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Review

The immune system of invertebrates and vertebrates ☆

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1. Introduction

All metazoans protect themselves from invasion of microorganisms, parasites, viruses and even cells from individuals of the same species. In all phyla, precise mechanisms of recognition allow for discrimination between self and non-self avoiding the danger of contamination. In that sense, all metazoans have an 'immune system'. This does not mean that the recognition events and the resulting effector reactions are mediated by homologous systems across metazoans. Some features may be conserved while some will be specific to one phylum or even one class within a phylum (Fig. 1 and review in Du Pasquier and Flajnik, 1999). This overview will summarise both the conserved and the divergent aspects of the various immune systems of metazoans throughout their history ultimately revealing the unique characteristics of the immune system in vertebrates.

2. Immune systems: Specific or non-specific? Innate or acquired? Somatic or germ-line? Polymorphic or not?

Before any response can be elicited, recognition of an external signal must be achieved. The initial step occurring on the surface of metazoan cells can be found under the term 'self-nonself discrimination'. They can be of many types and obey different principles. Are alien cells or substances simply recognised as being 'non-self', or are various 'non-self' recognised separately? Among all the recognition events, the recognition of allopolymorphic determinants on the surface of cells has fascinated immunologists for many years. It leads to histocompatibility reactions from sponges to vertebrates [(Humphreys and Reinherz, 1994; van de Vyver, 1975) reviewed in Du Pasquier and Flajnik, 1999]. Unfortunately, the recognition events in these reactions are not well understood in invertebrates and there is not yet a way to compare the structures and the sequences of all receptors involved in allorecognition from sponges to mammals.

An immune response is the result of a cascade of multiple convergent events leading, in the best scenario, to the elimination of the 'signal' that elicited the recognition reaction. The multiplicity of mechanisms can be great within one individual, within the various classes or within the phyla (Table 1). This multiplicity is a consequence of the importance of the task to be accomplished. It is rare that an important function is achieved by a single mechanism within an individual, as there are often fail-safe alternatives.

As opposed to vertebrates, invertebrate responses whether local or systemic, do not involve a clonal amplification of the cells producing a given effector molecule. The production of a given an-

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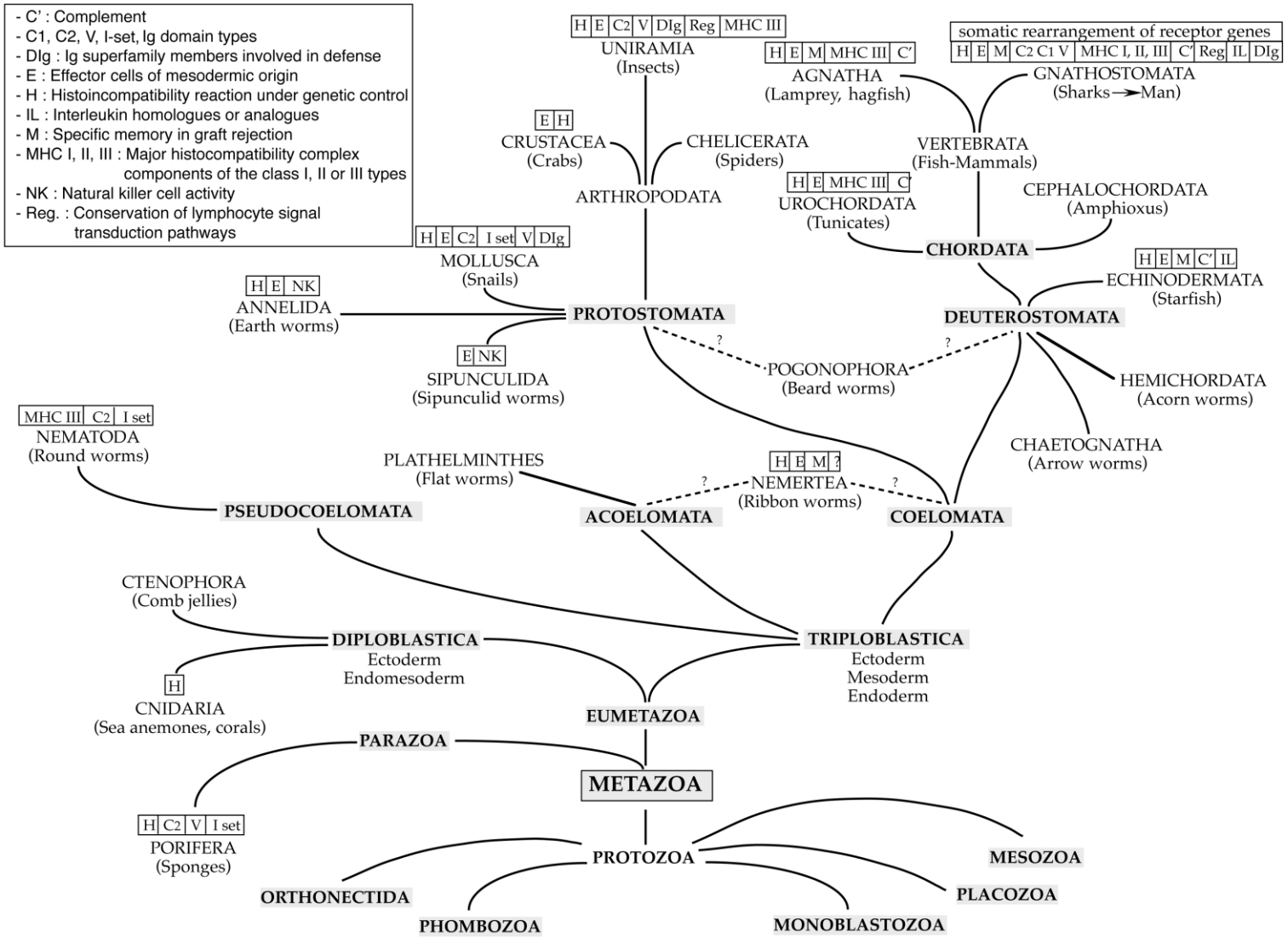
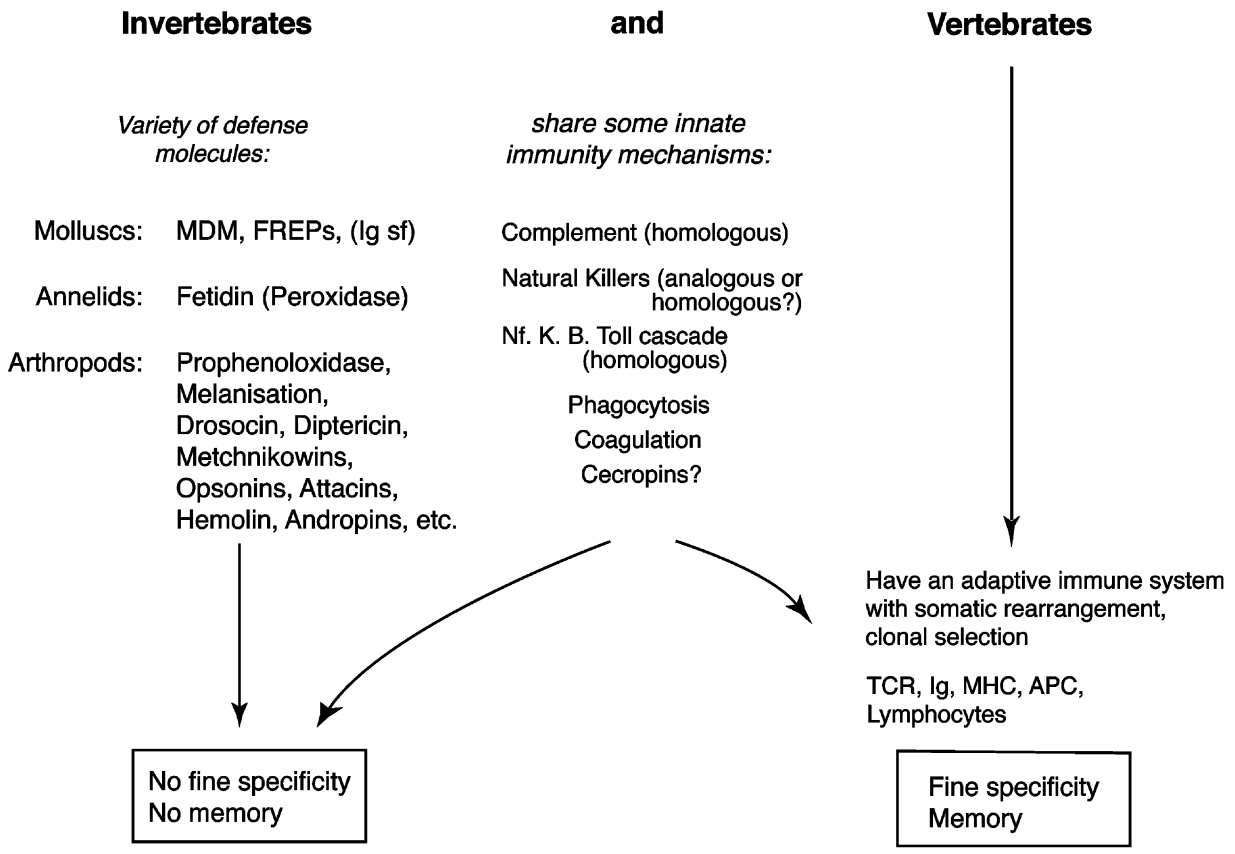


