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Review

Evolution of thymus organogenesis

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ABSTRACT

The thymus is the primary organ for functional T lymphocyte development in jawed vertebrates. A new study in the jawless fish, lampreys, indicates the existence of a primitive thymus in these surviving representatives of the most ancient vertebrates, providing strong evidence of co-evolution of T cells and thymus. This review summarizes the wealth of data that have been generated towards understanding the evolution of the thymus in the vertebrates. Progress in identifying genetic networks and cellular mechanisms that control thymus organogenesis in mammals and their evolution in lower species may inspire the development of new strategies for medical interventions targeting faulty thymus functions.

Contents

1. Introduction

The thymus, first discovered by Galen (130–200 AD), is the primary site of T lymphocyte development. It provides a unique microenvironment for the production of a diverse but self-MHC (Major Histocompatibility Complex)-restricted and self-tolerant T cell repertoire (Rodewald, 2008). T cell precursors enter the thymus via large vessels at the boundary between the cortex and medulla. During their traveling through the thymic cortex, they first commit to the T cell lineage, then sequentially rearrange and express T cell receptor (TCR) alpha and beta chains and undergo positive selection for the ability of TCR to interact with self-MHC. The thymocytes that survive from the positive selection migrate to the medullary regions, undergo negative selection to eliminate potentially autoreactive thymocytes and then exit from the thymus via vasculature in the cortico-medullary junction (Stritesky et al., 2011; Klein et al., 2009). This highly ordered migration and development of T cells requires the correct patterning and organization of thymic stromal components, including epithelial cells (cortical epithelial cells, cTEC, and medullary epithelial cells, mTEC), fibroblasts, nonfibroblastic mesenchymal cells, endothelial cells, dendritic cells, and macrophages. These elements constitute a complex and dynamic environment that is crucial for the optimal T cell development (Manley et al., 2011). Defects in the thymus stromal matrix can result in immunodeficiency or autoimmunity (Fletcher et al., 2011).

The thymus, together with the parathyroid glands, is derived from a single primordium in the pharyngeal pouches of the endodermal gut tube. This primordium is then patterned into the thymus- and parathyroid glands-specific domains, and eventually these two organs migrate to their final destination and separate

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during embryonic development (Gordon and Manley, 2011). Although it is no longer a topic of debate that the thymus epithelium, including cTEC and mTEC, has a common germ layer origin, endoderm, the details of the patterning of the primordium, the formation and migration of the thymus, and their appearance in evolution are poorly understood (Gordon and Manley, 2011). This review summarizes the recent understandings of the phylogeny of thymus organogenesis.

2. An overview of thymus development in the mouse model

The development of the thymus is best characterized in mouse models. In mice, the third pair of pharyngeal pouches each forms a common thymus-parathyroid primordium that will later develop into one thymus lobe and one parathyroid gland. The patterning of this pharyngeal pouch into organ-specific domains begins at embryonic day (E) 9.5 when the third pair of pharyngeal pouches begins to develop. Starting at E9.5, expression of glial cells missing homolog 2 (Gcm2), the earliest marker of parathyroid turns on in an anterodorsal domain within the third pouch endoderm (Gordon et al., 2001; Patel et al., 2006). Foxn1 (forkhead family transcription factor), the earliest known thymus-specific marker, however, does not begin to express in a discrete ventral domain until E11. As the role of Foxn1 in specifying the thymus fate is in question, when and what drives the thymus identity specification becomes more of a question of what initiates and maintains Foxn1 expression (Blackburn et al., 1996; Zamisch et al., 2005). Signaling molecules expressed in the endoderm that play key roles in regulating the tissue/organ formation were studied intensively. For instance, bone morphogenetic protein (Bmp) is expressed in the ventral domain of the pouch and expands with Foxn1 expression. Inhibition of BMP signaling caused loss of Foxn1 expression in the thymic anlage in zebrafish (Patel et al., 2006; Bleul and Boehm, 2005; Soza-Ried et al., 2008). Sonic hedgehog (Shh) signaling in the endoderm of the forming pharyngeal pouches may negatively regulate the thymus identity while positively regulate parathyroid identity in the pharyngeal epithelium. *Shh* null mouse shows defects in the pattern of the pharyngeal pouches, loss of Gcm2 expression while expansion of the neighboring *Bmp4* expressing presumptive thymus domain (Moore-Scott and Manley, 2005; Grevellec et al., 2011). Other factors, including those in the Hoxa3 (homeobox A2)-Eya1 (eyes absent homolog 1)-Pax1/9 (paired box protein 1/9)-Six1/4 (sine oculis homolog 1/4) network, Tbx1 (T-box 1), Wnt (wingless-int) and Fgf (fibroblast growth factor) pathways are also expressed prior to Foxn1 expression and are suggested to be involved in the initial patterning of the thymus (Manley and Condie, 2010; Gordon and Manley, 2011). But more direct in vivo evidence is required to link them to the thymus fate determination and/or Foxn1 regulation.

Neural crest cells (NCCs), an ectodermal cell population, is believed to play an important role in thymus organogenesis. NCCs were found to surround the thymic epithelium and form perivascular mesenchyme (Le Lievre and Le Douarin, 1975; Le Douarin and Jotereau, 1975). Normal thymus can develop from the pharynegeal endoderm that is transplanted prior to the migration of NCC into the pharyngeal region (Le Lievre and Le Douarin, 1975; Gordon et al., 2004). Mice that have a severe loss of NCCs (Splotch mutant (*Pax3*-null)) showed normal establishment of thymus and parathyroid identity until E11.5, when a dorsal shift in the border between parathyroid- and thymus-fated domains were found, giving rise to a larger thymus and smaller parathyroids (Griffith et al., 2009). Thus, the role of epithelial-mesenchymal interactions is probably not to determine the initial patterning of the common thymus-parathyroid primordium but to facilitate the establishment of the organ-specific domains in the endoderm of the third pharyngeal pouches. The molecular mechanisms underlying the epithelial-mesenchymal interactions is not clear but may involve Bmp, Shh, Fgf, and Wnt pathways (Bleul and Boehm, 2005; Patel et al., 2006; Gordon et al., 2010; Jeong et al., 2004; Moore-Scott and Manley, 2005; Frank et al., 2002; Balciunaite et al., 2002).

