CHAPTER 13

THE EVOLUTION OF ADAPTIVE IMMUNITY

Nadia Danilova
Department of Molecular, Cell and Developmental Biology, University of California; Los Angeles, Los Angeles, USA
Email: ndanilova@ucla.edu

Abstract: The concept of adaptive immunity suggests de novo generation in each individual of extremely large repertoires of diversified receptors and selective expansion of receptors that match the antigen/pathogen. Accordingly, adaptive immune system is also called "anticipatory". It allows each individual to have a unique repertoire of immune receptors corresponding to its life history. The memory of an antigen gets encoded in the clonal composition of the organism's immune cells instead of being encoded in the genome. Consequently, the immune response to repeated encounter with the same antigen becomes stronger, a phenomenon called immunological memory. Elements of adaptive immunity are found at all taxonomical levels, whereas in vertebrates, adaptive mechanisms have become the cornerstone of the immune system. In jaw vertebrates, adaptive immune receptors of T and B lymphoid cells belong to immunoglobulin superfamily and are created by rearrangement of gene segments. In jawless vertebrates lamprey and hagfish, recombination of leucine-rich repeat modules is used to form variable lymphocyte receptors. Striking functional similarity of the cellular and humoral branches of these systems suggests similar driving forces underlying their development.

INTRODUCTION

The main challenge for the immune system is to create in an economic way a repertoire of receptors able to discriminate between "self" molecules and cells and the vast arrays of pathogens. Immune system also needs to detect defective, damaged and transformed "self". Depending on the type of receptors, immune mechanisms are traditionally subdivided into innate and adaptive. The most ancient and universal innate immune mechanisms are based on germ-line encoded receptors that evolved to recognize molecular patterns common

Self and Nonself, edited by Carlos López-Larrea.

for large groups of pathogens or for defective "self".[1,2] Pathogens, however, constantly develop mechanisms for escaping immune surveillance and new pathogens arise. One of the ways pathogens escape immune system is by extensive diversification of their surface molecules. For example, the human malaria parasite *Plasmodium falciparum* generates diversity within the multi-copy variant antigen gene families by gene recombination.[3]

To meet the challenge, the host organisms need to maximize the scope of immune recognition. Extensive populational polymorphism is one strategy. In case of Drosophila melanogaster, variability in immune competence is associated with nucleotide polymorphism in at least 16 immunity genes.[4] Duplication of receptors is another strategy widely used by invertebrates.[5] Combinatorial use of receptors is employed to increase the range of recognition using the same number of receptors.[6] Somatic diversification of receptors such as gene recombination, conversion, or alternative splicing is used to increase the number of receptors produced from a limited number of genes. In insects and crustacean, large repertoire of receptors is generated through alternative splicing of the Downs syndrome adhesion molecule.[7,8] 185/333-gene family in the purple sea urchin is diversified by recombination and point mutations.[9] Highly adaptive nucleic acids based immune mechanisms evolved in all phyla, including bacteria.[10,11]

Somatic diversification of receptors became a dominant strategy in the adaptive immune system of jaw vertebrates (gnathostomes). From sharks to humans, antigen receptors of T and B lymphoid cells (TCR and BCR) are encoded as gene segments,[12] and functional genes are produced by rearrangement of these segments by recombination activated genes (RAGs).[13] Each lymphoid cell expresses unique receptors and can clonally expand in response to a matching antigen.[14] Rearranged BCRs can be further diversified by gene conversion, class switch recombination and somatic hypermutation, mechanisms dependent on activation-induced cytidine deaminase (AID).[15] Despite the similarity between TCR and BCR, T and B cells differ functionally and in the way they recognize antigens. B cells recognize antigens directly, in contrast, T cells recognize antigens in association with the major histocompatibility complex (MHC) proteins and related molecules serving as markers of "self".[16] T cells therefore combine recognition of self and nonself. T cells are responsible for the cellular immunity and regulatory functions, while B cells are involved in humoral immunity by producing antibodies.

Till recently, adaptive immune system was thought to be unique to jaw vertebrates.

However, recently a strikingly functionally similar, albeit based on a completely different type of receptors, adaptive immune system has been discovered in jawless vertebrates (cyclostomes) lamprey and hagfish.[17] In this system, leucine-rich repeat (LRR) modules are incorporated into noncomplete variable lymphocyte receptor (VLR) gene to form a functional gene. Two types of receptors, VLRA and VLRB are expressed in different cell populations that seem to divide functions, as do T and B cells of jaw vertebrates and to mediate, correspondently, the cellular and humoral immunity.[18] VLRB receptors can be secreted as antibodies. In contrast, VLRA resemble TCR in that they are found only in membrane-bound form and do not bind antigens directly. Moreover, VLRA positive lymphoid cells express genes typical for T cells of jaw vertebrates, while VLRB-bearing cells express B-cell types of genes. Remarkably, assembly of VLRs may depend on the agnatha's homologue of AID.[19]

This discovery changes the view of the evolution of adaptive immune system. If both jaw and jawless vertebrates have B- and T-like cells and AID is involved in diversification of receptors in both systems, then their common ancestor might have already had subdivision of lymphoid cells into two branches. It is also possible that AID

was already involved in diversification of some immune receptors. It is now clear that somatic diversification of antigen receptors takes place in many invertebrate species.[20] Therefore the ancestors of vertebrates already had a sophisticated immune system. Two events likely contributed the most to the divergent evolution of the immune systems in jaw and jawless vertebrates; whole genome duplication (WGD)[21] and the development of the Rag-mediated receptor diversification in jaw vertebrates.[22] The knowledge of two different adaptive immune systems opens new opportunities for understanding the principles of the underlying design and evolution of the immune mechanisms.

THE MAJOR FEATURES OF THE ADAPTIVE IMMUNE SYSTEM OF JAW VERTEBRATES

The adaptive immune system of jaw vertebrates combines strong conservation of its general features with fast evolution of immune genes. The structure of immune receptors of T and B cells, mechanisms of receptor diversification and signaling mechanisms are conserved from sharks to humans while sequences can have only residual similarity. The adaptive immune system is built around three major molecules, TCR, BCR and MHC. TCR and BCR are dimers.[16] BCR contains immunoglobulin (Ig) heavy (H) and light (L) chains; TCR is composed of alpha and beta chains in αβT cells or gamma and delta chains in γδT cells. Antigen-binding site is formed by hypervariable complimentarity determining regions (CDRs) from both chains. Two CDRs are encoded by variable segments while CDR3 is created at V(D)J junctions. The signaling from TCR and BCR depends on the immunoreceptor tyrosine-based activation motif (ITAM) in accessory molecules, which is CD3 chain for TCR and immunoglobulin alpha and beta chains for BCR.[23]

The variable regions of TCR and BCR are encoded as segments, variable (V), diversity (D) and joining (J) flanked by recombination signal sequences (RSSs)[12] (Fig. 1). During rearrangement, RAG1 and RAG2 together with other proteins complex on the RSS and nick the DNA at the border between the RSS and the gene segment.[13] This leads to a DNA hairpin at the end of the coding sequence. Then, proteins from the nonhomologous end joining repair pathway open and ligate the hairpins. During this process, nucleotides can be removed or added to the junctions, increasing the variability of receptors. In addition, nontemplated nucleotides can be added by terminal deoxynucleotidyl transferase (TdT).[24] Each lymphoid cell typically expresses only one type of receptor by a mechanism known as allelic exclusion.[25]

BCRs are diversified additionally by somatic hypermutation, gene conversion and class-switch recombination.[15,26,27] These processes start with deamination of cytidine to uridine by AID with subsequent steps performed by DNA repair enzymes. The RGYW motif (where R = purine, Y = pyrimidine and W = A or T) and the reciprocal WRCY motif are the preferred targets for AID.[28,29] Somatic hypermutations coupled with selection by antigen leads to evolution of BCR with high-affinity for antigen (Fig. 2). This process is especially efficient in mammals, while in cold-blooded vertebrates it is much slower. The structure of V regions has evolved to direct somatic hypermutations mostly to CDR1 by concentrating AID target sequences in this region.[30] However, the relative density of such targets in human CDR1 is much higher than in the corresponding zebrafish sequences.[31] It may be one of the reasons why maturation of immune response is slower in fish.

