nature reviews immunology

https://doi.org/10.1038/s41577-024-01083-9

Perspective

Check for updates

Understanding vertebrate immunity through comparative immunology

Thomas Boehm[1,2,3] ✉

Abstract

Evolutionary immunology has entered a new era. Classical studies, using just a handful of model animal species, combined with clinical observations, provided an outline of how innate and adaptive immunity work together to ensure tissue homeostasis and to coordinate the fight against infections. However, revolutionary advances in cellular and molecular biology, genomics and methods of genetic modification now offer unprecedented opportunities. They provide immunologists with the possibility to consider, at unprecedented scale, the impact of the astounding phenotypic diversity of vertebrates on immune system function. This Perspective is intended to highlight some of the many interesting, but largely unexplored, biological phenomena that are related to immune function among the roughly 60,000 existing vertebrate species. Importantly, hypotheses arising from such wide-ranging comparative studies can be tested in representative and genetically tractable species. The emerging general principles and the discovery of their evolutionarily selected variations may inspire the future development of novel therapeutic strategies for human immune disorders.

Sections

Introduction

A new era of comparative immunology

Alternative adaptive immune systems

Origin of vertebrate antigen receptors

The origin of lymphocytes

Gain and loss of lymphocyte lineages

Lymphoid organs

Antigen presentation

Tolerance

Evolution of vertebrate immunogenomes

Rewiring in vertebrate immune systems

Phenotypes and immune systems

Conclusion

[1]Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany. [2]Institute for Immunodeficiency, Center for Chronic Immunodeficiency, University Medical Center, Freiburg, Germany. [3]Max Planck Institute for Biology Tübingen, Tübingen, Germany. ✉e-mail: boehm@ie-freiburg.mpg.de

Introduction

The field of immunology was established by the seminal discoveries of Élie Metchnikoff on innate immune defence in the larvae of starfish in the late nineteenth century, and complementary work by Paul Ehrlich on the cytological differentiation of other immunologically relevant cell types and the description of antibody-mediated immune response in mammals around the turn of the twentieth century[1]. At that time, a possible functional interaction between and/or the interdependence of 'nonspecific' (Metchnikov-type) and 'specific' (Ehrlich-type) immune functions was not considered. Several decades later, it emerged that specific immunity was mechanistically more complex than initially thought, primarily as a result of detailed investigations on the origin and function of lymphocytes, a cell type that is unique to vertebrates. In the 1950s, clinical observations of patients suffering from (what is now called) Bruton's type of sex-linked recessive agammaglobulinaemia[2] suggested the existence of two arms of immune defence: one responsible for antibody formation and another underlying delayed hypersensitivity and allogeneic tissue graft rejection[3], a key concept of immunology that is still valid today. However, at this time, the anatomical and cellular basis of this apparent functional dichotomy of adaptive immunity was uncertain. Subsequent work by a number of investigators, using chicken, rabbits, guinea pigs and mice, led to the discovery of distinct lymphocyte lineages that underpin humoral and cellular immunity, the documentation of their functional cooperation and the discovery of the associated primary lymphoid organs[4].

A new era of comparative immunology

The early years of immunological research were primarily driven by the development and use of increasingly sophisticated in vivo and in vitro paradigms. Much of their success was due to the use of different animal models, each chosen to best suit the experimental question. Subsequently, the analysis of inherited immune deficiencies (primarily in humans), together with genetic screens, added a further dimension to our understanding of how immune systems function. Recent advances, such as high-resolution multiparameter imaging and sequencing techniques, the implementation of easy methods for genome engineering, such as those using CRISPR–Cas9 for sequence-specific modifications of DNA and the steadily increasing number of high-quality genome assemblies, now considerably extend the targets of immunological investigations. A detailed definition of the general principles of vertebrate immunity, spanning a period of more than 500 million years, is within reach, making it possible to also identify their evolutionarily selected variations. Particularly promising is the prospect of defining the details of how adaptive facilities such as lymphocytes and somatically assembled antigen receptors that emerged early in vertebrate evolution became integrated with more ancient innate immune functionalities[5] and how the co-evolutionary entanglement of these two principal arms of immunity unfolded (Fig. 1).

Alternative adaptive immune systems

Remarkably, during the early stages of vertebrate evolution, adaptive immune facilities emerged twice[6,7]: once in the branch leading to the extant jawless vertebrates (comprising about 200 species) and in the branch leading to the extant jawed vertebrates (comprising more than 60,000 species, including humans) (Fig. 1). Jawless and jawed vertebrates separated about 550 Ma (ref. 8); within the jawed vertebrate lineage, cartilaginous fishes emerged about 530 Ma, bony fishes about 450 Ma and mammals about 130 Ma (ref. 9) (Fig. 1). Although it is unclear why adaptive immunity has evolved in vertebrates, it has been suggested that it emerged because of the need to recognize and manage complex communities of beneficial microorganisms[10]; indeed, the microbiota contributes unique biosynthetic capacity to the vertebrate host[11,12]. Vertebrate adaptive immune systems are characterized by the presence of functionally distinct lymphocyte lineages with clonal expression of antigen receptors, dedicated primary and secondary lymphoid tissues, the use of genome editors to assemble functional antigen receptors and the presence of dedicated mechanisms for self-tolerance and maintenance of homeostasis. Apart from these general similarities, the adaptive immune systems of jawless and jawed vertebrates differ with respect to the molecular nature of the structural modules that make up the antigen receptors and the mechanisms by which incomplete genetic elements that encode the structural modules are assembled. To initiate the joining of distinct genetic elements during V(D)J recombination, jawed vertebrates depend on the activity of the RAG recombinase[13], which binds to the so-called recombination signal sequences that flank the individual genetic elements. The functionally similar adaptive arm of jawless vertebrates relies on the assembly of genomic cassettes that encode distinct leucine-rich modules; they are stitched together by a mechanism akin to gene conversion[14] that is guided by small patches of similar sequences at the ends of the individual cassettes. Although it is assumed that this assembly process requires the activity of cytidine deaminases (encoded by the *CDA* genes)[15], this has so far formally been demonstrated only for cytidine deaminase 2 (CDA2) and the *VLRB* loci in lampreys, representing one branch of jawless vertebrates[16]; no information is available yet for the other known lamprey VLR genes, nor is it known which, if any, CDA protein (or proteins) is responsible for *VLR* gene assembly in hagfishes, representing the second major branch of jawless vertebrates. Considering the currently available information, the most plausible evolutionary scenario is that the somatic diversification that underlies the vertebrate adaptive immunity was initiated by the 'domestication' of cytidine deaminases[17], a process that converted them from genotoxic enzymes to programmable editors of host genomes. According to this hypothesis, the RAG-based assembly process of antigen receptors was acquired later (perhaps by horizontal gene transfer via transposable elements) and is therefore specific to the jawed vertebrate lineage[18]. Phylogenetic and mechanistic evidence, coupled with structural analyses, indicates that ancient forms of RAG recombinases also experienced domestication, in this case a conversion from integrases to recombinases[19]. It is possible that both types of genome editing have evolved from ancient, possibly prokaryotic toxin–antitoxin systems[20]. Clearly, more work is required to understand their evolutionary history to reconstruct the components of the primordial adaptive immune system of ancestral vertebrate species[7].

Origin of vertebrate antigen receptors

One important question in comparative immunogenetics is the identification of primordial receptors that may have served as the starting point for the emergence of somatically rearranging receptors. In one strand of studies, still existing non-rearranging receptors are explored for their structural homologies to immunoglobulins and T cell receptors (TCRs) in jawed vertebrates, on the one hand, and the variable lymphocyte receptors (VLRs) of jawless vertebrates, on the other hand. The gene family that encodes immunoglobulins and the TCR is distinguished by two notable structural features: (i) the so-called VJ domain[21,22], which has been suggested to have been split into two parts (proto-V and proto-J segments) by the insertion of the RAG transposon into an exon of the immunoglobulin superfamily gene[23] early in the

evolution of jawed vertebrates; hence, the V and J elements are in need of re-assembly to become functional. (ii) The constant region domain (designated C1) has a peculiar structure that sets it apart from other C-like domains[21,24]. The fact that C1 domains are found in other immune-related molecules[25] gave rise to the speculation that immunoglobulin-related, TCR-related and MHC-related molecules have arisen in a genomic region that probably encoded several VJ-type and C1-type proteins[26]. Hence, the identification of an ancient linkage of these genes and presumptive primordial versions of these gene families would constitute an important step forward; for example, a recent report identified a gene encoding a protein with typical variable and constant regions in the MHC region of sharks[27]. With respect to the origin of split *VLR* genes, detailed sequence comparisons suggested that *VLR*s descended from a primordial component of the platelet glycoprotein receptor complex (a likely vertebrate-specific innovation), of which additional family members are encoded in the lamprey genome[15].

It is generally assumed that the founding members of the *Ig*/*TCR* and *VLR* gene families diversified through duplications and subsequent additional modifications. For lampreys, a total of five distinct *VLR* genes have been discovered[28,29], although their phylogenetic relationship across the jawless vertebrates has not been completely resolved.

Structurally, the heterodimeric TCR (typically composed of α/β or γ/δ chains) is equivalent to one arm (designated Fab, for 'fragment antigen binding') of a prototypical immunoglobulin. Each chain of the TCR and Fab contributes three antigen-binding surfaces (referred to as CDR for 'complementarity determining regions'). For immunoglobulins, several interesting variations have been discovered. For example, the so-called NAR (new antigen receptor) identified in nurse shark comprises a dimer of a single heavy chain[30], similar to the single-chain dimers of camelids[31], representing an instructive case of apparent convergent evolution. Immunoglobulin heavy chains in cattle were found to contain exceedingly long CDR3 regions[32], creating unprecedented conformational flexibility. Sharks appear to be particularly innovative with respect to the emergence of unconventional antigen receptors, as evidenced by the presence of an NAR-TCR, which is typified by the presence of a TCRδ constant region joined to two (instead of one) variable regions[33]. Moreover, early in development, cartilaginous fishes express antibody heavy chains encoded by 'germline-joined' rearranged VDJ elements[34], raising interesting questions about the repertoire structure early in vertebrate evolution. Moreover, in the human immune system, some broadly reactive antibodies of patients with malaria are generated by interchromosomal DNA transposition of non-antigen receptor gene-related sequences that extend the size and structure of the V region of the heavy chain[35]. In another unexpected twist, marsupials and monotremes were shown to generate a unique heterodimeric antigen receptor (termed TCRμ), which is composed of a TCRγ chain paired with an unusual immunoglobulin heavy chain-related molecule that consists of two V regions appended to a constant region[36–38].

Post-assembly modification of antigen receptor genes occurs primarily through the activity of activation-induced deaminase (AID), which is responsible for somatic hypermutation[39,40], and gene conversion processes of immunoglobulin genes in higher vertebrates[41]; the only known exception in which such events also take place at TCR loci are TCRα variable genes in cartilaginous fishes[42]. Whether somatic hypermutation also takes place in lamprey *VLR* genes is not yet known. Class-switch recombination at immunoglobulin gene loci[43] is another important function of AID; in humans, loss of AID function manifests itself in the hyper-IgM syndrome, a genetic condition whose detailed analysis was instrumental in the identification of the *AID* gene[44].