At E11.5 of mouse embryogenesis, the primordia in the third pharyngeal pouch are completely patterned into thymus and parathyroid domains (Gordon et al., 2001). At this stage, lymphoid progenitor cells start their first wave of immigration into the thymus domain by the coordinating guidance of CCR7- and CCR9-mediated chemokine signals (Liu et al., 2006; Owen and Ritter, 1969; Fontaine-Perus et al., 1981). Also during this time, the primordia initiate the detachment from the lateral surfaces of the pharynx via apoptosis (Gordon et al., 2004). NCCs are likely to be crucial in inducing organ-pharynx separation. Delayed or failure of pharyngeal detachment can be found in Splotch mutants that lack NCCs and in mice that have a NCC-specific deletion of Hoxa3 (Griffith et al., 2009; Chen et al., 2010). In addition, *Shh*-null, *Pax9*, and Frs2α (FGF pathway-associated docking protein) mutants also showed persistent attachment (Moore-Scott and Manley, 2005; Peters et al., 1998; Kameda et al., 2009).

By E12.0 of mouse development, the thymus-parathyroid primordia have completely detached from the pharynx, and have begun their separation into two discrete organs and their migration towards the anterior of the thoracic cavity. The parathyroids eventually stay adjacent to the thyroid gland whereas the thymus lobes continuously descend caudally and medially until reaching its final location at the midline, above the heart and behind the sternum. Evidence suggested that the interaction between the epithelia and NCC-derived mesenchyme again plays an essential role in the thymus-parathyroid organ separation and migration. Splotch mutants, *Bmp4* mutants, NCC-specific deletions of *ephrin B2* and *Hoxa3* all display delayed organ separation (Foster et al., 2010; Griffith et al., 2009; Chen et al., 2010; Gordon et al., 2010). A failure of thymus migration is also found in NCC-restricted deletion of *ephrin B2* (Foster et al., 2010).

3. The emergence of lymphocytes

As T cell development fails without the thymus, it is generally believed that the thymus appeared in evolution with the emergence of functional B and T lymphocyte lineages, the essence of the adaptive immune system. Virtually all vertebrates, including jawless and jawed animals, showed the existence of a dichotomy of lymphocyte lineages (Guo et al., 2009; Kishishita et al., 2010; Boehm, 2011; Heimberg et al., 2010; Mayer et al., 2002; Pancer et al., 2004). This prompts the assumption that the appearance of proto-lymphocyte-like cells may have existed prior to the divergence of cyclostomes and gnathostomes or even before the emergence of vertebrates. Indeed, some studies have identified cells that morphologically resemble lymphocytes in amphioxus and Botryllus schlosseri, two subphyla of invertebrate chordates (Han et al., 2010; Ballarin and Cima, 2005; Yu et al., 2005; Huang et al., 2007) and in starfish Asteria rubens that belongs to a sister phylum of chordates (Leclerc et al., 1980, 1981). Whether these cells possess lymphocyte functions await further investigation.

Interestingly, the mechanisms of somatic diversification of the antigen receptor genes between jawed and jawless vertebrates have revealed significant differences. Lymphocytes in lampreys express leucine-rich-repeat-containing variable lymphocyte receptors (VLRs) that use APOBEC-like cytidine deaminases to assemble VLR genes (Guo et al., 2009; Rogozin et al., 2007), whereas lymphocytes in jawed animals express TCR or immunoglobulin that use recombination activating gene (RAG) to rearrange the germline-encoded receptor genes. Both mechanisms give rise to lymphocytes with a vast immune repertoire.

4. The emergence of the thymus

In mice and human, the stepwise T cell development and migration within the thymus is well-studied. Proper organization of cortical and medullary epithelial cells and other stromal cells in the thymus is the basic requirement of such a microenvironment in instructing the developing T cells to differentiate, proliferate, or die. However, how does the thymus, a multi-component organ, co-evolve with T lymphocytes remains largely unsolved. The jawed cartilaginous fish (Chondrichthyes: sharks, skates, rays, and chimaeras), the oldest jawed vertebrates, possesses thymi with many structural and functional features of a true thymus. The thymi are situated dorsomedial to the gills and is composed of lobes with a clearly defined cortex and medulla (Wyffels et al., 2005; Criscitiello et al., 2010). The cortex contains densely packed cells with lymphocyte morphology. The medulla, however, is lack of Hassall's corpuscles. Similar to mammalian thymus, expression of TCRs, including α, β, γ, δTCRs, can be detected in the shark thymus. RAG1 and TdT expressions were found in the cortex whereas MHC class I and II expressing cells were detected in the cortex as well as in the medulla (Criscitiello et al., 2010; Zapata and Amemiya, 2000). Thus, sharks represent the oldest living vertebrates with the basic components of the thymus in mammals.