Fig. 1. Elements of the immune system of vertebrates. The Ig domain, C1, C2 and V domains, and the I-set correspond to the four types of domain. V domains are made of two β-pleated sheets, each consisting of four strands. The C2 domain and I-set have one β pleated sheet with three and four strands, respectively. The C1 domain, encountered only in vertebrates, has two β sheets made of three strands each. Complement component genes C′ have been cloned in echinoderms and urochordates. Activity has been known for a long time in echinoderms. Reg. Stands for 'regulation', and conservation of various activation cascades Toll, NF-Kappa B.



Scheme 1. Comparison of vertebrate and invertebrate immune systems.

timicrobial agent is amplified by regulation of transcription and there is usually no memory (Hultmark, 1993; Meister et al., 2000). On the contrary, clonal amplification of cells carrying a unique receptor (generated somatically) is at the core of the vertebrate immune system (Burnet, 1957; Tonegawa, 1983). Yet despite this important distinction, the different metazoan immune systems taken as complex ensembles do share certain elements between one phylum and another (Scheme 1). However, all the mechanisms conserved between invertebrates and vertebrates are related to innate immunity (Hoffmann et al., 1999). Phagocytosis for instance is conserved throughout metazoa. In addition, vertebrates have kept some cascade of events involved in inflammation that can be traced to protostomian invertebrates and perhaps even to plants. Signalling to the nucleus is the area where conservation is encountered. NF-kappa B/Rel transcription factors are central regulators of mammalian immunity and are also implicated in the induction of cecropins and other antibacterial peptides in insects. The NF-κB pathway originally discovered in B-cells has now been found in various insects where it is active during embryonic development and during differing immune responses (production of immune molecules in *Drosophila* and in the silkworm) (Engström et al., 1993; Lemaitre et al., 1996; Sun and Faye, 1992). To a certain extent, the Toll cascade, originally discovered in *Drosophila,* (Rosetto et al., 1995) has now been found in plants through vertebrates. However, the 'external' recognition events themselves, are not necessarily conserved (Medzhitov et al., 1997; Wilson et al., 1997). The presence of Rel proteins in *Drosophila* indicates that similar proteins were likely present in primordial immune systems and may serve as unique signalling functions. Different pathways can regulate different types of immunity (to bacteria or to fungi) thereby bringing some specificity in the responses that somehow compensate for the lack of an adaptive response. For instance, the kappa B motifs of the diptericin and cecropin genes are not functionally equivalent (Gross et al., 1996). In the same context, a

striking aspect of many vertebrate immune systems is the exceptionally high level of polymorphism they harbour. This polymorphism is probably driven by the diversity of pathogens that face selective pressures to evade attack by the host immune system. Are innate defence mechanisms, which use proteins with broad-spectrum bactericidal properties, going to be submitted to the same pressures? After all, pathogens will evolve resistance to insect defences however broad in specificity they are. Andropin and diptericin genes have patterns of variation that differ significantly from neutrality. Cecropin genes, however, are not exceptionally polymorphic (Clark and Wang, 1997).

The complement is a group of soluble proteins that complex foreign cell surface components and are capable of destroying those cells after the elaboration of a membrane attack complex. There are three pathways of complement activation: the classical pathway; the alternative pathway; and the lectin pathway (review in Sunyer and Lambris, 1998). The alternative pathway and the lectin pathway that do not involve the binding of complement components to an antigen–antibody complex exist in invertebrates. The echinoderm, or prochordate molecules, that are involved and that have been characterised so far (C3, factor B) are homologous to their mammalian counterpart (review in Nonaka, 2000). In the GenBank, there are many insect and nematode sequences belonging to this family. At this point it is difficult to decide whether they are related more to the proteinase inhibitor alpha 2 macroglobulin than to the complement components themselves. Whatever the circumstances, the origin of this family is ancient.

In general, invertebrates use a large diversity of bactericidal and fungicidal molecules even within a single species. Some of them are apparently conserved in vertebrates like cecropin consisting of little hydrophobic 'wedges' able to lyse bacteria. Cecropin, a family of antibacterial molecules, was originally described in the silkworm (Boman, 2000) but its homologous genes have now been found in *Drosophila* (Samakovlis et al., 1990), in *Anopheles* (Vizioli et al., 2000), in molluscs (accession number AF134472), in urochordates (Zhao et al., 1997) and in pigs (Lee et al., 1989). Cecropin related molecules could have evolved from ribosomal protein L1 of an ancestral intracellular pathogen that developed into a symbiont ending as an organelle (Putsep et al., 1999).

3. Uniqueness of the vertebrate immune system

The specific immune reactions of vertebrates start after an initial phase of inflammation involving the components of innate immunity. Specialised cells expressing the two main categories of major histocompatibility complex molecules class I and II achieve a complex processing of the antigen. Proteinic antigens, whether produced or engulfed by the cells, are processed and chopped into pieces. These pieces (on average, 9–12 amino acids long) are bound to the grove of the MHC molecule (most of the time class I for internal products, and class II for external products). This processing results in an appropriate presentation of the antigenic epitopes to the lymphocytes carrying the specific receptors (reviews in Paul, 1999). The immune system of vertebrates is unique because the antigen specific receptor, the *primun movens* in the cascade, is not the product of germ-line inherited complete genes. In fact, the receptors (one type per cell clone) are generated somatically during the ontogeny of the lymphocytes from segments of genes scattered in the genome. Vertebrates inherit a 'do-it-yourself kit' to be assembled and expressed in lymphocytes during ontogeny. The receptors are members of the immunoglobulin superfamily (Igsf) and are made of variable domain (V) and constant domain (C). The variable domains are generated somatically (reviews in Paul, 1999). In vertebrates, the constant domains associated with the receptors are all of the rather rare C1 type which is also shared by the MHC class II and class I molecules (Du Pasquier and Chrétien, 1996).

4. Origin of the receptors and of the rearranging machinery. The building of the immune system coevolving unit receptors / MHC

In innate immunity components, real cases of conservation can be encountered (see above). Whereas for the origin of the rearranging receptors, lineages have to be more subtly traced back through the phylogeny since the exact receptors themselves do not exist (Fig. 1 and Fig. 2). Without focusing systematically on genes expressed in