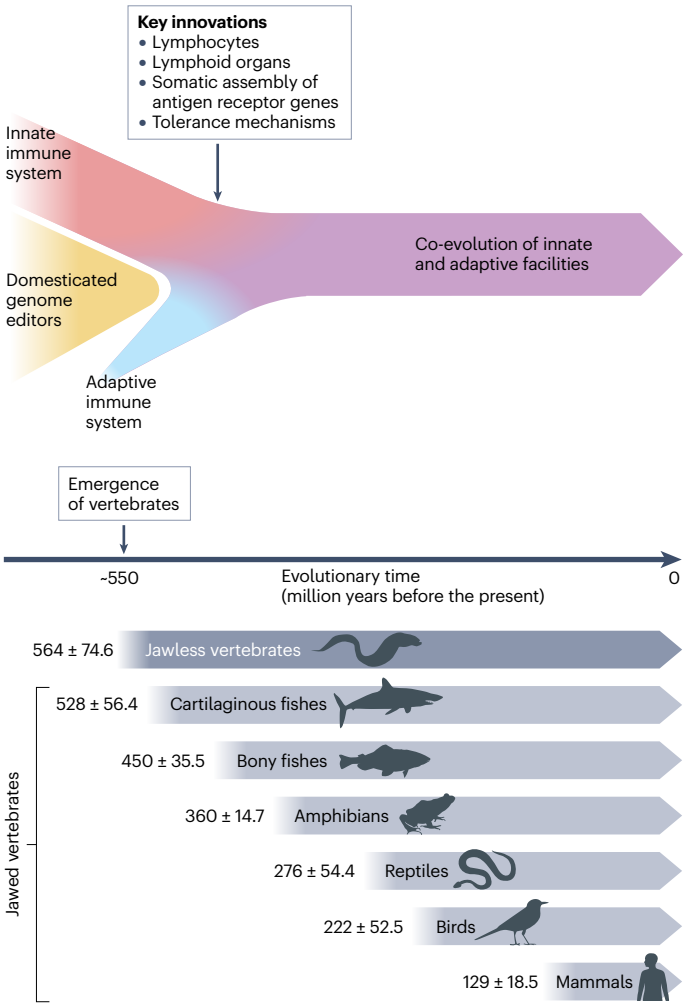

Fig. 1 | Evolutionary trajectory of vertebrate immune systems. About 550 Myr ago, adaptive immune facilities such as lymphocytes, lymphoid organs, mechanisms for somatic assembly of functional antigen receptor genes and tolerance mechanisms curtailing potential self-reactivity emerged in early vertebrates and became integrated into more ancient innate immune functions. Some of these ancient innate immune functions, such as DNA-sensing systems, genome editors and so on[5,202], might have originated in prokaryotes and became co-opted ('domesticated') by the innate and adaptive arms of the canonical vertebrate immune system, initiating an ever-increasing co-evolutionary process. The lower part of the figure illustrates the evolution of the major groups of jawless and jawed vertebrates, and their estimated emergence times (in millions years before the present)[9] are indicated.

The origin of lymphocytes

Comparative studies between jawless and jawed vertebrates suggest that the dichotomy of B cell-like and T cell-like lymphocyte lineages[4] probably predated the split of these two sister clades and therefore most likely emerged in the vertebrate ancestor[6,7,45]. It is possible therefore that, before the emergence of somatically diversifying antigen

receptors, such primordial lymphocytes (or Ur-lymphocytes) had features of innate lymphocytes. Innate lymphocytes have been identified in fish[46,47] and reportedly also exist in lampreys[48]. The presumptive common origin of innate and adaptive lymphocytes is reflected in their shared gene regulatory networks[49], although the reverse evolutionary sequence is also a distinct possibility, that is, that adaptive lymphocytes emerged before innate lymphocytes. In a perhaps more likely scenario, variegated expression of non-rearranging receptors may have sufficed to establish a primordial version of adaptive responses in the innate immune system[50,51] before the advent of modern-type adaptive lymphocytes (Fig. 2).

The question of the evolutionary origin of the lymphocyte has not yet been settled; cytological and transcriptional studies have failed to identify lymphocyte-like cells in a colonial tunicate[52], a representative of chordates, most closely related to vertebrates[53]. However, tunicates are a group of animals that undergo many phenotypic changes during development, which may make the detection of lymphocyte-like cells difficult. Interestingly, it has been shown that perturbations of DNA methylation patterns during differentiation of haematopoietic progenitor cells of zebrafish and mice predominantly affect the lymphoid lineage[54–57], supporting the view that myeloid cells represent a more ancient branch of immunologically important blood lineages and that a specific gene regulatory programme is required to establish and maintain the lymphoid lineage.

Gain and loss of lymphocyte lineages

Although lineage-specific expression of TCRαβ and TCRγδ genes has been demonstrated in many species, it is not yet clear whether the cells expressing the TCRμ receptor in marsupials and monotremes[36–38] represent a distinct third lineage of T cells. Interestingly, genetic evidence derived from the analysis of a zebrafish *ikzf1* mutant[58] suggests that the gene encoding an unusual immunoglobulin heavy chain gene, Igτ (also previously known as Igζ[59]), situated in the *Igm* cluster[60,61] of some but not all teleost species[62] defines a distinct B cell lineage that is most likely dedicated to defence at mucosal surfaces[63,64]. Hence, it appears that genetic networks underlying distinct branches of lymphoid differentiation pathways are subject to considerable evolutionary malleability (Fig. 2).

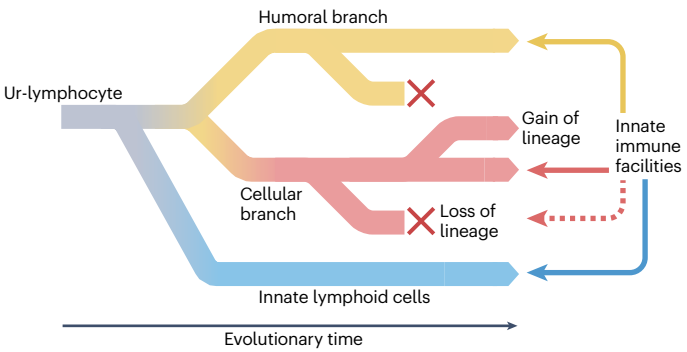

Fig. 2 | Malleability of vertebrate immune systems. Schematic illustrating the plasticity of adaptive immunity with respect to the emergence of new and loss of commonly used antigen receptors that is presumed to be associated with the loss and gain of lymphocyte lineages. Owing to their tight co-evolutionary entanglement, any changes in the adaptive arm are most likely accompanied by corresponding modifications of innate immune facilities, indicated by the coloured arrows. A key event in the evolution of lymphocyte lineages arising from the presumptive primordial lymphocyte (or Ur-lymphocyte) was the emergence of the dichotomy of humoral and cellular lymphoid systems. Whether innate lymphoid cells branched off before the emergence of their adaptive counterparts is uncertain.

With respect to VLR-expressing lymphocytes of jawless vertebrates, cell surface expression patterns have defined at least three separate lineages: B cell-like lymphocytes expressing VLRB and T cell-like cells expressing VLRA or VLRC[28,65,66]. Whether the newly discovered VLRD and VLRE receptors in lampreys are expressed by distinct T cell-like lineages as suggested[29] is not yet clear. If so, it will be interesting to determine whether distinct receptor expression is associated with distinct functions, perhaps akin to the clonotype bias that is associated with $CD4^+$ and $CD8^+$ T cell lineages in jawed vertebrates (for a recent discussion, see ref. 67).

Recent advances in single-cell and single-nuclear RNA sequencing offer the exciting prospect to determine the transcriptional diversity of cell types in individual species (for humans, see, for instance, refs. 68,69). Cross-species data integration will uncover similarities of particular cell types (for example, for neutrophils, see ref. 70), which, for innate effector cells, may be informed by studies of invertebrates (for haemocytes in mosquitoes, see ref. 71).

Strikingly, vertebrate species have been identified that lack one or more of the canonical set of antigen receptor genes. For example, squamates, a species-rich branch of vertebrates occupying various ecological niches, lack both TCRγ and TCRδ genes[72], indicating that their adaptive cellular immune defence relies entirely on the TCRαβ expressing T cell compartment, unless a recently discovered TCR (termed TCRε) that pairs with TCRα provides functional compensation[73]. Several species of the teleost family Gobiesocidae also do not possess TCRγ/δ genes and additionally lack immunoglobulin genes[74], suggesting that the requirement for 'rewiring' of their immune system is even greater. Finally, several species of deep-sea anglerfishes lack all TCR and immunoglobulin genes, associated with pseudogenization of the *RAG* genes[75] (Fig. 3). Although it is likely that this drastic reorganization of the immune system in this small group of vertebrates is related to the phenomenon of permanent joining between female and male fish, including the formation of a common blood circulation, it is not yet clear whether loss of RAG activity occurred before or after the emergence of this peculiar natural form of parabiosis[75]. Once high-quality genome assemblies are available for these species, it will be of interest to examine the mechanism (or mechanisms) by which these antigen receptor genes are pseudogenized and/or deleted.

Lymphoid organs

Lymphoid organs are commonly divided into two categories, primary and secondary lymphoid tissues, each exhibiting characteristic appositions of distinct stromal components and specific haematopoietic cell types that underlie their functional specializations. Although primary lymphoid organs, such as bone marrow and the thymus, foster the generation of the primary repertoire of B lymphocytes and T lymphocytes, secondary lymphoid tissues, such as lymph follicles or lymph nodes, specialize in the coordination of immune responses.

Thymus

Thymopoietic tissues have now been found in all vertebrates, most recently in lampreys[76], although it is unclear whether such a structure also exists in hagfishes. The latter question is particularly important for the reconstruction of the evolution of cellular and genomic facilities

of the ancestor that is common to all vertebrates. Previously, we have proposed that thymopoietic tissues evolve from a small placode of the *Foxn4* gene expressing pharyngeal endoderm, which was recognized in the cephalochordate amphioxus[77]. In jawed vertebrates, the thymic lobes develop from the endodermal tissues of the pharynx[78], a tissue site of astounding developmental and functional plasticity. The thymic epithelium emerges most often from the third pouch; the presence of additional ectopic (cervical) thymus tissue in mammals is variable across species and also within species[79,80]; the developmental origin of cervical thymuses from distinct pharyngeal structures has been elucidated[81,82]. The histological appearance of lamprey thymoid structures that are located at the tips of filaments throughout the gill basket[76] and the structure of 'mini-lobes' resulting from the activity of single thymic epithelial progenitors[83] suggest that the coalescence of thymic developmental units into a macroscopically recognizable tissue in jawed vertebrates is a derived feature of this primary lymphoid organ. It is unknown whether the thymoid of lampreys (and an equivalent structure in hagfishes, if it exists) undergoes the same kind of involution as that seen in jawed vertebrates[7,84–88]. Of note, the lifespan of adult lampreys after metamorphosis is relatively short, compared with the extended period of the larval stage; moreover, there may be differences between parasitic and non-parasitic ecotypes/species.