The process of receptor diversification brings the danger of autoimmunity since some of the newly generated receptors can be self-reactive. These receptors are modified by

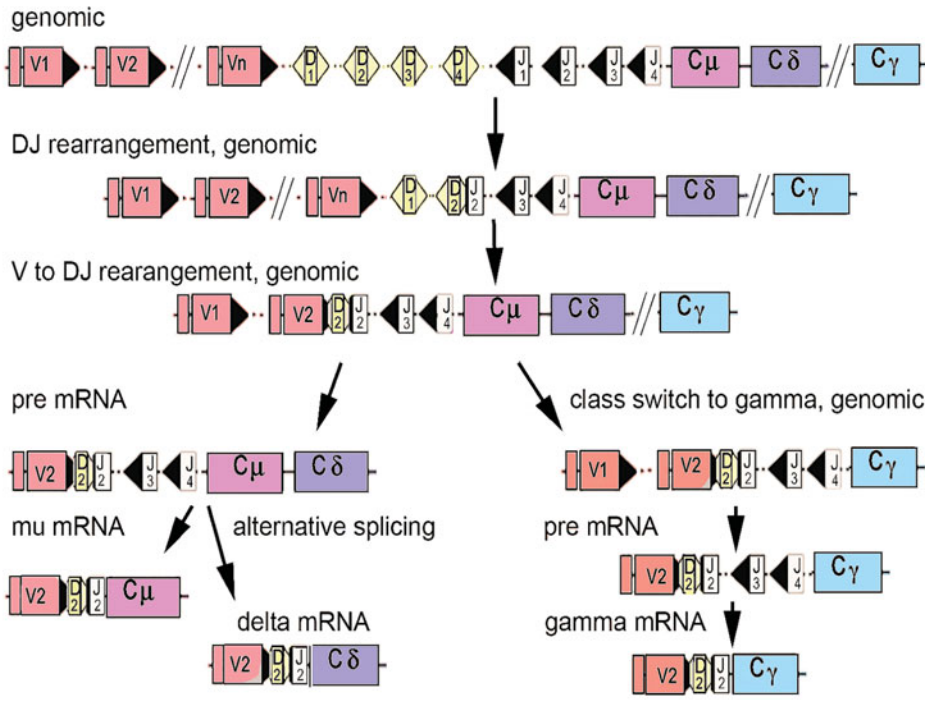

Figure 1. Diversification of immune receptors in jaw vertebrates. VDJ rearrangement of a hypothetical immunoglobulin heavy chain is shown.

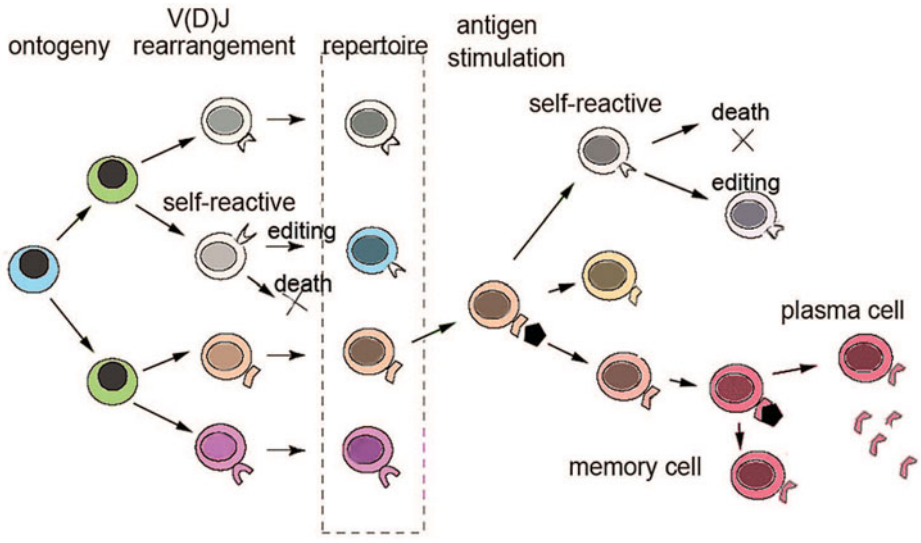

Figure 2. Highly specific antibody is a result of rounds of receptor modification and selection by antigen.

editing/revision when the V segment in a rearranged receptor is completely or partially replaced by another V segment. This process is mediated by RAGs proteins and depends on cryptic RSS sites in V segments.[32,33] Cells still bearing self-reactive receptors are removed by apoptotic death or silenced.

The variable and constant regions of lymphoid receptors evolve differently. V regions have evolved according to the "birth-and-death" model when duplication events together with lineage-specific gain and loss of individual members contribute to the rapid diversification of genes.[34] Constant regions are less constrained in the directions of their evolution, especially in immunoglobulins and have evolved to mediate various effector functions.

Interestingly, TCRα and TCRδ are expressed by the different types of T cells but are, nevertheless, encoded by a single locus with the delta DJC cluster proximal to the array of V segments followed by the alpha JC cluster.[16] This structure is preserved in evolution perhaps because it allows coordination of the expression of TCRδ and TCRα and, in this way, regulates the ratio of αβ to γδ T cells. TCRδ rearranges first and γδT cells are the first to appear during ontogeny. Combinatorial variability of the TCRδ is lower than that of the TCRα, because there are fewer DJ segments in the TCRδ cluster and because of restrictions on V regions rearrangement to TCRδ.[35] During the fetal life, the TdT enzyme is not active and junctions are not diversified, therefore early γδT cells have limited variability. However in adults, when TdT enzyme is involved in diversification, the potential number of different delta chains is very large, since the two D segments may participate simultaneously in V delta assembly and random nucleotides may be added at all three junctions.[36] This creates a sharp difference between the fetal and adult γδT repertoires, restricted in the fetus but variable in adults.

Immunoglobulins are much less conserved than TCRs with only IgM present in all species. The organization of Ig genomic loci also differs among species, especially that of immunoglobulin H chain. In cartilaginous fish, IgH are encoded in clusters with one V segment, two or three D segments and a single C region; separate clusters encode IgM, IgW and IgNAR.[37] Cluster organization of IgH is found only in cartilaginous fish, other species have IgH in translocon configuration with arrays of V, D and J segments encoded in the same locus. Light chains are encoded by the cluster type of loci in cartilaginous fish and by diverse types in other species.[38] Light chains are thought to have evolved to match heavy chains they pair up with in the BCR.

In teleost fish, three Ig classes are found, IgM, IgZ and IgD. Teleost IgH locus organization mimics the Tcrd-Tcra locus with an array of V segments followed by DJCzeta and DJCmuCdelta clusters.[39] V segments rearrange alternatively to zeta or mu clusters. Similarly to TCRδ, zeta has lower combinatorial variability and is expressed earlier in development than mu, however in adult fish, zeta chain has more N addition than mu. Therefore close functional parallels exist between pairs of IgZ/IgM B cells and δγ/αβ T cells. Teleost IgD is expressed by alternative splicing of DJCμCδ message often with inclusion of the first exon of Cμ.[40]

Coupling of mu and delta is preserved in tetrapods IgH loci. Contrary to teleost, tetrapods have several constant regions downstream from Cδ. These immunoglobulins are expressed by class switch recombination[41] (Fig. 1). This is a tetrapods-specific way of receptor diversification when CμCδ in the rearranged gene is replaced by one of the downstream constant regions. AID mediates this process through switch (S) regions located in front of each C region. Introduction of class switch recombination opened opportunities for evolution of various specialised immunoglobulins. For example IgA protects mucosal

surfaces and is adjusted for this function.[42] Even closely related tetrapod species often have different sets of specialised antibodies evolved to better perform specific tasks.

