How did our complex immune system evolve?

Max Cooper and Brantley Herrin discuss the evolution of innate and alternative adaptive immune systems for defence purposes and conclude that successful vaccines and other therapeutic manipulations of the immune system will require a composite strategy.

The more we learn about our immune system, the more amazed we are by its complexity. Just when immunologists thought the basic principles for the somatic diversification of antigen receptors for B and T cells had been solved, we began to appreciate the complexity of the innate immune response, its importance as a first level of protective immunity and its role in triggering antigen-specific responses by the adaptive immune system. Perhaps if we could work out the evolutionary history of innate and adaptive immunity, this could help us to understand the intricacies of how our immune system functions and sometimes dysfunctions.

Innate immune mechanisms can be found in representative species at almost every level of the evolutionary tree of life. This fact alone is evidence of the importance of innate immunity in the competitive 'struggle for existence'[1] that began with the appearance of single cell microorganisms on Earth more than 3.5 billion years ago. The ensuing evolution of diverse bacteria, archaea and eukaryotes was so successful that these microorganisms caused environmental changes, including an increase in the concentration of atmospheric oxygen, that fostered the development of multicellular organisms (metazoans) around 600 million years ago. The evolutionary burst of diversity in metazoan species over the next 50 million years presented new host opportunities for microbial pathogens. In turn, the need for new mechanisms of host defence might explain the remarkable diversity of innate defence mechanisms in plants and animals. Although many different innate immune mechanisms are deployed for host defence, a unifying theme of innate immunity is the use of germline-encoded pattern recognition receptors for pathogens or damaged self components, such as the Toll-like receptors, nucleotide-binding domain leucine-rich repeat (LRR)-containing receptors, retinoic acid-inducible gene I-like RNA helicases and C-type lectin receptors[2,3].

Another layer of complexity in immune defences emerged during chordate evolution with the appearance of adaptive immunity in vertebrates around 500 million years ago. The unique feature of an adaptive immune system is the somatic development of clonally diverse lymphocytes, each of which has a unique antigen recognition receptor that can be used to trigger its activation. The combinatorial generation of a highly diverse lymphocyte receptor repertoire allows vertebrates to recognize almost any potential pathogen or toxin and to mount antigen-specific responses to it. Antigen-activated lymphocytes undergo clonal expansion and differentiation into mature effector lymphocytes with cytotoxic and pro-inflammatory functions or into plasma cells that secrete antibodies (the soluble forms of the antigen-specific receptors). Moreover, the clonal expansion and long life of some antigen-primed cytotoxic lymphocytes and plasma cells provide protective memory to prevent reinvasion.

Effective cellular and humoral immune responses by T and B cells, respectively, require the participation of various phagocytic cells, dendritic cells (DCs), natural killer (NK) cells and other types of innate immune cell and humoral components, which have important inductive or effector roles to provide protective immunity[4]. Tracing the evolution of genes has become easier with the increasing availability of genomic sequences of representative species, but it is more difficult to discern the evolutionary history of the extensive network of individual cell types that must work together for effective immunity. For example, we do not currently know when some of the key immune players entered the evolutionary scene, such as DCs and NK cells. Moreover, continual evolutionary changes add to the confusion. A relevant example is the fairly recent evolution of entirely different types of NK cell receptors in mice and humans, who last shared a common ancestor around 65 million years ago; mouse NK cells use lectin receptors, whereas primate NK cells use immunoglobulin-based killer cell immunoglobulin-like receptors (KIRs) to recognize MHC class I-associated ligands, which control their activation[5].

As if understanding this complex evolutionary puzzle were not already sufficiently challenging, we have learned recently that two types of adaptive immune system have evolved in vertebrates: a recently recognized system in jawless vertebrates (hagfish and lamprey) and the more familiar adaptive immune system of jawed vertebrates[6]. These two systems use entirely different types of antigen recognition receptor, but they use similar lymphocyte

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differentiation strategies involving two lymphocyte lineages that somatically assemble highly diverse antigen receptor repertoires. Instead of the V(D)JC composition of immunoglobulin-based B cell receptors (BCRs) and T cell receptors (TCRs) in jawed vertebrates, the variable lymphocyte receptors (VLRs) of jawless vertebrates are composed of variable LRR sequences linked by a flexible invariant stalk region to the lymphocyte surface.