Considering the hypothesis that the primordial thymus evolved as a bi-potent lymphopoietic organ[89], it will be interesting to examine the structure of thymic tissue in teleosts that lack the T cell[74] or B cell[75] lineages or even both lineages together[75].

The cellular composition of the thymus has come under renewed scrutiny owing to the major advances in spatial transcriptomics and multiparameter immunophenotyping (for a recent example, see ref. 90). Likewise, given the unique advantages that imaging whole organisms provide, the seeding process of the thymus in zebrafish larvae has been recorded[91] and interrogated in situations of impaired lymphoid development to delineate different phases of colonization[92]. Such studies provide guidance for work in higher vertebrates[93].

Spleen and lymph nodes

The vast majority of vertebrates possess a spleen, which is considered to be a major secondary lymphoid organ[94,95]. However, there are exceptions. For instance, seahorses (family Syngnathidae) lack a spleen owing to a point mutation in the *Tlx1* gene[96]; mutations in mammalian *Tlx1* genes also cause asplenia[97]. Importantly, comparisons to closely related teleost species[98] offer the opportunity to examine the role of the spleen in the immune response and blood filtration; a potential caveat of such comparisons is that seahorses also feature some immunogenetic variations possibly associated with the phenomenon of male pregnancy[96].

Lymph nodes represent one of the true innovations of vertebrates, although considerable uncertainty surrounds their evolutionary origin. Lymph nodes are a general feature of mammals. Interestingly, however, the genetic underpinnings of their site-specific development within the organism differ[99], indicating that they have a different developmental fate. The zebrafish *Danio rerio* has no recognizable lymph node structures; instead, a tessellated lymphoid network is suggested to facilitate whole-body lymphocyte trafficking and antigen surveillance[100]. Advancing in evolutionary time, anatomically distinguishable lymph-node-like structures are found in the throat and axillary regions of the frog *Rana pipiens*[101] and in some birds[102]. By contrast, germinal centres that are associated with the initiation and maintenance of immune responses appear to be a more ancient feature of the vertebrate immune system (Box 1).

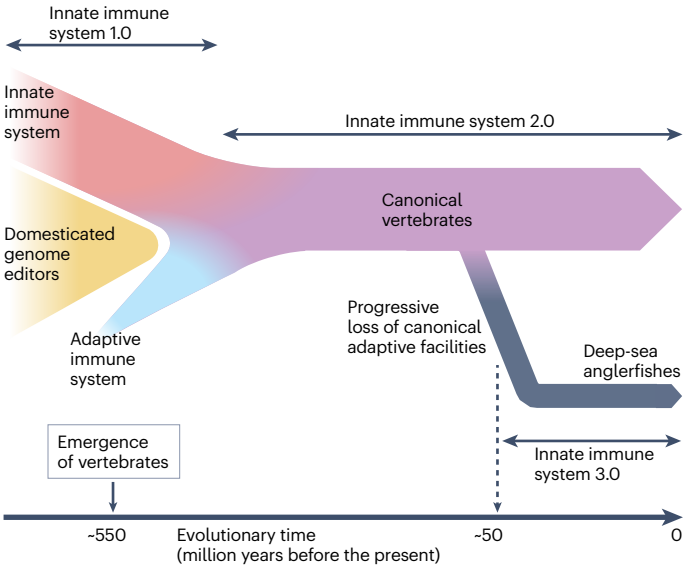

Fig. 3 | Plasticity of innate immunity. Schematic illustrating major changes of immune systems during vertebrate evolution, with a focus on the innate arm. The most dramatic change must have occurred when vertebrates evolved adaptive facilities, transforming invertebrate innate immunity type 1.0 to a prototypical vertebrate innate immunity type 2.0. The latter probably then changed in response to, for example, loss and gain of lymphocyte lineages (Fig. 2), but the characteristic co-evolutionary entanglement between the two arms remained and is characteristic for the overwhelming majority of extant ('canonical') vertebrates. In a small group of vertebrates, the deep-sea anglerfishes, drastic changes occurred in the adaptive arm of the immune system, culminating in the complete loss of the facility for somatic diversification through the loss of intact *Rag1* and *Rag2* genes[75] (about 50 Myr before the present[138]). This is suggested to have been accompanied by a second major reorganization of innate facilities, here referred to as innate immune system 3.0 (Box 3). Exploring its characteristics promises to reveal new avenues for immune reinforcement in patients with failing adaptive immunity.

The development of multiparametric morphological analysis of tissue structure adds unprecedented detail to our understanding of secondary lymphoid organ structure[103]; likewise, in vivo imaging of immune responses (albeit still limited in terms of the observable time span) adds new dimensions to our understanding of how immune cells interact (for instance, see ref. 104).

Antigen presentation

The various components of the MHC-based antigen processing machinery have been well characterized in various species of jawed vertebrates. Moreover, recent work has revealed details of the presumed organization of the ancestral MHC region in cartilaginous fish[105] and the structure of an ancient MHC type with features of both MHC class I and class II molecules[106]. The co-evolution of the TCR and MHC has also come into renewed focus[18,107], fuelled by suggestions that the genes encoding immunoglobulin-like and TCR-like antigen receptors may have emerged within or near the primordial MHC locus[27]. By contrast, although evidence has been presented for[108] and against[66] direct antigen binding by T cell lineage-related VLRs, nothing is yet known about the antigen presentation mechanism (if it exists) in the lamprey immune system, precluding comparative analyses with the MHC pathway of jawed vertebrates.

Box 1 | Germinal centres

After infection or immunization, germinal centres (GCs) develop within B cell follicles of the mammalian spleen and lymph nodes; they provide a dedicated microanatomical structure, fostering the production of high-affinity antibodies through repeated cycles of somatic hypermutation of immunoglobulin genes[203]. For decades, immunologists have debated whether such structures also exist in ectotherm vertebrates (such as fishes), given that immune responses in ectotherms tend to be slower than those in endotherm animals (such as mammals) and exhibit a reduced extent of affinity maturation of antibodies. Evidence for GC-like structures has now been obtained for cartilaginous fishes[204] and telosts[205]. However, the nature of the individual cellular components of GCs as defined in mammals (follicular dendritic cells, centrocyte B cells, antigen-specific T follicular helper cells) is a matter of debate[206], particularly with regard to functional equivalents of follicular dendritic cells[207]. Hence, it is possible that the slow immune response and reduced extent of antibody affinity maturation in ectotherms are the result of the peculiar cellular composition of their GCs[206]. It will be interesting to determine whether melanomacrophages, which are a frequent component of GC-like structures in fishes and are present also in jawless vertebrates, are likewise associated with evidence of post-assembly modifications of VLR antigen receptors.

Tolerance

It is conceivable that, when the facility of somatic diversification evolved in jawless and jawed vertebrates, the initial diversity of antigen receptor repertoires was much lower than that of existing vertebrates. Nonetheless, any type of somatically generated diversity carries the risk of self-reactivity and hence requires compensatory mechanisms to prevent autoimmune consequences. It has been argued that the numbers and precise sequence compositions of genomic substrates used for antigen receptor assembly are under Darwinian selection to (at least initially) restrict the overall diversity of the primordial repertoires[109]. The emergence of new and/or the repurposing of ancient antigen presentation pathways[110] is thought to have led to the plethora of central and peripheral tolerance mechanisms that characterize the present jawed vertebrate immune systems.

In terms of central tolerance induction during B cell development, several checkpoints have been identified in mammals[111]. Whether similar tolerance-inducing mechanisms operate in other jawed vertebrates, or indeed in jawless vertebrates, is unknown. They may be difficult to identify, given that vertebrate B cell development occurs in quite diverse tissue environments[112].

With respect to the process of T cell tolerance, several mechanisms have been discovered. First, it was shown that medullary epithelial cells, a major component of the thymic microenvironment[87], exhibit the phenomenon of ectopic (also known as promiscuous) gene expression, whereby a certain fraction of the genome is expressed by each medullary epithelial cell; in aggregate, this mosaic expression pattern[113] provides an intrathymic representation of the peripheral self. In addition, peripheral cell types in the thymus, such as muscle cells, were discovered more than 100 years ago[114] and suggested to have a role in tolerance induction[115]. Now collectively referred to as peripheral mimetic cells[116], they represent an additional way by which developing T cells can probe the specificity of their antigen receptors against peripheral self-antigens. The evolutionary roots of these (and perhaps other) mechanisms of tolerance induction have not yet been determined. Given the experimental challenges associated with genetic manipulation of jawless vertebrates, the identification of functionally equivalent mechanisms in jawless vertebrates will require the development of simple assay systems for self-reactivity. Although clonal deletion is a well-established mechanism that purges the T cell repertoire of potentially self-reactive clones in mice, the extent to which this occurs (particularly with respect to possible differences between $CD4^+$ and $CD8^+$ T cell lineages) in other jawed vertebrates is debated[117] and has not been investigated for lower jawed vertebrates. In lampreys, the repertoire of VLRC-expressing T cell-like cells was shown to be subject to changes upon development in the thymoid[118], hinting at the possibility that some form of tolerance and/or repertoire pruning mechanisms may also operate in these species. Of note, *Aire*, which encodes one of the master regulators of ectopic gene expression in the mammalian thymus[119], appears to be absent in the genomes of jawless vertebrates. The variable histological structure and anatomical distribution of thymopoietic tissues pose a number of questions related to the mechanistic aspects of central tolerance formation; similarly, the diverse microenvironments that support B cell development may be subject to similar mechanistic constraints (Box 2).

Interestingly, in addition to their well-established function in tolerance induction and maintenance[120–123], there is evidence that regulatory T cells orchestrate tissue regeneration[124–126], both by limiting tissue inflammation and by providing site-specific growth factors. It is possible that this latter feature represents the primordial function of such regulatory activities; clues may come from detailed phylogenetic studies of the FOXP family of transcription factors and their expression profiles in immune-related cell types. More generally, it will be of great interest to determine whether interference with the local availability of immuno-regulatory cytokines (similar to the control of IL-2 levels by mammalian regulatory T cells) is a jawed vertebrate-specific paradigm to maintain peripheral tolerance that also applies to jawless vertebrates. At present, although recent studies have examined the repertoire of cytokine receptors[127], the identification of their cytokine ligands in genomic sequences remains a challenging task.