Hybrid molecules that have a double V structure with the N-terminal V domains similar to Ig V domain while the rest of the molecule is similar to the conventional TCRδ have been found recently in sharks and marsupials.[43,44] These receptors are thought to be a result of a recombination between the TCR and immunoglobulin loci. They combine the properties of Ig-like antigen binding with TCR effector function. They might have been present in mammalian ancestors but later were lost from most mammals.

T and B cells undergo V(D)J rearrangement and selection for the absence of self-reactivity in the primary lymphoid organs, which is thymus for T cells in all species and variable tissues for B cells.[45] The newly formed T and B cells migrate via the bloodstream to peripheral lymphoid tissues, where, following antigen recognition, they undergo lymphoblastoid transformation, clonally expand and differentiate correspondently into effector cytotoxic or helper T-lymphocytes or plasma cells. T and B cells cannot function without MHC proteins, which are necessary for antigen presentation to T cells and communication of immune cells. MHC is discussed in Chapter 18 of this book.[46]

ADAPTIVE IMMUNE SYSTEM OF JAWLESS VERTEBRATES

For long time, it was known that the jawless fishes (lampreys and hagfish) have lymphocyte-like cells and produce antigen-specific agglutinins after immunization (reviewed in Amemiya and Saha[47]). However neither lymphoid organs similar to higher vertebrates no genes essential to the classical adaptive immunity such as Rags, immunoglobulins, or MHC have been found. More recent studies show that jawless fish have adaptive immune system based on variable lymphocyte receptors (VLR).[17]

VLR is encoded as an empty cassette containing only 5′ and 3′-ends of the gene (Fig. 3). Hundreds of various leucine-rich repeats (LRR) modules are encoded upstream and downstream of the VLR locus and variable numbers of them are recombined into the cassette to create a functional gene. A gene conversion mechanism is thought to

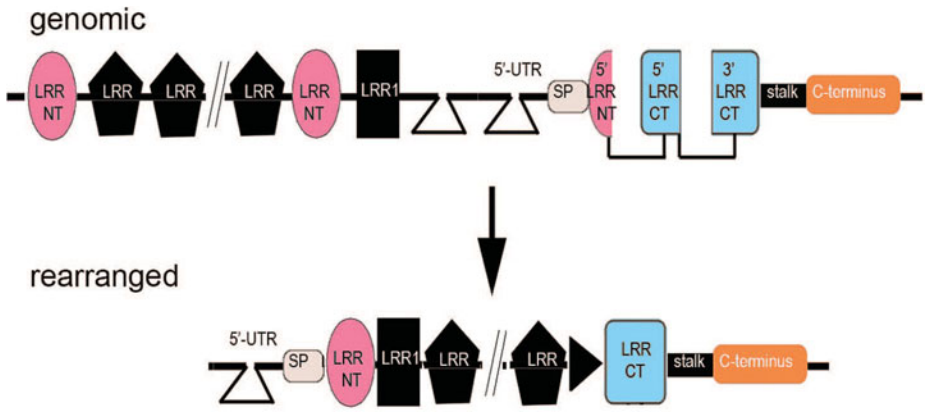

Figure 3. Adaptive immune receptors of jawless vertebrates are generated by assembly of LRR modules into incomplete VLR gene.

be responsible for copying the donor LRR sequences.[19] Lamprey lymphocytes express two putative deaminases of the AID-APOBEC family that may be involved in VLR diversification. The diversity of VLR antigen receptors is thought to be comparable to that generated in jaw vertebrates by Rag-mediated recombination.[19,48]

The structure of the VLR antibodies was predicted to resemble that of Toll-like receptors with typical LRR-containing solenoid structures.[17] Analysis of VLR sequences suggested that the concave surface of the VLR solenoid would be the binding site of the receptor since the patterns of amino acid substitutions indicated positive Darwinian selection.[48] This structure was validated after a VLR antibody was cocrystallized with the H antigen epitope from human "O" erythrocytes,[49] the same antigen that was found almost 40 years ago to elicit a specific agglutination response in lampreys.[50] Another structure resolved recently also demonstrated that an anti-lysozyme VLR was bound to the antigen by its concave surface.[51] In addition, presence of a loop that penetrated into the enzyme active site was noted.

Immunization of lamprey results in a shift in the flow activated cell-sorting (FACs) profile of lymphocytes, with increase in a population of larger, blast-like lymphocytes and a concomitant increase in *Vlr* mRNA levels.[48,52] Therefore, similar to the gnathostome lymphocytes, the agnathan lymphocytes undergo lymphoblastoid transformation following antigen and/or mitogen stimulation. Production of soluble VLRB was also noted. VLRB is bound to the membrane through glycosyl-phosphatidylinositol (GPI) anchor and the soluble protein can form via its cleavage. Immunization with several bacteria, polysaccharide antigens and some protein antigens elicited antibody with dissociation constants in picomolar range, equivalent to those of high-affinity IgG antibodies.[48,52,53]

VLRA and VLRB receptors are expressed by separate lymphocyte populations.[18] Cytosine deaminase 1 (CDA1) is expressed only in VLRA and CDA2 only in VLRB lymphocytes. Similar to receptors of the classical adaptive system, VLR receptors are expressed in monoallelic way. A surprising discovery was that VLRA and VLRB-expressing cells resemble functionally T and B cells of jaw vertebrates. Both VLRA and VLRB cells respond to antigenic stimulation by proliferation. However only VLRB lymphocytes bind native antigens and differentiate into VLR antibody-secreting cells while no direct binding of VLRA proteins to antigens could be detected and no soluble VLRA is present in lamprey plasma.[18] As a recombinant protein, VLRA is expressed exclusively as a transmembrane molecule similar to TCR. Another similarity to T cells is lymphoblastoid transformation of VLRA lymphocytes in response to phytohaemagglutinin while the response of VLRB cells is much weaker.

Gene expression profiles for VLRA and VLRB lymphocytes also resemble those of mammalian T and B cells. VLRA lymphocytes preferentially express a number of molecules characteristic of T cells in jawed vertebrates including GATA3, c-REL, aryl hydrocarbon receptor (AHR), BCL11b, NOTCH1, CD45, the IL-8 receptor CXCR2, IL-17 and MIF. In contrast, VLRB cells express CXCR4, TNFRSF14 that binds to LIGHT on T cells, two components of the BCR signaling cascade, SYK and the B-cell adaptor protein (BCAP), IL-8, the IL-17 receptor, TLR2, TLR7 and TLR10. Activated VLRB lymphocytes upregulate the expression of IL-8 while VLRA upregulate IL-17 and macrophage migration inhibitory factor (MIF).

Therefore it seems that VLRA lymphocytes recognize processed antigens and undergo selection in a manner analogous to the T-lymphocyte repertoire selection in jawed vertebrates. Lampreys lack the MHC that is used to present peptide fragments to T cells in jawed vertebrates. However, another, yet unknown molecules can perform this function in jawless fish.

The preferential expression of TLR2, TLR7 and TLR10 orthologues by the VLRB lymphocytes suggests that TLR ligands may facilitate activation of this population of lymphocytes in a manner similar to their roles in activating B lymphocytes. Some genes are expressed in a complementary way in VLRA and VLRB cells. Examples include expression of IL-17 in VLRA cells and IL-17 receptor in VLRB cells and expression of IL-8 in VLRB cells and IL-8 receptor in VLRA. Therefore VLRA and VLRB cells may communicate during immune response.

These data suggest that compartmentalization of lymphoid cells into the cellular and humoral branches have existed in ancestors of vertebrates before the appearance of different types of anticipatory receptors in jaw and jawless vertebrates and before separation of these vertebrate lineages.

ORIGIN OF THE REARRANGING IMMUNE RECEPTORS IN VERTEBRATES

Since the two vertebrate adaptive immune systems use the same type of immune cells the question arises: were these systems completely independent convergent evolutionary acquisitions,[18] did they coexist in vertebrate ancestors,[54] or did one precede the other?[55] There is currently some support for each of these hypotheses.