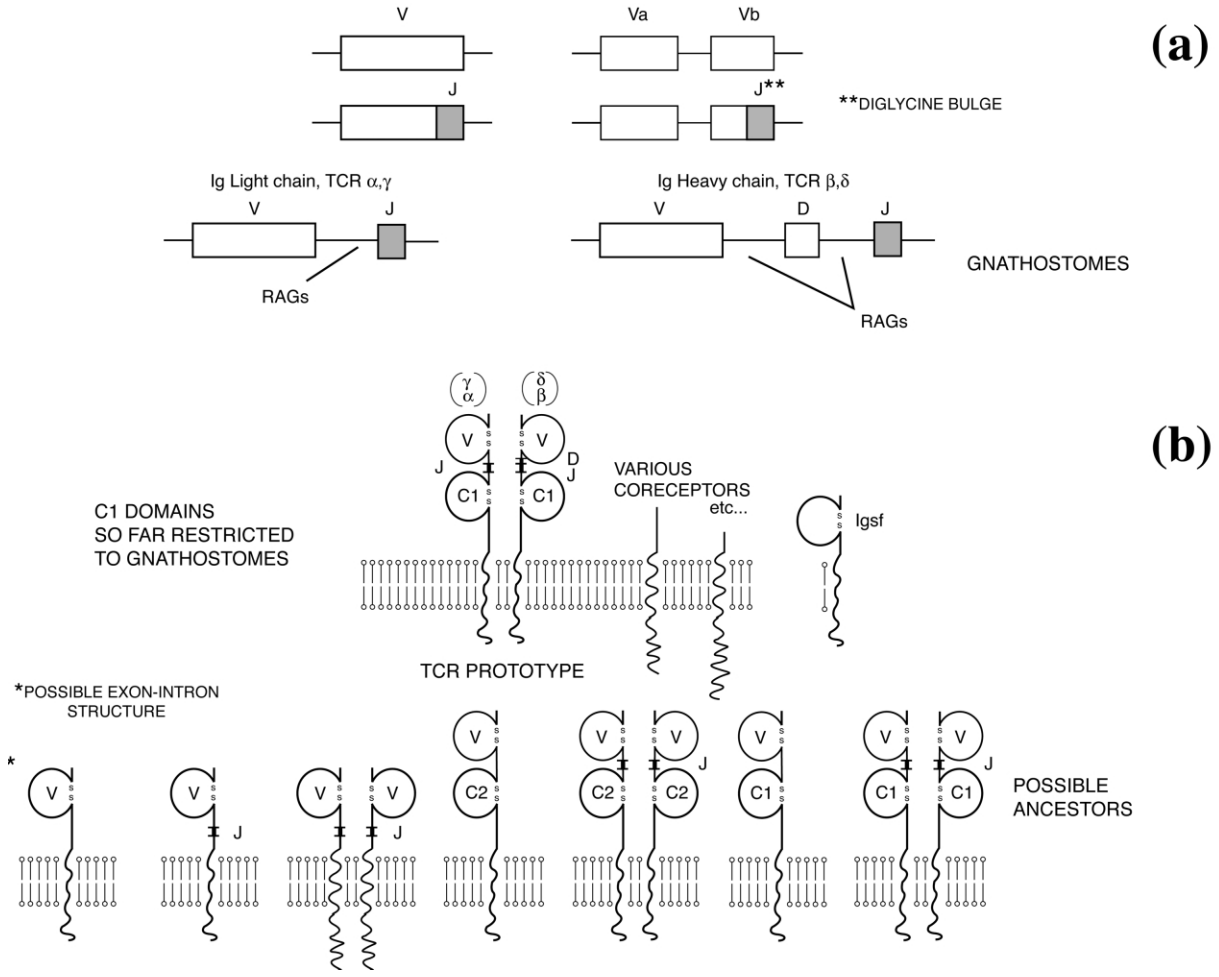


Fig. 2. Potential evolutionary pathways leading to the Ig or TCR. *Abbreviations*: V variable; J: joining segment; D: diversity segment; RAG: rearrangement associated genes; C: constant; Ig: immunoglobulin; TCR: T-cell receptor. (a) Generation of somatic rearrangement from Ig domains of the V type. The introduction of what nowadays behaves as a site sensitive to the RAG enzyme is supposed to have occurred via the introduction of a transposon (Rougeon, 1986; Thompson, 1995). (b) Typical configuration of a lymphocyte receptor and its possible precursors.

the immune systems, the most homologous sequences and gene architectures in the various metazoan phyla must be found. Perhaps the receptor ancestors were recruited from a pool of genes involved in other functions with a different tissue distribution (Du Pasquier, 2000a).

Somatic rearrangement is due to RAG1 and 2 (rearrangement associated genes1 and 2) enzymes. They are found only in gnathostome vertebrates. All putative homologues among DNA repair enzymes that one may find in other metazoan phyla (that do not have an adaptive immune system) or in the more primitive phyla of monocellular organisms, are far removed from each other. It is as if a 'horizontal' acquisition of a transposon had occurred at some point during the history of the vertebrates (Thompson, 1995). The receptor gene structure itself, in the region where the rearrangement takes place, suggests the introduction of a transposon. The recombination signal sequences flank the gene segments (V and J in the light chain for instance) in the same way as the LTR does in a transposon (Hansen and McBlane, 2000). In principle, RAG activity is confined to lymphocytes but the existence of germ-line rearrangements in light chain genes in the shark suggests that RAG (Lee et al., 2000) is still an active force changing the genome in some vertebrates. As the enzyme itself is not a good subject for phylogenetic analysis, one must turn to the history of the substrate i.e. the VC core of the receptor. Possible features of the immediate

or less immediate ancestor of the dimeric receptor of vertebrate are depicted in Fig. 2. The G strand of the V domains of Ig and TCR is encoded by a segment separated from the rest of the strands, A, B, C, D, E and F, and needs this rearrangement in order to build up a complete V region. In the ancestor that does not rearrange somatically, the G stand was probably an integral part of the V exon. Other introns might have penetrated the V domain creating a variety of V gene families (Fig. 2). There are many examples of this occurring in the history of the Ig superfamily, in the genes encoding CD4 and CTX, for instance (Du Pasquier, 2000a). The G strand of TCR and Ig provides another feature of interest for rearranging receptor phylogeny. A diglycine bulge, present in all cases, is thought to be either a beneficial adaptation or a cause for the dimeric nature of the receptors (Chothia et al., 1985). Therefore, monitoring this feature might reveal genes that had the ability to create a dimer similar to that of modern antigen specific receptors.

5. The V and VC 1 segments in evolution

The V domains recognise the antigenic epitope, and are therefore, the most important element for recognition. For this reason, it will be the first to be traced back in metazoan evolution by asking whether V domains exist in invertebrates. Domains with the typical V fold, whether true V set members or members of the I-set (Harpaz and Chothia, 1994), are now found in sponges to insects although they may not be necessarily involved in immune reactions. Among the molecules involved in the differentiation of the nervous system in invertebrates (e.g. amalgam, lachesin and fascicilin), the first ones were in fact, discovered by non-immunologists (reviewed in Du Pasquier, 2000a). Fig. 3 provides a summary of these molecules. Invertebrates also use Ig sf members in immunity but so far these molecules involved are not real V domains, but more I-set or C2, MDM (Hoek et al., 1996) and hemolin (Su et al., 1998; Sun et al., 1990). However, FREPS molecules produced by the mollusc *Biomphalani,* have a reasonable looking V domains at their distal end. They are involved in antiparasitic reactions (Adema et al., 1997). Fig. 3 further shows that none of the encountered molecules present a C1 domain. Up to now, these are restricted to gnathostomes, as though they were generated at the same time as the rearranging immune system and coevolved with it.

How does one attempt to find a non-somatic rearranging VC1? Perhaps some 'palaeontology' in the vertebrate genome itself will help. This would mean surveying the largest amount of data available, the human genome (Fig. 4). Indeed V, VC1 or C1 alone, can be found. Interestingly, many of them are encountered in the MHC class III region (human chromosome 6p21) or its paralogues (9, 12, 19, 1). Butyrophilin, CD83 and taDu Pasin all have a V domain and some have a C domain considered close to C1 (Abi Rached et al., 1999). More distant relatives with VC2 based architectures are present in this region as well, (RAGE, CTX) (Du Pasquier et al., 1999; Sugaya et al., 1994). The presence of several genes, related to those of rearranging receptor ancestors, on several paralogues suggests that the V-C1 core was generated early in vertebrate evolution subsequent to the emergence of the chordate superphylum. Among all these molecules, let us try to refine our quest. Butyrophilin is perhaps not on the direct track to antigen specific receptors. Its C domain, although proven to be C1 through its crystalline structure (Ikemizu et al., 2000), is more like a C2 at the primary sequence level, and belongs to the CD80/86 family, rather than the TCR. With this type of domain we might witness the building of the C1 domain. It would be interesting to discover its paralogues should it have any. So far, I detected only one (accession AL109659.20 | HSJ1024N4). Its location is on chromosome 1p32–33, a putative paralogous region of the MHC (Kasahara, 1999).

With respect to the variable domains, two gene segments stand out: a single V named NKP30; and a gene containing a VC1 core, tapasin, coding for a molecule involved in antigen processing (Ortmann et al., 1997).

