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The Evolution of Adaptive Immune Systems

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A clonally diverse anticipatory repertoire in which each lymphocyte bears a unique antigen receptor is the central feature of the adaptive immune system that evolved in our vertebrate ancestors. The survival advantage gained through adding this type of adaptive immune system to a pre-existing innate immune system led to the evolution of alternative ways for lymphocytes to generate diverse antigen receptors for use in recognizing and repelling pathogen invaders. All jawed vertebrates assemble their antigen-receptor genes through recombinatorial rearrangement of different immunoglobulin or T cell receptor gene segments. The surviving jawless vertebrates, lampreys and hagfish, instead solved the receptor diversification problem by the recombinatorial assembly of leucine-rich-repeat genetic modules to encode variable lymphocyte receptors. The convergent evolution of these remarkably different adaptive immune systems involved innovative genetic modification of innate-immune-system components.

Life began on our planet more than 3.5 billion years ago, and evolving single-cell organisms, eubacteria, archaebacteria, and eukaryotes, have flourished ever since. Around 600 million years ago, multicellular organisms (metazoans) began to form in conjunction with a dramatic increase in atmospheric oxygen levels. This development was followed by a remarkable diversification of metazoan species in such a relatively short time period that it has been termed the "evolutionary big bang." Each metazoan lineage that we recognize today, including the vertebrate lineage to which we belong, appeared more than 500 million years ago (Figure 1).

Vertebrates with jaws (gnathostomes) possess a remarkably adaptive immune system that can recognize and initiate a protective response against potentially lethal pathogens, including bacteria, viruses, fungi, and parasites. Our adaptive immune system also remembers previous pathogen encounters and can either repel a second invasion or quickly eliminate the recurrent invader by mobilizing a faster and more efficient immune response. Well before the evolution of an adaptive immune system, however, innate mechanisms of self-defense were acquired. Even single-cell organisms have heritable defense mechanisms, and every multicellular organism appears to have a complex innate immune system (Beutler, 2004). The basic protective strategy of an innate immune system is for the organism to constitutively produce generic receptors that recognize conserved patterns on different classes of pathogens to trigger an inflammatory response that limits pathogen invasion (Janeway and Medzhitov, 2002; Hoffmann, 2003; Akira et al., 2006 [this issue of *Cell*]). Specific adaptive immunity, by contrast, depends upon the somatic diversification of antigen-receptor genes to generate a vast repertoire of cells, each of which expresses a different antigen receptor. Lymphocytes, the specialized cell type of the adaptive immune system, use their cell-surface receptors to recognize antigenic configurations of specific pathogens and then respond to the antigen triggering by clonal amplification, cellular differentiation, and production of antibodies with the same antigen binding specificity (Burnet, 1959).

We are just beginning to understand when and how the adaptive immune system evolved to work in concert with the innate immune system. Through the work of evolutionary immunobiologists, we now know that the cardinal elements of our adaptive immune system are shared by all gnathostomes (Litman et al., 1999; Flajnik and Kasahara, 2001). Very recently, we have learned that vertebrates without jaws (agnathans) also have an adaptive immune system that is based on recombinatorial assembly of a different type of modular genetic units to generate a highly diverse repertoire of lymphocytes, each with a unique anticipatory receptor (Pancer et al., 2004a; Alder et al., 2005).

Minimalistic View of the Gnathosome Adaptive Immune System

The principal elements of our adaptive immune system are well known (Paul, 2003). Two major lineages of lymphocytes that can specifically recognize and respond to antigenic determinants of potentially hazardous pathogens and toxins are generated in the thymus and the bone marrow or the avian bursa of Fabricius (Cooper et al., 1965). These are called T (for thymus-derived) and B (for bursa- or bone-marrow-derived) lymphocytes. Like the other types of blood cells, the early progenitors of T and B