V(D)J recombination is very similar to the mechanisms of transpositional recombination and retroviral integration.[56,57] This led to the hypothesis that V(D)J recombination originated by integration of a bacterial transposon or a retrovirus into a genome of an ancestor of jaw vertebrates.[22] The integration resulted in cleavage of a gene encoding the V domain of an immune receptor into two recombinable fragments.[58,59] The intruder transposase evolved later into RAGs proteins. In support of this view, a fragment similar to the core region of RAG1 has been identified in transposases from Transib transposons.[60] The terminal inverted repeats of Transib transposons are very similar to RSSs used by RAGs. However, only the RAG1 core resembles Transib transposase, so it was suggested that the N-terminal domain was assembled from a different protein. One candidate is a mobile element from a mollusk.[61] *Rag1* gene is tightly linked with *Rag2* in all species. RAG2 protein bears no resemblance to transposases or any bacterial proteins, therefore it was suggested that RAG1 transposons landed in a vicinity of a primordial Rag2 gene and RAG2 was coopted by RAG1 to perform rearrangement. RAG1 core-like sequences were found in several protochordates. Strikingly, a *Rag1/2*-like linked gene pair is present in purple sea urchin genome that is similar in both sequence and genomic organization to the vertebrate *Rag1/2* pair.[22,62] Sea urchin RAGs are coexpressed during development and in adult tissues and form complex with RAG1 and RAG2 proteins from several vertebrate species. This discovery pushes the acquisition of the enzymes crucial for the origin of adaptive immunity many million years earlier than the origin of vertebrates. The role of sea urchin RAGs is unknown at present. Sea urchin RAGs are expressed in coelomocytes, which is consistent with the role of these proteins in immunity. No clusters of gene segments with similarity to the vertebrate V, D and J gene segments or RSS-like sequences have been identified within sea urchin genome. However if in sea urchin, RAGs act on diversification of a gene with only few gene segments, for example, a single V and a single J region, such a gene could be overlooked.

No RAGs have been identified in the C. intestinalis, which belongs to a group sister to vertebrates. However this species has a small genome and has likely underwent an

intensive gene loss. An alternative hypothesis is that a primordial herpes virus, rather than a transposon encoded the recombinase responsible for the origins of acquired immunity.[63] According to this hypothesis, the regulated expression of a viral recombinase in immune cells may have been positively selected for its ability to stimulate innate immunity to herpes virus infection. It provides a plausible explanation of the early and non-uniform appearance of RAGs in deuterostomata, although the sequence similarity between RAGs and herpes recombinases is low.

Another gene playing an important role in the adaptive immune system is AID.[64] AID and related APOBECs constitute a family of nucleic acid mutators.[19,64] APOBEC1 is a RNA-editing enzyme while the APOBEC3s are DNA mutators acting in defense against retroviruses. The AID/APOBECs are similar in structure and catalytic site to zinc-dependent deaminases, a large gene superfamily encoding enzymes involved in nucleic acid metabolism. The AID/APOBECs are thought to originate from tRNA adenosine deaminases (Tad/ADAT2) that edit adenosine to inosine in tRNAs in both eukaryotes and prokaryotes. The presence of two AID homologues in lamprey suggests that AID originated before the split of jaw and jawless vertebrates.[18] No full-length AID was identified in amphioxus; however, two regions exhibiting weak sequence identity to the N- and C-terminal regions of human AID have been found.[65] AID was suggested to develop in vertebrate ancestors as an antiviral protein.[19,59,66] In support, AID is induced outside the germinal centers in response to infection by certain retroviruses and contributes to antiviral defence. NF-kappaB binds the AID promoter and is required for the expression of a virus-induced AID.[67] NF-kappaB role in immune signaling is conserved in evolution, supporting the view that AID primordial role was antiviral defense. AID mutagenic activity could then become employed for somatic mutations in genes encoding immune receptors. Therefore the mechanism of deaminase-mediated diversification of antigen receptors could arise independently from V(D)J rearrangement. The AID/APOBECs family expanded in mammals and underwent complex gene duplications and positive selection.

TdT is one more molecule contributing to diversification of immune receptors by yet another unique mechanism, insertion of nontemplated nucleotides at V(D)J junctions. A closely related enzyme, polymerase μ, promotes repair of noncomplementary ends by nonhomologous end joining.[68] A gene homologous to TdT and polymerase μ is present in amphioxus and sea urchin.[65,69] Therefore many components of modern diversification machinery of the adaptive immune system are present in invertebrates.

Many candidates for a primordial receptor that was disrupted by a Rag-bearing transposon have been suggested from the members of IgSF involved in cell adhesion and innate immunity.[70] All major molecules involved in the adaptive immune system including Ig, TCR, MHC, tapasin and beta2-microglobulin have C1 type of constant domain. From other proteins with C1-like domains are signal-regulatory proteins (SIRPs).[71] SIRPs also encode V domains with a typical J motif and signal via ITAM-containing adaptor molecules. The expression of most members of this family is restricted to myeloid cells where they act as both inhibitory and activating receptors. SIRPα is an inhibitory receptor that interacts with the membrane protein CD47, which is a marker of self. CD47–SIRP interaction controls the effector functions of phagocytes protecting host cells against immune-mediated damage.[72]

In sea urchin, there are three V-C1-TM-cytoplasmic region genes.[69] Their role is currently unknown. The receptor disrupted by Rag-bearing transposon is expected to be involved in immune recognition. The somatic recombination of the receptor would

increase its repertoire and provide a selective advantage. To evolve into a typical TCR and BCR, the gene encoding this receptor would need to be duplicated to become a dimer and be duplicated again to form T and B-cell receptors. This could happen during the whole genome duplication (WGD).

WGD is likely to have played a pivotal role in evolution of the adaptive immune systems. WGD generates enormous amounts of genetic raw material available for acquisition of novel functions. Vertebrates were suggested to have undergone two rounds of WGD after the split of the urochordate and cephalochordate lineages.[21] Recent sequencing of invertebrate genomes has provided strong support for WGD hypothesis. In particular the genome of the cephalochordate amphioxus shows a high degree of synteny conservation with vertebrates. Amphioxus and the urochordate *Ciona* have a single MHC-like region that could be a precursor of the four MHC paralogons in jawed vertebrates.[65,73] Many more genes involved in modern adaptive immune system may originate as a result of WGDs.[54]

VLRs were suggested to originate from the GPIba, a part of the receptor complex GPIb-V-IX, which has a critical role in hemostasis.[19] This multifunctional receptor initiates platelet activation and thrombus formation at the sites of vascular injury and may control other vascular processes such as apoptosis, coagulation, inflammation and platelet-mediated tumor metastasis.[74] VLRs and GPIba share a unique insert between the a-helix and the first b-strand of the LRRCT module. This characteristic insert is absent from the LRRCT of other animal LRR-containing proteins.

The exact point in time when rearranging receptors originated is not known. The timing of divergence of cyclostomes from the gnathostome lineage is debated. Some molecular phylogenetic analyses suggest that the genome duplications occurred before the cyclostome-gnathostome split.[75] It can be speculated that RAG-rearranging receptor originated before the first WGD. After that, it was duplicated and became a dimer receptor. The second WGD led to duplication of the dimer receptor and created the grounds for the divergent evolution of TCR and BCR. The idea that all major components of both adaptive immune systems of vertebrates originated before the split of jawless vertebrates agrees with the presence in lamprey of such molecules as TCR-like, CD4-like, V-preB-like, CD3 epsilon etc.[76,77] Recently, two VLR-like genes have been identified in zebrafish suggesting that VLR receptors could also be in place at the time of the split.[55] This agrees with the idea that the vertebrate ancestor had both BCR/TCR and VLR precursors.[54] Their evolution might turn in opposite directions in jaw and jawless vertebrates with one system becoming dominant and the other being degenerated/lost.

