rearranging antigen receptors yet has the basic structure seen in Ig and TCR V domains (73). This type of V domain is considered to be ancestral (see Fig 2), predating antigen receptors and presumably invasion by the RAG transposon (74). It has been proposed that ancient ‘killer cells’ used receptors of this VJ-type and such killer cells scanned for foreign or stressed cell and eliminated them. Later in evolution, the insertion of RAG transposon split the VJ exon into separate V and J fragments, followed by recruitment of C1 domains to generate modern antigen receptors (75). NKp30 maps to the MHC where C1-type IgSF domains of MHC class I and class II are present. Finding a gene with VJ domain in the vicinity of C1 domain-containing MHC genes is likely not a coincidence and we predict that it is the primordial linkage state (Fig. 2). So far, the synteny of *NKp30* genes with any other gene is not known in the oldest jawed vertebrates, cartilaginous fish, but (at least) we predict linkage of shark *NKp30* to the MHC.

NCRs recognize virus-derived molecules such as HA from influenza, glycoprotein from vaccinia virus, and pp65 from HCMV (69). They also bind to self molecules such as BAT3 (76) [also known as BAG6 (77)] and PCNA (78)). Binding to ligands relays activating signals via ITAM-containing adapter molecules (e.g. CD3 ζ) associated in the cytoplasmic regions (79). NKp44 and NKp30 are also associated with DAMPs, which are often upregulated in cancer cells (69, 78). B7H6 is one of the known ligands for NKp30 which becomes upregulated in transformed cancer cells (80), and engagement of B7H6-NKp30 activates NK cells to kill B7H6-expressing transformed cells. This situation is reminiscent of the hypothetical function of ancient killer cells mentioned above. Recently, NKp30 binding was required for clearance of fungal infection such as *Cryptococcus neoformans* (81), and *Candida albicans*, pathogens that activate signaling via phosphatidylinositol 3-kinase (PI3K) and perforin release (82). These findings suggest that NKp30 also may function as a pattern recognition receptor. Indeed, the diverse V-IgSF domains in NKp30 genes of lower vertebrates indicate that they may be binding to broad array of pathogens in addition to self-derived B7H6 (69). Thus, NKp30 may have originated to bind foreign pathogens and later recruited to perform new functions such as immune regulation. Another example of this phenomenon may be the SLAMF genes that function in immune regulation (83), but are also known to bind to foreign antigen, perhaps their ancient function (84).

B7

The B7 family consists of about 10 genes with similar domain structure (85;86). The N-terminal most membrane proximal domain is V-type IgSF and followed by zero-two C-IgSF domain(s) (most B7 genes have one), and transmembrane domains with cytoplasmic tails with no known signaling capacity. The V domain of B7 ligands is used for the engagement with their receptors that leads to either activation or inhibition. Receptors for B7 molecules contain amino acid motifs in their cytoplasmic tails that signal differentially. Immune regulation by B7 ligands, and other systems such as TNF and SLAMF family members, are indispensable features of the immune system. As described above for NK cells, the balance between activation and inhibition influences the outcome of immune reactions. In mammals,

B7.1 and B7.2 (also known as CD80 and CD86) were the first to be discovered (87) and their functional capacity to activate naïve T cells is well characterized. The functions of more recently discovered B7 family members like B7-H1, B7x, and B7-H6, however, are poorly understood (88).

Studies of B7 molecules in lower vertebrates (89) have been mostly done in bony fish. It has been hypothesized that the bony fish B7R represents an ancestor of B7.1 and B7.2. Like B7.1 and B7.2, B7R is expressed in lymphocytes, upregulated upon LPS-stimulation leading to cytokines like IL2 (90) as well as their potential receptor CD28 (91), more recently in B cells (92). Functions of other B7 family were examined for B7H1/DC, B7H3, and B7H4 in *takifugu* and demonstrated their expression on monocytes, and T-cell activation or inhibition by measurement of cytokine production (93). All data in fish are so far consistent with B7 function in mammals. Since sharks possess most of B7 genes (TNF and SLAMF genes as well) except B7.1 and B7.2 (71), we expect that complex immune regulation existed at least 500 million years ago.