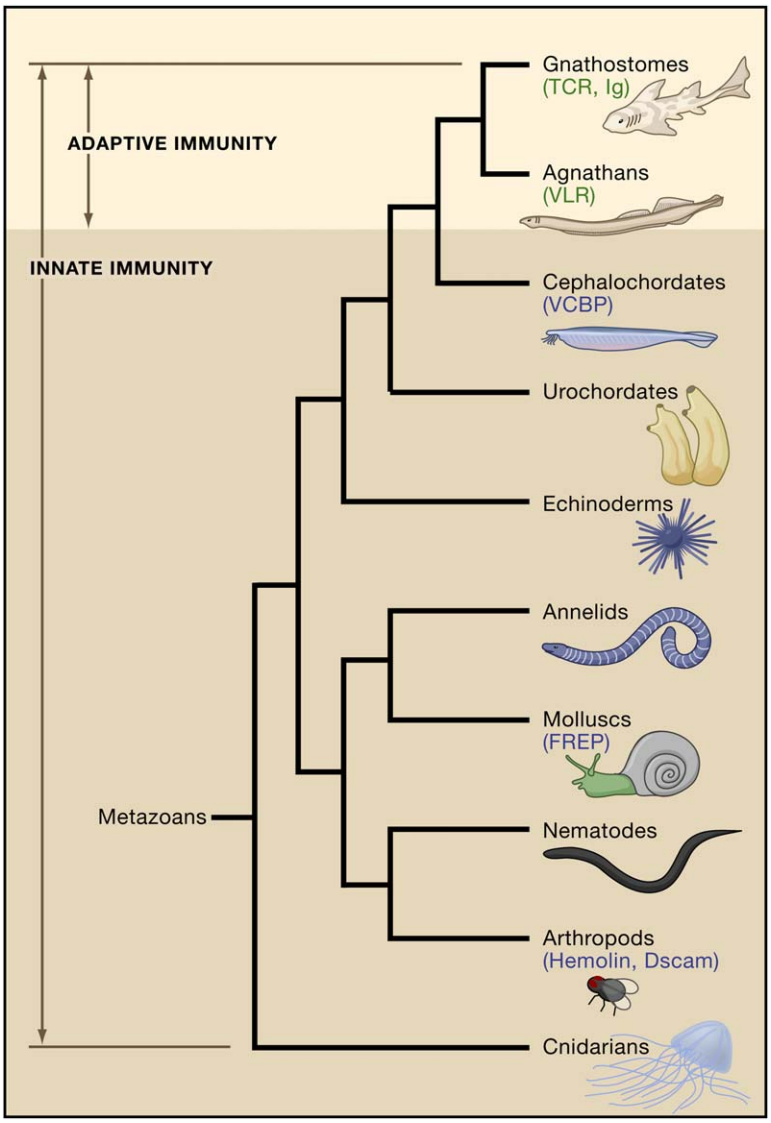

Figure 1. Phylogenetic Tree Indicating Theoretical Evolutionary Relationships of Metazoans and the Emergence of Adaptive Immunity in Conjunction with Innate Immunity

Families of immune molecules, other than Toll-like receptors, discussed in this review are indicated in blue: V type Ig domains and a chitin binding domain containing proteins (VCBP), fibrinogen-related proteins (FREPs), hemolin, and Down's syndrome cell adhesion molecule (Dscam). The recombinatorial-based immune receptors are indicated in green: T cell receptors (TCR), immunoglobulins (Ig), and variable lymphocyte receptors (VLR).

lymphocytes are derived from multipotent hematopoietic stem cells. During their early developmental stages, T and B lymphocyte progenitors rearrange different sets of prototypic immunoglobulin (Ig) variable (V), diversity (D), and joining (J) gene segments to generate the antigen binding regions of their T cell receptors (TCRs) and B cell receptors (BCRs). The antigen binding regions of the different V(D)J combinations are diversified further through the enzymatic addition of nonencoded nucleotides in the joints created during V(D)J segment assembly. The random nature of this diversification process results in the generation of some receptors that recognize self-antigens, but T and B lymphocytes bearing potentially harmful, self-reactive receptors may be deleted in their thymic and bone-marrow birthplaces or otherwise inactivated. The selected populations of long-lived T and B lymphocytes then enter the bloodstream to begin their patrol of the body via a migratory route that involves their entry into strategically located lymphoid tissues, where they may engage invading pathogens, and their subsequent return to the circulation via lymphatic channels. The TCRs recognize peptide fragments of antigens presented by other cells within cell-surface molecules encoded by the major histocompatibility complex (MHC) class I and class II genes. T lymphocytes therefore typically recognize antigens that have been partially digested by the antigen-presenting cells, primarily dendritic cells, phagocytic cells, and B lymphocytes. The membrane bound and secreted antibodies made by B lineage cells, by contrast, recognize exposed determinants (epitopes) of intact molecules, including surface protein and carbohydrate moieties of invasive microbes. In addition to the antigen binding chains of TCRs and BCRs, other transmembrane proteins in the TCR and BCR complexes trigger the intracellular signaling pathways leading to the expression of genes required for immune responses. For most antigen-induced responses,

B lymphocytes receive help from T lymphocytes in the activation process. Many different cell-surface molecules and secreted cytokines are used in this cellular interaction and to guide lymphocyte homing.