Very recently, a primitive type of thymus, thymoid, was identified in the jawless fish, lampreys (Bajoghli et al., 2011). The structures at the tips of the gill filaments in the gill basket of lampreys showed in close proximity the expression of *CDA1*, *FOXN1* (*FOXN4L* in lampreys), and Delta-like B (*DLL-B*), a Notch ligand of the Delta-like family. Also found in this particular region are high percentage of out-of-frame VLRA gene assemblies and caspase 3 positive cells, suggesting that expression of non-functional antigen receptor was associated to cell death, a process comparable to the one in jawed vertebrates. Similar to developing lymphocytes in jawed animals, T-like cells from this region failed to respond to activation signals such as the T-cell mitogen phytohemagglutinin that stimulate mature lymphocytes. Whether the thymoids in lamprey have distinct cortex and medulla structure has not been studied, but the expression of CDA1 was detected at outer edge in the thymoids suggesting a possible spatial differentiation in this tissue. Together, the evidence strongly supports the co-evolution of T lymphocytes and thymus in the jawless fish, the surviving representatives of the most ancient vertebrates. It remains to be seen if thymus-like tissue could be found in more ancient animals such as amphioxus or it is more of an evolutionary innovation of vertebrates.

5. The evolution of genetic networks of thymic epithelial cells

The evolution of the thymus was likely driven by changes at the gene-regulatory level. These may include deployment or co-option of ancestral gene batteries used elsewhere in the embryo, addition of new interactions, or invention of new genetic cascades. Novel developmental potential could be derived from one or some combination of these events. As lampreys and cartilaginous fish occupy key phylogenetic positions in the emergence of novelties and in the diversification of structures at the dawn of vertebrates, studies of thymus-related genes in these species may bring a better picture of the genetic evolution of this highly specialized tissue (Table 1) (Yu et al., 2007; Kozmik et al., 2001; Shimeld, 1999; Lin et al., 2009; Holland and Holland, 2001; Hetzer-Egger et al., 2000; Kozmik et al., 2007; Mahadevan et al., 2004; Sauka-Spengler et al., 2002; Petersen and Reddien, 2009).

FOXN1, an earliest thymus-specific marker that is required for the proliferation and differentiation of TECs, was identified in the pharyngeal area of lamprey (Bajoghli et al., 2011) suggesting that epithelial cells at the tips of the gill filaments have already committed to thymic epithelial cell fate. In cephalochordate amphioxus, *Foxn4* is also found positive in the pharyngeal endoderm as well as other sites (Bajoghli et al., 2009). However, the relationship between *Foxn4* expression and endodermal epithelial cells with a thymus-like fate remains to be established. The signaling pathways of BMP and Shh that appear to regulate thymus identity and/or FOXN1 expression may also be important to study in lampreys or even invertebrate chordates for pharyngeal pouch derivatives. At least the presence of BMP and Shh signaling has already been proved in amphioxus. Thus, further studies are required to investigate whether genes in these pathways function in amphioxus in facilitating the expression of Foxn1/Foxn4 and the appearance of primitive thymic epithelial cells or they gradually acquire new expression domains and new transcriptional regulation during invertebrate-to-vertebrate transition (Retaux and Kano, 2010).

In mammals, Foxn1 regulates the expression of Notch ligands that are essential for T cell identity. *Dll4* expression is absent in the mouse that have thymic epithelial cells lacking Foxn1 (Itoi et al., 2007). The expression of *FOXN1* and *DLL-B* were found in the same thymoid region of lampreys. The pharyngeal endoderm of amphioxus also showed coexpression of *Foxn4a* and *Dll* genes (Bajoghli et al., 2009), suggesting that a regulator-target relationship between Foxn1 and Dll has already been established in the common ancestor of vertebrates.

FOXN1 also regulates the expression of genes encoding thymopoietic chemokines. For instance, *ccl25a*, another target of FOXN1,

Table 1
Factors implicated in the genetic networks of thymic epithelial cells.

Molecules	Gnathostomes		Agnathans	Invertebrate chordates
	Bony fishes	Cartilaginous fishes	Lamprey	Amphioxus
Bone morphogenic protein (Bmp)	Yes	Yes	Yes	Yes
Sonic hedgehog (Shh)	Yes	Yes	Yes	Yes
Wingless homolog family (Wnt)	Yes	Yes	Yes	Yes
Forkhead box protein n (Foxn1)	Yes	Yes	Yes	Foxn4
Delta like ligand (Dll)	Yes	Yes	Yes	Yes
Chemokine ligand 25 (Ccl25)	Yes	Yes	0	0
Homeobox protein a (Hoxa)	Yes	Yes	Yes	Hox
Paired box protein 1/9 (Pax1/9)	Yes	Yes	Yes	Yes
Sine oculis homolog 1/4 (Six1/4)	Yes	Yes	Yes	Yes
Eyes absent homolog (Eya)	Yes	Yes	Yes	Yes
T-box 1 (Tbx1)	Yes	Yes	Yes	Yes
Major histocompatibility complex (MHC)	Yes	Yes	0	0
Thymoproteasome subunit, β5t	Yes	Yes	0	0
Autoimmune regulator (Aire)	Yes	0	0	0

was found in the genome of cartilaginous fish but not in amphioxus or lamprey genomes. Similarly, the receptor of *ccl25, ccr9*, first appears in cartilaginous fishes (Guo et al., 2009; Bajoghli et al., 2009). Evidence has shown that amphioxus lacks chemokine receptors in its genome whereas jawless fish contain homologues of *cxcr4* and *cxcl12*. In addition, a gene that is related in sequence to the mammalian chemokine receptors CCR7 and CCR9 was also detected from T-like cells in one species of lamprey (*Petromyzon marinus*) but not another (*Lampetra planeri*) (Guo et al., 2009; Bajoghli et al., 2009). It remains to be proved that these chemokine receptor-ligand pairs are involved in regulating the seeding of hematopoietic progenitors to the thymoids in lampreys. But these data suggest that in primitive vertebrates, novel transcriptional regulation of FOXN1 is quickly expanding and an evolutionarily novel genetic network of thymus is establishing.

