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Characterization of lamprey IL-17 family members and their receptors

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B.S., Zhejiang University, 2009

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Abstract

Characterization of lamprey IL-17 family members and their receptors

By Qifeng Han

Interleukin-17 is an ancient cytokine implicated in a variety of immune defense mechanisms. We have indentified five members of the sea lamprey *IL-17* family (*IL-17-1* to *IL-17-5*) and six *IL-17 receptor* genes (*IL-17R1* to *IL-17R6*), determined their relationship with mammalian orthologues, and examined their expression patterns and potential interactions in order to explore their potential roles in innate and adaptive immunity. The most highly expressed IL-17 family member is *IL-17-4* (mammalian IL-17D like), which was found to be preferentially expressed by epithelial cells of skin, intestine and gills and by the two types of lamprey T-like cells. IL-17-4 binds to recombinant IL-17R1 and to the surface of *IL-17R1*-expressing B-like cells and monocytes. Treatment of lamprey blood cells with recombinant IL-17-4 protein enhanced the transcription of genes expressed by B-like cells. These findings suggest an IL-17 mediated mechanism for coordinating the interaction of T-like cells with cells of the adaptive and innate immune systems in jawless vertebrates.

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Chapter 1

General Introduction

1.1 IL-17s and their receptors in mammals

IL-17 and IL-17 receptor structure

The interleukin-17s (IL-17s) cytokines are dimeric proteins: six homodimeric forms are designated IL-17A through IL-17F and IL-17A/F heterodimer is also known (Figure 1-1). The IL-17 receptor (IL-17R) family comprises five members (IL-17RA to IL-17RE) which are all membrane-bound proteins containing a single transmembrane domain (Figure 1-1). The crystal structures of free IL-17F, IL-17A dimer, IL-17A in complex with an antibody, an IL-17F/IL-17RA complex and an IL-17A/IL-17RA complex are available (Ely et al., 2009; Gerhardt et al., 2009; Hymowitz et al., 2001; Liu et al., 2013). The crystal structures of IL-17 dimer (IL-17A or IL-17F) show that the conserved cysteins in all members of IL-17 family form a classical cystine-knot fold found in the transforming growth factor β (TGF-β), bone morphogenetic protein, and nerve growth factor superfamilies; however, they lack another two cysteins required to form the canonical cystine-knot structure. The large cavity on the surface of IL-17F resembles that of nerve growth factor which binds its high affinity receptor, tropomyosin receptor kinase A (TrkA) (Hymowitz et al., 2001). The crystal structure of the IL-17F/IL-17RA complex reveals the interaction between the two type III fibronectin (FnIII) domains of IL-17RA and the IL-17F homodimer. The crystal structure of the IL-17A/IL-17RA complex shows three binding sites. Two sites are formed by the interaction between the FnIII of IL-17RA and either the N-terminal region or central β-strands of IL-17A, respectively. The third site is formed by the interaction between the IL-17RA FnIII and the C-terminal region of IL-17A. After IL-17A is bound by one IL-17RA molecule, the binding affinity for a second identical receptor is low whereas the affinity

for IL-17RC is higher. One study mentions that steric interference between two identical receptors may reduce the affinity for the second identical receptor (Ely et al., 2009). Another report finds that binding to IL-17RA at one side of the IL-17A molecule induces a conformational allosteric change in the second, symmetry-related receptor site of IL-17A (Liu et al., 2013). This change favors binding of a different receptor polypeptide to complete the cytokine-receptor complex.

IL-17Rs contain extracellular FnIII domains and a conserved cytoplasmic domain termed the SEFIR [SEF (similar expression to fibroblast growth factor genes) and IL-17R] domain (Novatchkova et al., 2003) (Figure 1-1). SEFIR domains share limited homology with the Toll/IL-1 receptor (TIR) domains of Toll-like receptors (TLRs) and IL-1R (Novatchkova et al., 2003). Act1 [NF-κB activator 1; also known as connection to IκB kinase and stress-activated protein kinase (CIKS)] also contains the SEFIR domain and is an essential component of IL-17 signaling (Chang et al., 2006; Claudio et al., 2009; Leonardi et al., 2000; Qian et al., 2007; Swaidani et al., 2009). The crystal structures of the SEFIR domain of IL-17RA and IL-17RB have recently been determined (Zhang et al., 2014, 2013). The crystal structure of IL-17RB SEFIR domain reveals that it adopts an overall architecture similar to TIR domains, but contains distinct structural elements. IL-17RA is unique among all IL-17Rs and contains the longest intracellular domain. Compared to the SEFIR domain of IL-17RB, the structure of IL-17RA SEFIR contains not only the complete SEFIR domain but also a short helical C-terminal extension, which stabilizes the folding. Therefore the IL-17RA SEFIR domain is believed to be structurally and functionally distinct from other SEFIR domains lacking C-terminal extensions.

Sources of IL-17

IL-17-producing $CD4^+$ T cells (Th17) are a novel T cell subset that is distinct from Th1 and Th2 cells (Figure 1-1). TGF-β, IL-6, IL-23, IL-1β, IL-21, prostaglandin E_2 (PGE_2), regulate development of this cell subset (Korn et al., 2009; Weaver et al., 2007). Transcription factors retinoic acid receptor-related orphan receptor-γt (RORγt), RORα, signal transducer and activator of transcription 3 (STAT3), interferon regulatory factor 4 (IRF4), aryl hydrocarbon receptor (AHR), basic leucine zipper transcription factor (BATF), Runt-related transcription factor 1 (RUNX1) and IκBξ are involved in the differentiation program of Th17 cells (Brüstle et al., 2007; Ivanov et al., 2006; Mudter et al., 2011; Okamoto et al., 2010; Schraml et al., 2009; Yang et al., 2008, 2007; Zhang et al., 2008). IL-17A-producing Th17 cells also produce IL-17F, IL-22, IFN-γ, tumor necrosis factor (TNF)-α, IL-6 and CCL20, which have both overlapping and distinct roles during inflammation and host defense (Gaffen, 2011; Miossec et al., 2009; Weaver et al., 2007). A subset of $CD8^+$ T cells called Tc17 cells is another adaptive source of IL-17A and IL-17F (Figure 1-1). Naive $CD8^+$ cells can be differentiated into a Tc1 subset, which secretes IFN-γ and IL-2 predominately, a Tc2 subset, which secretes IL-4, IL-5, IL-10, and a recently characterized IL-17-producing Tc17 subset (Liu et al., 2007; Mosmann et al., 1997; Sad et al., 1995). These Tc17 cells have decreased granzyme B expression and upregulated RORγt expression. Tc17 cells may play a role in host defense, inflammation and autoimmunity (Ciric et al., 2009; Hijnen et al., 2013; Huber et al., 2013; Nigam et al., 2011; Yen et al., 2009). More recently, B cells were shown to be a major source of IL-17A and IL-17F in response to *Trypanosoma cruzi* infection. The parasite-derived trans-

sialidase drives the formation of IL-17A- or IL-17F-producing B cells independent of the transcription factors RORγt and AHR (Bermejo et al., 2013).

Several innate immune cell populations, such as γδ T cells, iNKT cells, NK cells, LTi-like cells and neutrophils, have also been showed to produce IL-17A and IL-17F (Figure 1-1). IL-17-producing innate immune cells rapidly release IL-17A in response to pathogens or tissue injury (Cua and Tato, 2010). γδ T cells express pattern recognition receptors (PRRs) such as dectin-1 and TLR2, which allow for rapid IL-17A production in response to bacterial encounter (Martin et al., 2009). Splenic LTi-like cells produce IL-17A rapidly after stimulation with either exogenous IL-23 or the TLR2-agonist zymosan. A new IL-17- producing iNKT cell subset produces IL-17A after treatment with the glycolipid α-galactoceramide (α-GalCer) (Michel et al., 2007). Peritoneal NK cells isolated from *Toxoplasma gondii*-infected mice produce IL-17A in an IL-6- and IL-23-dependent manner (Passos et al., 2010).

Non-lymphoid cells may also produce and secrete IL-17A or IL-17F. Intestinal Paneth cells, which are highly specialized epithelial cells, can express IL-17A that may play a role in mucosal homeostasis and immunity (Cua and Tato, 2010) (Figure 1-1). Paneth cells activated in a model of TNF-induced shock have been shown to rapidly secrete IL-17A (Takahashi et al., 2008). In a model of acute kidney ischaemia-reperfusion injury, early production of IL-23 activates downstream IL-17-mediated pathways, including chemokine production and recruitment of neutrophils; most of the initial IL-17A is produced by bone marrow-derived neutrophils and not by lymphocytes (Li et al., 2010).

The sources of IL-25 include both immune cells [dendritic cells (DCs), alveolar macrophages, Th2 cells, $CD8^+$ T cells, mast cells, eosinophils and basophils] and non-immune cells (Paneth cells, endothelial and epithelial cells) (Gaffen, 2009; Gu et al., 2013; Iwakura et al., 2011) (Figure 1-1).

IL-17C is found to be expressed by epithelial cells, $CD4^+$ T cell, dendritic cells (DCs) and macrophages near inflammatory sites (Li et al., 2000; Ramirez-Carrozzi et al., 2011; Song et al., 2011) (Figure 1-1). The expression of IL-17B has been found in different tissues, such as pancreas, small intestine, stomach, embryonic limb buds and cell populations including chondrocytes and neurons (Li et al., 2000; You et al., 2005). Another study demonstrates that IL-17B protein is primarily localized to the neuronal cell bodies and axons (Moore et al., 2002). IL-17D is expressed at relatively high levels in skeletal muscle, brain, adipose, heart, lung, and pancreas tissues, and at lower levels by resting $CD4^+$ T cells and B cells in humans (Starnes et al., 2002).

Ligand-receptor interaction and IL-17 signaling

IL-17RA is shown to be a shared receptor for other IL-17 cytokines, with different receptor chains joining it in complex with the cytokines (Gaffen, 2009). 17RA has high affinity for IL-17A, intermediate affinity for the IL-17A/F heterodimer and around 100-fold lower affinity for IL-17F (Ely et al., 2009; Hymowitz et al., 2001; Wright et al., 2008, 2007). It has much weaker affinities for IL-17B, C, D and E (Gaffen, 2009). However, IL-17RA alone is insufficient for IL-17A, IL-17F and IL-17A/F mediated signaling and a heterodimeric receptor complex of IL-17RA and IL-17RC is

required for signaling (Ho et al., 2010; Hu et al., 2010; Kuestner et al., 2007; Wright et al., 2008) (Figure 1-1).

The SEFIR domain has been identified in all IL-17R family members and Act1 (Gaffen, 2009). Act1 is recruited to IL-17R through its SEFIR-SEFIR interaction in an IL-17 stimulation dependent manner (Chang et al., 2006; Qian et al., 2007). As an adaptor protein and U-box like E3 ligase, Act1 recruits and mediates Lys63-linked ubiquitination of TNF receptor-associated factor 6 (TRAF6) (Liu et al., 2009). The poly-ubiquitinated TRAF6 activates downstream TGFβ-activated kinase 1 (TAK1) for IL-17-induced NF-κB activation. A more refined domain mapping study showed that a CC' loop in the SEFIR domains of Act1 and IL-17R is responsible for the Act1-IL-17R interaction. In addition to the SEFIR domains, the "TIR-like loop"(TILL) motif and C-terminal region of IL-17RA are also required for IL-17 signaling (Gaffen, 2009; Ho et al., 2010; Onishi et al., 2010).

In addition to the TRAF6-dependent NF-κB, mitogen-activated protein kinases (MAPKs) and C/EBPs cascades (Gaffen, 2009), IL-17 can also act through inhibitor of nuclear factor κ-B kinase subunit i (IKKi)-TRAF2-TRAF5 dependent cascade to stabilize the proinflammatory mRNAs, such as CXCL1 induced by TNF-α (Hartupee et al., 2007; Sun et al., 2011). Upon IL-17A stimulation, IKKi is recruited to the IL-17R-Act1 complex and phosphorylates Act1 at Ser311. Phosphorylated Act1 recruits TRAF2 and TRAF5 without TRAF6 to form an Act1/TRAF2/TRAF5/alternative splicing factor (ASF or SF2) complex. This complex prevents ASF from binding to the 3′ UTR of CXCL1 mRNA for cleavage and therefore stabilizes CXCL1 mRNA (Bulek et al., 2011; Sun et al., 2011).

The signaling of IL-17E (also known as IL-25) is similar to that of IL-17A (Claudio et al., 2009; Maezawa et al., 2006; Swaidani et al., 2011, 2009). After IL-17E binds to a receptor complex of IL-17RA and IL-17RB, Act1 is recruited to the receptor complex through the SEFIR-SEFIR interaction (Figure 1-1). IL-17E stimulation leads to TRAF6-independent activation of MAPKs pathway. The expression of *IL-17RB* is detected in human kidney, pancreas, liver, brain, and intestines. IL-17RB transcripts are upregulated during intestinal inflammation in rats (Lee et al., 2001; Shi et al., 2000).

IL-17C binds to IL-17RA and IL-17RE receptor complex for signaling (Chang et al., 2011; Ramirez-Carrozzi et al., 2011; Song et al., 2011) (Figure 1-1). Act1 functions as an adaptor and IκBζ is activated in Th17 cells to potentiate the Th17 cell response (Chang et al., 2011). NF-κB and MAPKs pathways are activated by IL-17C in colon epithelial cells (Song et al., 2011). IL-17B binds specifically to IL-17RB, as determined by surface plasmon resonance analysis, flow cytometry, and co-immunoprecipitation experiments (Shi et al., 2000) (Figure 1-1). The receptor for IL-17D has yet to be identified.

IL-17RD (also known as Sef, similar expression to fibroblast growth factor) is first identified from a zebrafish embryo library. Its expression is positively regulated by fibroblast growth factor (FGF). Ectopic expression of *Sef* in zebrafish or *Xenopus laevis* embryos suppresses FGF signaling, which could be mediated through interaction with FGF receptors, FGFR1 and FGFR2 (Tsang et al., 2002). IL-17RD is highly expressed in human umbilical vein endothelial cells, highly vascularized tissues, and ductal epithelial cells of human salivary glands, seminal vesicles, and the collecting tubules of the kidney. Sef inhibits FGF-mediated ERK activation (Yang et al., 2003).

Another report shows that Sef interacts with TAK1 and activates c-Jun N-terminal kinases (JNK) (Yang et al., 2004). Mouse Sef has been shown to inhibit FGF-induced Akt activation, which is an important signaling molecule of phosphatidylinositol 3-kinase (PI3K) pathway (Kovalenko et al., 2003). Human Sef inhibits FGF2-induced PC-12 cell differentiation through the prevention of Ras-MAPK signaling (Xiong et al., 2003). An isoform of human Sef, hSef-b, generated by alternative splicing mechanism also inhibits FGF-induced cell proliferation and prevents the activation of MAPK (Preger et al., 2004). IL-17RD associates with IL-17RA and TRAF6 (Rong et al., 2009). IL-17RD without the intracellular domain dominant-negatively suppresses IL-17 signaling. IL-17RD is shown to differentially regulate the NF-κB and p38 MAPK signaling pathways of IL-17A signaling. IL-17RD deficient mice have reduced activation of p38 MAPK and neutrophil chemokine MIP-2 but enhanced NF-κB activation, IL-6 and keratinocyte chemoattractant expression induced by IL-17A. The mechanism could be that IL-17RD interacts with IL-17RA and Act1, and disrupts the interaction of Act1 and TRAF6 (Mellett et al., 2012). The ligand for IL-17RD is unknown.