Even this brief account makes clear the difficulty of tracing the evolution of every component of the complex adaptive immune system of gnathostomes. Immunologists therefore have concentrated their phylogenetic explorations on the identification of *TCR*, *BCR*, *RAG1/RAG2* (recombination-activating genes whose products initiate the V(D)J rearrangements), and *MHC class I* and *class II* genes as the key elements of the adaptive immune system. This search has led to the identification of all of these genes in every gnathostome species that has been carefully examined. Even the cartilaginous fish, like sharks, have *TCR* and *BCR* genes divided into V, D, J, and constant (C) region segments and *RAG1/RAG2*, *MHC I*, and *MHC II* genes (Cannon et al., 2004; Flajnik, 2002). Nonencoded nucleotides are also identifiable in the V(D)J joints of their rearranged *BCR* and *TCR* genes as evidence for terminal deoxynucleotidyl-transferase activity. The most notable difference in the antigen-receptor genes of these early gnathostomes is in the genomic organization of the *BCR* genes. The *Ig* heavy- and light-chain genes of cartilaginous fish are found in clusters of individual V(D)J and C region gene segments, thereby limiting recombinatorial diversification during *BCR* gene assembly. In contrast, our Ig V, D, and J gene segments are largely segregated into family groups located upstream of the C gene segments. While increasing complexity of the adaptive immune system is evident with vertebrate evolution, the same basic construction of the adaptive immune system is conserved throughout the gnathostome radiations. This suggests that the phylogenetic roots of our adaptive immune system were formed in early vertebrate ancestors.

Invertebrates Use Immunoglobulin Superfamily Genes in Immune Defense

Immunoglobulin superfamily (IgSF) members are key elements of the adaptive immune system in gnathostomes. The versatile Ig domains are used not only in the TCR, BCR, and MHC proteins but also by the invariant activating and inhibitor receptors employed by natural killer (NK) cells, Ig Fc receptors on phagocytic cells that can bind antibodies complexed to their antigens, cytokine receptors, cell-adhesion molecules, and other cell-surface molecules that govern T and B lymphocyte interactions with each other and with antigen-presenting and inflammatory cells. IgSF members have therefore received special attention in the search for the phylogenetic forerunners of our adaptive immune system.

IgSF members have been found in insects, and some of these, like the silk-moth hemolin, may have immune-defense function (Sun et al., 1990). One of the most interesting of these genes encodes the Down's syndrome cell adhesion molecule (Dscam), which was defined initially as having an important role in guiding neuronal wiring. *Dscam* is a large gene that contains clusters of variable exons flanked by constant exons (Watson et al., 2005). Highly variable alternative splicing of some *Dscam* exons may yield enough constant and variable exon combinations to generate more than 18,000 different extracellular domains. In contrast to the antigen binding loops of antibody molecules, Dscam diversity resides primarily in the A strand on the side of the Ig domain barrel. Nevertheless, its remarkable variability gives Dscam the potential to recognize diverse ligands and epitopes. Dscams are expressed both as transmembrane molecules on insect hemocytes and as secreted isoforms in the hemolymph. Moreover, the efficiency of bacterial uptake is impaired in phagocytic hemocytes that are Dscam deficient. This versatile IgSF member may thus participate in the innate immune responses of insects. Another interesting family of diverse IgSF molecules, the Ig domain-containing, fibrinogen-related proteins (FREPs), is found in snails (Zhang et al., 2004). FREP production is upregulated following parasitic invasion, and these proteins can bind the parasite invaders or their derivative products. Remarkable sequence variation is found in both the Ig and fibrinogen domains of FREPs, and individual snails may differentially express the alleles of different *FREP* subfamilies. While somatic variation of *FREP* genes could account for some of the diversity, FREP diversity is evident not only in hemocytes but in the nervous system and stomach muscle tissues as well. Yet another remarkably diverse multigene family has been found in the Florida lancelet, an amphioxus of the cephalochordate branch (Cannon et al., 2002). This large family of genes encodes proteins with V type Ig domains and a chitin binding domain (VCBP). The Ig V domains have extensive sequence diversity in their N-terminal regions, although there is no evidence of a somatic basis for generating this diversity. Since the VCBPs are secreted by intestinal cells and may bind microbial ligands, they could play a role in innate immunity against intestinal pathogens. These examples of invertebrate IgSF usage illustrate the remarkably versatility of Ig domains. Notably, however, they lack the distinctive structural and functional characteristics that would define them as lineal ancestors of the vertebrate *TCR* and *BCR* gene family.