Cartilaginous fishes already have a well-developed adaptive immune system. The major difference from teleost and tetrapods is the cluster organization of their IgH loci. Is cluster organization a primordial feature as often was suggested? It may be not. The structural and functional similarity of Tcrd-Tcra loci and teleost IgH loci may point to an ancestral type of organization of the locus encoding TCR and BCR that existed in jaw vertebrates before the divergence of the cartilaginous fish lineage. This scenario assumes that the IgH locus organization observed now in the teleost precedes the IgH cluster organization of cartilaginous fish.[31] Separation of IgM and IgZ loci in cartilaginous fish could occur by a translocation of one member of this pair into a different location. This would explain the origin of two ancient immunoglobulins of cartilaginous fish, IgM and IgW. Tetrapods could lose IgZ at some point in their evolution.

ORIGIN OF LYMPHOID CELLS AND ORGANS

An essential component of the adaptive immune system is a lymphoid cell able to live long to provide immunological memory and able to proliferate in response to antigen stimulation. Precisely at what time in evolution the lymphocytes had originated is unknown. Immune cells of invertebrates are mostly an unexplored area despite the long time since Metchnikoff' discovery of phagocytes in starfish larva in 1882. The oldest specialized immune cells that are found in almost all animals (metazoans) that have been studied are phagocytic cells. Moreover, immune-like phagocyte activity has been observed in the social amoebas that aggregate when starved to form a migrating slug. Cells called "S cells" engulf bacteria and sequester toxins circulating within the slug.[78] Phagocytes however, are relatively short-lived nondividing cells. Lymphocytes have emerged as a new type of immunocompetent cells. The evidence for two types of lymphocytes in lamprey and hagfish suggests that lymphocytes must have been present in the common ancestor of vertebrates. Segregation of the erythroid lineage also happened before the split of jaw from jawless vertebrates since lamprey has distinct erythroid cells.[79] Mussels have at least 3 types of hemocytes.[80] Ascidians have 5 discernible types of hemocytes including lymphocyte-like cells.[81]

Jaw vertebrates have a subpopulation of lymphoid cells, natural killer (NK) cells, which do not express rearranging receptors. Instead, they express innate receptors, activating and inhibitory.[82] Receptors recognizing MHCI on normal cells are inhibitory, those recognizing aberrant molecules are activating. NK response is the result of integration of activating and inhibitory signals. NK lyse virus infected and tumor cells. They also regulate other cell types through secretion of interferon (IFN)-γ and other cytokines. NK cells resemble adaptive cells in that they express a unique pattern of receptors creating a repertoire of specificities. Self-tolerance of NK cells must be established in each individual, which is also similar to T and B cells. Moreover, some data show that NK cells can expand in response to a specific antigen. For example, Ly49H+ NK cells expand after MCMV infection, similarly to how antigen-specific T cells proliferate after antigen stimulation.[83] Furthermore, recent data show that NK cells can produce memory response lasting at least two months after stimulation.[84,85] Therefore NK cells have characteristics of both innate and adaptive immune cells.

It is likely that cytotoxic lymphoid cells similar to NK cells developed quite early in evolution. Even the simplest animals such as corals, bryozoans and ascidians have systems for allorecognition although it is based on molecules other than MHC as "self" tags.[86] Urochordates *Botryllus schlosseri* cytotoxic cells express receptors related to mammalian NK CD94/NKR-P1 receptors.[87] Cytotoxic cells are present also in the sea urchin *Paracentrotus lividus*. The coelomic fluid from this species contains several coelomocyte types including amoebocytes and uncolored spherulocytes.[88] Cell population enriched in uncolored spherulocytes exert high cytotoxic activity against rabbit erythrocytes in the presence of amoebocytes or extracts from these cells pointing to cooperation of different cell types.

An important feature of lymphoid cells is their ability to clonally expand in response to immunological challenge. There are indications that sea urchin immune cells are a dynamic population. In response to lipopolysaccharide, they transiently increase in number.[89] The coelomocytes of individual sea urchins express scavenger receptor cysteine-rich genes from a multigene family encoding an estimated number of 1,200 SRCR domains in specific patterns unique to each individual. Their expression fluctuates up to 10-fold in

1 week and up to 30-fold over a period of 3 months.[90] It would be interesting to know is these cells can clonally expand in response to a specific antigen.

Knowledge of ontogeny of T and B cells may help to understand their phylogeny. However, the exact path from hematopoietic stem cells to lymphoid cells is not that apparent and has recently been a subject for debate. The classical model of hematopoiesis postulates early separation of lymphoid fate from erythroid/myeloid one and the existence of a common progenitor (CLP) for T and B lymphoid cells.[91] Some recent data support the existence of CLP population[92] while other data do not fit the CLP model. The thymic progenitors first lose the potential to generate B cells retaining a substantial macrophage potential as well as T, NK and dendritic cell potential.[93,94] About 30% of thymic macrophages are derived from early thymic progenitors. A close connection of B cells and macrophages was known for a while and fish and frog B cells have preserved the ability to phagocyte.[95] Recently, it was found that freshly isolated human peripheral blood γδ T cells can phagocyte, process and present antigens on MHCII.[96] It suggests a close connection of all immune cells. An alternative picture of hematopoesis suggests that both the innate (myeloid) and adaptive (lymphoid) lineages of the immune system arise from a common progenitor.[97,98] Many transcriptional factors involved in development of lymphoid and myeloid cells are shared.[99]

Until recently, it was believed that T cells are evolutionary older than B cells.[45] However B cell seems to be a default fate of lymphocyte development in absence of NOTCH signaling. NOTCH increases the frequency of multipotent progenitors; skews the T and NK potential of CLP and inhibits the differentiation of B cells.[100] Low doses of NOTCH ligands increase frequency of NK, whereas higher doses are required for increasing the frequency of T-cell clones. So it seems possible that B cells are evolutionary older than T cells.

T cells in all jaw vertebrates develop in thymus. Expression of NOTCH ligands by thymic epithelial cells is necessary for T-cell development. The fact that T-like cells in jawless fish express NOTCH suggests that development of these cells may be governed by the same signaling pathways as classical T cells. VLRA lymphocytes are enriched in the lamprey's gill regions suggesting that development of this cell population may take place in the same region, where T cell develop in jaw vertebrates.[18] *Foxn1* gene is expressed in epithelial cells of thymic primordium and is crucial for thymus development.[101] The agnatha ortholog of this gene, *Foxn4L*, is expressed in pharyngeal region in lamprey. Moreover, this expression overlaps NOTCH ligand DELTA-like 4, which is necessary for differentiation of lymphocyte progenitor cells into the T-cell lineage and is a downstream target of FOXN1.[101] In the same study, pharyngeal epithelial structures have been examined by in situ hybridization for the presence of lymphoid aggregates and none were found. This led to the conclusion that lamprey does not possess a lymphoid organ resembling the thymus of jawed vertebrates. However, the probe used for in situ hybridization was from *VLRB* gene, which is expressed in B-like cells. Usage of *VLRA* probe may clarify if T-like cells develop in lamprey's pharyngeal region.

The places where B cells develop vary among species and even among developmental stages in the same species. In jaw vertebrates, sites of B-cell development are often associated with the gut.[102,103] In developing lamprey, blood forms in the typhlosole (an invagination of the intestinal epithelium) and nephric fold, while in adults, blood forms in the protovertebral arch.[47] The development of cytotoxic T and NK cell in the gill region and antibody-producing B cells in the gut region may represent the ancient division of labor in protection of the regions most exposed to pathogens.

INNATE-ADAPTIVE INTERACTIONS

Adaptive immune system evolved from the innate immune mechanisms and it cannot function without their help.[104] Innate immune cells are the first to contact pathogens and they secrete various cytokines instructing adaptive cells about the nature of pathogen. For example, bacterial infections induce interleukin-12 (IL-12), while helminthes induce IL-4 and IL-13. In response, T cells develop into different subsets of helper cells; it is T-helper 1 (Th1) in case of intracellular bacteria, Th2 in case of helminthes and Th17 in case of extracellular bacteria and fungi.[105] Innate immune cells such as dendritic cells present antigens to T cells. Although B cells can recognize soluble antigens directly, antigen presentation to them is often mediated by macrophages and dendritic cells.[106] B-cell memory response is supported by basophils.[107] Therefore, innate immune modules control the adaptive cells so that the response is tailored to the pathogen, including the intensity of inflammation, duration, isotype of antibodies produced by B cells etc.