The distribution of most B7 genes in the human genome revealed an ancient connection to the MHC: B7 genes map to the MHC paralogous regions that were generated via the two rounds of genome duplication (2R), which occurred at the origin of vertebrate evolution (94). Phylogenetic analysis revealed that V-IgSF domains of various B7 members most resemble the V domain of tapasin (TAPBP), and B7H6 seems to be most like TAPBP (86). In addition, the C-IgSF domains of B7H6 are most divergent, suggesting that B7H6 may be the most related to the precursor of the B7 family (71). Furthermore, TAPBP (95) maps to MHC and is a functional component of MHC class I peptide loading complex. Therefore, ancestral B7 genes were likely in the precursor of the MHC (Fig. 2); as mentioned, finding B7 family members in MHC paralogous regions also is consistent with B7's connection to the MHC (12, 13). In the primordial MHC, TAPBP and B7 may have been generated from a shared common ancestor. So far, no B7-like genes have been found in the jawless vertebrates, lamprey and hagfish, or in lower chordates such as amphioxus. Thus, the emergence of B7 perfectly coincides with the presence of TCR, Ig, and MHC (96).

B7 and NKp30: an evolutionarily old NK system

NKp30 and B7H6 are both polygenic in lower vertebrates, and the number of genes of both, or the total absence of both, correlates perfectly, strongly suggesting co-evolution between NKp30 and B7H6 throughout vertebrate evolution (71) (Table 1). In most mouse species, B7H6 is entirely deleted from the genome, while NKp30 is a pseudogene by mutating one nucleotide to a stop codon, resulting in a premature stop codon (97).

Despite the evolutionary conservation of NKp30, we were puzzled that the *Xenopus* *NKp30* genes unexpectedly mapped outside the MHC (71). However, their homologs based on domain structure, the *XMIV* genes, are found at the exact location in the *Xenopus* MHC class III region where *NKp30* is found in the mammalian MHC (30). Like NKp30, XMIV contains VJ-type IgSF domain, followed by transmembrane and cytoplasmic tails. Although the V domains of NKp30 and XMIV did not co-cluster in phylogenetic trees, the recent *Xenopus* genome analysis revealed that *NKp30* mapped to the MHC paralogous regions

(manuscript in preparation). Therefore, we predict that NKp30 and XMIV are derived from a common ancestor and their root may predate the emergence of vertebrates.

The cytoplasmic tails from both *Xenopus NKp30* and *XMIV* genes are either long with ITIMs (98) or short with positive amino acids which may interact with ITAM-containing adapter molecules (79). This is a canonical feature of mammalian NKR, and suggests that NKp30 and XMIV are likely prominent in the *Xenopus* NK system.

There are fewer *XMIV* genes than *Xenopus NKp30* and *B7H6*, suggesting that XMIV may not bind to B7H6. Since *XMIV* genes map in the MHC, *XMIV* may rather co-evolve with MHC class Ia, like mammalian NKR. Such a linked receptor and ligand system is also found in the mammalian and bird NK systems (99–103). One puzzling question is how an inhibitory NKp30-B7H6 system functions in NK cells. Perhaps some B7H6 molecules are expressed ubiquitously and others are inducible, and receptors for ubiquitous B7H6 ligands will be activated by missing-self. We hypothesize that the B7H6-NKp30 system in lower vertebrates will reveal key features of modern NKR biology, especially in ectotherms.

Summary of part 3

Mouse and human NK cells use different families of NKR in their recognition of class Ia molecules, which set the precedent for NKR evolutionary studies. It has been nearly impossible to detect orthologous NKR across different vertebrate classes (65). Thus, it was a surprise to find that one particular NKR, NKp30, is evolutionarily conserved. Furthermore, the correlation between the numbers of genes for *NKp30* and *B7H6* is consistent with the discovery by Vivier and colleagues (80) that B7H6 is a stress-induced ligand for NKp30. Obviously less well understood is how NKp30 can be the receptor for B7H6 on one hand and recognize PAMPs on pathogens (like fungi) on the other! The study of the NKp30 genes that have been expanded in other species may be informative on this issue.