Appearance of Lymphocytes and Adaptive Immune Responses in Agnathans

Cells with phagocytic and other innate-immune-defense capabilities are present in many invertebrate species, but none of them have been shown to be long-lived migratory cells with clonally diverse anticipatory receptors and adaptive-immune-response capabilities. On the other hand, cells with the characteristic morphological features of lymphocytes and much of the molecular machinery possessed by gnathostome lymphocytes have been found in lampreys and hagfish (Uinuk-Ool et al., 2002; Mayer et al., 2002; Najakshin et al., 1999; Nagata et al., 2002), the only two living agnathan representatives. These

findings suggest that lymphocyte progenitors evolved in the most basal vertebrates, or possibly a protochordate ancestor.

Many of the genes for transcription factors involved in gnathostome lymphocyte development can be found in agnathans. *SPI-B*, *IKAROS*, *EBF*, *GATA*, *PAX-2/5/8*, and *BACH2* gene relatives have all been identified in the lamprey (Rothenberg and Pant, 2004). Since many of the signaling pathways involved in inflammatory responses, such as the NF-κB pathway, exist in insects, it is not surprising that lymphocyte-like cells in lampreys possess NF-κB and STAT signaling-cascade components. Many additional genes that our lymphocytes use for activation purposes are expressed by lamprey lymphocytes (Mayer et al., 2002; Pancer et al., 2004b). These include genes for the CD45 transmembrane protein tyrosine phosphatase, SYK protein tyrosine kinase, Src family members, and the HS-1 adaptor molecule. Lamprey lymphocyte-like cells also express relatives of the CXCR4 chemokine receptor and its SDF-1 ligand, the cytokine interleukin 8 (IL-8) and its receptor, and the IL-17 receptor. In keeping with their apparent lack of *MHC* genes, agnathans possess only the preduplication genes for each of the three proteasome subunit pairs needed for gnathostome immunoproteasomes to produce peptides that fit into the cleft of MHC class I molecules (Klein and Nikolaidis, 2005). The identification of this constellation of cellular components in lampreys suggested that, given the ability to make diverse anticipatory receptors, agnathan lymphocyte-like cells are potentially capable of mediating adaptive immune responses.

Identification of a Different Recombinatorial Immune System in Agnathans

As the nearest living phylogenetic relatives of gnathostomes, hagfish and lampreys were expected to have ancestral *TCR* and *BCR* genes. A transcriptome analysis of lamprey lymphocyte-like cells indeed revealed a *TCR-like* gene (Pancer et al., 2004b). The variable region of the lamprey *TCR-like* gene contains V- and J-like sequences, but only one copy of this gene could be found in the genome. Moreover, a single V region exon encodes its V- and J-like sequences, thereby precluding the possibility of recombinatorial diversification. A *VpreB-like* gene expressed by lymphocyte-like cells was also identified in lampreys (Cannon et al., 2005), and a family of paired Ig-like receptor genes encoding transmembrane proteins with activating and inhibitory potential has been identified recently in hagfish (Suzuki et al., 2005). The V type Ig domains of these molecules contain V and J regions, but they, too, are encoded in a single exon. What has not yet been found in agnathans are rearrangeable *TCR/BCR V(D)J* gene segments, *RAG1* and *RAG2*, and *MHC* genes.