Interactions between NCC-derived mesenchyme and thymic epithelial cells are essential in the organogenesis of embryonic thymus (Auerbach, 1960; Jenkinson et al., 2003; Bockman and Kirby, 1984; Griffith et al., 2009). The neural crest seems to co-evolve with the thymus in lampreys. However, a population of migratory pigment cells that expresses some neural crest markers was identified in the nonvertebrate, *ascidian Ecteinascidia* (Jeffery et al., 2004). Signaling molecules *BMP*, *Notch*, *Wnt*, and neural plate border specifiers *Msx, Zic,* and *Pax3/7* are also expressed in amphioxus in a pattern closely resembling that of vertebrates (Sauka-Spengler and Bronner-Fraser, 2008). This suggests that the initial steps of the neural tube border patterning and specification are already present in cephalochordates. At what time during evolution and by what molecular mechanism the functional interactions between NCCs and thymic epithelial cells were established remains elusive.

Thymocytes in mammals that express αβ TCRs are constrained by their requirement for recognition of antigen-presenting molecules (MHC or MHC-like molecules) on thymic epithelial cells and other cells. It is not clear how TCR-MHC interaction is evolved. Genes encoding MHC and MHC-like molecules are present in all jawed vertebrates (Flajnik and Kasahara, 2010; Litman et al., 2010; Rast et al., 1997). Evidence of alloresponse has also been obtained in the lamprey (Bajoghli et al., 2011), suggesting that primordial antigen-presenting molecules may present in the thymoids and may mediate the possible selection of antigen receptors (VLRA) on T-like cells. But genetic evidence of MHC-like molecules awaits detailed investigation in lampreys or even protochordates. Recently, a thymoproteasome subunit, β5t, was found to be involved in positive selection of a diverse repertoire of MHC class I-restricted T cells. The deficiency of this protein in cortical thymic epithelial cells resulted in significant reduction of CD8 single positive thymocytes (Murata et al., 2007; Nitta et al., 2010). Genes that are closely related to β5t homologues were found in the elephant shark *Callorhinchus milii* and bony fish, but not in the genomes of the cephalochordate *Branchiostoma floridae* and the jawless fish *P. marinus* (Boehm, 2009; Kandil et al., 1996). Thus, it appears that MHCs and β5t may become incorporated into the genetic network of thymic epithelial cells at similar time during the evolution of jawed vertebrates.

In mammals, developing T cells undergo negative selection to eliminate self-reactive cells. In order to fulfill this task, medullary thymic epithelial cells express a broad range of tissue-specific self-antigens (Kyewski and Klein, 2006). The expression of many of these antigens in the thymus requires the transcription factor autoimmune regulator (Aire) (Anderson et al., 2005; Metzger and Anderson, 2011). Defects in *Aire* result in T cell-mediated autoimmunity. Different from the expression of MHC molecules, *Aire* genes were not isolated from the cartilaginous fish, suggesting that an *Aire*-dependent mechanism of T cell tolerance may emerge at the appearance of bony fish (Saltis et al., 2008).

6. The thymus in different species

Given the length of evolutionary time (in the hundreds of millions of years), it is not surprised to find that the thymus possessed by vertebrates differs markedly among species. The molecular and cellular underpinnings of these differences, although poorly understood, must have also changed substantially. The thymus differences include the number per animal, anatomical position, the structure of the thymic lobes, the developmental origin and developmental processes. For instance, many teleost fish species have only one thymus composed of two bilateral lobes, whereas anuran amphibians (e.g., frogs), and eutherian mammals, birds and cold-blooded vertebrates have multiple thymuses (Rodewald, 2008).

The position of the thymus anlage along the pharyngeal endoderm also varies. In some species of fish several or almost every pouch develops thymic tissue (Bowden et al., 2005). In sharks, thymus can be found in the first to sixth pouch. In bony fish, birds, and mammals, however, the thymus primordia is located more specifically to the third and/or fourth pharyngeal pouches (Rodewald, 2008). This difference of the embryological origin of the thymus may reflect a specialization of certain arches during evolution (Grevellec and Tucker, 2010).

The developmental process of the thymus also differs significantly. In mice and most of the land mammalian animals, the thymus undergoes a multi-process development: initial thymus fate determination and morphogenesis of the thymus-parathyroid common primordium, followed by pharyngeal detachment and separation of the thymus and parathyroid, eventually thymus migration to the final location. In birds and fish, thymus does not completely separate from the pharynx and does not migrate throughout their lives (Le Douarin et al., 1984; Lam et al., 2002; Grevellec and Tucker, 2010). Thus, thymus migration is probably associated with the move to land and the need to internalize this crucial organ. Even with the occurrence of migration, the anatomical position of thymus in the neck (cervical thymus) and/or in the chest (thoracic thymus) can be different. Diprotodont marsupial species, including kangaroo, wallabies, and possums, have both cervical and thoracic thymuses, with the former being the dominant (Symington, 1898; Wong et al., 2011). Other mammals such as sheep, cattle, goat, pigs, and horse also possess distinct cervical thymuses (Jordan, 1976; Park, 1917). In certain strains of mice, the incidence of small cervical thymi could range from ~30% to 90% (Terszowski et al., 2006; Dooley et al., 2006). In humans, however, the thymus in the neck is often associated with diseases and is considered to be an asymptomatic pathological condition caused by failure of the thymus to migrate to its final location (Rodewald, 2008; Sturm-O'Brien et al., 2009). The locations of cervical thymus can range from the proximity of the thyroid or parathyroid tissue to underneath strings of muscle in the neck (Many et al., 1993; Terszowski et al., 2006; Dooley et al., 2006). The implication of the cervical thymus in the evolution and T cell development is largely unknown. But the transcriptome analysis of the cervical and thoracic thymi from a single tammar wallaby showed similar genetic signature (Wong et al., 2011). The cervical thymi in mice have also been shown to support positive and negative selection and egress of thymocytes (Terszowski et al., 2006; Dooley et al., 2006).