Conclusions

We have accumulated evidence that all of major families of genes of the adaptive immune system have their origins in a precursor immune complex (Fig. 2). Studies of homology and comparative analyses of vertebrate MHCs and MHC paralogous regions have suggested that the MHC, LRC, and NKC were originally in a syntenic region (1). The presence of C1 domains in class I and class II as well as the antigen receptors suggested, long ago, their divergence from a common ancestor, and the findings of VJ genes further supports the hypothesis. Studies of synteny in many vertebrates also strongly suggest a common origin for antigen receptors and class I/II (Ohta and Flajnik, unpublished data). NKR of the VJ and C-type lectin classifications are found in amphibians and birds, respectively, consistent with receptor-ligand pairs being encoded by linked genes. Furthermore, in *Xenopus* there is good evidence for C2 domain-containing IgSF molecules similar to KIR or FcR associated with the MHC (Ohta and Flajnik, unpublished data). The finding of B7-related genes in MHC and in MHC paralogues also suggests their origins. It has been known for a long time that TNF superfamily molecules are associated with the MHC (104), so that both TNF and IgSF costimulatory molecule families have their origins in the proto MHC.

The finding of *Imp* and *TAP* linked to the MHC in the early 1990s was unexpected and game-changing (14). Studies of non-mammalian vertebrates, especially chickens, have shown with little doubt that coevolution [and also coregulation (105)] were driving forces in the class I presentation system (3). We think such an idea can be extended to NKR (and eventually antigen receptor) recognition of class I, as well original interactions among other surface receptors such as molecules known as costimulators today (12).

Still unidentified after all these years is the emergence of the ever-elusive class I/II peptide-binding region (PBR). We thought that, by now, with the ever accruing crystal structures, there would be candidates for the original PBR. Keep looking immunofriends!

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Fig. 1. Original proposal for the location of class I, class II, class III, and antigen-processing genes in all vertebrates

Most non-mammalian vertebrates show a close linkage of *Imp*, TAP, and class Ia genes. Translocation of the class Ia genes to the 3' side of the class III region likely relaxed the coevolution of the processing and presenting genes in mammals (3). Green: class II, Gray: TAP, Pink, immunoproteasome or *Imp*, Red, class I, Dark gray, class III region.

Fig. 2. Hypothetical view of origins and coevolution within the MHC

In the forerunner of the MHC, we predict that ancestral genes with domains found in extant molecules (IgSF: V- and VJ-, C1- C2; TNF, PBR and C-type lectin families are shown in this figure). Early in vertebrate evolution, those gene fragments were ‘mixed and matched’ generating precursors of all modern immune genes involved in adaptive (and some innate) immunity. We further predict that many receptors and ligands were linked to each other and coevolved (some still are). Proteasome and (perhaps) transporter genes were also closely linked to the precursor MHC class Ia gene. Indeed, proteasome genes had been found in proto MHC in many invertebrate species, and the presence of lineages in many non-mammalian species suggests that they co-evolve with class Ia. Antigen receptors (AgR) are composed of VJ and C1 domains, like those found in NKp30 and class I/class II/tapasin, respectively. The C-type lectins class of NKR encoded in the NKC of mammals are still found in avian MHCs. B7 family members are present in MHC paralogues in many vertebrates, and are closely related to TAPBP. C2-domain containing molecules like KIR and FcR are MHC-associated in several ectotherms. Transporters are predicted to have been attracted to the MHC by ‘functional clustering,’ as homologues are not found in paralogous regions. Origins of the PBR: still a mystery.

Table I

Evolutionary conservation of NKp30 and B7H6 in vertebrates

	Human	Mouse	Rat	Opossum	Chicken	Xenopus frog	teleost Fish	Coelacanth	Shark
B7H6		---		---	---		---		
NKp30		pseudo		---	---		---		
NKp44				---	---	---		---	---
NKG2D					---	---	---	---	---
KIR				( ) ²⁴	---	?	---	---	---
LY49	pseudo			---	---	---	---	---	---

: single copy;   : multigene family; --- absence of gene; not found in genome/EST projects