Regulation of IL-17A signaling

TRAF4 is a negative regulator of IL-17 signaling. Enhanced IL-17 signaling pathways are observed in primary cells from TRAF4-deficient mice. TRAF4 competes with TRAF6 for the same TRAF binding sites on Act1, and therefore disrupts IL-17 signaling (Zepp et al., 2012). TRAF3 is another negative regulator of IL-17 signaling. TRAF3 suppresses IL-17 mediated NF-κB and MAPK activation and proinflammatory cytokine production. The binding of TRAF3 to IL-17R interferes with the formation of

signaling complex IL-17R-Act1-TRAF6 to suppress the downstream signaling (Zhu et al., 2010). The dual phosphorylation of C/EBPβ in its regulatory 2 domain at Thr188 and Thr179 by ERK and glycogen synthase kinase 3β (GSK3β) is another mechanism for regulating IL-17 signaling. The dual-phosphorylation of C/EBPβ triggered by IL-17 inhibits the expression of proinflammatory genes (Shen et al., 2009). USP25 is a deubiquitinating enzyme (DUB) that is found to negatively regulate IL-17 signaling. Overexpression of USP25 inhibit IL-17-mediated signaling, whereas in USP25 deficient cells, IL-17A treatment leads to increased phosphorylation of the IκBα and JNK, prolonged half-life for CXCL1 transcripts, and enhanced chemokine and cytokine production. USP25 deficient mice undergo IL-17-mediated inflammation in their lungs and autoimmune damages of central nervous system. The implied mechanism is that after IL-17 stimulation, USP25 associates with the TRAF5 and TRAF6, induces removal of ubiquitination in TRAF5 and TRAF6 and thus suppresses both TRAF6-dependent NF-κB activation and TRAF5-dependent mRNA stabilization in IL-17R signaling (Zhong et al., 2012). Another deubiquitinase, A20, is found to interact with TRAF6 in an IL-17-dependent manner and inhibits the activation of NF-κB and MAPKs (Garg et al., 2013). Protein degradation pathway also negatively regulates IL-17 signaling. IL-17 stimulation results in the recruitment of Act1 to SCF (Skp1-cullin-1-F-box)-type E3 ubiquitin ligase complexes containing β-transducin repeat-containing protein 1 (β-TrCP1) or β-TrCP2, which mediates the Lys 48-linked polyubiquitination and degradation of Act1 (Shi et al., 2011). MicroRNA miR-23b also acts as a suppressor of IL-17 signaling. IL-17 downregulates miR-23b expression in human fibroblast-like synoviocytes, mouse primary kidney cells and astrocytes, whereas miR-23b targets TGF-β-activated kinase

1/MAP3K7 binding protein 2 (TAB2), TAB3 and IKKα and suppresses IL-17-mediated NF-κB activation and inflammatory cytokine expression (Zhu et al., 2012). Transcription factor IκBζ is a positive regulator of IL-17 signaling (Karlsen et al., 2010). IκBζ is required for IL-17A induced expression of human β-defensin 2 and neutrophil gelatinase-associated lipocalin expression. C/EBPδ is demonstrated to be important for the functional cooperation between IL-17 and TNF-α. IL-17RD, has been shown to regulate IL-17A-induced signaling, as mentioned above (Mellett et al., 2012; Rong et al., 2009).

Biological functions of IL-17

IL-17A is the founding member of IL-17 family cytokines and was first identified from a murine cytotoxic T lymphocyte hybridoma cDNA library and named cytotoxic T lymphocyte-associated-8 (CTLA-8). A viral homologue of IL-17A was subsequently found in the open reading frame 13 of *Herpesvirus saimiri*, but the function of this homologue is unknown. IL-17F shares about 50% sequence similarity with IL-17A, which is the highest among IL-17 family members, and *IL-17F* gene is tightly linked with *IL-17A* in humans and mice, suggesting that they might arise from a gene duplication event and have similar functions (Rouvier et al., 1993; Yao et al., 1995). IL-17A was first found to stimulate IL-6 secretion in fibroblasts (Yao et al., 1995). IL-17A also induces the production of TNF-α, IL-1β, IL-10 and IL-12 in human macrophages (Jovanovic et al., 1998) and multiple chemokines, such as CXCL8 (IL-8), CXCL1, CXCL2, CXCL5, CCL20, CCL2 and CCL7, in different human cell types (Jones and Chan, 2002; Laan et al., 2001, 1999; Shen and Gaffen, 2008; Weaver et al., 2007) (Figure 1-1). In mouse and rat primary astrocytes, IL-17A treatment enhances the expression of inducible nitric

oxide synthase (iNOS) transcripts and the production of nitric oxide (NO) triggered by IFN-γ. IL-17A also synergizes with IL-1β and TNF-α for NO production in astrocytes (LeGrand et al., 2001; Trajkovic et al., 2001). In human articular chondrocytes, IL-17A stimulation induces the transcription and translation of iNOS, cyclooxygenase-2 (COX-2) and NO production. IL-1β, IL-6, and stromelysin, which are involved in inflammation and cartilage degradation, are also upregulated. MAPKs pathway and NF-κB pathway may be involved in this upregulation (Shalom-Barak et al., 1998). IL-17A has been shown to synergize with IFN-γ to enhance intercellular adhesion molecule 1 (ICAM-1) expression in bronchial epithelial cells and human keratinocytes (Kawaguchi et al., 2001, 1999; Teunissen et al., 1998). IL-17A stimulates the secretion of PGE_2 by human macrophages (Jovanovic et al., 1998). PGE_2 production is enhanced by adding IL-17 to TNF-α or IL-1β treated explants of the human osteoarthritic knee menisci (LeGrand et al., 2001). IL-17A upregulates the transcription and translation of granulocyte colony-stimulating factor (G-CSF) and synergistically enhances TNF-α-induced production of G-CSF in human bronchial epithelial cells and murine fibroblast cells (Cai et al., 1998; Jones and Chan, 2002). IL-17A also stimulates the release of granulocyte-macrophage colony-stimulating factor (GM-CSF) in human bronchial epithelial cells and venous endothelial cells (Laan et al., 2003). Through G-CSF, IL-17A can induce an increase of mature granulocytes in the spleen. G-CSF neutralization partially reverses IL-17A-induced splenic granulopoiesis, suggesting a G-CSF-independent effect of IL-17A on splenic granulopoiesis (Schwarzenberger et al., 2000). In primary human lung microvascular endothelial cells, IL-17F enhances the G-CSF production induced by IL-1β, but has an inhibitory effect on G-CSF production induced by TNF-α (Numasaki et al.,

2004). IL-17A significantly increases the transcription and translation of MMP1 in human periodontal and cardiac fibroblasts (Cortez et al., 2007; Shibata et al., 2014). IL-17A upregulates proMMP1 and MMP3 in gingival fibroblasts (Beklen et al., 2007). Treatment of mouse embryonic fibroblasts with IL-17A induces *MMP3* and *MMP13* expression (Park et al., 2005). Local stimulation with IL-17A increases the concentration of biologically active MMP9 as well as its precursor molecule in mouse airways (Prause, 2004). The secretion of MMP9 by osteoblasts is increased after IL-17A treatment (Zhang et al., 2011). IL-17A also stimulates membrane expression of receptor activator of NF-κB ligand (RANKL) which is a key regulator of osteoclastogenesis and bone resorption in osteoblasts (Kotake et al., 1999). IL-17A overexpression in the synovium of mice induces the expression of RANKL (Lubberts et al., 2003). Furthermore, IL-17A induces the expression of antimicrobial peptides including β-defensins and S100 proteins in airway epithelium and primary keratinocytes (Kao et al., 2004; Liang et al., 2006).

IL-17A and IL-17F have been shown to play important roles in host immune responses to extracellular bacteria, intracellular bacteria, fungi, virus and intracellular parasites (Figure 1-1). Several studies demonstrate the important function of IL-17A and IL-17F in response to extracellular bacteria. IL-17A is involved in the clearance of *Streptococcus pneumoniae* and neutrophil infiltration. IL-17RA-knockout mice are not protected against colonization in response to intranasal immunization with the killed *S. pneumoniae*. IL-17A expression in peripheral blood predicts protection in the immunized mice suggesting that IL-17A facilitates immunity to *S. pneumoniae* (Lu et al., 2008). In response to *Staphylococcus aureus* cutaneous infection, mice deficient in γδ T cells and IL-17RA have larger skin lesions and impaired neutrophil recruitment which is

dependent on IL-17A production (Cho et al., 2010). Mice deficient in IL-17RA are susceptible to intranasal *Klebsiella pneumoniae* infection and exhibit defective neutrophil recruitment and greater dissemination of the bacteria. This could be due to the reduction of G-CSF and MIP-2 production in IL-17RA deficient mice (Peng Ye et al., 2001). IL-17A and IL-17F produced in T cells, innate immune cells, and epithelial cells activate innate immune responses following mucoepithelial infection by *S. aureus* and *Citrobacter rodentium* (Ishigame et al., 2009).

IL-17A also helps in protection against intracellular bacteria, such as *Salmonella typhimurium, Listeria monocytogenes* and *Mycobacterium tuberculosis*. Impaired Th17 responses to *S. typhimurium* infection caused by depletion of Th17 cells after simian immunodeficiency virus (SIV) infection lead to increased bacterial dissemination. IL-17RA-deficient mice also have increased dissemination of *S. typhimurium* from the gut, suggesting an essential role of IL-17A in the mucosal immune response (Raffatellu et al., 2008). IL-17A is mainly produced by γδ T cells at the early stage of *L. monocytogenes* infection. IL-17A-deficient mice have a compromised protective response by γδ T cells to *L. monocytogenes* in liver infection, suggesting that IL-17A produced is important in the innate immune response against these bacteria. IL-17A is also important for adaptive immunity to *L. monocytogenes*. IL-17A produced by γδ T cells enhances cross-presentation of dendritic cells and promotes antigen-specific $CD8^+$ T cell proliferation and responses against *L. monocytogenes* infection (Hamada et al., 2008; Xu et al., 2010). IL-17A is shown to be an important cytokine in the neutrophil recruitment, optimal Th1 response and protective immunity against pulmonary infection by *Mycobacterium bovis* bacille Calmette-Guérin (BCG). IL-17A production by γδ T cells is induced by IL-23 and

other cytokines produced by *M. tuberculosis*-infected dendritic cells (Lockhart et al., 2006; Umemura et al., 2007).

In addition to anti-bacterial immunity, IL-17A is involved in controlling fungal, viral and parasitic infections. The administration of anti-IL-17A neutralizing antibody to wild-type mice infected with *Pneumocystis carinii* impairs chemokine responses and the clearance of infection. Similarly, it has been shown in IL-23p19 knockout mice, thereby suggesting that the IL-23-IL-17 axis mediates clearance of *Pneumocystis carinii* infection (Rudner et al., 2007). IL-17A-deficient and IL-17RA-deficient mice are highly susceptible to systemic *Candida albicans* infection and have impaired neutrophil recruitment. Dectin-2, which is a C-type lectin expressed by dendritic cells and macrophages, is important in inducing Th17 cell differentiation to combat infections. In response to oral candidiasis, IL-17A produced by Th17 cells is essential in the host response through its effect on neutrophils and antimicrobial factors. IL-17RC is also required in this response during oral mucosal infections (Conti et al., 2009; Ho et al., 2010; Huang et al., 2004; Saijo et al., 2010). Herpes simplex virus-1 (HSV-1) infection of the cornea leads to stromal keratitis. IL-17A is upregulated and mainly produced by γδ T cells in the early phase after HSV infection of the cornea. Knockout of IL-17RA or neutralization of IL-17A in wild-type mice leads to a decrease of disease severity, suggesting that IL-17A contributes to the pathogenesis of stromal keratitis (Suryawanshi et al., 2011). Compared to wild-type mice, IL-17A-deficient mice have greater susceptibility to *T. cruzi* infection featured by prolonged parasitemia, multiple organ failure, tissue injury and increased mortality. In the immune responses to *T. cruzi* infection, IL-17 production regulates the differentiation of Th1 cells, influx of

inflammatory cells to the heart and myocarditis (da Matta Guedes et al., 2010; Miyazaki et al., 2010). An interesting report indicates that B cells are the major source of rapid IL-17A production in response to *T. cruzi* infection (Bermejo et al., 2013)

Patients with autosomal dominant hyper-IgE syndrome (HIES, Job's syndrome) have a genetic mutation in the *STAT3* gene that impairs the Th17 immunity rendering them susceptible to severe pulmonary infections, the development of pneumatoceles, staphylococcal abscesses and mucocutaneous candidiasis (Milner et al., 2008). Patients with chronic mucocutaneous candidiasis who are also susceptible to *C. albicans* and *S. aureus* infections have an autosomal recessive deficiency in IL-17RA, autosomal dominant deficiency of IL-17F or either loss-of-function or gain-of-function mutations in *STAT1* (Liu et al., 2011; Puel et al., 2011).

IL-17A has also been associated with autoinflammatory and autoimmune diseases such as multiple sclerosis, psoriasis, asthma, inflammatory bowl diseases and rheumatoid arthritis (Korn et al., 2009) (Figure 1-1).

Commensal microbiota, which regulates the steady state differentiation of Th17 cells and Treg cells in the lamina propria is essential for gut homeostasis. A protective function for IL-17 has been demonstrated in a mouse colitis model. IL-17A modulates Th1 polarization and the production of Th1-type cytokines in colon tissue (O'Connor et al., 2009). Colonization of the small intestine of mice with segmented filamentous bacterium induces an increase in Th17 cells with upregulation of genes associated with inflammation and antimicrobial defenses in the lamina propria (Ivanov et al., 2009). Specific commensal microbiota is required for Th17 cell differentiation in the lamina propria of the small intestine. Antibiotic treatment inhibits this differentiation (Ivanov et

al., 2008). Polysaccharide A of gut commensal *Bacteroides fragilis* activates the TLR2 pathway and promotes tolerance by inducing Treg cells to establish host-microbial symbiosis (Round et al., 2011).

IL-25 shares around 15% sequence identity to the IL-17A protein (Gaffen, 2009). IL-25 facilitates Th2 responses by promoting the production of type 2 cytokines, including IL-4, IL-5, IL-13, and recruitment of eosinophils for host defense against helminth infections (Figure 1-1). IL-25 deficient mice are more susceptible to helminth infection (Fallon et al., 2006; Hurst et al., 2002; Kim et al., 2002; Owyang et al., 2006; Pan et al., 2001; Tamachi et al., 2006; Zhao et al., 2010). IL-25 also regulates proallergic responses (Angkasekwinai et al., 2007; Suzukawa et al., 2012; Wang et al., 2007). IL-25 produced by airway structural cells promotes activation of lung epithelial cells and eosinophils. In an allergen induced asthma model, IL-25 overexpression by lung epithelial cells increases mucus production and the infiltration of macrophages and eosinophils. Blocking IL-25 reduces Th2 cytokine production and airway inflammation. Another study shows IL-25 produced by eosinophils and basophils may also enhance the maintenance of adaptive Th2 memory cells to augment allergic inflammation (Wang et al., 2007).

IL-17B, IL-17C and IL-17D are not well characterized with regard to their expression and function (Figure 1-1). The amino acid identity between IL-17B (180 a.a.), IL-17C (197 a.a.), IL-17D (202 a.a.) and IL-17A is around 20-30%. Unlike other IL-17 family members which are disulfide-linked dimers, IL-17B forms a non-covalent dimer (Gaffen, 2009). IL-17C binds to the IL-17RA and IL-17RE receptor complex on epithelial cells. Bacterial challenge induces IL-17C production by epithelial cells. Similar

to IL-17A, IL-17C stimulates the expression of proinflammatory cytokines, chemokines and antimicrobial peptides and regulates innate epithelial immune responses in an autocrine manner (Ramirez-Carrozzi et al., 2011). In response to infection with *Citrobacter rodentium*, IL-17C is upregulated in colonic epithelial cells and induces antibacterial peptide expression in synergy with IL-22. IL-17C deficiency exaggerates experimental colitis in mice and this is associated with increased IL-17 expression by γδ T cells and Th17 cells (Reynolds et al., 2012). IL-17RE-deficient mice have decreased expression of antibacterial molecules and suffer from greater bacterial burden and early mortality (Song et al., 2011). IL-17C overproduction is also associated with some autoimmune diseases. Elevated protein levels of IL-17C are observed in psoriatic skin lesions, indicating a possible role of IL-17C in this disease. Endothelial cells treated with IL-17C produce increased TNF-α; keratinocytes treated with IL-17C and TNF-α upregulate gene expression in pattern similar to that elicited by IL-17A and TNF-α (Johansen et al., 2009; Johnston et al., 2013). *IL-17C* knockout mice show decreased severity of experimental autoimmune encephalomyelitis (EAE), a disease model of human multiple sclerosis (Chang et al., 2011). IL-17B and IL-17C enhance TNF-α and IL-1β production by a human leukemic monocytic cell line (Li et al., 2000). IL-17B and IL-17C have been shown to play an important role in the pathogenesis of autoimmune diseases. The production of IL-17B and IL-17C has been observed in the cartilage and other cell populations in collagen-induced arthritis (CIA) of mice, an animal model for rheumatoid arthritis. IL-17B and IL-17C induce TNF-α production by mouse peritoneal exudate cells. Increased serum TNF-α concentration and a high arthritis score have been observed in mice with bone marrow transplantation of *IL-17B* or *IL-17C* gene-transduced

bone marrow cells. In addition, neutralization of IL-17B suppresses the progression of arthritis in CIA mice (Yamaguchi et al., 2007). Intraperitoneal injection of IL-17B in mice induces neutrophil migration in a dose-dependent manner (Shi et al., 2000). IL-17B and IL-17RB have also been shown to be involved in cancer metastasis and malignancy. IL-17B signaling promotes pancreatic cancer cell invasion, in association with macrophage and endothelial cell recruitment via the ERK1/2 pathway (Wu et al., 2015). IL-17B and IL-17RB also promote tumorigenicity in breast cancer cells via the NF-κB pathway. Treatment with anti-IL-17RB or anti-IL-17B antibodies attenuates tumorigenicity of breast cancer cells (Huang et al., 2014). However, one report shows IL-17B maybe anti-angiogenic by inhibiting endothelial cell-matrix adhesion and cellular migration (Sanders et al., 2010). IL-17D, the largest IL-17 family member, suppresses myeloid progenitor cell proliferation, but enhances endothelial cell production of IL-6, IL-8 and GM-CSF (Starnes et al., 2002). IL-17D also promotes tumor rejection through recruitment of natural killer cells (O'Sullivan et al., 2014).