Many features of the adaptive immune system have developed to communicate with older innate mechanisms. For example, special regions of Ig evolved to bind the complement. B cells, in addition to adaptive receptors, express innate immune receptors such as TLRs. These receptors cooperate with BCRs in antigen recognition. B cells usually need T-cell help to be activated after BCR binds an antigen but if both BCR and TLR recognize the same antigen, B cells respond without T-cells help.[108]

On the other side, innate cells in species with adaptive immunity have also been extensively modified to work with adaptive mechanisms. All innate immune cells express MHCI and II genes, which are an integral part of the adaptive immune system. The machinery for antigen presentation was introduced in the innate immune cells. New cytokines to communicate with adaptive cells and the corresponding receptors have evolved. The complement system evolved to accept antibody-antigen complexes as activators. Phagocytes evolved receptors recognizing antibody bound to antigens. Introduction of T and B cells resulted in reduction of innate receptors. For comparison, there are 10 TLR in humans and 222 in sea urchin.[109]

Therefore innate modules in species with adaptive immune system differ from analogous modules in species without such system. Rightly, there are no pure innate or pure adaptive cells in our immune system, they turned into a nonseparable blend unable to function without one another. There is always some redundancy between individual immune mechanisms. In absence/defects of adaptive immune system, innate mechanisms are upregulated.[110] The relative contribution of innate and adaptive mechanisms into defense may vary between species. For example, zebrafish with mutation in *Rag1* gene and the corresponding absence of T and B cells, are viable and can be kept at usual condition;[111] while humans develop severe disease in absence of T and B cells.[112]

Recent studies have highlighted the role that helminth infection may have played in evolution of the adaptive immune system.[113] Helminth infections are very common in vertebrates. Helminths produce high levels of tissue damage, nevertheless the infection is usually well tolerated. Helminths modulate the host immune system by suppressing inflammatory reactions and skewing T-cell response to Th2 type. Th2 response is also involved in wound healing and tissue remodeling. Helminth-infected populations show increased susceptibility to microbial infection, while helminth-free populations have increased frequency of allergy and autoimmunity. A popular hypothesis is that the immune system evolved in the presence of helminths and developed a dependence on factors produced by the parasites. Helminths-derived products are intensively studied now as potential immunomodulators.[114]

CONCLUSION

There have been many speculations why the adaptive system evolved at all and why in vertebrates. The assumption has been that there should be something special in the vertebrate life-style of physiology that made diversification of receptors necessary. One hypothesis was that the predator life-style of early vertebrates created a necessity for gut protection.[115] Another hypothesis also assumes that there was a special need for adaptive immunity in vertebrates. It suggests that the maintenance of symbiotic microbial communities, especially in the gut, was the primary force driving the evolution of the adaptive immune system.[116,117] The basis for this hypothesis is that vertebrates harbor hundreds of symbiotic bacteria species while invertebrates do not form such relationships. Another idea is that the appearance of anticipatory immunity in vertebrates might have been driven by a need to facilitate the developmental and morphological plasticity in addition to increasing the scope of pathogen recognition.[118] Fewer offspring in jaw vertebrates in comparison to invertebrates was also suggested to play a role.[54]

The danger of self-reactivity, which accompanies the diversification of adaptive receptors and the fact that some species without adaptive immune system such as mollusks still have long life span is interpreted sometimes as evidence that the origin of adaptive immunity is just an evolutionary serendipity, which left us with an overly complex, costly and self-harmful immune system.[119] Yet the finding that the two surviving branches of vertebrate radiation jawless fish—lamprey and hagfish—and jawed vertebrates both developed adaptive immune systems based on different receptors yet strikingly similar functionally suggests that adaptive immunity provided a great survival benefit. Moreover, the existence of receptor diversification and memory in invertebrates suggests that similar driving forces act on all species leading to unique mechanisms of receptors diversification.[9,69,90]

REFERENCES

1. Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002; 20:197-216.
2. Matzinger P. The danger model: a renewed sense of self. Science 2002; 296:301-305.
3. Frank M, Kirkman L, Costantini D et al. Frequent recombination events generate diversity within the multi-copy variant antigen gene families of Plasmodium falciparum. Int J Parasitol 2008; 38:1099-1109.
4. Lazzaro BP, Sceurman BK, Clark AG. Genetic basis of natural variation in D. melanogaster antibacterial immunity. Science 2004; 303:1873-1876.
5. Messier-Solek C, Buckley KM, Rast JP. Highly diversified innate receptor systems and new forms of animal immunity. Semin Immunol 2010; 22:39-47.
6. Pal S, Wu LP. Lessons from the fly: pattern recognition in Drosophila melanogaster. Adv Exp Med Biol 2009; 653:162-174.
7. Watson FL, Puttmann-Holgado R, Thomas F et al. Extensive diversity of Ig-superfamily proteins in the immune system of insects. Science 2005; 309:1874-1878.
8. Brites D, McTaggart S, Morris K et al. The Dscam homologue of the crustacean Daphnia is diversified by alternative splicing like in insects. Mol Biol Evol 2008; 25:1429-1439.
9. Buckley KM, Terwilliger DP, Smith LC. Sequence variations in 185/333 messages from the purple sea urchin suggest posttranscriptional modifications to increase immune diversity. J Immunol 2008; 181:8585-8594.
10. Waterhouse PM, Wang MB, Lough T. Gene silencing as an adaptive defence against viruses. Nature 2001; 411:834-842.
11. Brouns SJ, Jore MM, Lundgren M et al. Small CRISPR RNAs guide antiviral defense in prokaryotes. Science 2008; 321:960-964.
12. Tonegawa S. Somatic generation of antibody diversity. Nature 1983; 302:575-581.
13. Schatz DG. V(D)J recombination. Immunol Rev 2004; 200:5-11.