Several biological systems lend themselves to comparative studies that may yield useful insights into the mechanisms that balance self versus non-self recognition. One such phenomenon is pregnancy. Although traditionally studied in placental mammals (for a recent review, see ref. 128), recent work has begun to focus on the evolution of the many forms of viviparity in other species[129,130]. Indeed, the placenta is considered to be a model to understand the evolution of a novel vertebrate organ[130]. One interesting hypothesis posits that the process of embryo implantation co-opted ancient inflammatory signalling pathways[131,132]. The remodelling of immune facilities has also been studied in seahorses and pipefish that exhibit the phenomenon of male pregnancy[133]. Many species in the live-bearing fish family Poeciliidae have independently evolved placenta-like organs[134,135], providing a rich source of data for comparative genome studies to identify the key drivers of placenta development and associated tolerance mechanisms at the cellular and molecular level. A spectacular phenomenon of natural parabiosis was discovered in certain species of deep-sea anglerfishes[136–138]. Attachment of dwarfed males to the much larger

females in these species can be transient or permanent, each associated with a dramatic remodelling of the immunogenome[75].

Evolution of vertebrate immunogenomes

Recent comparative genome studies have attempted to reconstruct the ancestral vertebrate genome[139,140], and work towards the genome assembly of thousands of vertebrates is ongoing. More specifically, the availability of genome assemblies of jawless vertebrates (lampreys[141–143] and hagfishes[144,145]) and jawed vertebrates at critical evolutionary transition points (cartilaginous fishes[146–151], non-teleost ray-finned fishes[152,153], lungfish[154,155], amphibians[156–158], birds[159], reptiles[160] and marsupials[161]) opens the door to genome-wide evolutionary studies of key effector molecules of both innate and adaptive facilities[162]. These studies will also provide a welcome framework for additional gene-centric studies, such as those of the RAG recombinase[163–166] and the AID-gene family[17,167]. Similarly, work has now begun to understand the evolutionary origin and trajectory of vertebrate innate immune-related receptors, such as TLRs[168], cGAS/STING and gasdermins[5], as well as cytokine[127] and chemokine[169] networks. A survey of the tumour necrosis factor superfamily of cytokines and receptors[170] will be particularly informative, as some of its members are known to be required for the development of lymph nodes[171]. Moreover, aligning vertebrate immunogenomes with their species-specific spectrum of infectious diseases[172] and/or the microbiota[173] will be a rich avenue for an eco-immunological synthesis of immune system diversity.

Rewiring in vertebrate immune systems

Historically, immunologists have focused on a handful of model species only, such as chicken, frog, rat and mouse. The advent of large-scale genome projects, the emergence of revolutionary techniques to determine gene expression patterns in space and time and the possibility to interfere with gene function, for instance, via CRISPR–Cas9 mutagenesis, now affords unprecedented opportunities for large-scale comparative and focused functional analyses. Immunologists can now explore species exhibiting unusual lifestyles and living in extreme habitats. A few examples should suffice to indicate that the rich biology of the approximately 60,000 vertebrate species is ripe for mechanistic studies disentangling phenotype–genotype associations, which will provide many examples attesting to the astounding flexibility of adaptive and innate immune facilities, according to the particular needs of a species.

Bats, a species-rich group of mammals, are known for their co-habitation with numerous viruses and thus represent a particular useful model system to understand the reciprocal interaction between virus and host and to identify the genetic footprints leading to the delicate balance of defence and tolerance[174–176]. Indeed, bats appear to have evolved numerous strategies to dampen inflammatory responses; one such example is the lack of certain cytoplasmic DNA sensors in some species[177]. Antarctic fishes represent another underexplored group of vertebrates likely to have evolved unique adaptations to their life in ice-cold water; with more and more genomes illuminating the astounding notothenioid radiation[178,179], ample opportunities exist for studies examining the sequence variation of immune-related molecules and possibly adaptations to challenges by a likely unique spectrum of microbial adversaries and macroparasites. Addressing cold-adapted immune regulators, a recent study examined amino acid changes associated with the inactivation of induced cytidine deaminase protein in cold-blooded fishes[180]. Similarly, sexual parasitism observed in a clade of deep-sea anglerfishes has been associated with dramatic reorganization of immune system facilities, culminating in the pseudogenization of the RAG genes[75], indicating that, unexpectedly, the co-evolution of adaptive and innate immune defences can be disentangled. Cavefish, for which surface and cave ecotypes have been extensively studied[181], represent an intriguing model for immune-related adaptations to different extents of parasite burden[182]. As a final example, naked mole-rats, a species distinguished by its cancer-free long life[183], have been found to lack natural killer cells and to exhibit an inverted myeloid/lymphoid ratio compared with other rodents[184]; it will be interesting to identify possible cellular back-up systems compensating for the innate cellular immune surveillance function. Collectively, these findings highlight the fact that the division of labour between adaptive and innate defence is subject to strong evolutionary forces, although the functional distinction between the two arms may not be as strict as previously thought[185]. Therefore, it is conceivable that whenever a specific lymphocyte lineage (as defined by the expression of its unique antigen receptor type) is lost or gained, both adaptive and innate aspects will change[72,74,75] (Fig. 2). Such reorganization of immune facilities is thought to be taken to its extreme in cases in which adaptive facilities are lost altogether, as exemplified by teleost fishes lacking intact *RAG* genes[75] (Fig. 3). To explore the compensatory changes in innate immune functions that accompany such reductions in adaptive receptor facilities, well-curated genome assemblies (based on sequence comparisons

Box 2 | Anatomical constraints that impact central tolerance

In mice, limited ectopic gene expression by scattered individual medullary epithelial cells[119] and the presence of the so-called mimetic cells masquerading as differentiated peripheral cell types in the medulla[116] ensure that developing thymocytes are exposed to the peripheral self, albeit in an inevitably stochastic manner. The long residence time of thymocytes (measured in days) and the seamless migration patterns of developing T cells within the microenvironment maximize the encounter with self-peptides and thus ensure efficient central tolerance. If, however, thymopoietic units are anatomically segregated, which by necessity restricts the number of self-presenting stromal cells, each developing T cell might be exposed to only a small share of the peripheral self. For the lamprey thymoid, this could mean that the emerging VLR repertoire has a low propensity of self-reactivity, that peripheral tolerance mechanisms can make up for the weaker central tolerance induction or that even a small stromal compartment can represent the full complement of peripheral self-antigens. Mice with an artificially reduced size of the thymic epithelium might help to distinguish between these alternatives.

In terms of central tolerance induction in the B cell lineage, several checkpoints have been identified during B cell development in mammals[111,208]; clonal deletion and a process referred to as receptor editing ensure the step-wise reduction of self-reactive B cell clones. Little is known about a similar mechanism in other jawed vertebrates or indeed in jawless vertebrates. The fact that B cell development occurs in quite diverse tissue environments[112] may complicate the identification of general mechanisms of tolerance induction.

and expression profiles) are required, to precisely determine the presence and composition of the many gene families that underpin much of innate immunity. This is not a trivial task, particularly because immune-related genes are known to be among the fastest diversifying genes; the evolution of receptors for natural killer cells provides an instructive example[186]. Hence, the comparison of genomes of different species based on sequence alone is complicated by uncertainties concerning the identification of orthologous genes and their possible functions.

Given the substantial changes observed in the composition of adaptive immune facilities, innate immune functions must have undergone similarly drastic changes. Primordial forms of innate immunity (here conceptualized as innate immunity 1.0) were subjected to co-evolutionary constraints with the advent of adaptive immunity, evolving into what might be broadly termed innate immunity 2.0. However, in the rare cases, in which some or all adaptive facilities were lost in a group of vertebrates, innate immunity had to change again, breaking the co-evolutionary companionship to evolve into innate immunity type 3.0 (Box 3).

Phenotypes and immune systems

Vertebrates come in many different shapes and sizes; some live for only a few weeks, others roam their habitat for hundreds of years. Studies on both aspects alone will greatly benefit from comparative studies.

It is well known that the lifespan of vertebrates varies considerably. Yet, investigations into associated changes of immune systems are best conducted by comparing closely related species[187]. Several such opportunities indeed exist. For example, a recent study examined the genomes of 88 species of Pacific Ocean rockfish, which have lifespans from around 10 years to more than 200 years[188]. Remarkably, in addition to evidence for positive selection in genes encoding components of DNA repair pathways, it was found that the copy numbers of butyrophylin genes increased in long-lived species. Considering the immunosuppressive function of butyrophylins[189], it is possible that this is one mechanism to reduce the extent of chronic inflammation, which is known to be strongly associated with organismal ageing[190]. The genome analysis of giant tortoises likewise identified species-specific variants in genes associated with genome stability, inflammatory mediators and cancer suppression[191]. For comparison, it will be interesting to study the immunogenomes of coral reef fish, which exhibit the shortest recorded lifespan of vertebrates[192]. For instance, one interesting question would be to see whether they still use AID for affinity maturation of immunoglobulins, given that this process would take a long time relative to the overall lifespan of an animal.

Whether the extended diapause[193] and subsequent short reproductive cycle of killifish[194] are associated with specific immunological adaptations remains to be seen. Another remarkable adaptation is the terrestrialization observed in lungfish, which survive in a granulocyte-rich mucus cocoon[195], pointing to an important role of innate defenses to long-term survival in such unusual organismal states. In ageing mammals, immune reactivity is thought to gradually decline[196,197]. Interestingly, over the course of their short lifespan, even killifish exhibit a contraction of antibody diversity[194], suggesting that the age-associated perturbations in the immune system align with individual life history, be it long or short.

Vertebrate body sizes differ by several orders of magnitude. *Paedocypris progenetica* is the smallest known fish[198] (body weight around 0.01 g) that possesses all the genetic hallmarks of a canonical adaptive immune system. Interestingly, the analysis of the clonotypic structure of their antigen receptor repertoires reveals their fractal nature, which is similar to those found in much larger vertebrates[199]. The somewhat smaller anglerfish *Photocorynus spiniceps*[200] has lost canonical adaptive immunity[75] and hence does not qualify for this comparison. Two species of the anuran genus *Paedophryne* are considered to be the smallest terrestrial vertebrates (body weight again around 0.01 g)[201]; however, the composition of their immunogenomes is unknown. At the other end of the spectrum are the teleost ocean sunfish *Mola mola* (body weight up to 1,000 kg), the cartilaginous whale

Box 3 | New forms of innate immune systems

It is likely that the emergence of adaptive immunity resulted in a substantial reorganization of ancient innate immune facilities, which are summarily referred to here as innate immunity 1.0. The onset of innate/adaptive co-evolution led to a new form of innate immunity (innate immunity 2.0). It probably lost certain functions to the newly emerging adaptive arm; for instance, it is conceivable that innate immunity no longer required the variegated cell surface expression of individual members of large antigen receptor families to achieve coverage of the antigenic space. Conversely, it must have gained new functions, for example, those enabling cooperative synchronization, for instance, through the emergence of new chemokines and their receptors as part of a sophisticated intercellular communication system. In reciprocal fashion, the gain or loss of adaptive recognition capabilities (such as the emergence of new antigen receptor types and/or lymphocyte lineages) most likely led to corresponding changes in the innate arm, most dramatically in the case in which canonical adaptive immunity was completely lost[75]. Although details are not yet known, such novel forms of innate immunity (innate immune system 3.0, to distinguish it from invertebrate innate immunity 1.0, and innate immunity 2.0, which co-evolved with adaptive immunity) may be explored by comparative analysis of a greater number of deep-sea anglerfishes, which represent one of the most species-rich family of the lophiiform fishes[137]. One interesting possibility is that crucial compensatory immune capabilities of innate immunity 3.0 may comprise more diversified myeloid cell activity, gain/loss of extracellular and intracellular pattern recognition receptors and/or gain/loss of certain cell types, such as natural killer cells.