1.2 IL-17s and their receptors in invertebrates and fish

Several *IL-17* genes have been identified in invertebrates. Three *IL-17* genes have been identified in *Ciona intestinalis*. They are expressed by hemocytes inside the pharynx tissues and are upregulated by LPS inoculation (Vizzini et al., 2014). IL-17 from pearl oyster *Pinctada fucata* is cloned and found to be involved in the immune response to LPS and poly(I:C) stimulation. The recombinant IL-17 protein could active the NF-κB signal pathway (S.-Z. Wu et al., 2013). In Pacific oyster, six IL-17 orthologues are identified

and cloned. They are highly expressed in the gill and digestive gland tissues and are significantly upregulated in hemocytes in response to pathogen-associated molecular patterns (PAMPs) (Li et al., 2014; Roberts et al., 2008). In Californian abalone (*Haliotis rufescens*), IL-17 orthologue is cloned and is found to be constitutively expressed in hemocytes. 12 hours after infection with *Vibrio anguillarum*, the transcription of *IL-17* is upregulated in hemocytes (Valenzuela-Muñoz and Gallardo-Escárate, 2014). Several putative *IL-17* genes are found in other invertebrates such as *Caenorhabditis elegans*, cephalochordate amphioxus (*Branchiostoma floridae*), lottia (*Lottia gigantea*) and sea urchin (*Strongylocentrotus purpuratus*) but cloning of the full-length genes has not been completed (Hibino et al., 2006; Huang et al., 2008; Kono et al., 2011; Li et al., 2014). Using the sequence information, amphioxus IL-17 is found to be upregulated 24 hours after *Escherichia coli* challenge in skin (Wu et al., 2011a).

In elephant shark *Callorhinchus milii*, three IL-17A-like sequences (GenBank accession no. NP_001279507, XP_007883573 and XP_007883503) and two IL-17C-like sequences (GenBank accession no. XP_007906115 and XP_007904597) have been identified by NCBI Eukaryotic Genome Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genome/annotation_euk/Callorhinchus_milii/100/). Multiple *IL-17* genes have been identified and characterized in teleost (Kono et al., 2011). Three zebrafish orthologues of mammalian IL-17A and IL-17F identified have been designated IL-17A/F (Gunimaladevi et al., 2006). IL-17A/F orthologues have also been found in rainbow trout and Japanese pufferfish (also known as fugu) (Korenaga et al., 2010; Monte et al., 2013). IL-17C orthologues have been reported in zebrafish, fugu, and rainbow trout (Gunimaladevi et al., 2006; Korenaga et al., 2010; Wang et al., 2010). IL-

17D orthologues have been identified in zebrafish, fugu, Atlantic salmon, grass carp, and channel catfish (Du et al., 2014; Gunimaladevi et al., 2006; Korenaga et al., 2010; Kumari et al., 2009; Roberts et al., 2008; Wang et al., 2014). By RT-PCR analysis, *IL-17D* expression is found to be typically evident in zebrafish spleen and gills; fugu *IL-17D* is lowly expressed in skin, brain, head kidney and the intestine. In Atlantic salmon and grass carp, *IL-17D* is expressed in skin, intestine by real-time PCR analysis. IL-17B and IL-17E orthologues have not been identified in teleost.

In invertebrates, *IL-17R* gene is found to be present in the *C. intestinalis* genome and transcripts of *IL-17R* has been detected in its hemocytes (Terajima et al., 2003; Vizzini et al., 2014). The IL-17Rs identified in *C. intestinalis* are IL-17RA-like (Wu et al., 2011b). Two *IL-17R*s have been identified in the genome of sea urchin (Hibino et al., 2006). IL-17RA and IL-17RD are identified in amphioxus and they might be the ancestors of vertebrate IL-17RA and IL-17RD (Wu et al., 2011b). The cloning and characterization of these invertebrate *IL-17R* genes is not available.

In cartilaginous fish, several incomplete sequences of IL-17Rs including IL-17RA, IL-17RB, IL-17RC and IL-17RD have been identified in shark *C. milii* (Wu et al., 2011b). IL-17RE (GenBank accession no. XP_007909458) has also been identified in elephant shark by NCBI Eukaryotic Genome Annotation Pipeline. In teleost fish, IL-17RA and IL-17RD have been identified in most species. *IL-17RA* mRNA sequence has been reported in fugu, Atlantic salmon and rainbow trout (Kono et al., 2011). By in silico cloning, the *IL-17RA* gene is found in the genome of fugu, stickleback, medaka and zebrafish (Kono et al., 2011). In another study, IL-17RA has been identified in zebrafish (three IL-17RAs), fugu (two IL-17RAs) and stickleback (two IL-17RAs) through homology search (Wu et

al., 2011). The *IL-17RD* gene has been found in zebrafish (Tsang et al., 2002); its existence is also confirmed in the fugu, green puffer, stickleback and medaka genome using in silico cloning analysis. In agreement with this, a single IL-17RD has been found in zebrafish, fugu and stickleback. IL-17RB is confirmed in the genome of stickleback and zebrafish. The *IL-17RC* gene is identified in the fugu, green puffer, zebrafish and stickleback genome, the existence of the *IL-17RE* gene is found in zebrafish, green puffer and stickleback. A group of IL-17R-like proteins in vertebrates which lack the intracellular conserved SEFIR domain but resemble the extracellular domain of IL-17RE have been recently identified and named IL-17RE-like (IL-17REL) proteins. IL-17REL has been found in zebrafish, fugu and stickleback.

1.3 lamprey VLR immune system

Petromyzon marinus

Sea lampreys (*Petromyzon marinus*) belong to jawless vertebrates which are the most basal group of vertebrates. This unique evolutionary position makes lampreys an ideal model for investigations on vertebrate evolution. Research comparing and contrasting the features among non-vertebrate chordates, jawless vertebrates and jawed vertebrates is performed to study the evolution of vertebrate characteristics, one of which is the adaptive immunity.

Invertebrates express various innate immune receptors that are employed to combat infections. One large group of these innate immune receptors contains immunoglobulin superfamily (IgSF) domain (Hirano et al., 2011). In amphioxus, an

IgSF-domain-containing molecule called V-region-containing chitin-binding protein (VCBP), of which the N-terminal region has extensive diversity, has been identified (Cannon et al., 2002). In the snail *Biomphalaria glabrata*, fibrinogen-related proteins (FREPs) which contain both IgSF domains and C-terminal fibrinogen domains are produced upon exposure to infection (Adema et al., 1997; Léonard et al., 2001; Zhang et al., 2001, 2004). In *Drosophila melanogaster,* IgSF-domain-containing receptors named Down's syndrome cell-adhesion molecules (Dscam) are expressed by immune-competent cells. The diversity of these receptors can be generated by alternative splicing (Watson et al., 2005). Another group of proteins widely used for immune defense in invertebrates contains leucine-rich repeat (LRR) motif. One example is TLRs which function as PRRs recognizing PAMPs.

All of the jawed vertebrates are shown to be capable of adaptive immunity. The molecular basis of their adaptive immunity, immunoglobulin-based TCR of T lymphocytes and BCR of B lymphocytes, is generated by V(D)J recombination initiated by recombination activating gene (RAG) protein in the thymus and hematopoietic tissues, respectively. The major histocompatibility complex (MHC) molecules are used in antigen presentation. However, immunoglobulin-based *BCR*, *TCR* and *MHC* genes could not be identified in jawless vertebrates. Instead, multiple *LRR* modules have been found in the sequences of cDNA clones isolated from antigen and mitogen stimulated lamprey lymphocytes (Pancer et al., 2004). The number of *LRR* modules on each cDNA clone and sequence of individual *LRR* modules are highly diverse. Because of the sequence diversity and the lymphocyte-restricted expression, these proteins are named variable lymphocyte receptors (VLRs). The mature *VLR*s in the lymphocytes are proposed to be

generated through somatic gene rearrangement by the copy-and-paste of diverse *LRR* modules from upstream and downstream cassettes into germline *VLR*, enabling the expression of functional VLRs (Pancer et al., 2004; Rogozin et al., 2007). Each VLR protein consists of a conserved signal peptide, N-terminal LRR (LRRNT) followed by variable number of LRRs, one connecting peptide LRR (LRRCP), C-terminal LRR (LRRCT) and the invariant threonine/proline-rich stalk region containing a glycosyl-phosphatidyl-inositol (GPI) cleavage site shown in Figure 1-2 (Hirano et al., 2011).

Three *VLR* genes have been identified and named *VLRA*, *VLRB* and *VLRC* respectively in lampreys. These three genes are expressed by three separate lymphocyte populations: $VLRA^+$, $VLRB^+$ and $VLRC^+$ lymphocytes found in the principal lymphoid tissues of lamprey larvae including blood, kidneys, typhlosole (a hematopoietic tissue) and gill region (the presumptive thymus equivalent in lamprey) (Alder et al., 2008; Guo et al., 2009; Hirano et al., 2013; Kasamatsu et al., 2010b). In the epithelium of skin and gills, $VLRA^+$ and $VLRC^+$ lymphocytes have been identified whereas $VLRB^+$ lymphocytes are rare at this site. Distinctive gene expression profiles have been observed in $VLRA^+$, $VLRB^+$ and $VLRC^+$ lymphocytes (Guo et al., 2009; Hirano et al., 2013). The genes expressed preferentially by $VLRA^+$ lymphocytes are orthologues of genes expressed by T cells in jawed vertebrates. They include transcription factor GATA2/3 implicated in T-cell differentiation, the chemokine receptor CCR9 involved in lymphocyte homing to the thymus, the T cell specification molecule Notch1, the tyrosine phosphatase receptor protein CD45 involved in TCR-mediated signaling, the IL-8 receptor CXCR2, proinflammatory cytokine IL-17 and macrophage migration inhibitory factor (MIF) (Figure 1-3) (Hirano et al., 2011). In contrast, $VLRB^+$ cells mainly express

orthologues of genes expressed by B cells in jawed vertebrates such as TLR homologues TLR2abc, TLR7 and TLR10, CXCR4 involved in hematopoietic progenitor homing, tumor necrosis factor receptor superfamily member 14 (TNFRSF14) which is a herpesvirus entry mediator, Syk and B cell adaptor protein (BCAP) involved in BCR-mediated signaling, the chemotactic inflammatory cytokine IL-8 and the IL-17 receptor. $VLRC^+$ transcriptional profiles largely resemble $VLRA^+$ lymphocytes. However, compared to $VLRA^+$, $VLRC^+$ lymphocytes preferentially express the SRY-box containing gene 13 (SOX13) encoding a fate-determining transcription factor used for γδ T-cell lineage commitment, an integrin αL (ITGAL) orthologue of one component of the heterodimeric lymphocyte function associated antigen 1 (LFA1), and integrins α4 and β1 (ITGA4 and ITGB1) orthologues of the two components of very late antigen 4 (VLA4), the expression of which is correlated with adherence of human γδ T cells to epithelial cells, TLR3 and IL-16 which is a modulator of T-cell activation. This analysis demonstrates that expression profiles of $VLRA^+$ cells and $VLRC^+$ resemble those of αβ T and γδ T cells in jawed vertebrates while $VLRB^+$ cells are similar to B cells in jawed vertebrates. This is also true in the analysis of functions of $VLRA^+$, $VLRB^+$ and $VLRC^+$ cells (Alder et al., 2008; Guo et al., 2009; Hirano et al., 2013). After immunization with *Bacillus anthracis* exosporium, *E. coli*, *S. typhimurium* or *S. pneumoniae*, the $VLRA^+$ cells proliferate but fail to secrete VLRA proteins that could bind these unmodified immunogens before or after immunization. $VLRC^+$ cells also demonstrate the proliferative responses after immunization with *Bacillus anthracis* exosporium. In response to stimulation by plant lectin phytohaemagglutinin (PHA), a classical T cell mitogen, $VLRA^+$ and $VLRC^+$ cells are preferentially activated and undergo vigorous

proliferation to become large lymphoblasts with limited endoplasmic reticulum but do not secret the VLRC proteins. The cell number of both populations in blood is increased. In addition, $VLRA^+$ cells upregulate *IL-17* transcription after PHA stimulation. These demonstrate that lamprey $VLRA^+$ and $VLRC^+$ cells are functionally equivalent to T cells in jawed vertebrates. Meanwhile, $VLRB^+$ cells resemble B cells in jawed vertebrates, upon immunization with antigens, $VLRB^+$ cells proliferate and differentiate into plasmacytes that secrete multimeric VLRB antibodies much like IgM antibodies of jawed vertebrates. These $VLRB^+$ cells can bind to cognate antigens while $VLRA^+$ cells are not able to bind the antigens. The VLRB antibody-secreting plasmacytes have expanded cytoplasm and rough endoplasmic reticulum which are similar to plasma cells of jawed vertebrates. In addition, the VLRB antibodies from immunized lampreys can interact with C1q and C3 proteins to mediate complement-dependent cytotoxicity for bacteria and tumor cells indicating that the VLRB antibodies are the counterparts of the immunoglobulin-based antibodies of jawed vertebrates used for complement activation (F. Wu et al., 2013). All these data suggest that $VLRA^+$ and $VLRC^+$ cells are T cell-like while $VLRB^+$ cells are B cell-like.

In mammals, some thymus-independent antigens induce antibody responses in the absence of helper T cells, whereas B cell responses to thymus-dependent antigens require antigen-specific T-cell help. In the T cell-B cell interactions, helper T cells recognize the peptide bound to MHC class II molecules on the B cell surface; CD40 ligand (CD40L) on the T cell surface interact with CD40 on the B cell surface. Activated B cells and T cells migrate to form a germinal center where antigen-specific T cells continue to provide help to the B cells which rapidly proliferate and different into either

antibody-secreting plasma cells or memory B cells. “Classical” germinal centers are only found in birds and mammals but not in cold-blooded vertebrates. In nurse shark *Ginglymostoma cirratum*, compartmentalization of B cells, T-like cells and dendritic cells is identified in the white pulp of spleen but mammalian-like germinal centre structures are not found, suggesting mammalian-like antigen activation of B cells by T cells via unknown mechanisms (Rumfelt et al., 2002). In channel catfish (*Ictalurus punctatus*), cell clusters which may be primordial germinal centers have been identified in the spleen and kidney using activation-induced cytidine deaminase (AID) as a marker (Saunders et al., 2010). Reptiles have a thymus (site of T cell maturation), spleen, gut-associated lymphoid tissue (GALT) and bone marrow (the site of hematopoiesis), but lack lymph nodes and germinal centers (Pettinello and Dooley, 2014; Zimmerman et al., 2010). Removal of the spleen suppresses the humoral response, suggesting the functional importance of the spleen in reptiles. Further studies exploring the interactions of T cells with B cells in the reptiles are needed. In lampreys, a thymus equivalent is found at the lymphoepithelial region at the tips of the gill filaments and the neighboring secondary lamellar in which *VLRA* or *VLRC* gene undergoes assembly. The lymphoid cells at this region express CDA1 which is an AID-apolipoprotein B mRNA editing catalytic component (APOBEC) family orthologue that may catalyze the gene assemblies (Bajoghli et al., 2011). The *VLRB* gene assembly takes place in the typhlosole which is a lamprey hematopoietic tissue. Cells expressing CDA2, which is another APOBEC family orthologue is abundant in typhlosole. Other principle lymphoid tissues in lamprey include kidneys and blood. Germinal centers have not been identified in the lampreys.