7. Concluding remarks

Based on studies performed over just a few years, our understandings of the divergence of lymphocytes and the presence of a dedicated lymphoepithelial tissue (thymus) for T cell development, two basic features of the adaptive immune system, have quickly extended to the most ancient vertebrates, jawless fish. Whether these features exist in the common vertebrate ancestor or are ver-

tebrate-specific innovations remains to be seen. Most of the efforts to understand the emergence of the thymus have focused on a limited numbers of markers that determine thymus specification and function. Many questions remain in elucidating the molecular and cellular details underlying the co-evolution of the thymus and T lymphocytes and the evolution of the T-epithelial and epithelial-NCC cellular interactions.

In addition, both thymus organogenesis and T cell development occur in a stepwise manner in mice and most of the land mammals. The former goes through thymus fate determination, morphogenesis, pharyngeal detachment, and migration to the final location; while the latter goes through T lineage specification, somatic diversification and expression of functional antigen receptor, positive selection for the capability of T cells interacting with MHCs, and negative selection to remove overly self reactive receptors. How this multi-process development and the genetic network underpinning these processes are gradually evolved in vertebrates will require more intensive investigation by comparative immunologists. The efforts to understand these questions may also inspire the development of new strategies for medical interventions targeting faulty thymus functions.

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References

Anderson, M.S., Venanzi, E.S., Chen, Z., Berzins, S.P., Benoist, C., Mathis, D., 2005. The cellular mechanism of Aire control of T cell tolerance. Immunity 23, 227–239.
Auerbach, R., 1960. Morphogenetic interactions in the development of the mouse thymus gland. Dev. Biol. 2, 271–284.
Bajoghli, B., Aghaallaei, N., Hess, I., Rode, I., Netuschil, N., Tay, B.H., Venkatesh, B., Yu, J.K., Kaltenbach, S.L., Holland, N.D., Diekhoff, D., Happe, C., Schorpp, M., Boehm, T., 2009. Evolution of genetic networks underlying the emergence of thymopoiesis in vertebrates. Cell 138, 186–197.
Bajoghli, B., Guo, P., Aghaallaei, N., Hirano, M., Strohmeier, C., McCurley, N., Bockman, D.E., Schorpp, M., Cooper, M.D., Boehm, T., 2011. A thymus candidate in lampreys. Nature 470, 90–94.
Balciunaite, G., Keller, M.P., Balciunaite, E., Piali, L., Zuklys, S., Mathieu, Y.D., Gill, J., Boyd, R., Sussman, D.J., Hollander, G.A., 2002. Wnt glycoproteins regulate the expression of FOXN1, the gene defective in nude mice. Nat. Immunol. 3, 1102–1108.
Ballarin, L., Cima, F., 2005. Cytochemical properties of Botryllus schlosseri haemocytes: indications for morpho-functional characterisation. Eur. J. Histochem. 49, 255–264.
Blackburn, C.C., Augustine, C.L., Li, R., Harvey, R.P., Malin, M.A., Boyd, R.L., Miller, J.F., Morahan, G., 1996. The nu gene acts cell-autonomously and is required for differentiation of thymic epithelial progenitors. Proc. Natl. Acad. Sci. USA 93, 5742–5746.
Bleul, C.C., Boehm, T., 2005. BMP signaling is required for normal thymus development. J. Immunol. 175, 5213–5221.
Bockman, D.E., Kirby, M.L., 1984. Dependence of thymus development on derivatives of the neural crest. Science 223, 498–500.
Boehm, T., 2009. The adaptive phenotype of cortical thymic epithelial cells. Eur. J. Immunol. 39, 944–947.
Boehm, T., 2011. Design principles of adaptive immune systems. Nat. Rev. Immunol. 11, 307–317.
Bowden, T.J., Cook, P., Rombout, J.H., 2005. Development and function of the thymus in teleosts. Fish Shellfish Immunol. 19, 413–427.
Chen, L., Zhao, P., Wells, L., Amemiya, C.T., Condie, B.G., Manley, N.R., 2010. Mouse and zebrafish Hoxa3 orthologues have nonequivalent in vivo protein function. Proc. Natl. Acad. Sci. USA 107, 10555–10560.
Criscitiello, M.F., Ohta, Y., Saltis, M., McKinney, E.C., Flajnik, M.F., 2010. Evolutionarily conserved TCR binding sites, identification of T cells in primary lymphoid tissues, and surprising trans-rearrangements in nurse shark. J. Immunol. 184, 6950–6960.
Dooley, J., Erickson, M., Gillard, G.O., Farr, A.G., 2006. Cervical thymus in the mouse. J. Immunol. 176, 6484–6490.
Flajnik, M.F., Kasahara, M., 2010. Origin and evolution of the adaptive immune system: genetic events and selective pressures. Nat. Rev. Genet. 11, 47–59.
Fletcher, A.L., Calder, A., Hince, M.N., Boyd, R.L., Chidgey, A.P., 2011. The contribution of thymic stromal abnormalities to autoimmune disease. Crit. Rev. Immunol. 31, 171–187.
Fontaine-Perus, J.C., Calman, F.M., Kaplan, C., le Douarin, N.M., 1981. Seeding of the 10-day mouse embryo thymic rudiment by lymphocyte precursors in vitro. J. Immunol. 126, 2310–2316.
Foster, K.E., Gordon, J., Cardenas, K., Veiga-Fernandes, H., Makinen, T., Grigorieva, E., Wilkinson, D.G., Blackburn, C.C., Richie, E., Manley, N.R., Adams, R.H., Kioussis, D., Coles, M.C., 2010. EphB-ephrin-B2 interactions are required for thymus migration during organogenesis. Proc. Natl. Acad. Sci. USA 107, 13414–13419.