The failure to identify the cardinal elements of our adaptive immune system in agnathans was puzzling, especially since adaptive types of immune responses had been reported earlier. In studies of lampreys and hagfish conducted more than 30 years ago (Finstad and Good, 1964; Hildemann and Thoenes, 1969), these agnathans were found to be capable of accelerated rejection of secondary skin allografts and delayed-type hypersensitivity reactions. Although convincing evidence has not been found for a thymus-like structure, the lamprey lymphocyte-like cells can undergo lymphoblast transformation after mitogen stimulation. Moreover, specific agglutinins were observed after stimulation with particulate antigens, and higher serum levels of agglutinins were seen after booster immunization. Biochemical analysis of the agnathan agglutinins, although inconclusive, failed to demonstrate an Ig-like structure (Litman et al., 1970).

In order to explore the underlying molecular basis for these immune-type responses, lampreys were injected repeatedly with a cocktail of particulate antigens, live bacteria, and plant mitogens. When transcriptomes of the activated lymphoblasts in the immunostimulated animals were surveyed, *TCR*, *BCR*, and *MHC* genes still were not found. Activated lamprey lymphoblasts were instead found to express transcripts for leucine-rich-repeat (LRR) proteins in great abundance and, surprisingly, these proved to have highly variable amino acid sequences (Pancer et al., 2004a). The diversity of these variable lymphocyte receptors (VLRs) is based on the variable numbers of sequence-diverse LRR modules that are sandwiched between the capping N-terminal and C-terminal LRR units, which also display sequence variability. The VLR molecules have a stalk-like region of invariant sequence, and they are tethered to the lymphocyte surface by a glycosyl-phosphatidyl-inositol (GPI) anchor. The GPI linkage allows the VLRs to be released from the lymphocyte by phospholipase-mediated cleavage. Molecular modeling based on sequence comparison with other LRR proteins whose structures are known predicts that the VLRs should have a solenoidal crescent-shape configuration, the concave surface of which has a β sheet lining.

A complex recombinatorial process provides the genetic basis for the VLR diversity (Figure 2). The lamprey genome contains a single *VLR* gene that has three coding regions separated by two large noncoding intervening sequences, which lack canonical splice sites. The germline *VLR* gene encodes only the signal peptide, partial N-terminal and C-terminal LRRs, and the invariant stalk region. Flanking this incomplete *VLR* gene are a large number of cassettes that encode one, two, or three of the different LRR modular units. In order to assemble the complete *VLR* gene, the flanking LRR coding units are randomly incorporated into the germline *VLR* gene via a multistep assembly process (Alder et al., 2005). During the stepwise completion of *VLR* genes, coding sequences to complete the capping LRR modular units are brought in first, and those for the internal LRR variable modules are added later. The two intervening sequences in the germline *VLR* gene are removed during this assembly process that occurs exclusively in lymphocytes. Although generated via a very different recombinatorial process than that used by gnathostomes to generate TCR and BCR diversity, the potential VLR repertoire has been estimated to be