14. Burnet FM. A modification of Jerne's theory of antibody production using the concept of clonal selection. Aust J Sci 1957; 20:67-69.
15. Maizels N. Immunoglobulin gene diversification. Annu Rev Genet 2005; 39:23-46.
16. Paul WE. Fundamental Immunology. 5 ed. Philadelphia: Lippincott Williams and Wilkins 2008.
17. Pancer Z, Amemiya CT, Ehrhardt GRC et al. Somatic diversification of variable lymphocyte receptors in the agnathan sea lamprey. Nature 2004; 430:174-180.
18. Guo P, Hirano M, Herrin BR et al. Dual nature of the adaptive immune system in lampreys. Nature 2009; 459:796-801.
19. Rogozin IB, Iyer LM, Liang L et al. Evolution and diversification of lamprey antigen receptors: evidence for involvement of an AID-APOBEC family cytosine deaminase. Nat Immunol 2007; 8:647-656.
20. Litman GW, Cannon JP, Rast JP. New insights into alternative mechanisms of immune receptor diversification. Adv Immunol 2005; 87:209-236.
21. Kasahara M. The 2R hypothesis: an update. Curr Opin Immunol 2007; 19:547-552.
22. Fugmann SD. The origins of the Rag genes-From transposition to V(D)J recombination. Semin Immunol 2010; 22:10-16.
23. Abram CL, Lowell CA. The expanding role for ITAM-based signaling pathways in immune cells. Sci STKE 2007; 2007:re2.
24. Alt FW, Baltimore D. Joining of immunoglobulin heavy chain gene segments: implications from a chromosome with evidence of three D-JH fusions. Proc Natl Acad Sci USA 1982; 79:4118-4122.
25. Cedar H, Bergman Y. Choreography of Ig allelic exclusion. Curr Opin Immunol 2008; 20:308-317.
26. Muramatsu M, Kinoshita K, Fagarasan S et al. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell 2000; 102:553-563.
27. Liu M, Schatz DG. Balancing AID and DNA repair during somatic hypermutation. Trends Immunol 2009; 30:173-181.
28. Rogozin IB, Kolchanov NA. Somatic hypermutagenesis in immunoglobulin genes. II. Influence of neighbouring base sequences on mutagenesis. Biochim Biophys Acta 1992; 1171:11-18.
29. Chaudhuri J, Basu U, Zarrin A et al. Evolution of the immunoglobulin heavy chain class switch recombination mechanism. Adv Immunol 2007; 94:157-214.
30. Chang B, Casali P. The CDR1 sequences of a major proportion of human germline Ig VH genes are inherently susceptible to amino acid replacement. Immunol Today 1994; 15:367-373.
31. Danilova N, Amemiya CT. Going adaptive: the saga of antibodies. Ann N Y Acad Sci 2009; 1168:130-155.
32. Gay D, Saunders T, Camper S et al. Receptor editing: an approach by autoreactive B-cells to escape tolerance. J Exp Med 1993; 177:999-1008.
33. Tiegs SL, Russell DM, Nemazee D. Receptor editing in self-reactive bone marrow B-cells. J Exp Med 1993; 177:1009-1020.
34. Ota T, Nei M. Divergent evolution and evolution by the birth-and-death process in the immunoglobulin VH gene family. Mol Biol Evol 1994; 11:469-482.
35. Uche UN, Huber CR, Raulet DH et al. Recombination signal sequence-associated restriction on TCRdelta gene rearrangement affects the development of tissue-specific gammadelta T-cells. J Immunol 2009; 183:4931-4939.
36. Elliott JF, Rock EP, Patten PA et al. The adult T-cell receptor delta-chain is diverse and distinct from that of fetal thymocytes. Nature 1988; 331:627-631.
37. Rumfelt LL, Marilyn Diaz M, Lohr RL et al. Unprecedented Multiplicity of Ig Transmembrane and Secretory mRNA Forms in the Cartilaginous Fish. J Immunol 2004; 173:1129–1139.
38. Criscitiello MF, Flajnik MF. Four primordial immunoglobulin light chain isotypes, including lambda and kappa, identified in the most primitive living jawed vertebrates. Eur J Immunol 2007; 37:2683-2694.
39. Danilova N, Bussmann J, Jekosch K et al. The immunoglobulin heavy-chain locus in zebrafish:identification and expression of a previously unknown isotype, immunoglobulin Z. Nat Immunol. 2005; 6:295-302.
40. Wilson M, Bengten E, Miller NW et al. A novel chimeric Ig heavy chain from a teleost fish shares similarities to IgD. Proc Natl Acad Sci USA 1997; 94:4593-4597.
41. Stavnezer J, Guikema JE, Schrader CE. Mechanism and regulation of class switch recombination. Annu Rev Immunol 2008; 26:261-292.
42. Fagarasan S. Evolution, development, mechanism and function of IgA in the gut. Curr Opin Immunol 2008; 20:170-177.
43. Criscitiello MF, Saltis M, Flajnik MF. An evolutionarily mobile antigen receptor variable region gene: doubly rearranging NAR-TcR genes in sharks. Proc Natl Acad Sci USA 2006; 103:5036-5041.
44. Parra ZE, Baker ML, Schwarz RS et al. A unique T-cell receptor discovered in marsupials. Proc Natl Acad Sci USA 2007; 104:9776-9781.
45. Boehm T, Bleul CC. The evolutionary history of lymphoid organs. Nat Immunol 2007; 8:131-135.
46. Martinez-Borra J, Lopez-Larrea C. The emergence of Major Histocompatibility Complex In: Lopez-Larrea C, ed. Self and Nonself. Austin: Landes Bioscience; 2011.

47. Amemiya CT, Saha NR, Zapata A. Evolution and development of immunological structures in the lamprey. Curr Opin Immunol 2007; 19(5):535-541.
48. Alder MN, Rogozin IB, Iyer LM et al. Diversity and function of adaptive immune receptors in a jawless vertebrate. Science 2005; 310:1970-1973.
49. Han BW, Herrin BR, Cooper MD et al. Antigen recognition by variable lymphocyte receptors. Science 2008; 321:1834-1837.
50. Pollara B, Litman GW, Finstad J et al. The evolution of the immune response. VII. Antibody to human "O" cells and properties of the immunoglobulin in lamprey. J Immunol 1970; 105:738-745.
51. Velikovsky CA, Deng L, Tasumi S et al. Structure of a lamprey variable lymphocyte receptor in complex with a protein antigen. Nat Struct Mol Biol 2009; 16:725-730.
52. Alder MN, Herrin BR, Sadlonova A et al. Antibody responses of variable lymphocyte receptors in the lamprey. Nat Immunol 2008; 9:319-327.
53. Tasumi S, Velikovsky CA, Xu G et al. High-affinity lamprey VLRA and VLRB monoclonal antibodies. Proc Natl Acad Sci USA 2009; 106:12891-12896.
54. Flajnik MF, Kasahara M. Origin and evolution of the adaptive immune system: genetic events and selective pressures. Nat Rev Genet 2009; 11:47-59.
55. Saha NR, Smith J, Amemiya CT. Evolution of adaptive immune recognition in jawless vertebrates. Semin Immunol 2010; 22:25-33.
56. Spanopoulou E, Zaitseva F, Wang FH et al. The homeodomain region of Rag-1 reveals the parallel mechanisms of bacterial and V(D)J recombination. Cell 1996; 87:263-276.
57. van Gent DC, Mizuuchi K, Gellert M. Similarities between initiation of V(D)J recombination and retroviral integration. Science 1996; 271:1592-1594.
58. Sakano H, Huppi K, Heinrich G et al. Sequences at the somatic recombination sites of immunoglobulin light-chain genes. Nature 1979; 280:288-294.
59. Schatz DG. Antigen receptor genes and the evolution of a recombinase. Semin Immunol 2004; 16:245-256.
60. Kapitonov VV, Jurka J. RAG1 core and V(D)J recombination signal sequences were derived from Transib transposons. PLoS Biol 2005; 3:e181.
61. Panchin Y, Moroz LL. Molluscan mobile elements similar to the vertebrate Recombination-Activating Genes. Biochem Biophys Res Commun 2008; 369:818-823.
62. Fugmann SD, Messier C, Novack LA et al. An ancient evolutionary origin of the Rag1/2 gene locus. Proc Natl Acad Sci USA 2006; 103:3728-3733.
63. Dreyfus DH. Paleo-immunology: evidence consistent with insertion of a primordial herpes virus-like element in the origins of acquired immunity. PLoS One 2009; 4:e5778.
64. Conticello SG. The AID/APOBEC family of nucleic acid mutators. Genome Biol 2008; 9:229.
65. Holland LZ, Albalat R, Azumi K et al. The amphioxus genome illuminates vertebrate origins and cephalochordate biology. Genome Res 2008; 18:1100-1111.
66. Flajnik MF. Comparative analyses of immunoglobulin genes: surprises and portents. Nat Rev Immunol 2002; 2:688-698.
67. Gourzi P, Leonova T, Papavasiliou FN. Viral induction of AID is independent of the interferon and the Toll-like receptor signaling pathways but requires NF-kappaB. J Exp Med 2007; 204:259-265.
68. Davis BJ, Havener JM, Ramsden DA. End-bridging is required for pol mu to efficiently promote repair of noncomplementary ends by nonhomologous end joining. Nucleic Acids Res 2008; 36:3085-3094.
69. Rast JP, Messier-Solek C. Marine invertebrate genome sequences and our evolving understanding of animal immunity. Biol Bull 2008; 214:274-283.
70. Du Pasquier L. Speculations on the origin of the vertebrate immune system. Immunol Lett 2004; 92:3-9.
71. van den Berg TK. On the origin and function of immune 'self' recognition. Immunol Lett 2009; 128:24-25.
72. Tsai RK, Discher DE. Inhibition of "self" engulfment through deactivation of myosin-II at the phagocytic synapse between human cells. J Cell Biol 2008; 180:989-1003.
73. Olinski RP, Lundin LG, Hallbook F. Conserved synteny between the Ciona genome and human paralogons identifies large duplication events in the molecular evolution of the insulin-relaxin gene family. Mol Biol Evol 2006; 23:10-22.
74. Canobbio I, Balduini C, Torti M. Signalling through the platelet glycoprotein Ib-V-IX complex. Cell Signal 2004; 16:1329-1344.
75. Kuraku S, Meyer A, Kuratani S. Timing of genome duplications relative to the origin of the vertebrates: did cyclostomes diverge before or after? Mol Biol Evol 2009; 26:47-59.
76. Pancer Z, Mayer WE, Klein J et al. Prototypic T-cell receptor and CD4-like coreceptor are expressed by lymphocytes in the agnathan sea lamprey. Proc Natl Acad Sci USA 2004; 101:13273-13278.
77. Cannon JP, Haire RN, Pancer Z et al. Variable domains and a VpreB-like molecule are present in a jawless vertebrate. Immunogenetics 2005; 56:924-929.
78. Chen G, Zhuchenko O, Kuspa A. Immune-like phagocyte activity in the social amoeba. Science 2007; 317:678-681.