The discovery of lamprey T-like and B-like cells leads to the question whether an evolutionarily conserved form of T-like and B-like cell interactions exists during an immune response. The identification of the T-like and B-like arms implies there might be crosstalk between different populations in response to the antigen stimulation. As abovementioned, an IL-17 homolog which is a proinflammatory cytokine and the IL-8 receptor CXCR2 are expressed in T-like cells while an IL-17R homolog and the chemotactic inflammatory cytokine IL-8 are expressed by B-like cells. Upon stimulation by PHA, T-like cells upregulate the expression of IL-17 while B-like cells enhance the expression of IL-8, indicating the functional interaction between T-like and B-like cells (Figure 1-3) (Hirano et al., 2011). IL-17 has been shown to help B cell responses in mammals. In the BXD2 mouse model of autoimmunity, IL-17A upregulates the B cell expression of regulator of G-protein signaling 13 and 16 (Rgs13 and Rgs16) via NF-κB pathways to reduce the chemotactic response to the chemokine CXCL12 and to promote the formation of autoreactive germinal centers. Blocking IL-17 signaling by an inhibitory adenovirus construct disrupts the formation of germinal centers and IL-17R knockout mice have reduced germinal center B cell development and autoantibodies production. (Hsu et al., 2008; Xie et al., 2010b). In K/BxN mouse model of inflammatory arthritis, treatment with anti-IL-17 monoclonal antibodies (mAbs) decreases the serum autoantibody titers and blocks disease progression. IL-17 is required for efficient germinal center formation in the K/BxN model via a direct effect on B cells shown by transfer experiments. The percentage of B cells from IL-17RA knockout mice in germinal centers is decreased compared to B cells from wild-type mice (Wu et al., 2010). In addition, IL-17A promotes immunoglobulin isotype switching in mice (Mitsdoerffer et

al., 2010). It is interesting to examine whether lamprey IL-17 is involved in coordinating T-like and B-like cells in helping B-like cell response to antigens.

1.4 Summary of aims of the study

Lamprey T- and B-like lymphocyte lineages resemble the adaptive immune system of jawed vertebrates, which implies the cooperative interactions between the lamprey T-like and B-like cells during an immune response. Cytokines could be one of the ways to mediate the cooperative the cell-cell interactions. Indeed, reciprocal expression of cytokine and cytokine receptor expression has been demonstrated for lamprey T-like and B-like lymphocytes. A lamprey orthologue of mammalian IL-17 family, which contains critical players in normal immune responses and inflammatory diseases, have been identified. Prior studies show that transcripts of lamprey *IL-17* orthologue are expressed preferentially by T-like lymphocytes, and a lamprey *IL-17R* orthologue is primarily expressed by the B-like lymphocytes in lampreys (Guo et al., 2009). Enhanced expression of *IL-17* in T-like lymphocytes after activation coupled with the expression of *IL-17R* by B-like lymphocytes suggests functional interactions between the T-like and B-like lymphocyte populations. However, the *IL-17* and *IL-17R* gene orthologues in lampreys are presently incompletely defined, and the function of their protein products is unknown. Therefore, we propose to define the IL-17 and IL-17R family in lampreys and test the hypothesis that lamprey IL-17 and IL-17R family members are used for effective adaptive immune response against antigens by coordinating T-like and B-like lymphocyte populations. Two specific aims of this project

have been proposed to study the function of IL-17 and IL-17R family in lampreys. The first aim is to characterize the expression of IL-17 and IL-17R family members in the different cell types and tissues of lampreys, and the second aim is to determine the biological activities of the IL-17 family members. We hypothesize that lamprey IL-17 and IL-17R family members are used for effective adaptive immune response against antigens by coordinating T-like and B-like lymphocyte populations. The long term goal of this project is to elucidate the immune roles of IL-17 and IL-17R family members in lampreys. Success in this study will demonstrate an evolutionarily conserved form of cell-cell interactions during an immune response and the comparative studies of IL-17s and IL-17Rs will provide insights into the phylogenetic emergence of IL-17 and IL-17R family members.

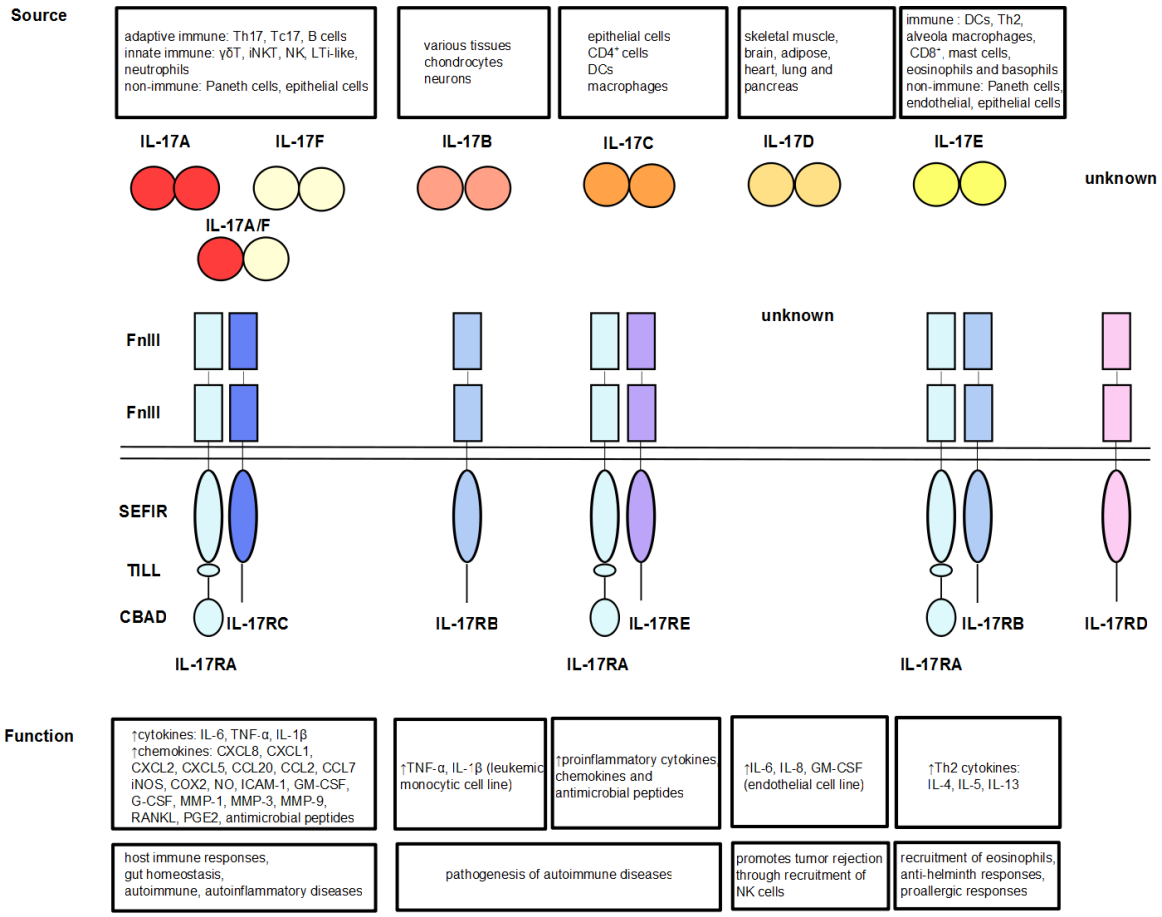

Source
adaptive immune: Th17, Tc17, B cells
innate immune: γδT, iNKT, NK, LTi-like, neutrophils
non-immune: Paneth cells, epithelial cells
various tissues
chondrocytes
neurons
epithelial cells
CD4+ cells
DCs
macrophages
skeletal muscle, brain, adipose, heart, lung and pancreas
immune : DCs, Th2, alveola macrophages, CD8+, mast cells, eosinophils and basophils
non-immune: Paneth cells, endothelial, epithelial cells
IL-17A
IL-17F
IL-17A/F
IL-17B
IL-17C
IL-17D
IL-17E
unknown
unknown
FnIII
FnIII
SEFIR
TILL
CBAD
IL-17RC
IL-17RA
IL-17RB
IL-17RE
IL-17RA
IL-17RB
IL-17RA
IL-17RD
Function
↑cytokines: IL-6, TNF-α, IL-1β
↑chemokines: CXCL8, CXCL1, CXCL2, CXCL5, CCL20, CCL2, CCL7
iNOS, COX2, NO, ICAM-1, GM-CSF, G-CSF, MMP-1, MMP-3, MMP-9, RANKL, PGE2, antimicrobial peptides
↑TNF-α, IL-1β (leukemic monocytic cell line)
↑proinflammatory cytokines chemokines and antimicrobial peptides
↑IL-6, IL-8, GM-CSF (endothelial cell line)
↑Th2 cytokines: IL-4, IL-5, IL-13
host immune responses, gut homeostasis, autoimmune, autoinflammatory diseases
pathogenesis of autoimmune diseases
promotes tumor rejection through recruitment of NK cells
recruitment of eosinophils, anti-helminth responses, proallergic responses

Figure 1-1. IL-17 and IL-17R family members in mammals. The IL-17 family consists of six members from IL-17A to IL-17F, while the IL-17R family comprises five members from IL-17RA to IL-17RE. Both IL-17A and IL-17F form covalent homodimers and also IL-17A-IL-17F heterodimers. IL-17A and IL-17F bind IL-17RC and IL-17RA. IL-17RB can bind IL-17B; it can also pair with IL-17RA to bind IL-17E. IL-17C is the ligand for IL-17RA and IL-17RE. The ligand for IL-17RD and the receptor for IL-17D are unknown. The sources and functions of IL-17 family members are also shown. CBAD, C/EBP activation domain; FN, fibronectin III-like domain; SEFIR, SEF/IL-17R; TILL, TIR-like loop.


VLR protein
SP
LRR NT
LRR 1
LRR V
LRR V
LRR V
LRR V
LRR V
LRR V
LRR V
LRR V
LRR Ve
CP
LRR CT
Stalk
C-term

Figure 1-2. The structure of the mature VLR protein. Functional VLR protein consists of a SP, an LRRNT, an LRR1, up to eight LRRV cassettes, an LRRVe, a CP LRR, an LRRCT, an invariant stalk region containing a GPI cleavage site, and a C-terminal HP. SP, signal peptide; CP, connecting peptide; HP, hydrophobic peptide. (Hirano et al., 2011)

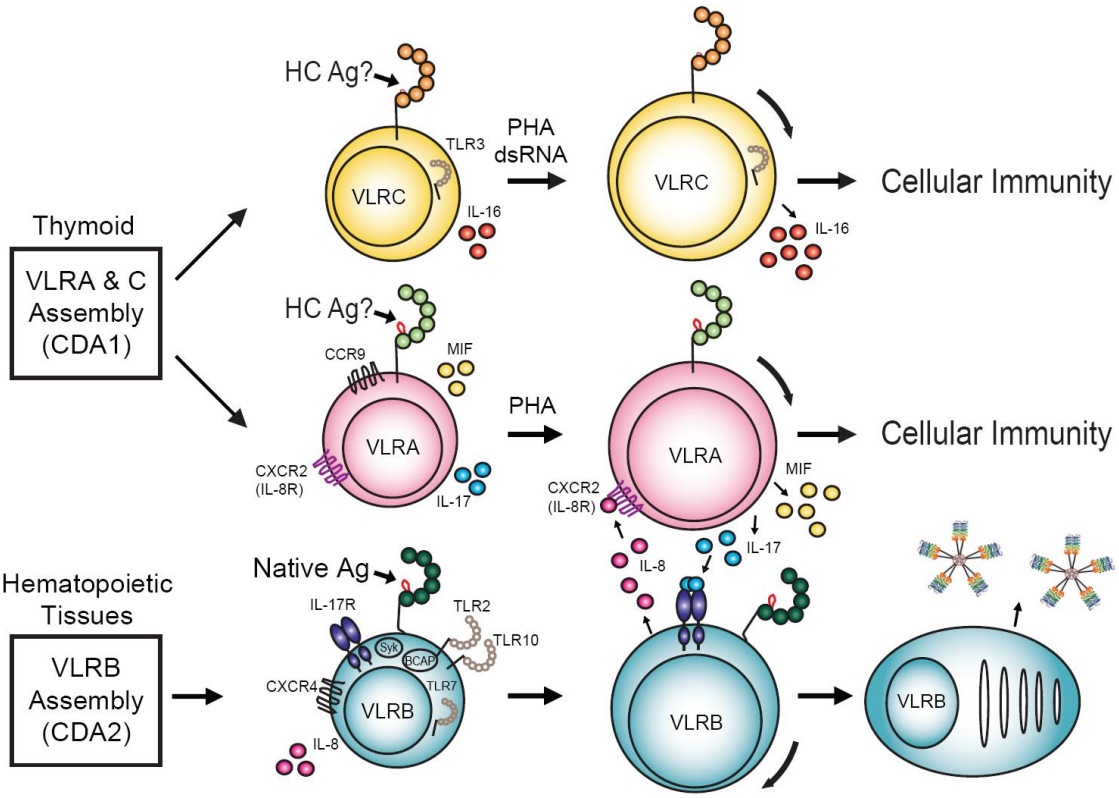

Thymoid
VLRA & C Assembly (CDA1)
HC Ag?
TLR3
VLRC
IL-16
PHA dsRNA
VLRC
IL-16
Cellular Immunity
HC Ag?
CCR9
MIF
VLRA
CXCR2 (IL-8R)
IL-17
PHA
VLRA
CXCR2 (IL-8R)
MIF
IL-17
IL-8
Cellular Immunity
Hematopoietic Tissues
VLRB Assembly (CDA2)
Native Ag
IL-17R
TLR2
TLR10
Syk
BCAP
CXCR4
VLRB
TLR7
IL-8
VLRB
VLRB

Figure 1-3. VLR lymphocytes in lamprey. Distinct gene expression profiles have been observed for $VLRA^+$, $VLRB^+$ and $VLRC^+$ cells. $VLRA^+$ and $VLRC^+$ T-like lymphocytes express orthologues of molecules which are important in T cell development and function in jawed vertebrates. Meanwhile, $VLRB^+$ B-like lymphocytes express orthologues of molecules critical for B cell development and function in jawed vertebrates. Upton PHA stimulation, $VLRA^+$ cells upregulate the expression of IL-17 while $VLRB^+$ cells increase the expression of IL-8. The expression of IL-17R in $VLRB^+$ and IL-8R in $VLRA^+$ cells suggest functional interactions between these two populations. Modified image (Hirano et al., 2011).

Chapter 2

Characterization of lamprey IL-17 family members

This chapter includes work submitted to Journal of Immunology as Han, Q.F., Das, S., Hirano, M., Holland, S., McCurley, N., Guo, P., Boehm, T. and Cooper, M.D.. Characterization of lamprey IL-17 family members and their receptors. The majority of the work was done by Qifeng Han. Sabyasachi Das and Masyuki Hirano helped with experimental design and data analysis. Max D. Cooper and Thomas Boehm edited the manuscript for publication.

2.1 Abstract

A lamprey *interleukin-17* (*IL-17*) orthologue was shown earlier to be expressed preferentially by T-like lymphocytes, whereas B-like lymphocytes express transcripts for an *IL-17 receptor*. Here, we have characterized five members of the lamprey IL-17 family (*IL-17-1* to *IL-17-5*) as a first step in exploring their roles in T-like, B-like and innate cell interactions. Lamprey *IL-17-1, IL-17-2, IL-17-3* and *IL-17-5* were expressed primarily in intestine and gills at much lower levels than *IL-17-4* (*IL-17D* like), which was expressed by T-like cells and epithelial cells of skin, intestine and gills. IL-17-4 protein was also detected in skin, gills and intestine by Western blot analysis with an IL-17-4-specific mAb. *IL-17-4* transcription by blood leukocytes was upregulated after injection of bacteria and yeast. These data suggest the diversity of the lamprey IL-17 family members and the potential function of *IL-17-4* in antibacterial and antifungal responses in lampreys.

2.2 Introduction

IL-17 is an ancient cytokine, orthologues of which have been identified in invertebrates that employ innate immune responses for protection against pathogens (Hibino et al., 2006; Huang et al., 2008; Roberts et al., 2008; Vizzini et al., 2014; S.-Z. Wu et al., 2013). In mammals, six members of the IL-17 family (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F) have been characterized, where they play important roles in a variety of immune responses. IL-17A and IL-17F are essential for adaptive immune responses against extracellular bacteria and fungi in humans, and they contribute to the

pathogenesis of inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis (Korn et al., 2009). IL-17B and IL-17C were shown to enhance TNF-α and IL-1β production, both important immune regulators, by a human leukemic monocytic cell line (Li et al., 2000). With an extended C-terminal domain, the IL-17D protein is the largest IL-17 family member; it is expressed at relatively high levels in skeletal muscle, brain, adipose, heart, lung, and pancreas tissues, and at lower levels by resting $CD4^+$ T cells and B cells in humans(Starnes et al., 2002). IL-17D suppresses myeloid progenitor cell proliferation, but enhances endothelial cell production of IL-6 and IL-8. IL-17D also promotes tumor rejection through recruitment of natural killer cells (O'Sullivan et al., 2014). IL-17E, also called IL-25, promotes production of Th2 cell cytokines and recruitment of eosinophils to provide protection against nematode infection (Fort et al., 2001).