Within the jawless vertebrate branch, no species is yet known to lack cytidine deaminase-related genome editors; however, more detailed phylogenetic studies may reveal a clearer picture of the co-evolution of *VLR* and *CDA* gene families. Of note, analysis of primary immunodeficiencies in humans indicates that the causal mutations affecting innate immunity genes are, in relative terms, close to seven times less frequent than those affecting adaptive immunity genes[209], indicating that the functional networks of the descendants of ancient innate facilities are more robust than those commonly associated with adaptive immunity[210]. It will be interesting to determine whether this ratio also applies to other vertebrate species.

Glossary

Camelids
A group of large herbivorous mammalian species.

Class-switch recombination
A DNA rearrangement process by which the constant region (isotype) of an immunoglobulin gene is exchanged, maintaining the variable antigen binding part.

Constant region domain
A structurally invariant segment that is fused to structurally variable regions of antigen receptors and is responsible for effector functions.

CRISPR–Cas9
The CRISPR–Cas9 ribonucleoprotein of prokaryotic origin can be engineered to recognize specific sequences in DNA and introduce double-stranded breaks.

Cytidine deaminases
A family of enzymes that catalyse hydrolytic deamination of cytidine to uridine or deoxycytidine to deoxyuridine.

Domestication
An evolutionary process in which proteins are repurposed to carry out a function that is useful to the host organism.

FOXP family
A group of transcription factors that belong to a larger family that shares a DNA-binding domain characterized by butterfly loops ('wings') connecting DNA recognition helices.

Gene conversion
A process by which genetic material is copied from one site to another site of related sequence.

Genome assembly
A computational process by which short fragments of genomic DNA are aligned to recreate the sequence of entire chromosomes.

Genome editor
A summary term for enzymes such as recombinases or base modifiers that are used to change a DNA sequence by insertion, deletion, modification or replacement.

Integrases
A family of enzymes that facilitate the integration of DNA into the genome.

Leucine-rich modules
A structural unit of 24 amino acid residues that is the building block of many proteins, including the variable lymphocyte receptors of jawless vertebrates.

Notothenioid
Name of a group of fishes that mainly inhabit oceans with water temperatures between −2 °C and 4 °C.

Placode
An embryonic structure that gives rise to a discrete anatomical structure.

Public clonotypes
Antigen receptor sequences that can be found in more than one individual of the same species.

RAG recombinase
An enzyme consisting of two components, RAG1 and RAG2, that are required for the rearrangement of genes encoding immunoglobulins and T cell receptors.

RAG transposon
A mobile genetic element that encodes the presumptive ancestor of the RAG recombinase of jawed vertebrates.

Recombinases
A family of enzymes that catalyse the recombination of DNA sequences.

Somatic hypermutation
A somatic process that introduces mutations primarily into the variable parts of assembled antigen receptor genes.

Toxin–antitoxin systems
Systems of functionally linked effectors that antagonize each other, which are prevalent in prokaryotes and often encoded by closely linked genes.

VJ domain
A structural module connecting two segments: one segment stabilized by a disulfide bridge and the other characterized by a short glycine-rich region important for dimerization.

VLR genes
Genes that encode a variable lymphocyte receptor of jawless vertebrates that is anchored to the cell membrane.

shark *Rhincodon typus* (body weight up to 15,000 kg), the blue whale *Balaenoptera musculus* (body weight around 200,000 kg) and the African bush elephant *Loxodonta africana* (body weight about 10,000 kg). When studying the relationship between body mass and the structure of the immune system, it is important to compare representatives of similar clades; for example, a body weight difference of $\geq 10^7$ applies to the comparison of *Paedocypris* sp. with the largest known species of the cyprid family (giant barb, *Catlocarpio siamensis*, body weight up to 300 kg). Among terrestrial mammals, for example, a factor of $\geq 10^6$ applies to the comparison of the Etruscan shrew (*Suncus etruscus*; body weight about 2 g) to the elephant. Far fewer species of jawless vertebrates are known, associated with a much narrower range of the body sizes. The sea lamprey *Petromyzon marinus* can attain a body weight of roughly 2 kg, whereas fresh water lampreys (for example, European brook lamprey *Lampetra planeri*) reach 3–5 g of body weight (scaling factor of $\geq 10^2$).

Considering antigen receptor repertoires, as an example, it is conceivable that body size and perhaps also lifespan leave an evolutionarily recognizable imprint on both the generative rules of diversity and the clonal structure of adaptive lymphocytes. So far, many questions are mostly unanswered. Do lymphocyte numbers scale with body size? Are the generative rules of somatic assembly of antigen receptor genes the same irrespective of lifespan and body size? Is the receptor diversity in the shrew as great as it is in the elephant? Are lymphocyte clones of similar size in all vertebrates? Is the fraction of public clonotypes the same? The discovery of the fractal nature of antigen receptor repertoires has important ramifications for the efficient design of the process of tolerance induction and the ability of antigen recognition and discrimination. Asking similar questions about all other major components of vertebrate immunity (molecules, cells and organs) will allow unique insights into the overall structure of vertebrate immunity.

Conclusion

Over the past 500 million years, vertebrates appear to have been extraordinarily creative in modulating innate and adaptive immune facilities to suit their specific needs and to accommodate extraordinarily diverse lifestyles. It is hoped that such wide-ranging comparative studies will provide a much more detailed picture of common and species-specific features of vertebrate immune systems and may even inspire the development of novel therapeutic strategies for human immune disorders.