Frank, D.U., Fotheringham, L.K., Brewer, J.A., Muglia, L.J., Tristani-Firouzi, M., Capecchi, M.R., Moon, A.M., 2002. An Fgf8 mouse mutant phenocopies human 22q11 deletion syndrome. Development 129, 4591–4603.
Gordon, J., Bennett, A.R., Blackburn, C.C., Manley, N.R., 2001. Gcm2 and Foxn1 mark early parathyroid- and thymus-specific domains in the developing third pharyngeal pouch. Mech. Dev. 103, 141–143.
Gordon, J., Manley, N.R., 2011. Mechanisms of thymus organogenesis and morphogenesis. Development 138, 3865–3878.
Gordon, J., Patel, S.R., Mishina, Y., Manley, N.R., 2010. Evidence for an early role for BMP4 signaling in thymus and parathyroid morphogenesis. Dev. Biol. 339, 141–154.
Gordon, J., Wilson, V.A., Blair, N.F., Sheridan, J., Farley, A., Wilson, L., Manley, N.R., Blackburn, C.C., 2004. Functional evidence for a single endodermal origin for the thymic epithelium. Nat. Immunol. 5, 546–553.
Grevellec, A., Graham, A., Tucker, A.S., 2011. Shh signalling restricts the expression of Gcm2 and controls the position of the developing parathyroids. Dev. Biol. 353, 194–205.
Grevellec, A., Tucker, A.S., 2010. The pharyngeal pouches and clefts: development, evolution, structure and derivatives. Semin. Cell Dev. Biol. 21, 325–332.
Griffith, A.V., Cardenas, K., Carter, C., Gordon, J., Iberg, A., Engleka, K., Epstein, J.A., Manley, N.R., Richie, E.R., 2009. Increased thymus- and decreased parathyroid-fated organ domains in Splotch mutant embryos. Dev. Biol. 327, 216–227.
Guo, P., Hirano, M., Herrin, B.R., Li, J., Yu, C., Sadlonova, A., Cooper, M.D., 2009. Dual nature of the adaptive immune system in lampreys. Nature 459, 796–801.
Han, Y., Huang, G., Zhang, Q., Yuan, S., Liu, J., Zheng, T., Fan, L., Chen, S., Xu, A., 2010. The primitive immune system of amphioxus provides insights into the ancestral structure of the vertebrate immune system. Dev. Comp. Immunol. 34, 791–796.
Heimberg, A.M., Cowper-Sal-lari, R., Semon, M., Donoghue, P.C., Peterson, K.J., 2010. MicroRNAs reveal the interrelationships of hagfish, lampreys, and gnathostomes and the nature of the ancestral vertebrate. Proc. Natl. Acad. Sci. USA 107, 19379–19383.
Hetzer-Egger, C., Schorpp, M., Boehm, T., 2000. Evolutionary conservation of gene structures of the Pax1/9 gene family. Biochim. Biophys. Acta 1492, 517–521.
Holland, L.Z., Holland, N.D., 2001. Evolution of neural crest and placodes: amphioxus as a model for the ancestral vertebrate? J. Anat. 199, 85–98.
Huang, G., Xie, X., Han, Y., Fan, L., Chen, J., Mou, C., Guo, L., Liu, H., Zhang, Q., Chen, S., Dong, M., Liu, J., Xu, A., 2007. The identification of lymphocyte-like cells and lymphoid-related genes in amphioxus indicates the twilight for the emergence of adaptive immune system. PLoS One 2, e206.
Itoi, M., Tsukamoto, N., Amagai, T., 2007. Expression of Dll4 and CCL25 in Foxn1-negative epithelial cells in the post-natal thymus. Int. Immunol. 19, 127–132.
Jeffery, W.R., Strickler, A.G., Yamamoto, Y., 2004. Migratory neural crest-like cells form body pigmentation in a urochordate embryo. Nature 431, 696–699.
Jenkinson, W.E., Jenkinson, E.J., Anderson, G., 2003. Differential requirement for mesenchyme in the proliferation and maturation of thymic epithelial progenitors. J. Exp. Med. 198, 325–332.
Jeong, J., Mao, J., Tenzen, T., Kottmann, A.H., McMahon, A.P., 2004. Hedgehog signaling in the neural crest cells regulates the patterning and growth of facial primordia. Genes Dev. 18, 937–951.
Jordan, R.K., 1976. Development of sheep thymus in relation to in utero thymectomy experiments. Eur. J. Immunol. 6, 693–698.
Kameda, Y., Ito, M., Nishimaki, T., Gotoh, N., 2009. FRS2alpha is required for the separation, migration, and survival of pharyngeal-endoderm derived organs including thyroid, ultimobranchial body, parathyroid, and thymus. Dev. Dyn. 238, 503–513.
Kandil, E., Namikawa, C., Nonaka, M., Greenberg, A.S., Flajnik, M.F., Ishibashi, T., Kasahara, M., 1996. Isolation of low molecular mass polypeptide complementary DNA clones from primitive vertebrates. Implications for the origin of MHC class I-restricted antigen presentation. J. Immunol. 156, 4245–4253.
Kishishita, N., Matsuno, T., Takahashi, Y., Takaba, H., Nishizumi, H., Nagawa, F., 2010. Regulation of antigen-receptor gene assembly in hagfish. EMBO Rep. 11, 126–132.
Klein, L., Hinterberger, M., Wirnsberger, G., Kyewski, B., 2009. Antigen presentation in the thymus for positive selection and central tolerance induction. Nat. Rev. Immunol. 9, 833–844.
Kozmik, Z., Holland, L.Z., Schubert, M., Lacalli, T.C., Kreslova, J., Vlcek, C., Holland, N.D., 2001. Characterization of Amphioxus AmphiVent, an evolutionarily conserved marker for chordate ventral mesoderm. Genesis 29, 172–179.
Kozmik, Z., Holland, N.D., Kreslova, J., Oliveri, D., Schubert, M., Jonasova, K., Holland, L.Z., Pestarino, M., Benes, V., Candiani, S., 2007. Pax-Six-Eya-Dach network during amphioxus development: conservation in vitro but context specificity in vivo. Dev. Biol. 306, 143–159.