The protein structure of IL-17 family members is distinguished by the characteristic cystine-knot with four C-terminal cysteines forming two intramolecular disulfide bonds as indicated by the crystal structure of human IL-17F (Hymowitz et al., 2001; Liu et al., 2013). This cystine-knot motif, originally identified in studies on the nerve growth factors (McDonald et al., 1991), is highly conserved. Orthologues of mammalian IL-17A and IL-17F have been described in zebrafish (Gunimaladevi et al., 2006), rainbow trout and Japanese pufferfish (also known as fugu) (Korenaga et al., 2010; Monte et al., 2013). IL-17C orthologues have been reported in zebrafish, fugu, and rainbow trout (Gunimaladevi et al., 2006; Korenaga et al., 2010; Wang et al., 2010). IL-17D orthologues were identified in zebrafish, fugu, Atlantic salmon, grass carp, channel catfish and Japanese lamprey (Du et al., 2014; Gunimaladevi et al., 2006; Korenaga et al.,

2010; Kumari et al., 2009; Roberts et al., 2008; Tsutsui et al., 2007; Wang et al., 2014). Studies on the expression patterns of IL-17 homologues indicate their tissue specificity. By RT-PCR analysis, *IL-17D* expression is evident in zebrafish spleen and gills (Gunimaladevi et al., 2006); fugu *IL-17D* is expressed at low levels in skin, brain, head kidney and the intestine (Korenaga et al., 2010); Japanese lamprey *IL-17D* expression is most prominent in skin, gills, and intestine (Tsutsui et al., 2007). In Atlantic salmon and grass carp, *IL-17D* is expressed in skin, intestine by real-time PCR analysis (Du et al., 2014; Kumari et al., 2009). A novel IL-17 ligand named IL-17N has only been identified in bony fish including zebrafish, medaka, fugu and stickleback (Kono et al., 2011; Korenaga et al., 2010). In elephant shark *Callorhinchus milii*, three IL-17A-like sequences (GenBank accession no. NP_001279507, XP_007883573 and XP_007883503) and two IL-17C-like sequences (GenBank accession no. XP_007906115 and XP_007904597) have been identified by NCBI Eukaryotic Genome Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genome/annotation_euk/Callorhinchus_milii/100/). Putative *IL-17* genes have also been identified in invertebrates, including vase tunicate, pearl oyster, Pacific oyster and Californian abalone (Li et al., 2014; Roberts et al., 2008; Valenzuela-Muñoz and Gallardo-Escárate, 2014; Vizzini et al., 2014; S.-Z. Wu et al., 2013). Several *IL-17*-like genes are found in other invertebrates, such as amphioxus (*Branchiostoma floridae*), sea urchin (*Strongylocentrotus purpuratus*), lottia (*Lottia gigantea*) and *Caenorhabditis elegans*, although full-length sequences for these genes are not yet available (Hibino et al., 2006; Huang et al., 2008; Kono et al., 2011; Li et al., 2014).

An IL-17 orthologue has also been identified in lampreys (Guo et al., 2009; Tsutsui et al., 2007). These jawless vertebrates have an alternative adaptive immune system in which leucine-rich repeat (LRR)-based proteins named variable lymphocyte receptors (VLRs) are used for antigen recognition (Alder et al., 2008; Guo et al., 2009; Hirano et al., 2013; Kasamatsu et al., 2010b). Three *VLR* genes have been identified; the *VLRA* and *VLRC* genes are expressed by T-like $VLRA^+$ and $VLRC^+$ cells respectively, and *VLRB* genes are expressed by B-like $VLRB^+$ cells. $VLRA^+$ lymphocytes were found to preferentially express *IL-17* transcripts, whereas *IL-17R* transcripts were expressed by $VLRB^+$ lymphocytes (Guo et al., 2009), suggesting the functional interactions of lamprey T-like and B-like cells. In the present studies, we have defined the members of the lamprey IL-17 family, determined their expression patterns in the steady state, and examined the upregulation after bacteria and yeast challenge.

2.3 Materials and Methods

Antigens

Escherichia coli (ATCC 25922 strain), S*treptococcus pneumoniae* (R36A strain) and *Saccharomyces cerevisiae* (EBY100 strain) were provided by D. E. Briles, W. H. Benjamin, Jr (University of Alabama at Birmingham) and E.V. Shusta (University of Wisconsin). Bacteria and yeast were heat-killed by incubation at 60 °C for 1 h. 1×10^8 heat-killed *E. coli*, 1×10^8 *S. pneumoniae* and 1×10^7 *S. cerevisiae* were used for immunization.

Animal maintenance and immunization.

Petromyzon marinus larvae (outbred, 8–15 cm long and 2–4 years of age) were from Great Lakes of North America. *Lampetra planeri* larvae (outbred, about 10cm long) were obtained from the tributaries of the Rhine river in Germany. Larvae were maintained in sand-lined aquariums at 18 °C and were fed brewer's yeast. Adult sea lampreys were maintained in temperature-controlled tanks (16-18 °C). Fish were maintained in sand-lined aquariums at 18 °C and were fed brewer's yeast. Larvae were anaesthetized with 0.1g/l ethyl 3-aminobenzoate methanesulphonate (MS-222) and 0.14g/l sodium bicarbonate (Sigma) and were given 30 μl intracoelomic injections of bacterial, yeast or protein prepared in sterile 0.67× PBS buffer. Animals were sacrificed in 1 g/1 MS-222 and 1.4g/l sodium bicarbonate followed by exsanguination. Peripheral blood was collected in 0.66×PBS/30mM EDTA, layered on top of 55% percoll and subjected to density centrifugation (400×g, 20 min, no brake). Adult lamprey blood was collected by cutting the tail and leukocytes were separated by Lymphoprep. Subsequently, the lamprey lymphocytes were collected for following studies. Cells were isolated from kidney, intestine and gills by disrupting tissues between frosted glass slides and 400×g centrifugation. The cell pellets were lysed in 1% NP-40 lysis buffer (with 5μg/ml leupeptin, 1μg/ml pepstatin, 5μg/ml aprotinin, 10μg/ml soybean trypsin inhibitor, 40μg/ml PMSF). The tissue lysates were used for immunoblot assays. All studies have been reviewed and approved by Institutional Animal Care and Use Committee at Emory University.

Transcriptome assembly of lampreys

Total RNA was extracted from the kidney, gills, blood and intestines (containing typhlosole) of lampreys, treated with DNAse and Illumina Hi-Seq compatible, mRNA enriched, stranded cDNA libraries were generated using Illumina TruSeq RNA (stranded) kit, following the manufacturer's instructions. Transcriptomes were assembled from 100bp paired-end reads (generated on the x sequencer) using the Trinity suite of programs (Grabherr et al., 2011) and a kmer size of 25bp. Reads were assembled in a strand-specific manner with assembly time reduced through in-silico normalization of reads to a maximum of 30x coverage using the in-built Trinity parameter. Open reading frames (ORFs) were extracted using Trinity's Transdecoder option to select the best open reading-frame for each transcript in a strand-specific manner, with identical ORFs being collapsed into a single sequence.

Identification of IL-17 sequences from lamprey genome

Known mammalian IL-17 sequences were used as queries for tBLASTn search against sea lamprey (http://petromyzon.msu.edu) and Japanese lamprey genome sequences (http://jlampreygenome.imcb.a-star.edu.sg). Unique ORF collections (Holland *et al*., manuscript in preparation) from European brook lamprey transcriptomes were interrogated using IL-17 sequences of various species. The signal peptide and putative domains were predicted by SMART software (http://smart.embl-heidelberg.de) and conserved domain database (CDD) of NCBI. The conserved IL-17 domain of IL-17s was used in the phylogenetic analysis. Multiple protein sequence alignments were conducted using the ClustalW program (Larkin et al., 2007). The phylogenetic trees were constructed by (i) neighbor-joining (NJ) and (ii) maximum likelihood (ML) methods

using the MEGA5.0 program (Tamura et al., 2011). The evolutionary distances were computed by the JTT matrix-based method (Jones et al., 1992). The reliability of the tree was assessed by bootstrap resampling with a minimum of 1000 replications.

Cloning and sequencing sea lamprey (*Petromyzon marinus*) *IL-17-4*

Total RNA was extracted from lymphoid tissues of lamprey larvae. First-strand cDNA was synthesized with oligo(dT) primer by Superscript III (Invitrogen). PCR primers were designed according to the sea lamprey genome sequences and nested PCR was performed to clone the *IL-17-4* gene. Primers used for nested PCR are all listed in Table 1.

Real-time PCR

Tissues (skin, kidneys, intestine, gills and blood) from lamprey larvae were extracted for RNA isolation. RNA was purified from lamprey tissues using RNeasy kits with on-column DNA digestion by DNase I (QIAGEN). First-strand cDNA was synthesized with random hexamer primers by Superscript III (Invitrogen). Quantitative real-time PCR was performed using SYBR Green on 7900HT ABI Prism (Applied Biosystems). All samples were run in three replicates. Cycling conditions were 1 cycle at 50 ℃ for 2 min, 1 cycle at 95 ℃ for 10 min, followed by 40 cycles of denaturation at 95 ℃ for 15 s and annealing/extension at 60 ℃ for 1 min. The values for the *IL-17* genes were normalized to the expression of *GAPDH*. Primers used in this experiment are listed in Table 1.

In situ hybridization analysis

RNA *in situ* hybridization was performed with digoxigenin (DIG)-labelled RNA riboprobes as described previously (Bajoghli et al., 2011). The probe sequence for *IL-17-4* is listed in Table 1.

IL-17-4 expression in HEK-293T cells

HEK-293T cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% FBS, 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin at 37 ℃ in 5% CO_2. *IL-17-4* cDNA was cloned into a fusion vector made from pIRESpuro2 (Invitrogen, Grand Island, NY) harboring a puromycin resistance gene to produce a fusion protein IL-17-4/IgG1-Fc which is composed of IL-17-4 and the Fc region of human immunoglobulin-G1 (IgG1-Fc) (Figure 2-5). The IgG1-Fc portion facilitated detection of the fusion protein with anti-IgG antibodies and its purification by protein A chromatography. The plasmids were transfected into HEK-293T cells using linear polyethylenimine (PEI), MW 25,000 (Polysciences, Inc.) at a 3:1 (PEI:DNA) ratio. After 60 hours, cells were harvested by centrifugation at 300g for 3 minutes. The supernatant were collected. The cell pellets were washed once with PBS and lysed in 1% NP-40 lysis buffer (with 5μg/ml leupeptin, 1μg/ml pepstatin, 5μg/ml aprotinin, 10μg/ml soybean trypsin inhibitor, 40μg/ml PMSF). The whole cell lysates and the supernatants were used for immunoblot assays.

Purification of recombinant IL-17-4/IgG1-Fc proteins

Recombinant IL-17-4/IgG1-Fc proteins were purified from supernatants via the human IgG1-Fc portion using protein A-agarose column (GE Healthcare). 500mL cell culture supernatants were collected from stably transfected HEK-293T cells under selection of 2μg/ml puromycin (Sigma). The supernatants were then loaded onto the protein A-agarose column, and washed with 10 column volumes of PBS. The non-specific proteins were removed by 15 column volumes of 50mM citrate at pH4.5 and recombinant 17D/IgG1-Fc proteins were eluted with 15 column volumes of 50mM citrate, 50mM NaCl at pH3. The eluate was dialyzed in PBS and concentrated by centrifugation using an Amicon Ultra-15 30K Centrifugal Filter Device (Millipore).

Production of anti-IL-17-4 mAbs

BALB/c mice were hyper-immunized with the IL-17-4/IgG1-Fc fusion proteins and the lymphocytes were isolated from the draining lymph nodes of these mice and subsequently fused with the Ag8.653 myeloma cell line using PEG-1500 (Roche). The hybridomas were screened by ELISA and Western blot and the positive clones were subcloned by serial dilutions. The ELISA and Western blot assay (Figure 2-5) of a representative mouse anti-IL-17-4 mAb (clone114-178, IgG2a) was shown.

Immunoblotting

Samples treated with 2-mercaptoethanol were separated on 10% SDS–PAGE gels and then transferred onto nitrocellulose membranes (Millipore) by semi-dry transfer method. Membranes were blocked with 7% skimmed milk for an hour and incubated with primary antibodies overnight at 4 degree. The primary antibodies used in this study

include horseradish peroxidase (HRP) conjugated goat anti-human Fc (1:5000, Southern Biotech), rabbit anti-γ-Tubulin (AK-15) antibody (1:1000, Sigma). After three washes with TBS-1% Tween-20, membranes were incubated with secondary antibodies including HRP conjugated goat anti-mouse Ig (1:10000, Dako), HRP conjugated goat anti-rabbit Ig (1:10000, Dako). Secondary antibody incubation is not needed for HRP conjugated goat anti-human Fc. After three washes, blots were developed using SuperSignal West Pico chemiluminescent substrate (Thermo Scientific) for 5 minutes.

Immunofluorescence.

For analysis of lymphocyte distribution in *P. marinus* larvae, dissected tissues were fixed for 12 h in 0.67× PBS containing 2% paraformaldehyde at 4 °C, cryopreserved in 30% sucrose, embedded in OCT compound (Tissue-Tek, Sakura) and sectioned at 7 μm on a cryostat (Thermo). Sections were permeabilized in PBS containing 10% goat serum, 0.5% saponin, 10 mM HEPES buffer and 10 mM glycine. They were stained for 1 h with mouse anti-lamprey IL-17-4 mAb (clone 114-178), followed by 1 h with the Alexa-Fluor-conjugated secondary antibody (Invitrogen) before being mounted in ProLong Gold with 4’,6-diamidino-2-phenylindole (DAPI) solution (Invitrogen). Fluorescence microscopy was performed with an Axiovert 200M microscope (Zeiss), equipped with a ×40 objective (numerical aperture, 0.6; ocular magnification, ×10). Images were processed with Adobe Photoshop (Adobe Systems).

Flow cytometric analysis and sorting

Leukocytes isolated from blood were stained for flow cytometry as described. Briefly, leukocytes from blood were stained with primary antibodies including anti-VLRA rabbit polyclonal serum (R110), anti-VLRB mouse mAb (4C4), and anti-VLRC mouse mAb (3A5). Secondary antibodies were matched. Staining and washes were in 0.67 × PBS with 1% BSA. Cells were gated using forward scatter-A (FSC-A) vs side scatter-A (SSC-A) (lymphocytes), FSC-A vs FSC-H (singlets), and negative propidium iodide (Sigma P4864) staining (live cells). Flow cytometric analysis was performed on a CyAn ADP (Dako) flow cytometer. $VLRA^+$, $VLRB^+$, $VLRC^+$, and VLR triple-negative cells were sorted on BD FACS Aria II (BD Bioscience) for real-time PCR analysis. The purity of the sorted cells was over 90%.

Statistical analysis

For western blotting analysis and flow cytometry staining, data were generated from 3 or more experiments. All data are expressed as the mean ± SEM (n = 3 or more). Real-time PCR data were analyzed using one-way repeated measures ANOVA performed with GraphPad Prism Version 5.0 (GraphPad Software). If a significant difference between multiple means was reported, ANOVAs were followed by Tukey's post-hoc test. A p value of <0.05 was considered significant. The significant difference is denoted with * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.001$).

2.4 Results

Phylogenetic relationships of IL-17 sequences identified in lampreys

By conducting similarity searches and homology inferences from genome and transcriptome data, we identified five different *IL-17* genes in the draft genome sequences of sea lamprey (*Petromyzon marinus*), eight *IL-17* genes in Japanese lamprey (*Lethenteron japonicum*) genome and five different *IL-17* sequences in transcriptomes of European brook lamprey (*Lampetra planeri*) (Table 2). Our phylogenetic analysis of the derived IL-17 C-terminal amino acid sequences from the three different lamprey species suggests that IL-17s in lampreys can be classified into five groups designated as IL-17-1, IL-17-2, IL-17-3, IL-17-4 and IL-17-5 (Figure 2-1, Figure 2-2). Among the eight sequences identifies in Japanese lamprey, IL-17-1, IL-17-3 and IL-17-4 have one to one orthologous relationship with the corresponding sequences in sea lamprey and European brook lamprey. Three additional IL-17 sequences of the Japanese lamprey (IL-17-2a, IL-17-2b, IL-17-2c) group with IL-17-2 sequences; two other IL-17 sequences (IL-17-5a, IL-17-5b) are related to IL-17-5 of sea lamprey and European brook lamprey (Figure 2-1, Figure 2-2). As the sea lamprey genome sequence is incomplete and the European brook lamprey genome sequence is not available, it is not possible to arrive at a definitive conclusion about possible duplication or deletion events affecting *IL-17* genes in each lineage after divergence of these three lamprey species. Compatible with the presumed cystine-knot structure of IL-17 proteins, the IL-17 sequences identified in three lamprey species all possess the four characteristic conserved cysteines (Figure 2-2). Based on phylogeny and conservation in amino acid residues, relative to the well-defined IL-17 family members in mammals, the lamprey IL-17-4 and IL-17-1 sequences are close to mammalian IL-17D, whereas IL-17-2, IL-17-3 and IL-17-5 sequences are similar to mammalian IL-17B, IL-17E and IL-17C sequences, respectively (Figure 2-1, Figure 2-2).