NKP30, made of a single Ig domain of the V type, is an activating receptor of natural killer cells (Neville and Campbell, 1999; Pende et al., 1999). It is of special interest, as it offers a link to a cell type encountered (analogue or homologue?) in invertebrates (Boiledieu and Valembois, 1976). Its ligand is unknown. It could be a relative of an ancient receptor, distinct from the inhibitory receptor, whose history is linked to the emergence of MHC class II and class I. In order to resemble a probable ancestor, the V domain of NKP30

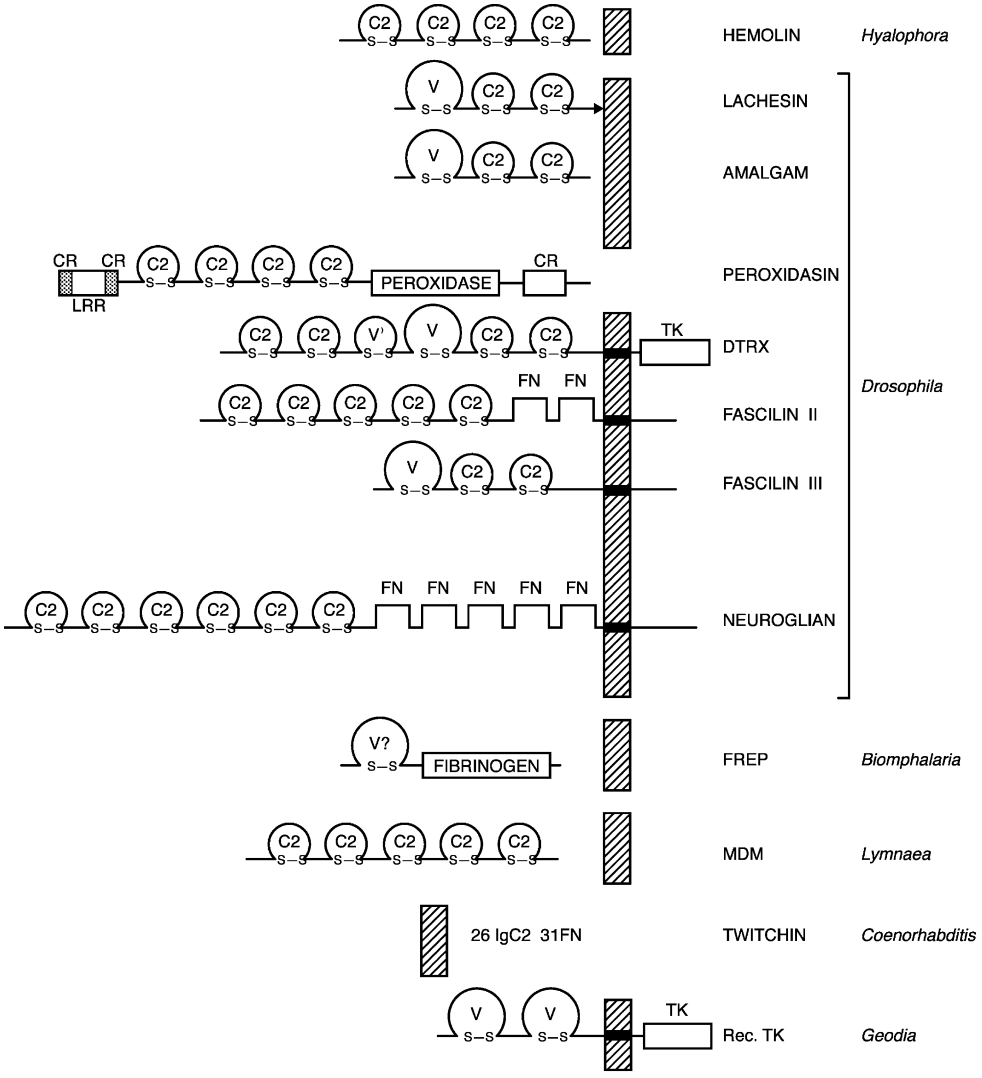


Fig. 3. Immunoglobulin superfamily V-C2 domains in invertebrates. *Abbreviations*: TK: tyrosine kinase; DTRK: *Drosophilia* receptor tyrosine kinase; FREP: fibrinogen related protein; MDM: mollusc defence molecule; FN: fibronectin; RecTK: receptor tyrosine kinase; CR: cystein rich; LRR: leucin rich region. Hemolin, MDM, and FREP can be involved in 'immune' responses. V domains defined by their strand composition (predicted from primary sequences with computer programs) are ancient and not necessarily linked to an immune function. The hatched boxes represent the cell membrane. The twitchin is entirely intracellular.

would need only to be hooked up to a C1 single domain. In fact, a C1 single domain is also encountered in this region of the genome, the preT-cell receptor alpha (Fehling et al., 1995). In silico, the two form a structure with similarity to the remaining VC1 gene encountered within tapasin genes. Aside from Ig and TCR, taDu Pasin is one of the rare cases, if not the only other case, of a gene segment with a VC1 structure existing on several paralogous linkage groups. In other words, while this gene is related to the rearranging receptor structure, it is undoubtedly very old. It predated the gene duplication that led to the gnathostomes. Another set of molecules, the SIRPs also have a distal VC1C1 segment as does the Poliovirus receptor (VC1 C2) (both reviewed in Du Pasquier, 2000a) related sequences. They represent another group that could be linked

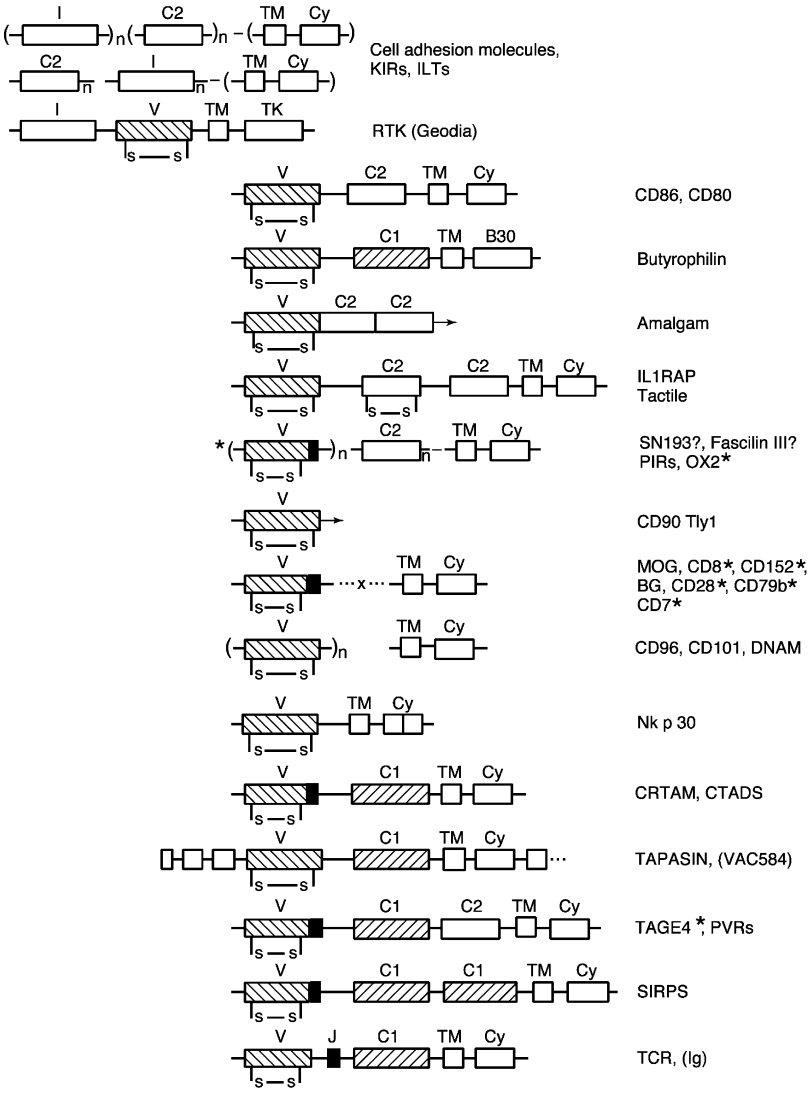


Fig. 4. Molecules with V domains. The various architectures of the Igsf members and genes with V domains not generated by somatic rearrangement compared to TCR. The molecules are classified in function of the intron-exon organization or the composition, i.e. their association with I-set C2 or C1 domains. Only examples are given. Asterisk: molecules with G strand resembling a J segment with a diglycine bulge; Black: J segment-like sequences; KIR: killer inhibitory receptors; RTK: receptor tyrosine kinase; IL1 RAP, IL1 receptor accessory molecule; FREP: fibrinogen related protein; Tage4: tumor associated glycoprotein E4; SIRP: signal regulatory protein (Kharitonenkov et al., 1997); DNAM: DNAX accessory molecule-1 expressed on peripheral lymphocytes (Shibuya et al., 1996); ILT: Ig-like transcripts (Cella et al., 1997); PVR: poliovirus receptor; TM: transmembrane; Cy: cytoplasmic domain; MOG: myelin/oligodendrocyte glycoprotein (Linington and Lassmann, 1987); PIR: paired Ig-like receptors (Kubagawa et al., 1997).

to the history of the Ig and T cell receptor (Du Pasquier, 2000a,b).