Kyewski, B., Klein, L., 2006. A central role for central tolerance. Annu. Rev. Immunol. 24, 571–606.
Lam, S.H., Chua, H.L., Gong, Z., Wen, Z., Lam, T.J., Sin, Y.M., 2002. Morphologic transformation of the thymus in developing zebrafish. Dev. Dyn. 225, 87–94.
le Douarin, N.M., Dieterlen-Lievre, F., Oliver, P.D., 1984. Ontogeny of primary lymphoid organs and lymphoid stem cells. Am. J. Anat. 170, 261–299.
le Douarin, N.M., Jotereau, F.V., 1975. Tracing of cells of the avian thymus through embryonic life in interspecific chimeras. J. Exp. Med. 142, 17–40.
le Lievre, C.S., le Douarin, N.M., 1975. Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. J. Embryol. Exp. Morphol. 34, 125–154.
Leclerc, M., Brillouet, C., Luquet, G., 1980. The starfish axial organ: an ancestral lymphoid organ. Dev. Comp. Immunol. 4, 605–615.
Leclerc, M., Brillouet, C., Luquet, G., Agogue, P., Binaghi, R.A., 1981. Properties of cell subpopulations of starfish axial organ: in vitro effect of pokeweed mitogen and evidence of lymphokine-like substances. Scand. J. Immunol. 14, 281–284.
Lin, Y., Cai, Z., Huang, S., Yang, L., Wang, C., Liu, Z., Cao, J., An, Y., Zhang, H., 2009. Ptc, Smo, Sufu, and the Hedgehog signaling pathway in amphioxus. Evol. Dev. 11, 710–718.
Litman, G.W., Rast, J.P., Fugmann, S.D., 2010. The origins of vertebrate adaptive immunity. Nat. Rev. Immunol. 10, 543–553.
Liu, C., Saito, F., Liu, Z., Lei, Y., Uehara, S., Love, P., Lipp, M., Kondo, S., Manley, N., Takahama, Y., 2006. Coordination between CCR7- and CCR9-mediated chemokine signals in prevascular fetal thymus colonization. Blood 108, 2531–2539.
Mahadevan, N.R., Horton, A.C., Gibson-Brown, J.J., 2004. Developmental expression of the amphioxus Tbx1/10 gene illuminates the evolution of vertebrate branchial arches and sclerotome. Dev. Genes Evol. 214, 559–566.
Manley, N.R., Condie, B.G., 2010. Transcriptional regulation of thymus organogenesis and thymic epithelial cell differentiation. Prog. Mol. Biol. Trans. Sci. 92, 103–120.
Manley, N.R., Richie, E.R., Blackburn, C.C., Condie, B.G., Sage, J., 2011. Structure and function of the thymic microenvironment. Front. Biosci. 17, 2461–2477.
Many, M.C., Drexhage, H.A., Denef, J.F., 1993. High frequency of thymic ectopy in thyroids from autoimmune prone nonobese diabetic female mice. Lab. Invest. 69, 364–367.
Mayer, W.E., Uinuk-Ool, T., Tichy, H., Gartland, L.A., Klein, J., Cooper, M.D., 2002. Isolation and characterization of lymphocyte-like cells from a lamprey. Proc. Natl. Acad. Sci. USA 99, 14350–14355.
Metzger, T.C., Anderson, M.S., 2011. Control of central and peripheral tolerance by Aire. Immunol. Rev. 241, 89–103.
Moore-Scott, B.A., Manley, N.R., 2005. Differential expression of Sonic hedgehog along the anterior–posterior axis regulates patterning of pharyngeal pouch endoderm and pharyngeal endoderm-derived organs. Dev. Biol. 278, 323–335.
Murata, S., Sasaki, K., Kishimoto, T., Niwa, S., Hayashi, H., Takahama, Y., Tanaka, K., 2007. Regulation of CD8+ T cell development by thymus-specific proteasomes. Science 316, 1349–1353.
Nitta, T., Murata, S., Sasaki, K., Fujii, H., Ripen, A.M., Ishimaru, N., Koyasu, S., Tanaka, K., Takahama, Y., 2010. Thymoproteasome shapes immunocompetent repertoire of CD8+ T cells. Immunity 32, 29–40.
Owen, J.J., Ritter, M.A., 1969. Tissue interaction in the development of thymus lymphocytes. J. Exp. Med. 129, 431–442.
Pancer, Z., Mayer, W.E., Klein, J., Cooper, M.D., 2004. Prototypic T cell receptor and CD4-like coreceptor are expressed by lymphocytes in the agnathan sea lamprey. Proc. Natl. Acad. Sci. USA 101, 13273–13278.
Park, E.A., 1917. Extirpation of the thymus in the guinea pig. J. Exp. Med. 25, 129–152.
Patel, S.R., Gordon, J., Mahbub, F., Blackburn, C.C., Manley, N.R., 2006. Bmp4 and Noggin expression during early thymus and parathyroid organogenesis. Gene. Expr. Patterns 6, 794–799.