The jawless vertebrates have two types of VLR and diverse repertoires of each receptor type are expressed by separate populations of lymphocytes that resemble our B and T cells[7,8]. The germline *vlra* and *vlrb* genes are both incomplete in that they encode only portions of the amino- and carboxy-terminal LRRs plus the complete stalk region. Hundreds of different LRR sequences flank both germline VLR genes, and these are randomly selected as templates to be copied in a piece-by-piece manner to complete a *vlra* or *vlrb* gene during lymphocyte development[9–11]. Monoallelic *vlra* assembly is associated with expression of an AID–APOBEC (activation-induced cytidine deaminase–apolipoprotein B mRNA-editing enzyme, catalytic polypeptide) orthologue, known as cytidine deaminase 1 (CDA1), and monoallelic *vlrb* assembly is associated with CDA2 expression, which indicates that these enzymes might differentially participate in a gene conversion mechanism for VLR assembly. Although VLRA$^+$ and VLRB$^+$ lymphocytes use the same basic constituents and assembly mechanisms to generate extensive repertoires of anticipatory receptors, they use the two types of receptor differently, much as T and B cells use their TCRs and BCRs in different ways. VLRA$^+$ lymphocytes respond to antigenic stimulation but do not secrete their receptors, whereas antigen-triggered VLRB$^+$ lymphocytes undergo proliferation and differentiation into plasma cells, which secrete multimeric VLRB antibodies that resemble our IgM antibodies, except that the basic subunit is a single polypeptide chain[12].

The VLRA$^+$ lymphocytes have other T cell-like features. They express orthologues of genes that mammalian cells use for T cell lineage commitment, differentiation and pro-inflammatory cytokine production. There is even some indication that VLRA$^+$ and VLRB$^+$ lymphocytes are functionally interactive. For example, activated VLRA$^+$ cells upregulate expression of an interleukin-17 (*Il17*) orthologue and VLRB$^+$ cells preferentially express an IL-17 receptor (*Il17r*) orthologue. Conversely, activated VLRB$^+$ cells upregulate expression of IL-8 and VLRA$^+$ cells preferentially express IL-8R. The T and B cell analogy for the VLRA$^+$ and VLRB$^+$ lymphocytes of jawless vertebrates extends to differences in their antigen receptor repertoires in that only the VLRB$^+$ cells seem to bind unprocessed antigens. The surprising resemblance of the two arms of the adaptive immune system in lamprey to the T and B cell lineages in jawed vertebrates raises a large number of questions. Where is the thymus equivalent in jawless vertebrates? Is the VLRA repertoire selected for self versus non-self recognition? If so, what is the histocompatibility gene? Do the VLRA$^+$ and VLRB$^+$ cells cooperate to provide protective immunity and to avoid autoimmunity? How does the VLR-based adaptive immune system interface with innate immune mechanisms?

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The evolution of alternative adaptive immune systems was facilitated by two rounds of whole genome duplication (Supplementary information S1 (figure)), which enabled the original function of a gene to be maintained while allowing evolutionary selection of modifications of additional gene copies for new purposes[13]. The common ancestor of lamprey and hagfish probably emerged between the first and second rounds of genome duplication, as amphioxus and tunicates have single gene copies, lamprey have two gene copies and jawed vertebrates typically have four copies of retained genes[14]. We suspect that the immunoglobulin-based adaptive immune system evolved later and independently of the VLR-based adaptive immune system, in that none of the immunoglobulin-based BCR, TCR or MHC receptor genes is found in jawless vertebrates and VLR genes have not been found in jawed vertebrates[15]. Such convergent evolution of mechanisms for the generation of diverse antigen receptors after the split in jawless and jawed vertebrate ancestry raises the question of whether the two pathways of lymphocyte differentiation arose in a common vertebrate ancestor.

With so many still unanswered questions, one could ask whether we have learned anything useful from the attempts to solve this evolutionary puzzle. An obvious conclusion is that the extraordinary complexity of our integrated innate and adaptive immune systems is the result of powerful and enduring selection, most probably to improve the odds for successfully combating pathogens. However, the emergence of an adaptive immune system featuring a large randomly generated receptor repertoire expressed by lymphocytes with pro-inflammatory potential would inevitably pose the threat of autoimmunity. This leads to the conjecture that two interactive lymphocyte arms are a fundamental feature of the adaptive immune system that was selected to provide balance and self-regulation. Given that the individual components of our complex immune system must work together to invoke protective immunity, vaccine strategies clearly need to be designed with this principle in mind. This holistic view is also important for understanding inflammatory and autoimmune diseases and for designing ways to alleviate harmful immune responses. Finally, it seems highly probable that lamprey VLRB antibodies will be useful for biomedical purposes because of their ease of genetic engineering, stability, avidity and ability to recognize antigen epitopes that are inaccessible to mammalian antibodies.

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Competing interests statement
The authors declare no competing financial interests.

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