6. A possible scenario leading to the immune system of vertebrates

A scenario emerges that places the generation of somatically rearranging receptor genes at the top of the cascade of evolutionary events that lead to the shaping of the vertebrate immune system. In the following, I have assumed that variation precedes selection as in most phylogenetic pathways. What is probable to have originally occurred was the introduction of somatic variability into a gene segment that was presumably derived from one of the above mentioned V-C1 ancestors, and present in the MHC

class III region. This region is apparently ancient since conservation of the linkage of several of its loci is visible from *C. elegans* to vertebrates (Kasahara, 2000; Rast et al., 2000). It was already involved in some aspects of innate immunity as the MHC or its paralogues contained conserved homologous complement components (C4, C3, C5, and alpha2 macroglobulin), some of which exist in echinoderm and prochordates, in linkage with factor B (review in Nonaka, 2000; Rast et al., 2000).

Although somatic rearrangement offered an impressive repertoire of recognition structures, it was a 'Damocles Sword', as with the random generation of diversity, anti-self structures might be generated. This created an immediate need for selecting the useful, and discharging of the dangerous. The MHC molecules (in this model, the class I, ubiquitously expressed and dealing with self-products came first) were recruited in the immune system and within the MHC core where the ancestors of the receptors had once been. This phenomenon probably occurred rapidly, otherwise the cost of losing individuals to autoimmunity may have caused the expulsion of the transposon from the pre-gnathostome vertebrate genome. This is probably the reason that the generation of all the coevolving units of the immune system seems to have been so abrupt (Bernstein et al., 1996). The integration of a transposon, suggested by some to explain the genesis of the somatic rearrangement, was risky. This would only have been possible if early vertebrates had already devoted a lineage of cells to the expression of Ig-like receptors. An accident due to the imperfection of early rearrangement mechanisms (e.g. rearranging genes segments that were not supposed to be rearranged) would stay confined to one clone of cells and would not necessarily threaten the life of the species. Yet in some offspring of primitive vertebrates, the chondrichthyes, a remnant of an ancestral situation is still visible. Immunoglobulin gene segments rearranged in the germ line indicate that rearrangement was not entirely confined to the lymphoid lineage (Lee et al., 2000).

All of these receptors could have been generated from a pool of genes present in the MHC III region. Perhaps at some stage, a general control of transcription over this region was useful. The C1 domains present in this region may have been selected for specific function in the adaptive immune system, these include: the binding to co-receptor CD4 and CD8 for the MHC; CD3 for the TCR; and the alpha beta for the immunoglobulin.

The origin of the peptide-binding region remains a mystery. One assumes it to have been imported from another set of genes, by means of exon shuffling, rather than via differentiation from an Ig superfamily member (Flajnik et al., 1991). The recent discovery of new molecules which look like $\alpha 1\ \alpha 2$ domains recognised by some lectin-like NK receptors in the mouse opens new avenues in the quest for the pBR ancestor (Diefenbach et al., 2000). Another phase not considered in the present model is the duplication that led to the separation of TCR-Ig. Which one came first?

7. The evolution of the immune system within jawed vertebrates

Whatever the causes for, and the order of appearance of its essential elements, the immune system became constituted in the ancestors of the jawed vertebrates. It formed a coevolving unit that was unlikely to allow for dramatic variations. In fact, some variations only occur in the usage of the genes, in the number of genes, and in the architecture of the various loci, but not in the fundamental principle of the immune system (Du Pasquier and Flajnik, 1999, Figs. 5 and 6). The difference in gene organisation, gene usage and gene number for Ig genes are depicted in Fig. 6. Rearrangement can take place both throughout life or only during certain stages. Diversification of the repertoire of immunoglobulins can involve either combinatorial joining at the moment of rearrangement, or gene conversion following the unique rearrangement that occurred early in B-cell ontogeny (Reynaud et al., 1987). No combinatorial joining is possible in elasmobranchs as the genes are arranged in clusters (Litman et al., 1999). The imprecision of junction during the rearrangement can, however, still generate a lot of diversity. None of these organisations or modes of usage are specific to a vertebrate class (Du Pasquier and Flajnik, 1999). Cluster organisation can be encountered in elasmobranchs, teleosts, and mammals. Gene conversion can operate in birds and mammals. Given that the amount of diversity generated is high whether or not combinatorial joining is used (in fact none of them will ever have enough cells to fully express their diver-

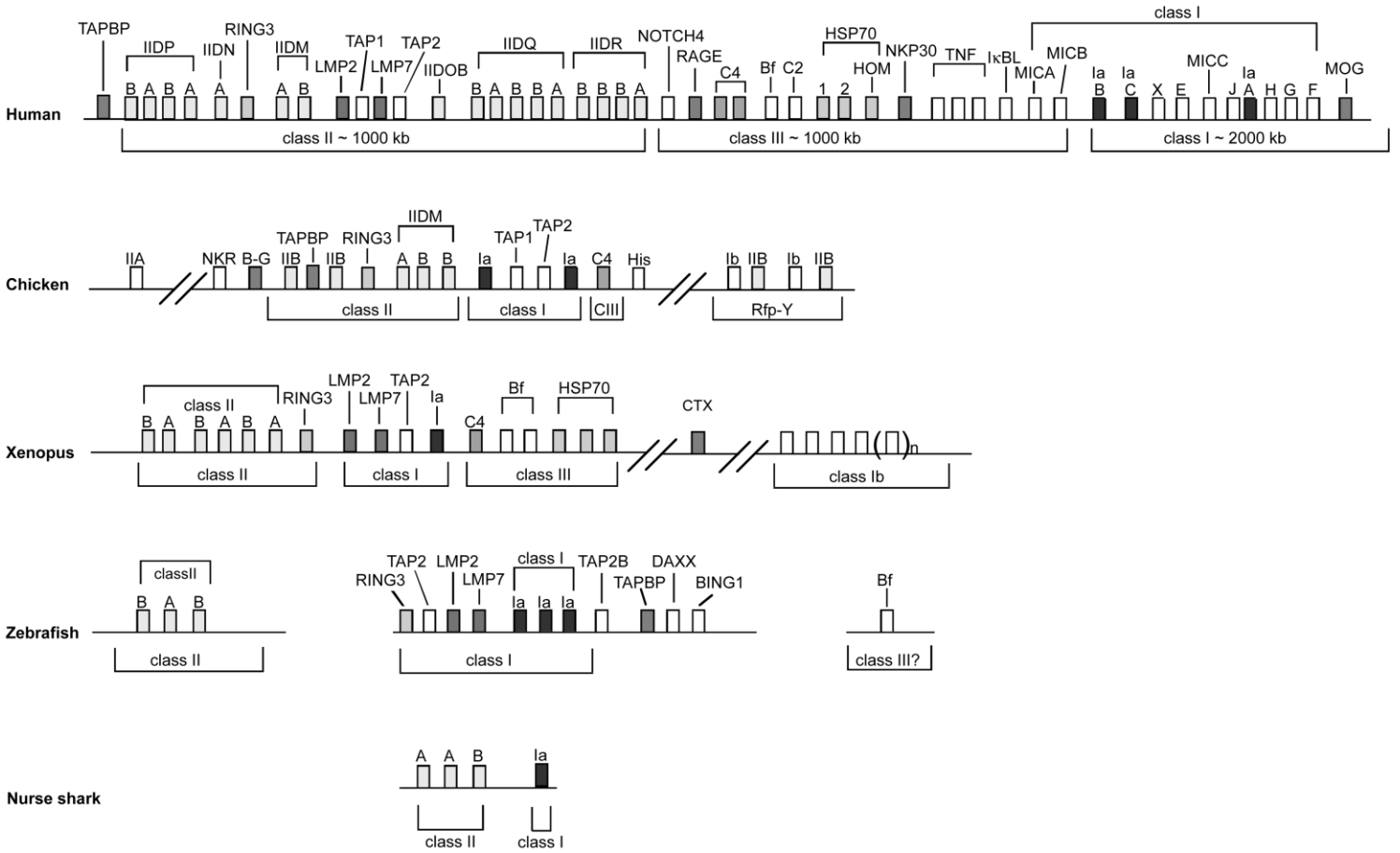


Fig. 5. The MHC linkage maps of human, chicken, *Xenopus* species, zebrafish and nurse shark (updated from Du Pasquier and Flajnik, 1999). Distances between the genes are not to scale but the relative order of the genes is as displayed except for *Xenopus* species, for which the order and even the potential 'regions' are not well known. Not all MHC linked genes are indicated. Large slash marks indicate linkage on the same chromosome but not in the same clusters. Separation of clusters in zebrafish indicates nonlinkage of the regions shown. References: human (Consortium, 1999), chicken (Kaufman et al., 1999), *Xenopus* (Nonaka et al., 1997), Zebrafish (Sultmann et al., 2000), nurse shark (Ohta et al., 2000). *Abbreviations*: TAPBP tapasin, II DQ DN DM DO DR human class II genes (A alpha chain, B beta chain), RING3 nucleolar serin threonine kinase, TAP transport associated protein, LMP low molecular weight proteasome, RAGE receptor for advanced glycosilation end products, C4 and 2 complement components 4 and 2, Bf complement component factor B, HSP heat shock protein, NKP-30 activating receptor of natural killer cells (also known as 1C7), TNF tumor necrosis factor, MOG myelin/oligodendrocyte glycoprotein.