Peters, H., Neubuser, A., Kratochwil, K., Balling, R., 1998. Pax9-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. Genes Dev. 12, 2735–2747.
Petersen, C.P., Reddien, P.W., 2009. Wnt signaling and the polarity of the primary body axis. Cell 139, 1056–1068.
Rast, J.P., Anderson, M.K., Strong, S.J., Luer, C., Litman, R.T., Litman, G.W., 1997. Alpha, beta, gamma, and delta T cell antigen receptor genes arose early in vertebrate phylogeny. Immunity 6, 1–11.
Retaux, S., Kano, S., 2010. Midline signaling and evolution of the forebrain in chordates: a focus on the lamprey Hedgehog case. Integr. Comp. Biol. 50, 98–109.
Rodewald, H.R., 2008. Thymus organogenesis. Annu. Rev. Immunol. 26, 355–388.
Rogozin, I.B., Iyer, L.M., Liang, L., Glazko, G.V., Liston, V.G., Pavlov, Y.I., Aravind, L., Pancer, Z., 2007. Evolution and diversification of lamprey antigen receptors: evidence for involvement of an AID-APOBEC family cytosine deaminase. Nat. Immunol. 8, 647–656.
Saltis, M., Criscitiello, M.F., Ohta, Y., Keefe, M., Trede, N.S., Goitsuka, R., Flajnik, M.F., 2008. Evolutionarily conserved and divergent regions of the autoimmune regulator (Aire) gene: a comparative analysis. Immunogenetics 60, 105–114.
Sauka-Spengler, T., Bronner-Fraser, M., 2008. Insights from a sea lamprey into the evolution of neural crest gene regulatory network. Biol. Bull. 214, 303–314.
Sauka-Spengler, T., le Mentec, C., Lepage, M., Mazan, S., 2002. Embryonic expression of Tbx1, a DiGeorge syndrome candidate gene, in the lamprey *Lampetra fluviatilis*. Gene. Expr. Patterns 2, 99–103.
Shimeld, S.M., 1999. The evolution of the hedgehog gene family in chordates: insights from amphioxus hedgehog. Dev. Genes Evol. 209, 40–47.
Soza-Ried, C., Bleul, C.C., Schorpp, M., Boehm, T., 2008. Maintenance of thymic epithelial phenotype requires extrinsic signals in mouse and zebrafish. J. Immunol. 181, 5272–5277.
Stritesky, G.L., Jameson, S.C., Hogquist, K.A., 2011. Selection of self-reactive T cells in the thymus. Annu. Rev. Immunol. [Epub ahead of print].
Sturm-O'Brien, A.K., Salazar, J.D., Byrd, R.H., Popek, E.J., Giannoni, C.M., Friedman, E.M., Sulek, M., Larrier, D.R., 2009. Cervical thymic anomalies – the Texas children's hospital experience. Laryngoscope 119, 1988–1993.
Symington, J., 1898. The thymus gland in the Marsupialia. J. Anat. Physiol. 32, 278–291.
Terszowski, G., Muller, S.M., Bleul, C.C., Blum, C., Schirmbeck, R., Reimann, J., Pasquier, L.D., Amagai, T., Boehm, T., Rodewald, H.R., 2006. Evidence for a functional second thymus in mice. Science 312, 284–287.
Wong, E.S., Papenfuss, A.T., Heger, A., Hsu, A.L., Ponting, C.P., Miller, R.D., Fenelon, J.C., Renfree, M.B., Gibbs, R.A., Belov, K., 2011. Transcriptomic analysis supports similar functional roles for the two thymuses of the tammar wallaby. BMC Genomics 12, 420.
Wyffels, J.T., Walsh, C.J., Luer, C.A., Bodine, A.B., 2005. In vivo exposure of clearnose skates, Raja eglanteria, to ionizing X-radiation: acute effects on the thymus. Dev. Comp. Immunol. 29, 315–331.
Yu, C., Dong, M., Wu, X., Li, S., Huang, S., Su, J., Wei, J., Shen, Y., Mou, C., Xie, X., Lin, J., Yuan, S., Yu, X., Yu, Y., Du, J., Zhang, S., Peng, X., Xiang, M., Xu, A., 2005. Genes "waiting" for recruitment by the adaptive immune system: the insights from amphioxus. J. Immunol. 174, 3493–3500.
Yu, J.K., Satou, Y., Holland, N.D., Shin, I.T., Kohara, Y., Satoh, N., Bronner-Fraser, M., Holland, L.Z., 2007. Axial patterning in cephalochordates and the evolution of the organizer. Nature 445, 613–617.
Zamisch, M., Moore-Scott, B., Su, D.M., Lucas, P.J., Manley, N., Richie, E.R., 2005. Ontogeny and regulation of IL-7-expressing thymic epithelial cells. J. Immunol. 174, 60–67.
Zapata, A., Amemiya, C.T., 2000. Phylogeny of lower vertebrates and their immunological structures. Curr. Top. Microbiol. Immunol. 248, 67–107.