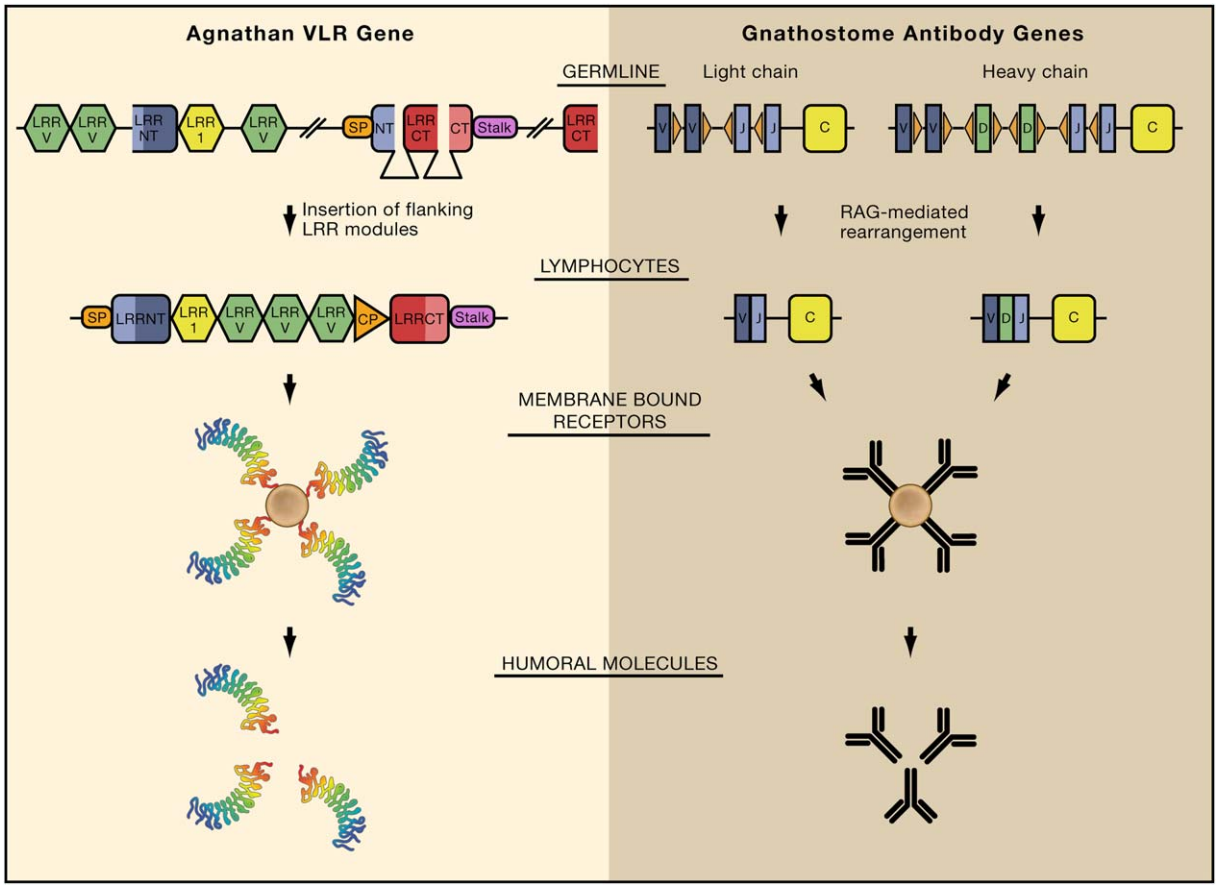

Figure 2. Two Recombinatorial Systems Used for Generating Diverse Antigen Receptors in Vertebrates

This illustration contrasts the assembly of leucine-rich-repeat (LRR) modular genetic units in agnathan lymphocytes to generate variable lymphocyte receptor (VLR) genes versus the rearrangement of Ig gene segments in gnathostome B lymphocytes to generate diverse antibody genes. The following abbreviations are used: variable 24 amino acid LRR (LRRV, green), N-terminal capping LRR (LRRNT, blue), variable 18 amino acid LRR (LRR1, yellow), signal peptide (SP, orange), first 6 amino acids of LRRNT (NT, light blue), C-terminal capping LRR (LRRCT, red), last 9 amino acids of LRRCT (CT, pink), variable 13 amino acid connecting peptide (CP, orange), and invariant VLR stalk (Stalk, purple). The small orange triangles adjacent to the representative V (blue), D (green), and J (light blue) gene segments represent recombination signal sequences (RSSs). The C (yellow) indicates the immunoglobulin constant region. This diagram is a modification of illustrations in articles by Pancer et al. (2004a) and Flajnik (2004).

essentially equivalent to that of the antibody repertoire (>10^{14}). An important difference between the two recombinatorial systems is the absence of recombinatorial signal sequences bordering the modular LRR units used to complete the *VLR* gene. Short homology sequences at the ends of the modular LRR units may instead serve as anchorage sites for the VLR assembly process. These characteristics suggest to us that gene conversion is an attractive candidate mechanism for the *VLR* diversification.

The VLR recombinatorial system is used to generate a highly diverse lymphocyte repertoire in both lampreys and hagfish, although more is known about the lamprey VLR repertoire (Pancer et al., 2005; Alder et al., 2005). Each lamprey lymphocyte typically expresses a unique *VLR* gene in monoallelic fashion. The diversity of the anticipatory repertoire therefore is limited only by the number of lymphocytes. Correspondingly, this is also true for the actual lymphocyte-receptor repertoire in gnathostomes. Use of the VLR repertoire in an immune response to a particulate antigen is characterized by lymphocyte activation and the release of soluble VLRs with antigen binding specificity, but much more information is needed about how the agnathan VLR system functions in adaptive immune responses and for protective immunity.