Published online: 24 September 2024

References

1. Kaufmann, S. H. Immunology's foundation: the 100-year anniversary of the Nobel Prize to Paul Ehrlich and Elie Metchnikoff. *Nat. Immunol.* **9**, 705–712 (2008).
2. Bruton, O. C. Agammaglobulinemia. *Pediatrics* **9**, 722–728 (1952).
3. Porter, H. M. The demonstration of delayed-type reactivity in congenital agammaglobulinemia. *Ann. N. Y. Acad. Sci.* **64**, 932–935 (1957).
4. Cooper, M. D., Peterson, R. D. & Good, R. A. Delineation of the thymic and bursal lymphoid systems in the chicken. *Nature* **205**, 143–146 (1965).
5. Wein, T. & Sorek, R. Bacterial origins of human cell-autonomous innate immune mechanisms. *Nat. Rev. Immunol.* **22**, 629–638 (2022).
6. Cooper, M. D. & Alder, M. N. The evolution of adaptive immune systems. *Cell* **124**, 815–822 (2006).
7. Boehm, T. et al. Evolution of alternative adaptive immune systems in vertebrates. *Annu. Rev. Immunol.* **36**, 19–42 (2018).
8. Janvier, P. Facts and fancies about early fossil chordates and vertebrates. *Nature* **520**, 483–489 (2015).
9. Kumar, S. & Hedges, S. B. A molecular timescale for vertebrate evolution. *Nature* **392**, 917–920 (1998).
10. McFall-Ngai, M. Adaptive immunity: care for the community. *Nature* **445**, 153 (2007).
11. Shine, E. E. & Crawford, J. M. Molecules from the microbiome. *Annu. Rev. Biochem.* **90**, 789–815 (2021).
12. Chung, H. et al. Gut immune maturation depends on colonization with a host-specific microbiota. *Cell* **149**, 1578–1593 (2012).
13. Schatz, D. G., Oettinger, M. A. & Baltimore, D. The V(D)J recombination activating gene, RAG-1. *Cell* **59**, 1035–1048 (1989).
14. Nagawa, F. et al. Antigen-receptor genes of the agnathan lamprey are assembled by a process involving copy choice. *Nat. Immunol.* **8**, 206–213 (2007).
15. Rogozin, I. B. et al. Evolution and diversification of lamprey antigen receptors: evidence for involvement of an AID-APOBEC family cytosine deaminase. *Nat. Immunol.* **8**, 647–656 (2007).
16. Morimoto, R. et al. Cytidine deaminase 2 is required for *VLRB* antibody gene assembly in lampreys. *Sci. Immunol.* **5**, eaba0925 (2020).
17. Krishnan, A., Iyer, L. M., Holland, S. J., Boehm, T. & Aravind, L. Diversification of AID/APOBEC-like deaminases in metazoa: multiplicity of clades and widespread roles in immunity. *Proc. Natl Acad. Sci. USA* **115**, E3201–E3210 (2018).
18. Tsakou-Ngouafo, L., Paganini, J., Kaufman, J. & Pontarotti, P. Origins of the RAG transposome and the MHC. *Trends Immunol.* **41**, 561–571 (2020).
19. Liu, C., Zhang, Y., Liu, C. C. & Schatz, D. G. Structural insights into the evolution of the RAG recombinase. *Nat. Rev. Immunol.* **22**, 353–370 (2022).
20. Boehm, T., Morimoto, R., Trancoso, I. & Aleksandrova, N. Genetic conflicts and the origin of self/nonself-discrimination in the vertebrate immune system. *Trends Immunol.* **44**, 372–383 (2023).
21. Du Pasquier, L., Zucchetti, I. & De Santis, R. Immunoglobulin superfamily receptors in protochordates: before RAG time. *Immunol. Rev.* **198**, 233–248 (2004).
22. Dornburg, A. & Yoder, J. A. On the relationship between extant innate immune receptors and the evolutionary origins of jawed vertebrate adaptive immunity. *Immunogenetics* **74**, 111–128 (2022).
23. Sakano, H., Hüppi, K., Heinrich, G. & Tonegawa, S. Sequences at the somatic recombination sites of immunoglobulin light-chain genes. *Nature* **280**, 288–294 (1979).
24. Zucchetti, I., De Santis, R., Grusea, S., Pontarotti, P. & Du Pasquier, L. Origin and evolution of the vertebrate leukocyte receptors: the lesson from tunicates. *Immunogenetics* **61**, 463–481 (2009).
25. Williams, A. F. & Barclay, A. N. The immunoglobulin superfamily-domains for cell surface recognition. *Annu. Rev. Immunol.* **6**, 381–405 (1998).
26. Ohta, Y., Kasahara, M., O'Connor, T. D. & Flajnik, M. F. Inferring the 'primordial immune complex': origins of MHC class I and antigen receptors revealed by comparative genomics. *J. Immunol.* **203**, 1882–1896 (2019).
27. Flajnik, M. F. et al. An ancient MHC-linked gene encodes a nonrearranging shark antibody, UrIg, convergent with IgG. *J. Immunol.* **211**, 1042–1051 (2023).
28. Hirano, M. et al. Evolutionary implications of a third lymphocyte lineage in lampreys. *Nature* **501**, 435–438 (2013).
29. Das, S. et al. Evolution of two distinct variable lymphocyte receptors in lampreys: VLRD and VLRE. *Cell Rep.* **42**, 112933 (2023).
30. Greenberg, A. S. et al. A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks. *Nature* **374**, 168–173 (1995).
31. Hamers-Casterman, C. et al. Naturally occurring antibodies devoid of light chains. *Nature* **363**, 446–448 (1993).
32. Wang, F. et al. Reshaping antibody diversity. *Cell* **153**, 1379–1393 (2013).
33. Criscitiello, M. F., Saltis, M. & Flajnik, M. F. An evolutionarily mobile antigen receptor variable region gene: doubly rearranging NAR-TcR genes in sharks. *Proc. Natl Acad. Sci. USA* **103**, 5036–5041 (2006).
34. Rumfelt, L. L. et al. A shark antibody heavy chain encoded by a nonsomatically rearranged VDJ is preferentially expressed in early development and is convergent with mammalian IgG. *Proc. Natl Acad. Sci. USA* **98**, 1775–1780 (2001).
35. Tan, J. et al. A LAIR1 insertion generates broadly reactive antibodies against malaria variant antigens. *Nature* **529**, 105–109 (2016).
36. Morrissey, K. A. et al. The molecular assembly of the marsupial γμ T cell receptor defines a third T cell lineage. *Science* **371**, 1383–1388 (2021).
37. Parra, Z. E. et al. A unique T cell receptor discovered in marsupials. *Proc. Natl Acad. Sci. USA* **104**, 9776–9781 (2007).
38. Morrissey, K. A., Stammnitz, M. R., Murchison, E. & Miller, R. D. Comparative genomics of the T cell receptor μ locus in marsupials and monotremes. *Immunogenetics* **75**, 507–515 (2023).
39. Muramatsu, M. et al. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* **102**, 553–563 (2000).
40. Petersen-Mahrt, S. K., Harris, R. S. & Neuberger, M. S. AID mutates *E. coli* suggesting a DNA deamination mechanism for antibody diversification. *Nature* **418**, 99–103 (2002).
41. Harris, R. S., Sale, J. E., Petersen-Mahrt, S. K. & Neuberger, M. S. AID is essential for immunoglobulin V gene conversion in a cultured B cell line. *Curr. Biol.* **12**, 435–438 (2022).
42. Ott, J. A., Harrison, J., Flajnik, M. F. & Criscitiello, M. F. Nurse shark T-cell receptors employ somatic hypermutation preferentially to alter alpha/delta variable segments associated with alpha constant region. *Eur. J. Immunol.* **50**, 1307–1320 (2020).
43. Chaudhuri, J. Evolution of the immunoglobulin heavy chain class switch recombination mechanism. *Adv. Immunol.* **94**, 157–214 (2007).
44. Revy, P. et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). *Cell* **102**, 565–575 (2000).
45. Boehm, T. Design principles of adaptive immune systems. *Nat. Rev. Immunol.* **11**, 307–317 (2011).
46. Hernández, P. P. et al. Single-cell transcriptional analysis reveals ILC-like cells in zebrafish. *Sci. Immunol.* **3**, eaau5265 (2018).
47. Suffiotti, M., Carmona, S. J., Jandus, C. & Gfeller, D. Identification of innate lymphoid cells in single-cell RNA-seq data. *Immunogenetics* **69**, 439–450 (2017).
48. Vivier, E., van de Pavert, S. A., Cooper, M. D. & Belz, G. T. The evolution of innate lymphoid cells. *Nat. Immunol.* **17**, 790–794 (2016).
49. Saini, A., Ghoneim, H. E., Lio, C. J., Collins, P. L. & Oltz, E. M. Gene regulatory circuits in innate and adaptive immune cells. *Annu. Rev. Immunol.* **40**, 387–411 (2022).
50. Rückert, T. & Romagnani, C. Extrinsic and intrinsic drivers of natural killer cell clonality. *Immunol. Rev.* **323**, 80–106 (2024).
51. Qin, Y., Mace, E. M. & Barton, J. P. An inference model gives insights into innate immune adaptation and repertoire diversity. *Proc. Natl Acad. Sci. USA* **120**, e2305859120 (2023).
52. Rosental, B. et al. Complex mammalian-like haematopoietic system found in a colonial chordate. *Nature* **564**, 425–429 (2018).
53. Delsuc, F., Brinkmann, H., Chourrout, D. & Philippe, H. Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature* **439**, 965–968 (2006).
54. Bröske, A. M. et al. DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. *Nat. Genet.* **41**, 1207–1215 (2009).
55. Iwanami, N. et al. Epigenetic protection of vertebrate lymphoid progenitor cells by Dnmt1. *iScience* **23**, 101260 (2020).
56. Iwanami, N. et al. Transgenerational inheritance of impaired larval T cell development in zebrafish. *Nat. Commun.* **11**, 4505 (2020).
57. Lawir, D. F. et al. Antagonistic interactions safeguard mitotic propagation of genetic and epigenetic information in zebrafish. *Commun. Biol.* **7**, 31 (2024).
58. Schorpp, M. et al. Conserved functions of Ikaros in vertebrate lymphocyte development: genetic evidence for distinct larval and adult phases of T cell development and two lineages of B cells in zebrafish. *J. Immunol.* **177**, 2463–2476 (2006).
59. Dornburg, A. et al. From IgZ to IgT: a call for a common nomenclature for immunoglobulin heavy chain genes of ray-finned fish. *Zebrafish* **18**, 343–345 (2021).
60. Danilova, N., Bussmann, J., Jekosch, K. & Steiner, L. A. The immunoglobulin heavy-chain locus in zebrafish: identification and expression of a previously unknown isotype, immunoglobulin Z. *Nat. Immunol.* **6**, 295–302 (2005).
61. Hansen, J. D., Landis, E. D. & Phillips, R. B. Discovery of a unique Ig heavy-chain isotype (IgT) in rainbow trout: implications for a distinctive B cell developmental pathway in teleost fish. *Proc. Natl Acad. Sci. USA* **102**, 6919–6924 (2005).
62. Mirete-Bachiller, S. & Gambon-Deza, F. Tracing the origin of fish immunoglobulins. *Mol. Immunol.* **153**, 146–159 (2023).
63. Salinas, I., Zhang, Y. A. & Sunyer, J. O. Mucosal immunoglobulins and B cells of teleost fish. *Dev. Comp. Immunol.* **35**, 1346–1365 (2011).
64. Weir, H. et al. DNP-KLH yields changes in leukocyte populations and immunoglobulin isotype use with different immunization routes in zebrafish. *Front. Immunol.* **6**, 606 (2015).
65. Pancer, Z. et al. Somatic diversification of variable lymphocyte receptors in the agnathan sea lamprey. *Nature* **430**, 174–180 (2004).
66. Guo, P. et al. Dual nature of the adaptive immune system in lampreys. *Nature* **459**, 796–801 (2009).
67. Camaglia, F. et al. Quantifying changes in the T cell receptor repertoire during thymic development. *eLife* **12**, e81622 (2023).
68. Shilts, J. et al. A physical wiring diagram for the human immune system. *Nature* **608**, 397–404 (2022).
69. Suo, C. et al. Mapping the developing human immune system across organs. *Science* **376**, eabo0510 (2022).
70. Kirchberger, S. et al. Comparative transcriptomics coupled to developmental grading via transgenic zebrafish reporter strains identifies conserved features in neutrophil maturation. *Nat. Commun.* **15**, 1792 (2024).
71. Raddi, G. et al. Mosquito cellular immunity at single-cell resolution. *Science* **369**, 1128–1132 (2020).
72. Morrissey, K. A. et al. Comparison of reptilian genomes reveals deletions associated with the natural loss of γδ T cells in squamates. *J. Immunol.* **208**, 1960–1967 (2022).