Notably, lamprey orthologues of mammalian IL-17A and IL-17F were not found. A full length clone of *IL-17-4* was derived by use of nested PCR.

Expression patterns of sea lamprey *IL-17* genes

The full length open reading frame of *IL-17-4* was cloned by nested PCR. After obtaining the full-length *IL-17-4* sequence and partial sequences for other *IL-17* family members, the expression patterns of *IL-17* genes were determined by real-time PCR analysis after validating the specificities of primers by sequencing amplicons generated by reverse-transcription PCR (data not shown). We found that *IL-17-4* was the most prominently expressed family member, and that it was expressed at highest levels in skin and gills, with lower levels being observed in the kidneys and white blood cells (WBC) (Figure 2-3). The other IL-17s in lampreys, *IL-17-1*, *IL-17-2*, *IL-17-3* and *IL-17-5*, were expressed at much lower levels, primarily in the intestine and gills. RNA *in situ* hybridization analysis indicated that the epithelial cells in the larval skin, gill filaments and intestine expressed *IL-17-4* transcripts (Figure 2-4). In a prior study, $VLRA^+$ lymphocytes were found to preferentially express an *IL-17* that corresponds to the presently defined *IL-17-4* member (Guo et al., 2009). In the present studies, we sorted the $VLRA^+$, $VLRB^+$, $VLRC^+$ and triple-negative cells within the lymphocyte gate and examined their expression of *IL-17-4*. The $VLRA^+$ and $VLRC^+$ lymphocytes were both found to express *IL-17-4* preferentially, whereas minimal *IL-17-4* expression was demonstrated for $VLRB^+$ lymphocytes and the triple-negative cells (Figure 2-3). Our findings thus indicate that *IL-17-4* is preferentially expressed by epithelial cells at barrier

surfaces in the gills, skin and intestine and by circulating $VLRA^+$ and $VLRC^+$ T cell-like lymphocytes.

Expression of IL-17-4 protein

Since *IL-17-4* proved to be the most highly expressed *IL-17* family member in lampreys, the following studies focused on its cytokine product. To obtain reagents for these studies, the coding sequence of the *IL-17-4* gene was subcloned in-frame with the Fc region of human IgG1 (IgG1-Fc) in a hybrid expression vector that was then used to transfect HEK-293T cells. The transfectants secreted the IL-17-4/IgG1-Fc fusion protein into the supernatant (Figure 2-5). The IgG1-Fc portion facilitated detection of the fusion protein with anti-IgG antibodies and its purification by protein A chromatography. The recombinant IL-17-4/IgG1-Fc fusion protein was then used as an immunogen to produce mouse mAbs, the IL-17-4 specificity of which were indicated by ELISA and Western blot assays. In keeping with our transcriptional analysis, IL-17-4 expression was demonstrated in skin, gills and intestine of lamprey larvae by Western blot analysis with an IL-17-4-specific mAb (clone 114-178) (Figure 2-6). The immunofluorescence staining of IL-17-4 by this mouse monoclonal anti-IL-17-4 antibody showed that it is expressed in the thymoid region at the tips of gill filaments (Figure 2-7). The expression is localized in the cytoplasm at the apical side of the tips where secretory vesicles have been shown by transmission electron microscopy (Bajoghli et al., 2011). The thymoid contains both epithelial cells (expression of *FOXN1* and *DLL-B* which is one of the key target genes of FOXN1), and lymphocytes in close proximity (expression of *CDA1* which is predicted to be associated with somatic diversification of the *VLRA* locus).

Activation of *IL-17-4* transcription by bacteria and yeast injection

To determine whether bacterial and yeast injection could induce *IL-17-4* transcription, real-time PCR was used to detect the transcripts in blood leukocytes and intestine sampled at different time points. At 24 hrs following injection of bacteria and yeast, a significant increase in *IL-17-4* and *IL-8* transcripts of blood leukocytes were detected; at day 7, the transcription decreased to the resting level (Figure 2-8). In the intestine, the *IL-17-4* transcripts were not significantly changed in all time points following injection. Interestingly, the increase of *IL-8* transcripts in the blood and intestine was detected at 24 hours and 48 hours respectively after bacterial and yeast injection, but not in the blood leukocytes.

2.5 Discussion

In our analysis of the lamprey *IL-17* ligand and receptor gene families, we have identified five members of the *IL-17* family of ligands (*IL-17-1* through *IL-17-5*). All five lamprey *IL-17*s encode the conserved C-terminal region with four cysteines that likely form the characteristic cystine-knot noted in human IL-17F; other cysteine residues in lamprey IL-17s may participate in homodimer or heterodimer formation via interchain disulfide bonds. It is noteworthy that, by examining genomic and transcriptome datasets of three lamprey species, we were unable to identify lamprey orthologues of mammalian IL-17A and IL-17F.

IL-17-4 (mammalian IL-17D like) was found to be highly expressed at both mRNA and protein levels in skin, intestine and gills of sea lamprey larvae, thereby

resembling the tissue distribution observed for *IL-17D* expression in teleost fish. *IL-17-4* expression was localized to epithelial cells in these barrier tissues, an expression pattern also noted for IL-17 family members in mammals. In mice and humans, epithelial cell secretion of IL-17C is induced by pathogen products and proinflammatory cytokines (Pappu et al., 2012). Furthermore, colonic epithelial cells express IL-17E in conventionally-reared mice, but not in germ-free mice (Zaph et al., 2008). The expression of IL-17 cytokines by epithelial cells is thought to be regulated through PRR sensing of pathogens or commensal microbiota (Wells et al., 2011). TLR expression in gills, intestine, kidney and other lamprey tissues (Kasamatsu et al., 2010a) potentially could affect the basal production of IL-17-4 to control infections and promote the VLR-mediated adaptive immune responses. The sensitivity of RNA in situ hybridization proved to be insufficient to detect the lower levels of *IL-17-4* expression in the lamprey blood cells. However, by real-time PCR, the T-like $VLRA^+$ and $VLRC^+$ cells were shown to express *IL-17-4*. In mammals, Th17 cells, γδ T cells and innate lymphoid cells have all been shown to produce proinflammatory IL-17 (Cua and Tato, 2010; Weaver et al., 2007).

IL-17-4 protein was expressed in the thymoid region shown by the immunofluorescence staining. The transcripts were also detected in the thymoid region at the tips of the gill filaments but the signal is much stronger in the epithelial cells of the other regions of gill filaments. The reason could be the translation of the IL-17-4 only takes place in the thymoid region or simply due to the cross-reactivity of the mouse monoclonal anti-IL-17-4 antibody (clone 114-178) in the immunofluorescence stain. More characterization of the specificity of this mAb is needed.

IL-17-4 transcripts were significantly increased in blood leukocytes in a time-dependent manner after infection that suggested IL-17-4 is involved in the antibacterial and antifungal immune defenses. On the other hand, *IL-8* was upregulated in the intestine suggesting its important roles in mucosal immune responses. This result is in accordance with the crucial role of IL-17 in antibacterial response in mammals (P Ye et al., 2001) and the findings in Pacific oyster and rainbow trout (Monte et al., 2013; Roberts et al., 2008).

In summary, we have identified five IL-17 family members in sea lamprey. *IL-17-4* was mainly expressed in the epithelium of skin, gills and intestine. In lamprey lymphocytes, $VLRA^+$ and $VLRC^+$ lymphocytes are the major producers of *IL-17-4*. After bacteria and yeast injection, *IL-17-4* and *IL-8* transcription are activated in blood leukocytes.

Table 1. List of primers used in the study

Primer name	Primer sequence (5'→3')	Use
qRT_IL-17-1_F4	gttcccgtcacccaggccg	real-time PCR
qRT_IL-17-1_R4	cacggcgatgttgtgcgtgtc	real-time PCR
qRT_IL-17-2_F2	gtctccgtgcccgtcat	real-time PCR
qRT_IL-17-2_R2	acggcgatctcctccca	real-time PCR
qRT_IL-17-3_F	gagcgctccctcgtctcc	real-time PCR
qRT_IL-17-3_R	cagctccggggctatctt	real-time PCR
qRT_IL-17-4_F	caagattccttgcgttaccc	real-time PCR
qRT_IL-17-4_R	tgtctctaggtccggcttgt	real-time PCR
qRT_IL-17-5_F1	acaggatcgtggagaacc	real-time PCR
qRT_IL-17-5_R1	gttgacctcggtcgtctc	real-time PCR
qRT_IL-17R1_F1	cgaggctgctgtatgtgaaa	real-time PCR
qRT_IL-17R1_R1	ctggaacggtgtgcatattgc	real-time PCR
qRT_IL-17R2_F	cagcagttggatcaaagcaa	real-time PCR
qRT_IL-17R2_R	tcttcccgaaattccacttg	real-time PCR
qRT_IL-17R3_F	gctctctcgcctcgtaccta	real-time PCR
qRT_IL-17R3_R	cctgcagccagttcatgtta	real-time PCR
qRT_IL-17R4_F	gccactgatatgtgccgact	real-time PCR
qRT_IL-17R4_R	aacgtcggggatgtctttct	real-time PCR
qRT_IL-17R5_F2	cttcacgtactcgtgtttct	real-time PCR
qRT_IL-17R5_R2	actctggcacggtgtagt	real-time PCR
qRT_IL-17R6_F6	caagacggtgaacgtgac	real-time PCR
qRT_IL-17R6_R6	cgtacaccacgtacgactc	real-time PCR
qRT_IL-8_F	cacagagctccaactgcaag	real-time PCR
qRT_IL-8_R	aatgtggctgatcaccttcc	real-time PCR
E2A_F1	ccaagctgggaatccttcac	real-time PCR
E2A_R1	cagctttcggattcaagttcct	real-time PCR
Pax5_F1	gacaatgtttgcctgggagatc	real-time PCR
Pax5_R1	ttccgttccgtgtgattctg	real-time PCR
Blimp1_F1	cctaccagtgtcaggtgtgc	real-time PCR
Blimp1_R1	ggagcttcaggtggatgaac	real-time PCR
Bcl6_F1	acacgggagagaagccctat	real-time PCR
Bcl6_R1	accttggtgtttgtgatggc	real-time PCR
Syk_F1	caagccctacccgaagatgaaag	real-time PCR
Syk_R1	cgggcaatgttccggttt	real-time PCR
BCAP_F1	gcccagcgtgtaaacccata	real-time PCR
BCAP_R1	acggcaacatgcacagtacct	real-time PCR
GAPDH_F	ttgaggatgggaagctgttga	real-time PCR
GAPDH_R	gggtcacgctccgagtagac	real-time PCR
clone IL-17R1_F3	gctgctgttgaatgagaacg	cloning
clone IL-17R1_R1	gcatcttgatattcgtgcattg	cloning
clone_IL-17R2_F1	tggtcttcgtggggtgtaga	cloning
clone_IL-17R2_F2	tagagggcctgcgtttatgg	cloning
clone_IL-17R2_R1	tgtgtgcttacgatggtacagttg	cloning

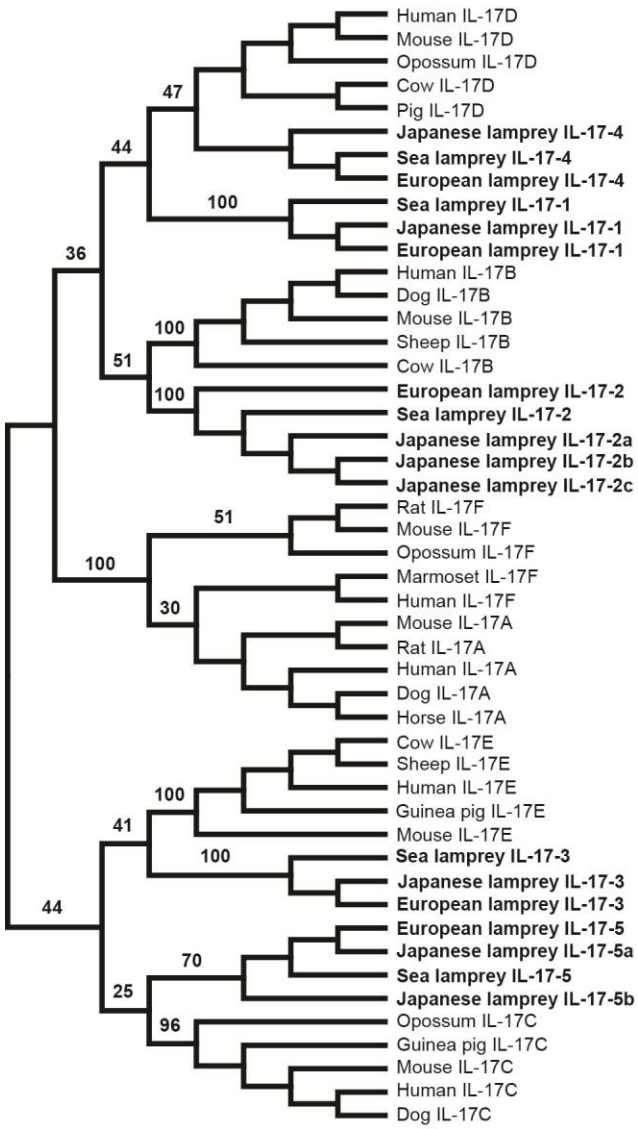
clone_IL-17R2_R2	tggacacgtagttttgggagttc	cloning
clone_IL-17R3_F1	atttcccgactgctggagtt	cloning
clone_IL-17R3_F2	actcgcgcagaggatagagg	cloning
clone_IL-17R3_R1	cccatgcaaaagaagttcaaca	cloning
clone_IL-17R3_R2	gctgatctcgatttcggtcac	cloning
clone IL-17-4_F2	acggcaggagagatacagga	cloning
clone IL-17-4_F3	agctggcacgttcttttgat	cloning
clone IL-17-4_Rout	cccacaggtccttctctcct	cloning
clone IL-17-4_R	ggggagaatggcaatcca	cloning
3RACE_IL-17R1_F	tggtccttgacctctggcaagtgaa	RACE
5RACE_IL-17R1_R	cgactttgcgtcctccttgagtgtg	RACE
3RACE_IL-17R2_F	gacagggaggatgcagtggctgatg	RACE
5RACE_IL-17R2_R	ctgagggcggggctgtacatgtct	RACE
3RACE_IL-17R3_F	ggaaggagaggcctccagcagagaa	RACE
5RACE_IL-17R3_R	gcagcgccatgcgtaatatctcagg	RACE
***IL-17-4* sense probe**	accession no. KR059941	in-situ hybridization

Table 2. List of *IL-17* and *IL-17R* genes in sea lamprey and Japanese lamprey

Lamprey species	Gene name	Source	Scaffold/Contig /Accession no.	Start	Stop	Strand
Sea Lamprey	*IL-17-1*	Genome	Sca2697	18751	18990	F
Sea Lamprey	*IL-17-2*	Genome	Sca995	164419	164667	R
Sea Lamprey	*IL-17-3*	Genome	Sca1481	2056	2310	F
Sea Lamprey	*IL-17-4*	Cloning	KR059941			
Sea Lamprey	*IL-17-5*	Transcriptome	KR059956			
Sea Lamprey	*IL-17R1*	Cloning	KR059942			
Sea Lamprey	*IL-17R2*	Cloning	KR059943			
Sea Lamprey	*IL-17R3*	Cloning	KR059944			
Sea Lamprey	*IL-17R4*	Genome	Sca1205	18952	19704	R
Sea Lamprey	*IL-17R5**	Genome	Sca14325	1319	6044	F
Sea Lamprey	*IL-17R6**	Genome	Sca563	63041	63220	R
European brook lamprey	*IL-17-1*	Transcriptome	KR059945			
European brook lamprey	*IL-17-2*	Transcriptome	KR059946			
European brook lamprey	*IL-17-3*	Transcriptome	KR059947			
European brook lamprey	*IL-17-4*	Transcriptome	KR059948			
European brook lamprey	*IL-17-5*	Transcriptome	KR059949			
European brook lamprey	*IL-17R1*	Transcriptome	KR059950			
European brook lamprey	*IL-17R2*	Transcriptome	KR059951			
European brook lamprey	*IL-17R3*	Transcriptome	KR059952			
European brook lamprey	*IL-17R4*	Transcriptome	KR059953			
European brook lamprey	*IL-17R5*	Transcriptome	KR059954			
European brook lamprey	*IL-17R6*	Transcriptome	KR059955			
Japanese Lamprey	*IL-17-1*	Genome	Sca00061	1103835	1104062	F
Japanese Lamprey	*IL-17-2a*	Genome	Sca00085	123306	124634	R
Japanese Lamprey	*IL-17-2b*	Genome	Sca00265	24268	25632	R
Japanese Lamprey	*IL-17-2c*	Genome	Sca00321	463705	463929	F
Japanese Lamprey	*IL-17-3*	Genome	Sca00508	279777	280001	F
Japanese Lamprey	*IL-17-4*	Genome	Sca00087	1656028	1658572	F
Japanese Lamprey	*IL-17-5a*	Genome	Sca02272	3558	4163	F
Japanese Lamprey	*IL-17-5b*	Genome	Con116163	186	419	R
Japanese Lamprey	*IL-17R1*	Genome	Sca415	633	4676	F
Japanese Lamprey	*IL-17R2*	Genome	Sca00374	461849	484143	F
Japanese Lamprey	*IL-17R3*	Genome	Sca00138	1586662	1590219	F
Japanese Lamprey	*IL-17R4*	Genome	Con062298	2	703	F
Japanese Lamprey	*IL-17R5*	Genome	Sca00976	58113	75959	R
Japanese Lamprey	*IL-17R6*	Genome	Sca00003	3120453	3209856	R

Note: * The sequence encoding conserved domain was partially identified or not found due to incompleteness of the genome sequence.