Separate Evolutionary Roots for the Two Recombinatorial Immune Systems in Vertebrates

The identification of two very different recombinatorial systems for generating a diverse repertoire of anticipatory receptor-bearing lymphocytes in agnathans and gnathostomes raises interesting questions concerning their phylogenetic relationship. Since paleontological analysis indicates that agnathans are basal to the gnathostomes (Forey and Janvier, 1993), the failure to find evidence of

a TCR/BCR recombinatorial system in living agnathan representatives suggests that the VLR recombinatorial system may have evolved earlier. However, the defining VLR characteristics differ from those expected for a precursor of the BCR/TCR recombinatorial system.

An abundance of ancestral LRR genes were available for the evolutionary development of the VLR recombinatorial immune system seen in lampreys and hagfish. LRR proteins are found in unicellular organisms and throughout the animal and plant kingdoms, where they are used for a variety of purposes, including microbial invasion and host defense responses (Buchanan and Gay, 1996). Well-known examples include the bacterial internalins (Cabanes et al., 2002), plant disease-resistance R proteins (Dangl and Jones, 2001), and Toll-like receptors of the innate immune system (Akira et al., 2006). Due to the large interaction areas provided by their elongated and curved shape, LRR proteins may bind their ligands with very high affinities. These favorable structural and functional characteristics, coupled with the presence of a large number of LRR genes in cephalochordates (Pancer and Cooper, 2006), would have made the different LRR modular units an attractive substrate for generating diversity given the development of a recombinatorial mechanism for randomly assembling them in an agnathan ancestor.

There are many precedents for the use of gene shuffling to generate protein diversity in plants and animals. Among the mechanisms employed for these phenotypic changes, gene conversion may be used for generating diversity of cell-surface proteins in trypanosomes (Borst, 2002) and bacteria (Zhang et al., 1997) to enhance their chance for survival in immunocompetent hosts. Gene conversion is also one of the mechanisms used for generating antibody diversity (Thompson and Neiman, 1987; Reynaud et al., 1987). The employment of a gene-conversion type of mechanism for the assembly of modular LRR genetic units therefore could have provided an agnathan ancestor with the means for generating a diverse VLR repertoire for use in adaptive immune responses. Presently, however, the *activation-induced deaminase* (*AID*) gene (Muramatsu et al., 2000), which is essential for the gene-conversion mechanism used for antibody diversification, has been identified only in gnathostomes (Conticello et al., 2005).

As a parallel solution for generating diverse anticipatory receptors, the versatility of the Ig domain structure in ligand binding and an increasing number of IgSF members, contributed by the genomic duplication events postulated to have occurred before and after the agnathan lamprey and hagfish phylogenetic branch (Ohno, 1999; Kasahara et al., 2004), would have provided an attractive alternative substrate. Here, again, the acquisition of a mechanism for gene rearrangement and assembly of the coding units for different Ig domain segments would have been crucial. Discovery that the *BCR* and *TCR* genes consist of multiple V, D, J, and C gene segments yielded important insight into how this recombinatorial system might have evolved (Tonegawa, 1983; Yanagi et al., 1984; Hedrick et al., 1984). The RAG1/RAG2 proteins were later shown to recognize the recombination signal sequences flanking the V(D)J gene segments to initiate the double-stranded DNA breaks and recruitment of other proteins required for their recombination (Oettinger et al., 1990; Jung and Alt, 2004). The subsequent demonstration that RAG1 and RAG2 form a transposase that can excise a piece of DNA containing recombination signal sequences and reinsert it elsewhere supports the currently favored theory that *RAG1/RAG2* were once components of a transposable element (Agrawal et al., 1998; Hiom and Gellert, 1997). In this scenario, an ancestral RAG transposon consisting of recombination signal sequences flanking *RAG1-like* and *RAG2-like* genes was mobilized and inserted into an exon of a receptor gene much like the lamprey *TCR-like* gene. The recipient gene could then be expressed when the inserted transposon was excised by the RAG proteins and the two exon ends rejoined by double strand DNA break repair factors. This type of split gene would have a structure analogous to that of the genes for Ig light chains and the TCRα and γ chains. A second transposon insertion into the same exon could split it again, this time into V and D gene fragments to yield the tripartite structure characteristic of the Ig heavy chain and the TCRβ and δ chain variable-region genes. Alternatively, the D segment may have arisen through germline recombination events resulting in the formation of signal joints with junctional insertions (Lee et al., 2000; Lewis and Wu, 2000). Duplications of the V, D, and J gene segments and retention of the *RAG1* and *RAG2* genes elsewhere in the genome would then yield the basic recombinatorial immune system of gnathostomes (Figure 2).