73. Gambón-Deza, F. A new T cell receptor in squamata reptiles. Preprint at *bioRxiv* https://doi.org/10.1101/2023.09.04.556186 (2023).
74. Mirete-Bachiller, S., Olivieri, D. N. & Gambón-Deza, F. *Gouania willdenowi* is a teleost fish without immunoglobulin genes. *Mol. Immunol.* **132**, 102–107 (2021).
75. Swann, J. B., Holland, S. J., Petersen, M., Pietsch, T. W. & Boehm, T. The immunogenetics of sexual parasitism. *Science* **369**, 1608–1615 (2020).
76. Bajoghli, B. et al. A thymus candidate in lampreys. *Nature* **470**, 90–94 (2011).
77. Bajoghli, B. et al. Evolution of genetic networks underlying the emergence of thymopoiesis in vertebrates. *Cell* **138**, 186–197 (2009).
78. Gordon, J. Hox genes in the pharyngeal region: how Hoxa3 controls early embryonic development of the pharyngeal organs. *Int. J. Dev. Biol.* **62**, 775–783 (2018).
79. Dooley, J., Erickson, M., Gillard, G. O. & Farr, A. G. Cervical thymus in the mouse. *J. Immunol.* **176**, 6484–6490 (2006).
80. Terszowski, G. et al. Evidence for a functional second thymus in mice. *Science* **312**, 284–287 (2006).
81. Corbeaux, T. et al. Thymopoiesis in mice depends on a Foxn1-positive thymic epithelial cell lineage. *Proc. Natl Acad. Sci. USA* **107**, 16613–16618 (2010).
82. Li, J., Liu, Z., Xiao, S. & Manley, N. R. Transdifferentiation of parathyroid cells into cervical thymi promotes atypical T-cell development. *Nat. Commun.* **4**, 2959 (2013).
83. Bleul, C. C. et al. Formation of a functional thymus initiated by a postnatal epithelial progenitor cell. *Nature* **441**, 992–996 (2006).
84. Steinmann, G. G., Klaus, B. & Müller-Hermelink, H. K. The involution of the ageing human thymic epithelium is independent of puberty. A morphometric study. *Scand. J. Immunol.* **22**, 563–575 (1985).
85. Henry, L. & Anderson, G. Epithelial-cell architecture during involution of the human thymus. *J. Pathol.* **152**, 149–155 (1987).
86. Cowan, J. E., Takahama, Y., Bhandoola, A. & Ohigashi, I. Postnatal involution and counter-involution of the thymus. *Front. Immunol.* **11**, 897 (2020).
87. Rodewald, H. R. Thymus organogenesis. *Annu. Rev. Immunol.* **26**, 355–388 (2008).
88. Boehm, T. & Swann, J. B. Thymus involution and regeneration: two sides of the same coin? *Nat. Rev. Immunol.* **13**, 831–838 (2013).
89. Swann, J. B. et al. Conversion of the thymus into a bipotent lymphoid organ by replacement of FOXN1 with its paralog, FOXN4. *Cell Rep. B* **8**, 1184–1197 (2014).
90. Yayon, N. et al. A spatial human thymus cell atlas mapped to a continuous tissue axis. Preprint at *bioRxiv* https://doi.org/10.1101/2023.10.25.562925 (2023).
91. Kissa, K. et al. Live imaging of emerging hematopoietic stem cells and early thymus colonization. *Blood* **111**, 1147–1156 (2008).
92. Hess, I. & Boehm, T. Intravital imaging of thymopoiesis reveals dynamic lympho-epithelial interactions. *Immunity* **36**, 298–309 (2012).
93. McIntyre, L. L., Lutes, L. K. & Robey, E. A. Studying T cell development in neonatal and adult thymic slices. *Methods Mol. Biol.* **2580**, 233–247 (2023).
94. Lenti, M. V. et al. Asplenia and spleen hypofunction. *Nat. Rev. Dis. Prim.* **8**, 71 (2022).
95. Zapata, A. G. The fish spleen. *Fish Shellfish Immunol.* **144**, 109280 (2024).
96. Liu, Y. et al. Immunogenetic losses co-occurred with seahorse male pregnancy and mutation in *tlx1* accompanied functional asplenia. *Nat. Commun.* **13**, 7610 (2022).
97. Brendolan, A., Rosado, M. M., Carsetti, R., Selleri, L. & Dear, T. N. Development and function of the mammalian spleen. *Bioessays* **29**, 166–177 (2007).
98. Hughes, L. C. et al. Comprehensive phylogeny of ray-finned fishes (Actinopterygii) based on transcriptomic and genomic data. *Proc. Natl Acad. Sci. USA* **115**, 6249–6254 (2018).
99. van de Pavert, S. A. & Mebius, R. E. New insights into the development of lymphoid tissues. *Nat. Rev. Immunol.* **10**, 664–674 (2010).
100. Robertson, T. F. et al. A tessellated lymphoid network provides whole-body T cell surveillance in zebrafish. *Proc. Natl Acad. Sci. USA* **120**, e2301137120 (2023).
101. Zapata, A., Villena, A. & Cooper, E. L. Ultrastructure of the jugular body of *Rana pipiens*. *Cell Tissue Res.* **221**, 193–202 (1981).
102. Sugimura, M., Hashimoto, Y. & Nakanishi, Y. H. Thymus- and bursa-dependent areas in duck lymph nodes. *Jpn. J. Vet. Res.* **25**, 7–16 (1977).
103. Radtke, A. J. et al. IBEX: a versatile multiplex optical imaging approach for deep phenotyping and spatial analysis of cells in complex tissues. *Proc. Natl Acad. Sci. USA* **117**, 33455–33465 (2020).
104. Kienle, K. et al. Neutrophils self-limit swarming to contain bacterial growth in vivo. *Science* **372**, eabe7729 (2021).
105. Veríssimo, A. et al. An ancestral major histocompatibility complex organization in cartilaginous fish: reconstructing MHC origin and evolution. *Mol. Biol. Evol.* **40**, msad262 (2023).
106. Okamura, K. et al. Discovery of an ancient MHC category with both class I and class II features. *Proc. Natl Acad. Sci. USA* **118**, e2108104118 (2021).
107. Martin, R. & Kaufman, J. Some thoughts about what non-mammalian jawed vertebrates are telling us about antigen processing and peptide loading of MHC molecules. *Curr. Opin. Immunol.* **77**, 102218 (2022).
108. Deng, L. et al. A structural basis for antigen recognition by the T cell-like lymphocytes of sea lamprey. *Proc. Natl Acad. Sci. USA* **107**, 13408–13413 (2010).
109. Giorgetti, O. B., O'Meara, C. P., Schorpp, M. & Boehm, T. Origin and evolutionary malleability of T cell receptor α diversity. *Nature* **619**, 193–200 (2023).
110. Boehm, T. Co-evolution of a primordial peptide-presentation system and cellular immunity. *Nat. Rev. Immunol.* **6**, 79–84 (2006).
111. Wardemann, H. et al. Predominant autoantibody production by early human B cell precursors. *Science* **301**, 1374–1377 (2003).
112. Boehm, T., Hess, I. & Swann, J. B. Evolution of lymphoid tissues. *Trends Immunol.* **33**, 315–321 (2012).
113. Derbinski, J. & Kyewski, B. How thymic antigen presenting cells sample the body's self-antigens. *Curr. Opin. Immunol.* **22**, 592–600 (2010).
114. Pappenheimer, A. M. A contribution to the normal and pathological histology of the thymus gland. *J. Med. Res.* **22**, 1–74 (1910).
115. Mackay, I. R. & Goldstein, G. Thymus and muscle. *Clin. Exp. Immunol.* **2**, 139–140 (1967).
116. Michelson, D. A., Hase, K., Kaisho, T., Benoist, C. & Mathis, D. Thymic epithelial cells co-opt lineage-defining transcription factors to eliminate autoreactive T cells. *Cell* **185**, 2542–2558 (2022).
117. Yu, W. et al. Clonal deletion prunes but does not eliminate self-specific αβ CD8(+) T lymphocytes. *Immunity* **42**, 929–941 (2015).
118. Holland, S. J. et al. Selection of the lamprey VLRC antigen receptor repertoire. *Proc. Natl Acad. Sci. USA* **111**, 14834–14839 (2014).
119. Herzig, Y. et al. Transcriptional programs that control expression of the autoimmune regulator gene Aire. *Nat. Immunol.* **18**, 161–172 (2017).
120. Quintana, F. J. et al. Adaptive autoimmunity and Foxp3-based immunoregulation in zebrafish. *PLoS ONE* **5**, e9478 (2010).
121. Sugimoto, K., Hui, S. P., Sheng, D. Z., Nakayama, M. & Kikuchi, K. Zebrafish FOXP3 is required for the maintenance of immune tolerance. *Dev. Comp. Immunol.* **73**, 156–162 (2017).
122. Josefowicz, S. Z., Lu, L. F. & Rudensky, A. Y. Regulatory T cells: mechanisms of differentiation and function. *Annu. Rev. Immunol.* **30**, 531–564 (2012).
123. Sakaguchi, S. et al. Regulatory T cells and human disease. *Annu. Rev. Immunol.* **38**, 541–566 (2020).
124. Hui, S. P. et al. Zebrafish regulatory T cells mediate organ-specific regenerative programs. *Dev. Cell* **43**, 659–672.e5 (2027).
125. Muñoz-Rojas, A. R. & Mathis, D. Tissue regulatory T cells: regulatory chameleons. *Nat. Rev. Immunol.* **21**, 597–611 (2021).
126. Loffredo, L. F., Savage, T. M., Ringham, O. R. & Arpaia, N. Treg-tissue cell interactions in repair and regeneration. *J. Exp. Med.* **221**, e20231244 (2024).
127. Boulay, J. L., Du Pasquier, L. & Cooper, M. D. Cytokine receptor diversity in the lamprey predicts the minimal essential cytokine networks of vertebrates. *J. Immunol.* **209**, 1013–1020 (2022).
128. Rizzuto, G. & Erlebacher, A. Trophoblast antigens, fetal blood cell antigens, and the paradox of fetomaternal tolerance. *J. Exp. Med.* **219**, e20211515 (2022).
129. Blackburn, D. G. Evolution of vertebrate viviparity and specializations for fetal nutrition: a quantitative and qualitative analysis. *J. Morphol.* **276**, 961–990 (2015).
130. Griffith, O. W. & Wagner, G. P. The placenta as a model for understanding the origin and evolution of vertebrate organs. *Nat. Ecol. Evol.* **1**, 72 (2017).
131. Griffith, O. W. et al. Embryo implantation evolved from an ancestral inflammatory attachment reaction. *Proc. Natl Acad. Sci. USA* **114**, E6566–E6575 (2017).
132. Hilgers, L. et al. Inflammation and convergent placenta gene co-option contributed to a novel reproductive tissue. *Curr. Biol.* **32**, 715–724.e4 (2022).
133. Roth, O. et al. Evolution of male pregnancy associated with remodeling of canonical vertebrate immunity in seahorses and pipefishes. *Proc. Natl Acad. Sci. USA* **117**, 9431–9439 (2020).
134. Furness, A. I., Avise, J. C., Pollux, B. J. A., Reynoso, Y. & Reznick, D. N. The evolution of the placenta in poeciliid fishes. *Curr. Biol.* **31**, 2004–2011 (2011).
135. Safian, D. et al. Repeated independent origins of the placenta reveal convergent and divergent organ evolution within a single fish family (Poeciliidae). *Sci. Adv.* **9**, eadf3915 (2023).
136. Regan, C. T. Dwarfed males parasitic on the females in oceanic angler-fishes (*Pediculati ceratioidea*). *Proc. R. Soc. B* **97**, 386–400 (1925).
137. Pietsch, T. W. *Oceanic Anglerfishes* (Univ. California Press, 2009).
138. Brownstein, C. D. et al. Synergistic innovations enabled the radiation of anglerfishes in the deep open ocean. *Curr. Biol.* https://doi.org/10.1016/j.cub.2024.04.066 (2024).
139. Simakov, O. et al. Deeply conserved synteny resolves early events in vertebrate evolution. *Nat. Ecol. Evol.* **4**, 820–830 (2020).
140. Nakatani, Y. et al. Reconstruction of proto-vertebrate, proto-cyclostome and proto-gnathostome genomes provides new insights into early vertebrate evolution. *Nat. Commun.* **12**, 4489 (2021).
141. Timoshevskaya, N. et al. An improved germline genome assembly for the sea lamprey *Petromyzon marinus* illuminates the evolution of germline-specific chromosomes. *Cell Rep.* **42**, 12263 (2023).
142. Zhu, T. et al. Chromosome-level genome assembly of *Lethenteron reissneri* provides insights into lamprey evolution. *Mol. Ecol. Resour.* **21**, 448–463 (2021).
143. Hess, J. E. et al. Genomic islands of divergence infer a phenotypic landscape in Pacific lamprey. *Mol. Ecol.* **20**, 3841–3856 (2020).
144. Marlétaz, F. et al. The hagfish genome and the evolution of vertebrates. *Nature* **627**, 811–820 (2024).
145. Yu, D. et al. Hagfish genome elucidates vertebrate whole-genome duplication events and their evolutionary consequences. *Nat. Ecol. Evol.* **8**, 519–535 (2024).
146. Yamaguchi, K. et al. Elasmobranch genome sequencing reveals evolutionary trends of vertebrate karyotype organization. *Genome Res.* **33**, 1527–1540 (2023).
147. Wagner, C. I. et al. Characteristics of the spiny dogfish (*Squalus acanthias*) nuclear genome. *G3* **13**, jkad146 (2023).
148. Stanhope, M. J. et al. Genomes of endangered great hammerhead and shortfin mako sharks reveal historic population declines and high levels of inbreeding in great hammerhead. *iScience* **26**, 105815 (2022).