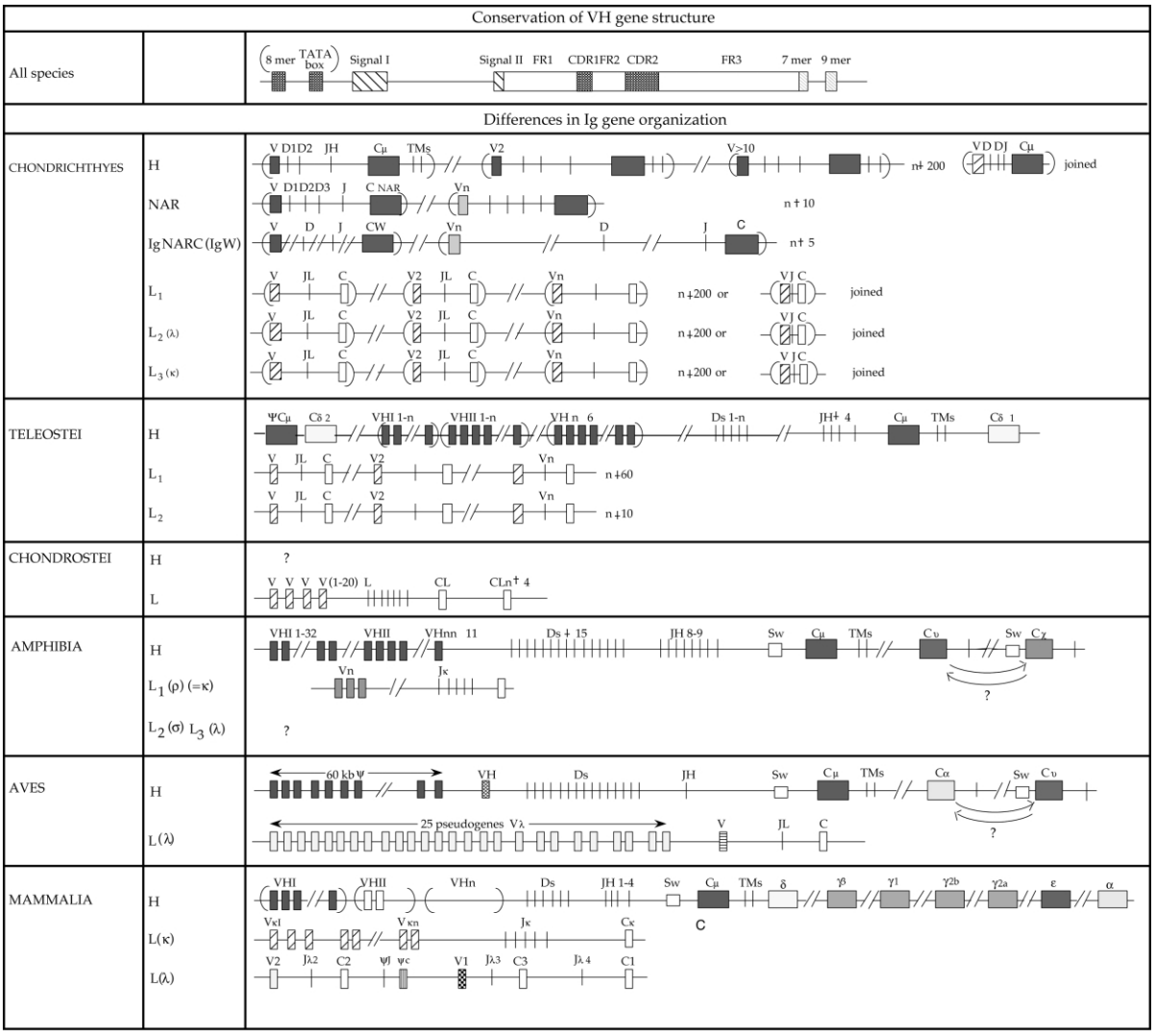


Fig. 6. Immunoglobulin loci in vertebrates (modified from Du Pasquier and Flajnik, 1999). *Abbreviations*: FR framework; CDR complimentarity determining region; 7, 8, 9 mer-hepta, octa, nonamers; H heavy chain; L light chain; J joining segment; D diversity; TM transmembrane + cytoplasmic segments; SW switch region; NAR New (or Nurse shark) antigen receptor; NARC new antigen receptor from cartilaginous fish.

sity), all vertebrates should be able to mount equally good responses. This, however, is not the case. Only mammals, and to a certain extent birds, show the highest quality in their response. A good response is characterised by the existence of an increase in antibody affinity towards the antigen during the progression of the response. The reason seems to be that only mammals exploit maximum potentialities of somatic diversification after antigenic challenge via somatic hypermutation. Somatic hypermutation in the Ig locus of other receptor genes (e.g. the NAR locus of the shark (Greenberg et al., 1995) occurs from shark to mammals. Yet the selection of mutants is a difficult task. Finding one cell with better affinity amongst millions of other cells and allowing enough time to select and deliver a signal for proliferation requires specific cell types and an adapted lymphoid organ architecture (follicular dendritic cells and germinal centres developed only in mammals). Apparently, these elements are missing in cold-blooded vertebrates and use of somatic mutants of immunoglobulin may not be optimal (Fig. 7, review in Du Pasquier et al., 1998; Wilson et al., 1992). Most likely, the optimal exploitation of somatic mutants in mammals is