79. Hagerstrand H, Danieluk M, Bobrowska-Hagerstrand M et al. The lamprey (Lampetra fluviatilis) erythrocyte; morphology, ultrastructure, major plasma membrane proteins and phospholipids and cytoskeletal organization. Mol Membr Biol 1999; 16:195-204.
80. Le Foll F, Rioult D, Boussa S et al. Characterisation of Mytilus edulis hemocyte subpopulations by single cell time-lapse motility imaging. Fish Shellfish Immunol 2010; 28:372-386.
81. de Barros CM, de Carvalho DR, Andrade LRP et al. Nitric oxide production by hemocytes of the ascidian Styela plicata. Cell Tissue Res 2009; 338:117-128.
82. Cooper MA, Colonna M, Yokoyama WM. Hidden talents of natural killers: NK cells in innate and adaptive immunity. EMBO Rep 2009; 10:1103-1110.
83. Dokun AO, Kim S, Smith HR et al. Specific and nonspecific NK cell activation during virus infection. Nat Immunol 2001; 2:951-956.
84. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. Nature 2009; 457:557-561.
85. Cooper MA, Elliott JM, Keyel PA, Y et al. Cytokine-induced memory-like natural killer cells. Proc Natl Acad Sci USA 2009; 106:1915-1919.
86. Nicotra ML, Powell AE, Rosengarten RD et al. A hypervariable invertebrate allodeterminant. Curr Biol 2009; 19:583-589.
87. Khalturin K, Becker M, Rinkevich B et al. Urochordates and the origin of natural killer cells: identification of a CD94/NKR-P1-related receptor in blood cells of Botryllus. Proc Natl Acad Sci USA 2003; 100:622-627.
88. Arizza V, Giaramita FT, Parrinello D et al. Cell cooperation in coelomocyte cytotoxic activity of Paracentrotus lividus coelomocytes. Comp Biochem Physiol A Mol Integr Physiol 2007; 147:389-394.
89. Brockton V, Henson JH, Raftos DA et al. Localization and diversity of 185/333 proteins from the purple sea urchin—unexpected protein-size range and protein expression in a new coelomocyte type. J Cell Sci 2008; 121:339-348.
90. Pancer Z. Dynamic expression of multiple scavenger receptor cysteine-rich genes in coelomocytes of the purple sea urchin. Proc Natl Acad Sci USA 2000; 97:13156-13161.
91. Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell 1997; 91:661-672.
92. Inlay MA, Bhattacharya D, Sahoo D et al. Ly6d marks the earliest stage of B-cell specification and identifies the branchpoint between B-cell and T-cell development. Genes Dev 2009; 23:2376-2381.
93. Wada H, Masuda K, Satoh R et al. Adult T-cell progenitors retain myeloid potential. Nature 2008; 452:768-772.
94. Bell JJ, Bhandoola A. The earliest thymic progenitors for T-cells possess myeloid lineage potential. Nature2008; 452:764-767.
95. Li J, Barreda DR, Zhang YA et al. B-lymphocytes from early vertebrates have potent phagocytic and microbicidal abilities. Nat Immunol 2006; 7:1116-1124.
96. Wu Y, Wu W, Wong WM et al. Human gamma delta T-cells: a lymphoid lineage cell capable of professional phagocytosis. J Immunol 2009; 183:5622-5629.
97. Laslo P, Pongubala JM, Lancki DW et al. Gene regulatory networks directing myeloid and lymphoid cell fates within the immune system. Semin Immunol 2008; 20:228-235.
98. Kawamoto H, Katsura Y. A new paradigm for hematopoietic cell lineages: revision of the classical concept of the myeloid-lymphoid dichotomy. Trends Immunol 2009; 30:193-200.
99. Spooner CJ, Cheng JX, Pujadas E et al. A recurrent network involving the transcription factors PU.1 and Gfi1 orchestrates innate and adaptive immune cell fates. Immunity 2009; 31:576-586.
100. Benne C, Lelievre JD, Balbo MH et al. Notch increases T/NK potential of human hematopoietic progenitors and inhibits B-cell differentiation at a pro-B stage. Stem Cells 2009; 27:1676-1685.
101. Bajoghli B, Aghaallaei N, Hess I et al. Evolution of genetic networks underlying the emergence of thymopoiesis in vertebrates. Cell 2009; 138:186-197.
102. Glick B, Chang TS, Jaap RG. The bursa of Fabricius and antibody production. Poult. Sci 1956; 35:224.
103. Pospisil R, Mage RG. Rabbit appendix: a site of development and selection of the B-cell repertoire. Curr Top Microbiol Immunol 1998; 229:59-70.
104. Palm NW, Medzhitov R. Pattern recognition receptors and control of adaptive immunity. Immunol Rev 2009; 227:221-233.
105. Zhou L, Chong MM, Littman DR. Plasticity of CD^{4+} T-cell lineage differentiation. Immunity 2009; 30:646-655.
106. Batista FD, Harwood NE. The who, how and where of antigen presentation to B-cells. Nat Rev Immunol 2009; 9:15-27.
107. Denzel A, Maus UA, Rodriguez Gomez M et al. Basophils enhance immunological memory responses. Nat Immunol 2008; 9(7):733-742.
108. Leadbetter EA, Rifkin IR, Hohlbaum AM et al. Chromatin-IgG complexes activate B-cells by dual engagement of IgM and Toll-like receptors. Nature 2002; 416:603-607.
109. Rast JP, Smith LC, Loza-Coll M et al. Genomic insights into the immune system of the sea urchin. Science 2006; 314:952-956.

110. Jima DD, Shah RN, Orcutt TM et al. Enhanced transcription of complement and coagulation genes in the absence of adaptive immunity. Mol Immunol 2009; 46:1505-1516.
111. Wienholds E, Schulte-Merker S, Walderich B et al. Target-selected inactivation of the zebrafish rag1 gene. Science 2002; 297:99-102.
112. Karaca NE, Aksu G, Genel F et al. Diverse phenotypic and genotypic presentation of RAG1 mutations in two cases with SCID. Clin Exp Med 2009; 9:339-342.
113. Jackson JA, Friberg IM, Little S et al. Review series on helminths, immune modulation and the hygiene hypothesis: immunity against helminths and immunological phenomena in modern human populations: coevolutionary legacies? Immunology 2009; 126:18-27.
114. Steinfelder S, Andersen JF, Cannons JL et al. The major component in schistosome eggs responsible for conditioning dendritic cells for Th2 polarization is a T2 ribonuclease (omega-1). J Exp Med 2009; 206:1681-1690.
115. Matsunaga T, Rahman A. What brought the adaptive immune system to vertebrates?—The jaw hypothesis and the seahorse. Immunol Rev 1998; 166:177-186.
116. McFall-Ngai M. Adaptive immunity: care for the community. Nature 2007; 445:153.
117. Sansonetti PJ, Medzhitov R. Learning tolerance while fighting ignorance. Cell 2009; 138:416-420.
118. Pancer Z, Cooper MD. The Evolution of Adaptive Immunity. Annu. Rev. Immunol 2006; 24:497-518.
119. Hedrick SM. The acquired immune system: a vantage from beneath. Immunity 2004; 21:607-615.