A


Human IL-17D
Mouse IL-17D
Opossum IL-17D
Cow IL-17D
Pig IL-17D
Japanese lamprey IL-17-4
Sea lamprey IL-17-4
European lamprey IL-17-4
Sea lamprey IL-17-1
Japanese lamprey IL-17-1
European lamprey IL-17-1
Human IL-17B
Dog IL-17B
Mouse IL-17B
Sheep IL-17B
Cow IL-17B
European lamprey IL-17-2
Sea lamprey IL-17-2
Japanese lamprey IL-17-2a
Japanese lamprey IL-17-2b
Japanese lamprey IL-17-2c
Rat IL-17F
Mouse IL-17F
Opossum IL-17F
Marmoset IL-17F
Human IL-17F
Mouse IL-17A
Rat IL-17A
Human IL-17A
Dog IL-17A
Horse IL-17A
Cow IL-17E
Sheep IL-17E
Human IL-17E
Guinea pig IL-17E
Mouse IL-17E
Sea lamprey IL-17-3
Japanese lamprey IL-17-3
European lamprey IL-17-3
European lamprey IL-17-5
Japanese lamprey IL-17-5a
Sea lamprey IL-17-5
Japanese lamprey IL-17-5b
Opossum IL-17C
Guinea pig IL-17C
Mouse IL-17C
Human IL-17C
Dog IL-17C
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100
30
100
41
100
44
70
25
96

B

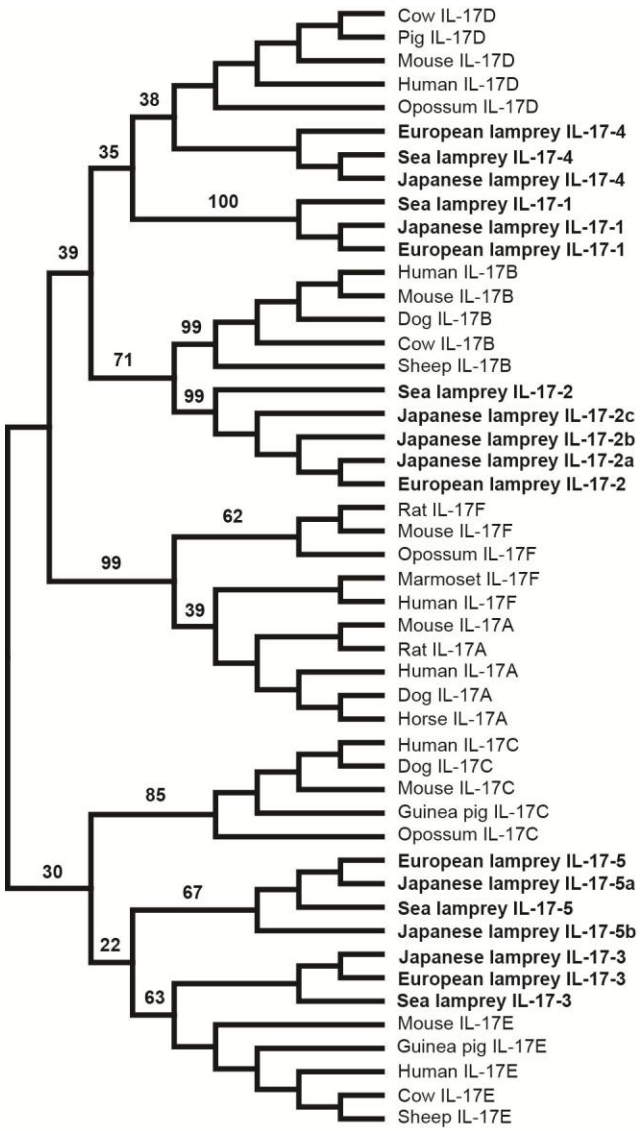

Cow IL-17D
Pig IL-17D
Mouse IL-17D
Human IL-17D
Opossum IL-17D
European lamprey IL-17-4
Sea lamprey IL-17-4
Japanese lamprey IL-17-4
Sea lamprey IL-17-1
Japanese lamprey IL-17-1
European lamprey IL-17-1
Human IL-17B
Mouse IL-17B
Dog IL-17B
Cow IL-17B
Sheep IL-17B
Sea lamprey IL-17-2
Japanese lamprey IL-17-2c
Japanese lamprey IL-17-2b
Japanese lamprey IL-17-2a
European lamprey IL-17-2
Rat IL-17F
Mouse IL-17F
Opossum IL-17F
Marmoset IL-17F
Human IL-17F
Mouse IL-17A
Rat IL-17A
Human IL-17A
Dog IL-17A
Horse IL-17A
Human IL-17C
Dog IL-17C
Mouse IL-17C
Guinea pig IL-17C
Opossum IL-17C
European lamprey IL-17-5
Japanese lamprey IL-17-5a
Sea lamprey IL-17-5
Japanese lamprey IL-17-5b
Japanese lamprey IL-17-3
European lamprey IL-17-3
Sea lamprey IL-17-3
Mouse IL-17E
Guinea pig IL-17E
Human IL-17E
Cow IL-17E
Sheep IL-17E
38
35
100
39
99
71
99
62
99
39
85
30
67
22
63

Figure 2-1. Phylogenetic comparison of lamprey IL-17s with mammalian IL-17s. The C-terminal conserved domain was used in the analysis. The phylogenetic tree is constructed by NJ (A) method and ML (B) method. The numbers indicate bootstrap confidence values obtained for each node after 1000 replications. The accession numbers for sequences used in this analysis were as follows: Human IL-17A, NP 002181; Dog IL-17A, NP 001159350; Mouse IL-17A, NP 034682; Rat IL-17A, NP 001100367; Horse IL-17A, NP 001137264; Monkey IL-17F, XP 002746687; Rat IL-17F, NP 001015011; Mouse IL-17F, NP 665855; Opossum IL-17F, XP 001370182; Human IL-17F, NP 443104; Human IL-17B, NP 055258; Dog IL-17B, XP 546311; Mouse IL-17B, NP 062381; Cow IL-17B, NP 001178974; Sheep IL-17B, XP 004009006; Human IL-17C, NP 037410; Mouse IL-17C, NP 665833; Guinea pig IL-17C, XP 003460965; Dog IL-17C, XP 851256; Opossum IL-17C, XP 007477360; Human IL-17D, NP 612141; Mouse IL-17D, NP 665836; Cow IL-17D, XP 871741; Pig IL-17D, XP 005653873; Opossum IL-17D, XP 001375129; Human IL-17E, NP 073626; Cow IL-17E, XP 605190; Guinea pig IL-17E, XP 003474349; Sheep IL-17E, NP 001182148; Mouse IL-17E, NP 542767.

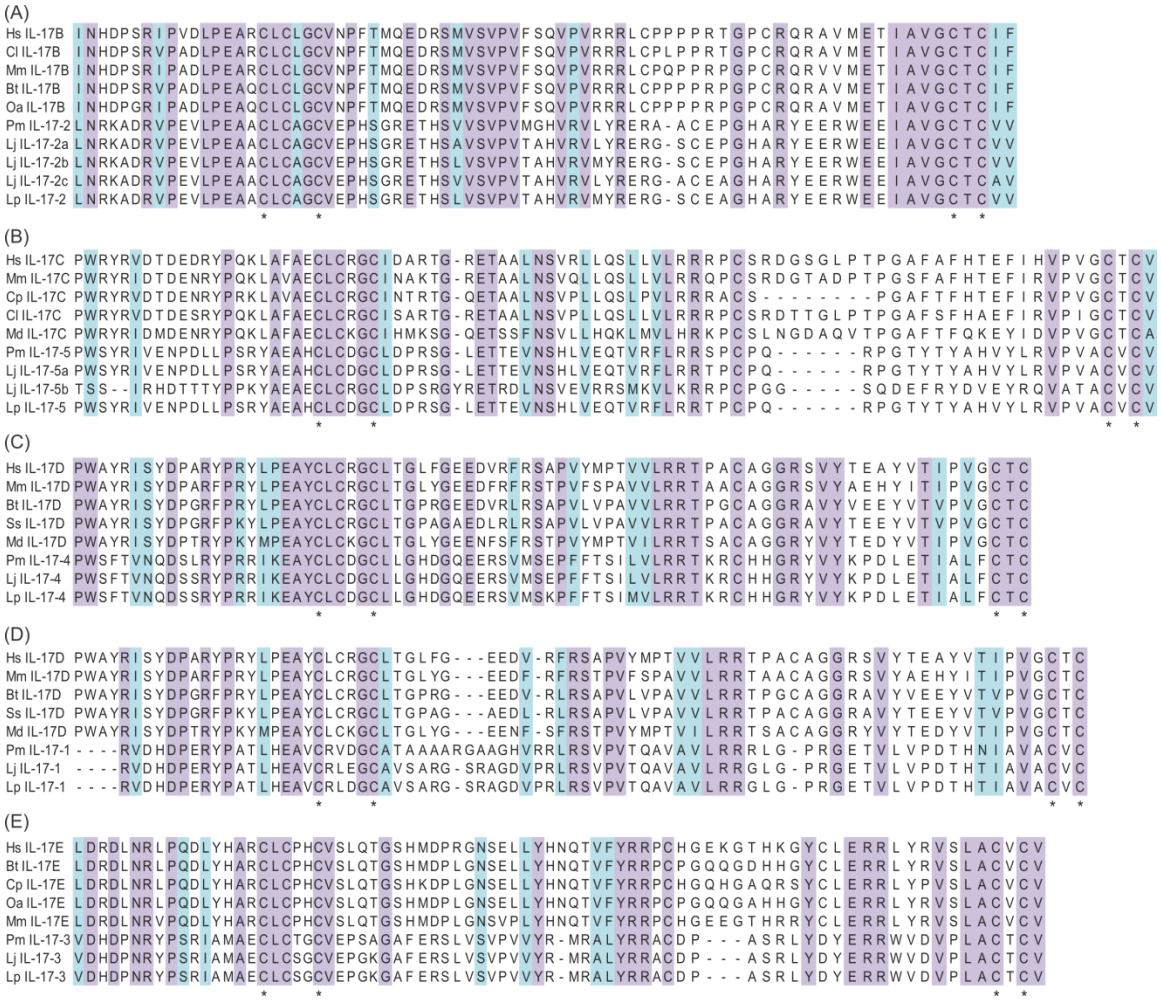
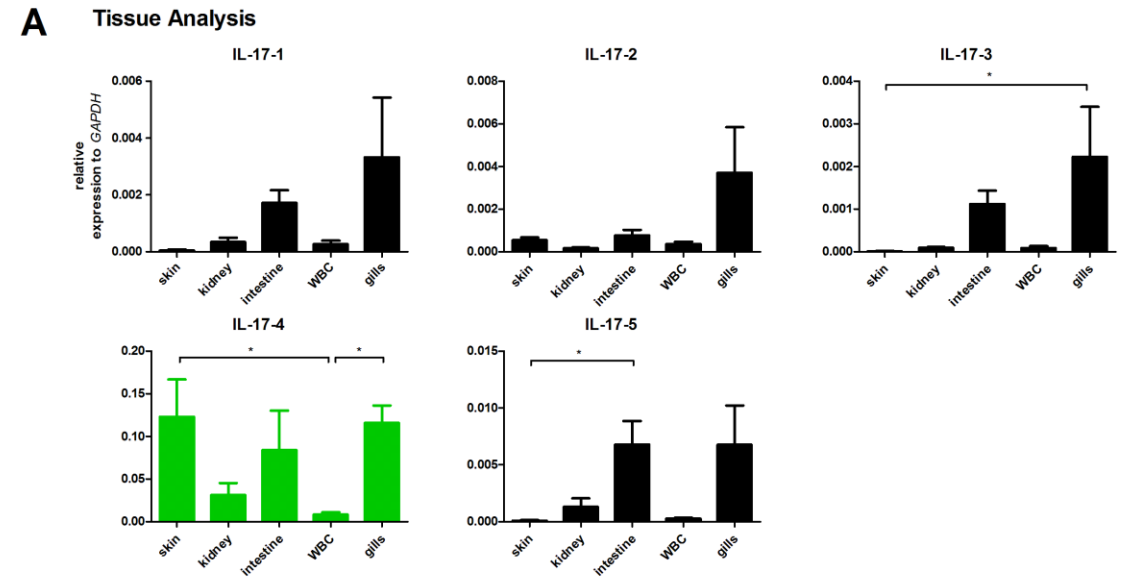
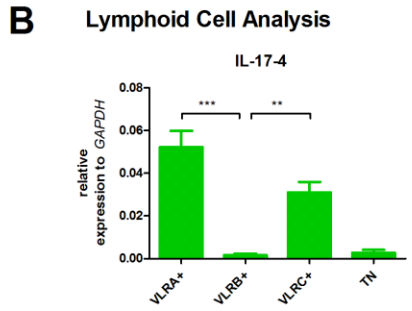