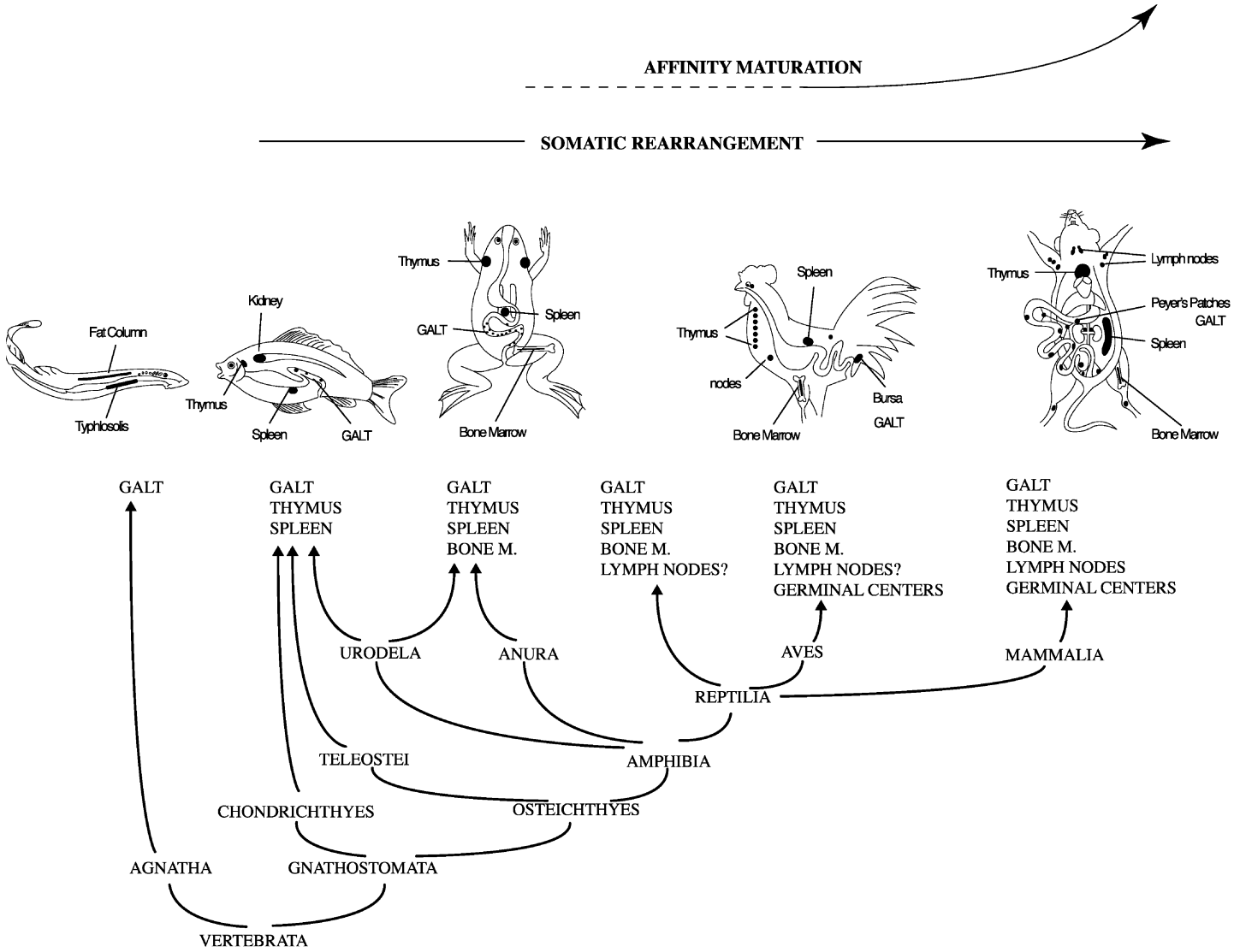


Fig. 7. Evolution of lymphoid systems. BM: bone marrow; L: node, lymph node; affinity maturation, ability to increase antibody affinity after immunisation; somatic rearrangement, antigen receptor variable region genes generated by somatic rearrangement of gene segments mediated by RAG enzymes. Modified from (Du Pasquier and Flajnik, 1999).

due to more than one cause. One of them could be the great value of a single individual in the relatively small mammalian progenies. In this way each of them, the survival of which is precious, receives a tool that can adapt to any situation and the genetic bias of inheriting a bad gene is tempered. In homeotherms, one can count on the system to operate predictably because of the stability provided by a constant temperature. In amphibians and fish, where responses can suddenly be impaired by low temperature, perhaps natural selection operates positively on other facets of the system.

8. Summary

All Metazoa need some sort of system to protect their individuality. Recognition of allopolymorphisms and of foreign substances is universal. However, the molecular mechanisms underlying their function are not necessarily conserved. A great variety of mechanisms has been generated from Porifera to Chordata.

Several mechanisms mediating innate immunity can be conserved from protostomian to vertebrates but no convincing cases of specific memory nor clonal expansion, nor of somatic generation of repertoire of receptors have been detected in any invertebrate phylum. The generation of an adaptive immune system is seen within vertebrates. It seems to have occurred somewhat abruptly in the direct ancestors of jawed vertebrates. This scenario stems from the analysis of the sequence of the components of the immune system and from linkage studies. Clearly, molecules resembling Ig, or TCR were present in invertebrates before the somatic rearrangement was introduced. Once created, the system with its T-cell receptors, immunoglobulins and major histocompatibility complex did not evolve much. 'Complexfication' is seen at the level of lymphoid organs that seems to result, in mammals, in a better way of exploiting the different somatic mechanisms (somatic mutations, isotype switch) that allow an improvement of the response with time after immunisation.

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References

Abi Rached, L., McDermott, M.F., Pontarotti, P., 1999. The MHC big bang. Immunol. Rev. 167, 33–44.

Adema, C.M., Hertel, L.A., Miller, R.D., Loker, E.S., 1997. A family of fibrinogen-related proteins that precipitates parasite-derived molecules is produced by an invertebrate after infection. Proc. Natl. Acad. Sci. USA 94, 8691–8696.

Bernstein, R.M., Schluter, S.F., Bernstein, H., Marchalonis, J.J., 1996. Primordial emergence of the recombination activating gene 1 (RAG1): sequence of the complete shark gene indicates homology to microbial integrases. Proc. Natl. Acad. Sci. USA 93, 9454–9459.

Boiledieu, D., Valembois, P., 1976. Etude in vitro de l'activité cytotoxique des leucocytes de Siponcles à l'encontre d'erythrocytes allogéniques et xénogéniques. C. R. Acad. Sci. Hebd. Seances Acad. Sci. D. 283, 247–249.

Boman, H.G., 2000. Innate immunity and the normal microflora. Immunol. Rev. 173, 5–16.

Burnet, F.M., 1957. A modification of Jerne's theory of antibody production using the concept of clonal selection. Austral. J. Science 20, 67–69.

Cella, M., Döhring, C., Samaridis, J. et al., 1997. A novel inhibitory receptor (ILT3) expressed on monocytes, macrophages, and dendritic cells involved in antigen processing. J. Exp. Med. 185, 1743–1751.

Chothia, C., Novotny, J., Bruccoleri, R., Karplus, M., 1985. Domain association in immunoglobulin molecules. The packing of variable domains. J. Mol. Biol. 186, 651–663.

Clark, A.G., Wang, L., 1997. Molecular population genetics of *Drosophila* immune system genes. Genetics 147, 713–724.

Consortium, M.S., 1999. Complete sequence and gene map of a human major histocompatibility complex. The MHC sequencing consortium (see comments). Nature 401, 921–923.

Diefenbach, A., Jamieson, A.M., Liu, S.C., Shastri, N., Raulet, D.H., 2000. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. Nature Immunol. 1, 119–126.

Du Pasquier, L., 2000a. The phylogenetic origin of antigen-specific receptors. Curr. Top. Microbiol. Immunol. 248, 160–185.

Du Pasquier, L., 2000b. Relationships among the genes encoding MHC molecules and the specific antigen

receptors. In: Kasahara, M. (Ed.), Major Histocompatibility Complex, Evolution, Structure and Function. Springer, Tokyo.

Du Pasquier, L., Chrétien, I., 1996. CTX, a new lymphocyte receptor in *Xenopus* and the early evolution of Ig domains. Res. Immunol. 147, 218–226.

Du Pasquier, L., Courtet, M., Chrétien, I., 1999. Duplication and MHC linkage of the CTX family of genes in *Xenopus* and in mammals. Eur. J. Immunol. 29, 1729–1739.

Du Pasquier, L., Flajnik, M.F., 1999. Origin and evolution of the vertebrate immune system. In: Paul, W.E. (Ed.), Fundamental immunology. Lippincott–Raven, Philadelphia.

Du Pasquier, L., Wilson, M., Greenberg, A., Flajnik, M.F., 1998. Somatic mutation in ectothermic vertebrates: musing on selection and origins. M.F. Flajnik and G. Kelsoe (Eds.), Springer, Miami, Curr. Top. Microbiol. Immunol..

Engström, Y., Kadalayil, L., Sun, S.C., Samakovlis, C., Hultmark, D., Faye, I., 1993. kB-like motifs regulate the induction of immune genes in *Drosophila*. J. Mol. Biol. 232, 327–333.

Fehling, H.J., Laplace, C., Mattei, M.G., Saint-Ruf, C., von Boehmer, H., 1995. Genomic structure and chromosomal location of the mouse pre-T-cell receptor alpha gene. Immunogenetics 42, 275–281.

Flajnik, M.F., Canel, C., Kramer, J., Kasahara, M., 1991. Which came first, MHC class I or class II? Immunogenetics 33, 295–300.

Greenberg, A.S., Avila, D., Hughes, M., Hughes, A., McKinney, E.C., Flajnik, M.F., 1995. A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks. Nature 374, 168–173.

Gross, I., Georgel, P., Kappler, C., Reichhart, J.M., Hoffmann, J.A., 1996. *Drosophila* immunity: a comparative analysis of the Rel proteins dorsal and Dif in the induction of the genes encoding diptericin and cecropin. Nucleic Acids Res. 24, 1238–1245.

Hansen, J.D., McBlane, J.F., 2000. Recombination-activating genes, transposition, and the lymphoid-specific combinatorial immune system: a common evolutionary connection (In Process Citation). Curr. Top. Microbiol. Immunol. 248, 111–135.

Harpaz, Y., Chothia, C., 1994. Many of the immunoglobulin superfamily domains in cell adhesion molecules and surface receptors belong to a new structural set which is close to that containing variable domains. J. Mol. Biol. 238, 528–539.