The search for the evolutionary origins of *RAG1* and *RAG2* genes and V(D)J recombination signal sequences has led to the recent identification of a RAG1 core region sequence with resemblance to the transposase encoded by DNA transposons belonging to the eukaryote *Transib* superfamily (Kapitonov and Jurka, 2005). The *Transib* transposons are flanked by 5 bp target-site duplications, much like those characterized for RAG1/RAG2-mediated transpositions. The identification of *RAG1* core-like sequences in the sea urchin, lancelet, sea anemone, and hydra genomes reinforces the hypothesis of a transposon-related origin of the V(D)J rearrangement machinery. This idea is strengthened by the demonstration that another phylogenetically conserved transposon, the fly *HERMES* transposon of the *hAT* superfamily, can induce a double-strand break via a hairpin-formation mechanism similar to that used in V(D)J recombination (McBlane et al., 1995; Zhou et al., 2004).

Speculation on Why and When the Two Recombinatorial Immune Systems Evolved

The convergent evolution of two types of recombinatorial immune systems is consistent with the idea that encounter with new infectious agents in different environmental niches provided a compelling impetus for the development of adaptive immunity. In support of the assumption that an adaptive immune system provided added value

to the innate immune system in promoting survival, inherited immunodeficiency diseases, like those caused by loss-of-function mutations in the *RAG1*, *RAG2*, and *MHC* genes, lead to a fatal outcome following infection by a variety of pathogens (Fischer, 2004). Having evolved in the presence of well-established innate immunity, our adaptive immune system is designed to work well in conjunction with innate immune mechanisms (Pulendran and Ahmed, 2006 [this issue of *Cell*]). The coevolution of innate and adaptive immune systems over the last 500 million years has ensured the inextricable linkage of their coordinate roles in mediating host protection. Given the likelihood that the agnathan VLR recombinatorial system is also used for protective immunity, it too must function in concert with the innate immune system.

The resource material needed to trace the most informative roots of our V(D)J recombinatorial immune system may have been lost with the extinction of agnathans other than lampreys and hagfish around 400 million years ago (Forey and Janvier, 1993). Agnathans with dermal skeletons (ostracoderms) that had acquired many of the structural characteristics of the gnathostomes are known only through their fossil remains. It is thought that one of these diverse ostracoderm branches gave rise to the gnathostomes (Janvier, 1999). According to this view, the BCR/TCR V(D)J recombinatorial system likely represents a convergent evolutionary development in an ostracoderm branch that was antecedent to the gnathostome lineage.

In that molecular phylogeny favors a monophyletic origin for lampreys and hagfish (Takezaki et al., 2003), the VLR LRR-based recombinatorial system may have evolved in an ancestor shared only by these two agnathans, thereby potentially accounting for their selective survival. In this highly speculative scenario, other agnathans, including those sharing common ancestry with the gnathostomes, may have fallen prey to pathogen-mediated extinction because they failed to acquire a recombinatorial immune system. Alternatively, the VLR recombinatorial system could have evolved in an ancestor common to both agnathans and gnathostomes. In this case, the subsequent acquisition of a V(D)J recombinatorial system may have led, at least initially, to the coexistence of VLR and V(D)J recombinatorial mechanisms for lymphocyte-receptor diversification. Inevitably, the random nature of the two recombinatorial mechanisms working concurrently would result in the generation of lymphocytes having antigen receptors of both self-and non-self-specificity. The predicted detrimental consequences of mixed signals for lymphocyte activation, such as autoimmune disease, would thus have favored the loss of one or the other recombinatorial immune system. This hypothetical model predicts that tell-tale remnants of the VLR system may be found in jawed vertebrates. Many challenging issues clearly remain unresolved about how and why very different components of the innate immune system were co-opted for use in the evolutionary construction of two distinctive recombinatorial immune systems.

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