149. Tan, M. et al. The whale shark genome reveals patterns of vertebrate gene family evolution. *eLife* **10**, e65394 (2021).
150. Hara, Y. et al. Shark genomes provide insights into elasmobranch evolution and the origin of vertebrates. *Nat. Ecol. Evol.* **2**, 1761–1771 (2018).
151. Venkatesh, B. et al. Elephant shark genome provides unique insights into gnathostome evolution. *Nature* **505**, 174–179 (2014).
152. Thompson, A. W. et al. The bowfin genome illuminates the developmental evolution of ray-finned fishes. *Nat. Genet.* **53**, 1373–1384 (2021).
153. Braasch, I. et al. The spotted gar genome illuminates vertebrate evolution and facilitates human–teleost comparisons. *Nat. Genet.* **48**, 427–437 (2016).
154. Meyer, A. et al. Giant lungfish genome elucidates the conquest of land by vertebrates. *Nature* **590**, 284–289 (2021).
155. Wang, K. et al. African lungfish genome sheds light on the vertebrate water-to-land transition. *Cell* **184**, 1362–1376 (2021).
156. Streicher, J. W. Wellcome Sanger Institute Tree of Life Programme, Wellcome Sanger Institute Scientific Operations: DNA Pipelines Collective, Tree of Life Core Informatics Collective & Darwin Tree of Life Consortium. The genome sequence of the common frog, *Rana temporaria* Linnaeus 1758. *Wellcome Open Res.* **6**, 286 (2021).
157. Streicher, J. W. Wellcome Sanger Institute Tree of Life Programme, Wellcome Sanger Institute Scientific Operations: DNA Pipelines Collective, Tree of Life Core Informatics Collective & Darwin Tree of Life Consortium. The genome sequence of the common toad, *Bufo bufo* (Linnaeus, 1758). *Wellcome Open Res.* **6**, 281 (2021).
158. Gable, S. M. et al. The state of squamate genomics: past, present, and future of genome research in the most speciose terrestrial vertebrate order. *Genes* **14**, 1387 (2023).
159. Stiller, J. et al. Complexity of avian evolution revealed by family-level genomes. *Nature* https://doi.org/10.1038/s41586-024-07323-1 (2024).
160. Card, D. C., Jennings, W. B. & Edwards, S. V. Genome evolution and the future of phylogenomics of non-avian reptiles. *Animals* **13**, 471 (2023).
161. Deakin, J. E. & O'Neill, R. J. Evolution of marsupial genomes. *Annu. Rev. Anim. Biosci.* **8**, 25–45 (2020).
162. Vinkler, M. et al. Understanding the evolution of immune genes in jawed vertebrates. *J. Evol. Biol.* **36**, 847–873 (2023).
163. Martin, E. C. et al. Insights into RAG evolution from the identification of 'missing link' family A RAGL transposons. *Mol. Biol. Evol.* **40**, msad232 (2023).
164. Martin, E. C. et al. Identification of RAG-like transposons in protostomes suggests their ancient bilaterian origin. *Mob. DNA* **11**, 17 (2020).
165. Zhang, Y. et al. Transposon molecular domestication and the evolution of the RAG recombinase. *Nature* **9**, 9–84 (2019).
166. Huang, S. et al. Discovery of an active RAG transposon illuminates the origins of V(D)J recombination. *Cell* **166**, 102–114 (2016).
167. Holland, S. J. et al. Expansions, diversification, and interindividual copy number variations of AID/APOBEC family cytidine deaminase genes in lampreys. *Proc. Natl Acad. Sci. USA* **115**, E3211–E3220 (2018).
168. Carlson, K. B., Nguyen, C., Wcisel, D. J., Yoder, J. A. & Dornburg, A. Ancient fish lineages illuminate toll-like receptor diversification in early vertebrate evolution. *Immunogenetics* **75**, 465–478 (2023).
169. Nomiyama, H., Osada, N. & Yoshie, O. Systematic classification of vertebrate chemokines based on conserved synteny and evolutionary history. *Genes Cell* **18**, 1–16 (2012).
170. Collette, Y., Gilles, A., Pontarotti, P. & Olive, D. A co-evolution perspective of the TNFSF and TNFRSF families in the immune system. *Trends Immunol.* **24**, 387–394 (2023).
171. Jeucken, K. C. M., Koning, J. J., Mebius, R. E. & Tas, S. W. The role of endothelial cells and TNF-receptor superfamily members in lymphoid organogenesis and function during health and inflammation. *Front. Immunol.* **10**, 2700 (2019).
172. Hedrick, S. M. Understanding immunity through the lens of disease ecology. *Trends Immunol.* **38**, 888–903 (2017).
173. Hooper, L. V., Littman, D. R. & Macpherson, A. J. Interactions between the microbiota and the immune system. *Science* **336**, 1268–7123 (2012).
174. Irving, A. T., Ahn, M., Goh, G., Anderson, D. E. & Wang, L. F. Lessons from the host defences of bats, a unique viral reservoir. *Nature* **589**, 363–370 (2021).
175. Déjosez, M. et al. Bat pluripotent stem cells reveal unusual entanglement between host and viruses. *Cell* **186**, 957–974 (2023).
176. Demian, W. L., Cormier, O. & Mossman, K. Immunological features of bats: resistance and tolerance to emerging viruses. *Trends Immunol.* **45**, 198–210 (2024).
177. Gorbunova, V., Seluanov, A. & Kennedy, B. K. The world goes bats: living longer and tolerating viruses. *Cell Metab.* **32**, 31–43 (2020).
178. Daane, J. M. & Detrich, H. W. Adaptations and diversity of Antarctic fishes: a genomic perspective. *Annu. Rev. Anim. Biosci.* **10**, 39–62 (2022).
179. Bista, I. et al. Genomics of cold adaptations in the Antarctic notothenioid fish radiation. *Nat. Commun.* **14**, 412 (2023).
180. Ghorbani, A. et al. Ancestral reconstruction reveals catalytic inactivation of activation-induced cytidine deaminase concomitant with cold water adaption in the Gadiformes bony fish. *BMC Biol.* **20**, 293 (2022).
181. Swaminathan, A., Xia, F. & Rohner, N. From darkness to discovery: evolutionary, adaptive, and translational genetic insights from cavefish. *Trends Genet.* **40**, 24–38 (2024).
182. Peuß, R. et al. Adaptation to low parasite abundance affects immune investment and immunopathological responses of cavefish. *Nat. Ecol. Evol.* **4**, 1416–1430 (2020).
183. Tian, X. et al. High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. *Nature* **499**, 346–349 (2013).
184. Hilton, H. G. et al. Single-cell transcriptomics of the naked mole-rat reveals unexpected features of mammalian immunity. *PLoS Biol.* **17**, e3000528 (2019).
185. Santosa, E. K. & Sun, J. C. Cardinal features of immune memory in innate lymphocytes. *Nat. Immunol.* **24**, 1803–1812 (2023).
186. Vilches, C. & Parham, P. KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. *Annu. Rev. Immunol.* **20**, 217–251 (2002).
187. O'Connor, E. A. & Cornwallis, C. K. Immunity and lifespan: answering long-standing questions with comparative genomics. *Trends Genet.* **38**, 650–661 (2022).
188. Kolora, S. R. R. et al. Origins and evolution of extreme life span in Pacific Ocean rockfishes. *Science* **374**, 842–847 (2021).
189. Burke, K. P., Chaudhri, A., Freeman, G. J. & Sharpe, A. H. The B7:CD28 family and friends: unraveling coinhibitory interactions. *Immunity* **57**, 223–244 (2014).
190. Hotamisligil, G. S. Inflammation, metaflammation and immunometabolic disorders. *Nature* **542**, 177–185 (2017).
191. Quesada, V. et al. Giant tortoise genomes provide insights into longevity and age-related disease. *Nat. Ecol. Evol.* **3**, 87–95 (2019).
192. Depczynski, M. & Bellwood, D. R. Shortest recorded vertebrate lifespan found in a coral reef fish. *Curr. Biol.* **15**, R288–R289 (2005).
193. Reichard, M., Cellerino, A. & Valenzano, D. R. Turquoise killifish. *Curr. Biol.* **25**, R741–R742 (2015).
194. Bradshaw, W. J., Poeschla, M., Placzek, A., Kean, S. & Valenzano, D. R. Extensive age-dependent loss of antibody diversity in naturally short-lived turquoise killifish. *eLife* **11**, e65117 (2022).
195. Heimroth, R. D. et al. The lungfish cocoon is a living tissue with antimicrobial functions. *Sci. Adv.* **7**, eabj0829 (2021).
196. Goronzy, J. J. & Weyand, C. M. Mechanisms underlying T cell ageing. *Nat. Rev. Immunol.* **19**, 573–583 (2019).
197. Nikolich-Žugich, J. The twilight of immunity: emerging concepts in aging of the immune system. *Nat. Immunol.* **19**, 10–19 (2018).
198. Kottelat, M., Britz, R., Hui, T. H. & Witte, K. E. *Paedocypris*, a new genus of Southeast Asian cyprinid fish with a remarkable sexual dimorphism, comprises the world's smallest vertebrate. *Proc. Biol. Sci.* **273**, 895–899 (2006).
199. Giorgetti, O. B. et al. Antigen receptor repertoires of one of the smallest known vertebrates. *Sci. Adv.* **7**, eabd8180 (2021).
200. Miya, M. et al. Evolutionary history of anglerfishes (Teleostei: Lophiiformes): a mitogenomic perspective. *BMC Evol. Biol.* **10**, 58 (2010).
201. Rittmeyer, E. N., Allison, A., Gründler, M. C., Thompson, D. K. & Austin, C. C. Ecological guild evolution and the discovery of the world's smallest vertebrate. *PLoS ONE* **7**, e29797 (2012).
202. Trancoso, I., Morimoto, R. & Boehm, T. Co-evolution of mutagenic genome editors and vertebrate adaptive immunity. *Curr. Opin. Immunol.* **65**, 32–41 (2020).
203. Victora, G. D. & Nussenzweig, M. C. Germinal centers. *Annu. Rev. Immunol.* **40**, 413–442 (2022).
204. Matz, H. et al. Organized B cell sites in cartilaginous fishes reveal the evolutionary foundation of germinal centers. *Cell Rep.* **42**, 112664 (2023).
205. Shibasaki, Y. et al. Cold-blooded vertebrates evolved organized germinal center-like structures. *Sci. Immunol.* **8**, eadf1627 (2023).
206. Matz, H. & Dooley, H. 450 million years in the making: mapping the evolutionary foundations of germinal centers. *Front. Immunol.* **14**, 1245704 (2023).
207. Boehm, T. The chilling origin of germinal centers. *Sci. Immunol.* **8**, eadl1470 (2023).
208. Nemazee, D. Mechanisms of central tolerance for B cells. *Nat. Rev. Immunol.* **17**, 281–294 (2017).
209. Fischer, A. & Rausell, A. Primary immunodeficiencies suggest redundancy within the human immune system. *Sci. Immunol.* **1**, eaah5861 (2016).
210. Fischer, A. & Rausell, A. What do primary immunodeficiencies tell us about the essentiality/redundancy of immune responses? *Semin. Immunology* **36**, 13–16 (2018).

Acknowledgements
The author is grateful to the members of the group for insightful discussions and their contributions to the work from the laboratory cited here. The author is supported by the Max Planck Society, the Deutsche Forschungsgemeinschaft and the European Research Council (grant no. 834302).

Competing interests
The author declares no competing interests.

Additional information
Peer review information *Nature Reviews Immunology* thanks Robert Miller, Jeffrey A. Yoder and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.