Hoek, R.M., Smit, A.B., Frings, H., Vink, J.M., de Jong-Brink, M., Geraerts, W.P.M., 1996. A new Ig-superfamily member, molluscan defence molecule (MDM) from *Lymnaea stagnalis*, is down-regulated during parasitosis. Eur. J. Immunol. 26, 939–944.

Hoffmann, J.A., Kafatos, F.C., Janeway, C.A., Ezekowitz, R.A., 1999. Phylogenetic perspectives in innate immunity. Science 284, 1313–1318.

Hultmark, D., 1993. Immune reactions in *Drosophila* and other insects: a model for innate immunity. Trends Genet. 9, 178–183.

Humphreys, T., Reinherz, E.L., 1994. Invertebrate immune recognition, natural immunity and the evolution of positive selection. Immunol. Today 15, 316–320.

Ikemizu, S., Gilbert, R.J., Fennelly, J.A. et al., 2000. Structure and dimerization of a soluble form of B7-1. Immunity 12, 51–60.

Kasahara, M., 1999. The chromosomal duplication model of the major histocompatibility complex. Immunol. Rev. 167, 17–32.

Kasahara, M., 2000. Genome paralogy: a new perspective on the organization and origin of the major histocompatibility complex (In Process Citation). Curr. Top. Microbiol. Immunol. 248, 53–66.

Kaufman, J., Milne, S., Gobel, T.W. et al., 1999. The chicken B locus is a minimal essential major histocompatibility complex (see comments). Nature 401, 923–925.

Kharitonenkov, A., Chen, Z., Sures, I., Wang, H., Schilling, J., Ullrich, A., 1997. A family of proteins that inhibit signalling through tyrosine kinase receptors. Nature 386, 181–186.

Kubagawa, H., Burrows, P.D., Coopers, M.D., 1997. A novel pair of immunoglobulin-like receptors expressed by B cells and myeloid cells. Proc. Natl. Acad. Sci. USA 94, 5261–5266.

Lee, J.Y., Boman, A., Sun, C.X. et al., 1989. Antibacterial peptides from pig intestine: isolation of a mammalian cecropin. Proc. Natl. Acad. Sci. U.S.A. 86, 9159–9162.

Lee, S.S., Fitch, D., Flajnik, M.F., Hsu, E., 2000. Rearrangement of immunoglobulin genes in shark germ cells (see comments). J. Exp. Med. 191, 1637–1648.

Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.M., Hoffmann, J.A., 1996. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. Cell 86, 973–983.

Linington, C., Lassmann, H., 1987. Antibody responses in chronic relapsing experimental allergic encephalomyelitis: correlation of serum demyelinating activity with antibody titre to the myelin/oligodendrocyte glycoprotein (MOG). J. Neuroimmunol. 17, 61–69.

Litman, G.W., Anderson, M.K., Rast, J.P., 1999. Evolution of antigen binding receptors. Ann. Rev. Immunol. 17, 109–147.

Medzhitov, R., Preston-Hurlburt, P., Janeway, C.A.J., 1997. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. Nature 388, 394–397.

Meister, M., Hetru, C., Hoffmann, J.A., 2000. The antimicrobial host defence of *Drosophila*. Curr. Top. Microbiol. Immunol. 248, 17–36.

Neville, M.J., Campbell, R.D., 1999. A new member of the Ig superfamily and a V-ATDu Pase G subunit are among the predicted products of novel genes close to the TNF locus in the human MHC. J. Immunol. 162, 4745–4754.

Nonaka, M., 2000. Origin and evolution of the complement system (In Process Citation). Curr. Top. Microbiol. Immunol. 248, 37–50.

Nonaka, M., Namikawa, C., Kato, Y., Sasaki, M., Salter-Cid, L., Flajnik, M.F., 1997. Major histocompatibility complex gene mapping in the amphibian *Xenopus* implies a primordial organization. Proc. Natl. Acad. Sci. USA 94, 5789–5791.

Ohta, Y., Okamura, K., McKinney, E.C., Bartl, S., Hashimoto, K., Flajnik, M.F., 2000. Primitive synteny of vertebrate major histocompatibility complex class I and class II genes. Proc Natl Acad Sci U S A 97, 4712–4717.

Ortmann, B., Copeman, J., Lehner, P.J. et al., 1997. A critical role for taDu Pasin in the assembly and function of multimeric MHC class I-TAP complexes. Science 277, 1306–1309.

Paul, W.E., 1999. Fundamnetal Immunology. Lippincott–Raven, Philadelphia, New York.

Pende, D., Parolini, S., Pessino, A. et al., 1999. Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. J. Exp. Med. 190, 1505–1516.

Putsep, K., Normark, S., Boman, H.G., 1999. The origin of cecropins; implications from synthetic peptides derived from ribosomal protein L1. FEBS Lett. 451, 249–252.

Rast, J.P., Pancer, Z., Davidson, E.H., 2000. New approaches towards an understanding of deuterostome immunity. Curr. Top. Microbiol. Immunol. 248, 3–16.

Reynaud, C.A., Dahan, A., Weill, J.C., 1987. A gene conversion program during the ontogenesis of chicken B cells. Trends Genet. 3, 248–251.

Rosetto, M., Engstrom, Y., Baldari, C.T., Telford, J.L., Hultmark, D., 1995. Signals from the IL-1 receptor homolog, Toll, can activate an immune response in a *Drosophila hemocyte* cell line. Biochem. Biophys. Res. Commun. 209, 111–116.

Rougeon, F., 1986. La diversité des anticorps. La Recherche 17, 680–689.

Samakovlis, C., Kimbrell, D.A., Kylsten, P., Engstrom, A., Hultmark, D., 1990. The immune response in *Drosophila*: pattern of cecropin expression and biological activity. EMBO J. 9, 2969–2976.

Shibuya, A., Campbell, D., Hannum, C. et al., 1996. DNAM-1, a novel adhesion molecule involved in the cytolytic function of T lymphocytes. Immunity 4, 573–581.

Su, X.D., Gastinel, L.N., Vaughn, D.E., Faye, I., Poon, P., Bjorkman, P.J., 1998. Crystal structure of hemolin: a horseshoe shape with implications for homophilic adhesion. Science 281, 991–995.

Sugaya, K., Fukagawa, T., Matsumoto, K. et al., 1994. Three genes in the human MHC class III region near the junction with the class II: gene for receptor of advanced glycosylation end products, PBX2 homeobox gene and a notch homolog, human counterpart of mouse mammary tumor gene int-3. Genomics 23, 408–419.

Sultmann, H., Murray, B.W., Klein, J., 2000. Identification of seven genes in the major histocompatibility complex class I region of the zebrafish. Scand. J. Immunol. 51, 577–585.

Sun, S.C., Faye, I., 1992. Affinity purification and characterization of CIF, an insect immunoresponsive factor with NF-κ B-like properties. Comp. Biochem. Physiol. 103, 225–233.

Sun, S.C., Lindstrom, I., Boman, H.G., Faye, I., Schmidt, O., 1990. Hemolin: an insect-immune protein belonging to the immunoglobulin superfamily. Science 250, 1729–1732.

Sunyer, J.O., Lambris, J.D., 1998. Evolution and diversity of the complement system of poikilothermic vertebrates. Immunol. Rev. 166, 39–57.

Thompson, C.B., 1995. New insights into V(D)J recombination and its role in the evolution of the immune system. Immunity 3, 531–539.

Tonegawa, S., 1983. Somatic generation of antibody diversity. Nature 302, 575–581.

van de Vyver, G., 1975. La non conflunce intraspécifique chez les spongiaires et la notion d'individu. Ann. Embryol. Morphol. 3, 251–262.

Vizioli, J., Bulet, P., Charlet, M. et al., 2000. Cloning and analysis of a cecropin gene from the malaria vector mosquito, *Anopheles gambiae*. Insect. Mol. Biol. 9, 75–84.

Wilson, I., Vogel, J., Somerville, S., 1997. Signalling pathways: a common theme in plants and animals? Curr. Biol. :rr178 7, 175.

Wilson, M., Hsu, E., Marcuz, A. et al., 1992. What limits affinity maturation of antibodies in *Xenopus*-the rate of somatic mutation or the ability to select mutants? EMBO J. 11, 4337–4347.

Zhao, C., Liaw, L., Lee, I.H., Lehrer, R.I., 1997. cDNA cloning of three cecropin-like antimicrobial peptides (Styelins) from the tunicate, *Styela clava*. FEBS Lett. 412, 144–148.