(A)
Hs IL-17B INHDPSRIPVDLPEARCLCLGCVNPFTMQEDRSMVSVPVFSQVPVRRRLCPPPPRTGPCRQRAVMETIAVGCTCIF
Cl IL-17B INHDPSRVPADLPEARCLCLGCVNPFTMQEDRSMVSVPVFSQVPVRRRLCPLPPRTGPCRQRAVMETIAVGCTCIF
Mm IL-17B INHDPSRIPADLPEARCLCLGCVNPFTMQEDRSMVSVPVFSQVPVRRRLCPQPPRPGPCRQRVVMETIAVGCTCIF
Bt IL-17B INHDPSRVPADLPEAQCLCLGCVNPFTMQEDRSMVSVPVFSQVPVRRRLCPPPPRPGPCRQRAVMETIAVGCTCIF
Oa IL-17B INHDPGRIPADLPEAQCLCLGCVNPFTMQEDRSMVSVPVFSQVPVRRRLCPPPPRPGPCRQRAVMETIAVGCTCIF
Pm IL-17-2 LNRKADRVPEVLPEAACLCAGCVEPHSGRETHSVVSVPVMGHVRVLYRERA-ACEPGHARYEERWEEIAVGCTCVV
Lj IL-17-2a LNRKADRVPEVLPEAACLCAGCVEPHSGRETHSAVSVPVTAHVRVLYRERG-SCEPGHARYEERWEEIAVGCTCVV
Lj IL-17-2b LNRKADRVPEVLPEAACLCAGCVEPHSGRETHSLVSVPVTAHVRVMYRERG-SCEPGHARYEERWEEIAVGCTCVV
Lj IL-17-2c LNRKADRVPEVLPEAACLCAGCVEPHSGRETHSVVSVPVTAHVRVLYRERG-ACEAGHARYEERWEEIAVGCTCAV
Lp IL-17-2 LNRKADRVPEVLPEAACLCAGCVEPHSGRETHSLVSVPVTAHVRVMYRERG-SCEAGHARYEERWEEIAVGCTCVV
(B)
Hs IL-17C PWRYRVDTDEDRYPQKLAFAECLCRGCIDARTG-RETAALNSVRLLQSLLVLRRRPCSRDGSGLPTPGAFAFHTEFIHVPVGCTCV
Mm IL-17C PWRYRIDTDENRYPQKLAVAECLCRGCINAKTG-RETAALNSVQLLQSLLVLRRQPCSRDGTADPTPGSFAFHTEFIRVPVGCTCV
Cp IL-17C PWRYRVDTDENRYPRKLAVAECLCRGCINTRTG-QETAALNSVPLLQSLPVLRRRACS--------PGAFTFHTEFIRVPVGCTCV
Cl IL-17C PWRYRVDTDESRYPQKLAFAECLCRGCISARTG-RETAALNSVPLLQSLLVLRRRPCSRDTTGLPTPGAFSFHAEFIRVPIGCTCV
Md IL-17C PWRYRIDMDENRYPQKLAFAECLCKGCIHMKSG-QETSSFNSVLLHQKLMVLHRKPCSLNGDAQVTPGAFTFQKEYIDVPVGCTCA
Pm IL-17-5 PWSYRIVENPDLLPSRYAEAHCLCDGCLDPRSG-LETTEVNSHLVEQTVRFLRRSPCPQ------RPGTYTYAHVYLRVPVACVCV
Lj IL-17-5a PWSYRIVENPDLLPSRYAEAHCLCDGCLDPRSG-LETTEVNSHLVEQTVRFLRRTPCPQ------RPGTYTYAHVYLRVPVACVCV
Lj IL-17-5b TSS--IRHDTTTYPPKYAEAECLCRGCLDPSRGYRETRDLNSVEVRRSMKVLKRRPCPGG-----SQDEFRYDVEYRQVATACVCV
Lp IL-17-5 PWSYRIVENPDLLPSRYAEAHCLCDGCLDPRSG-LETTEVNSHLVEQTVRFLRRTPCPQ------RPGTYTYAHVYLRVPVACVCV
(C)
Hs IL-17D PWAYRISYDPARYPRYLPEAYCLCRGCLTGLFGEEDVRFRSAPVYMPTVVLRRTPACAGGRSVYTEAYVTIPVGCTC
Mm IL-17D PWAYRISYDPARFPRYLPEAYCLCRGCLTGLYGEEDFRFRSTPVFSPAVVLRRTAACAGGRSVYAEHYITIPVGCTC
Bt IL-17D PWAYRISYDPGRFPRYLPEAYCLCRGCLTGPRGEEDVRLRSAPVLVPAVVLRRTPGCAGGRAVYVEEYVTVPVGCTC
Ss IL-17D PWAYRISYDPGRFPKYLPEAYCLCRGCLTGPAGAEDLRLRSAPVLVPAVVLRRTPACAGGRAVYTEEYVTVPVGCTC
Md IL-17D PWAYRISYDPTRYPKYMPEAYCLCKGCLTGLYGEENFSFRSTPVYMPTVILRRTSACAGGRYVYTEDYVTIPVGCTC
Pm IL-17-4 PWSFTVNQDSLRYPRRIKEAYCLCDGCLLGHDGQEERSVMSEPFFTSILVLRRTKRCHHGRYVYKPDLETIALFCTC
Lj IL-17-4 PWSFTVNQDSSRYPRRIKEAYCLCDGCLLGHDGQEERSVMSEPFFTSILVLRRTKRCHHGRYVYKPDLETIALFCTC
Lp IL-17-4 PWSFTVNQDSSRYPRRIKEAYCLCDGCLLGHDGQEERSVMSKPFFTSIMVLRRTKRCHHGRYVYKPDLETIALFCTC
(D)
Hs IL-17D PWAYRISYDPARYPRYLPEAYCLCRGCLTGLFG---EEDV-RFRSAPVYMPTVVLRRTPACAGGRSVYTEAYVTIPVGCTC
Mm IL-17D PWAYRISYDPARFPRYLPEAYCLCRGCLTGLYG---EEDF-RFRSTPVFSPAVVLRRTAACAGGRSVYAEHYITIPVGCTC
Bt IL-17D PWAYRISYDPGRFPRYLPEAYCLCRGCLTGPRG---EEDV-RLRSAPVLVPAVVLRRTPGCAGGRAVYVEEYVTVPVGCTC
Ss IL-17D PWAYRISYDPGRFPKYLPEAYCLCRGCLTGPAG---AEDL-RLRSAPVLVPAVVLRRTPACAGGRAVYTEEYVTVPVGCTC
Md IL-17D PWAYRISYDPTRYPKYMPEAYCLCKGCLTGLYG---EENF-SFRSTPVYMPTVILRRTSACAGGRYVYTEDYVTIPVGCTC
Pm IL-17-1 ----RVDHDPERYPATLHEAVCRVDGCATAAAARGAAGHVRRLRSVPVTQAVAVLRRRLG-PRGETVLVPDTHNIAVACVC
Lj IL-17-1 ----RVDHDPERYPATLHEAVCRLEGCAVSARG-SRAGDVPRLRSVPVTQAVAVLRRGLG-PRGETVLVPDTHTIAVACVC
Lp IL-17-1 ----RVDHDPERYPATLHEAVCRLDGCAVSARG-SRAGDVPRLRSVPVTQAVAVLRRGLG-PRGETVLVPDTHTIAVACVC
(E)
Hs IL-17E LDRDLNRLPQDLYHARCLCPHCVSLQTGSHMDPRGNSELLYHNQTVFYRRPCHGEKGTHKGYCLERRLYRVSLACVCV
Bt IL-17E LDRDLNRLPQDLYHARCLCPHCVSLQTGSHMDPLGNSELLYHNQTVFYRRPCPGQQGDHHGYCLERRLYRVSLACVCV
Cp IL-17E LDRDLNRLPQDLYHARCLCPHCVSLQTGSHKDPLGNSELLYHNQTVFYRRPCHGQHGAQRSYCLERRLYPVSLACVCV
Oa IL-17E LDRDLNRLPQDLYHARCLCPHCVSLQTGSHMDPLGNSELLYHNQTVFYRRPCPGQQGAHHGYCLERRLYRVSLACVCV
Mm IL-17E LDRDLNRVPQDLYHARCLCPHCVSLQTGSHMDPLGNSVPLYHNQTVFYRRPCHGEEGTHRRYCLERRLYRVSLACVCV
Pm IL-17-3 VDHDPNRYPSRIAMAECLCTGCVEPSAGAFERSLVSPVVYR-MRALYRRACDP---ASRLYDYERRWVDVPLACTCV
Lj IL-17-3 VDHDPNRYPSRIAMAECLCSGCVEPGKGAFERSLVSPVVYR-MRALYRRACDP---ASRLYDYERRWVDVPLACTCV
Lp IL-17-3 VDHDPNRYPSRIAMAECLCSGCVEPGKGAFERSLVSPVVYR-MRALYRRACDP---ASRLYDYERRWVDVPLACTCV

Figure 2-2. Sequence alignment of C-terminal residues of lamprey IL-17s with mammalian IL-17s. The alignment was achieved by using Clustal W (Larkin et al., 2007). Identical residues are boxed in purple and highly conserved residues in blue. The four cysteines that may form the cystine-knot structure in the IL-17 family are marked with asterisks. Pm IL-17s: sea lamprey IL-17s; Lj IL-17s: Japanese lamprey IL-17s; Lp: European brook lamprey IL-17s. The accession numbers for sequences used in this analysis are listed in Fig. 2-1.


A
Tissue Analysis
IL-17-1
IL-17-2
IL-17-3
IL-17-4
IL-17-5
relative expression to GAPDH
skin
kidney
intestine
WBC
gills


B
Lymphoid Cell Analysis
IL-17-4
relative expression to GAPDH
VLRA+
VLRB+
VLRC+
TN

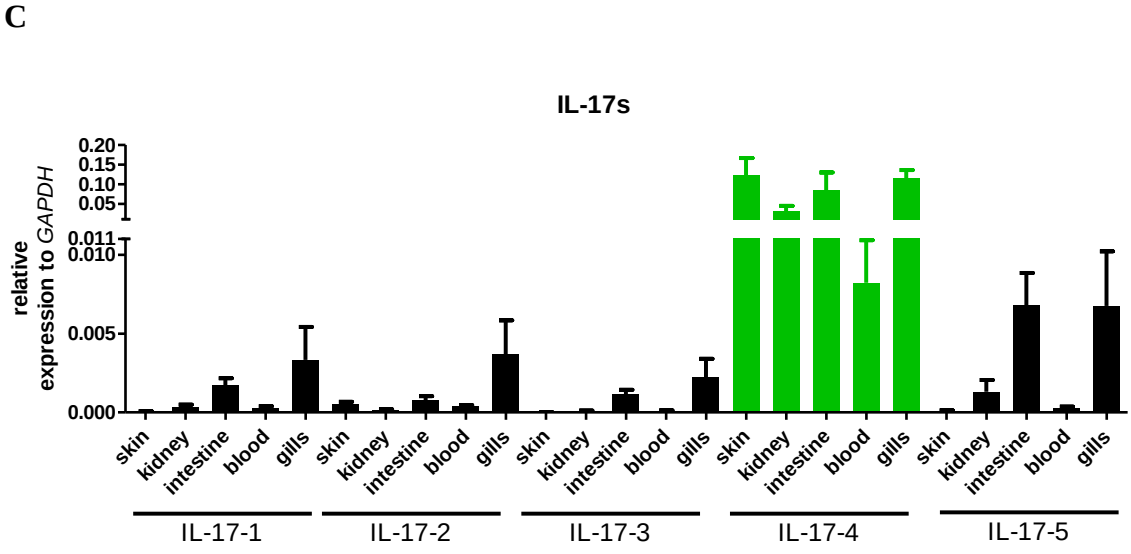

C
IL-17s
relative expression to GAPDH
skin
kidney
intestine
blood
gills
IL-17-1
IL-17-2
IL-17-3
IL-17-4
IL-17-5

Figure 2-3. Sea lamprey expression of *IL-17* transcripts. (A) Tissue distribution. Relative expression levels of *IL-17-1*, *IL-17-2*, *IL-17-3*, *IL-17-4* and *IL-17-5* transcripts were determined by real-time PCR analysis of skin, kidneys, intestine, white blood cells (WBC) and gills of sea lamprey larvae (n=6 larvae). (B) *IL-17-4* expression by sorted $VLRA^+$, $VLRB^+$, $VLRC^+$ and triple negative (TN) lymphocyte populations (n=6 larvae). Relative expression level of *IL-17-4* was determined by real-time PCR. mRNA abundance relative to that of GAPDH: $2^{-\Delta Ct}$, $\Delta Ct = Ct_{\text{target gene}} - Ct_{GAPDH.}$ Error bars indicate SEM. The scales of the y-axis are different on each graph. The data are plotted on the same scale (fragmented y-axis) in (C).

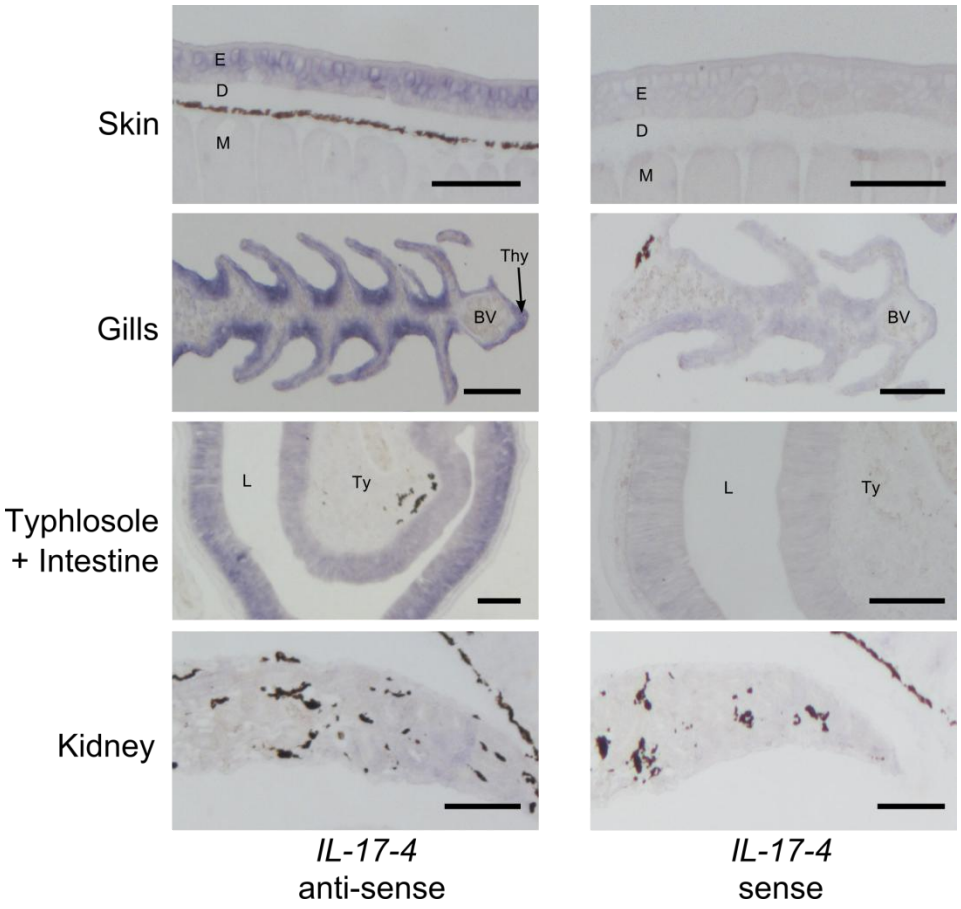

Skin
Gills
Typhlosole
+ Intestine
Kidney
E
D
M
Thy
BV
L
Ty
IL-17-4
anti-sense
IL-17-4
sense

Figure 2-4. Cellular expression of *IL-17-4* transcripts in different tissues. Tissue sections of skin, gills, kidney, typhlosole and intestine of lamprey larvae were hybridized with sense (control) and anti-sense riboprobes. Positive hybridization signals with *IL-17-4* riboprobes are indicated by blue color. E, epidermis; D, dermis; M, muscle; Thy, thymoid; BV, blood vessel; L, lumen; Ty, typhlosole. Scale bars, 100 μm.

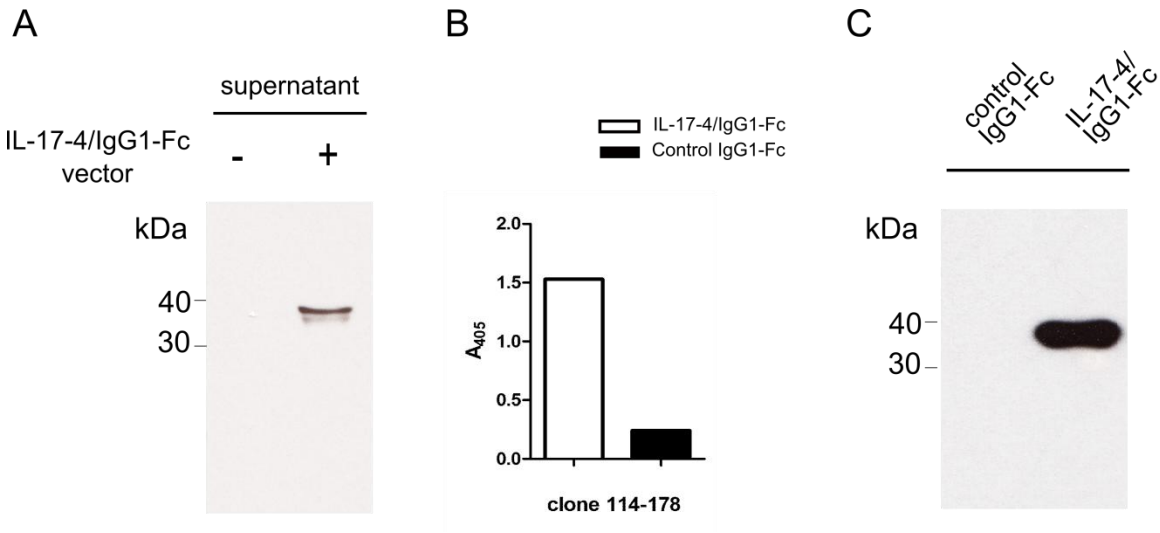

A
supernatant
IL-17-4/IgG1-Fc vector
-
+
kDa
40
30
B
IL-17-4/IgG1-Fc
Control IgG1-Fc
2.0
1.5
1.0
0.5
0.0
A405
clone 114-178
C
control IgG1-Fc
IL-17-4/ IgG1-Fc
kDa
40
30

Figure 2-5. Production of IL-17-4/IgG1-Fc fusion protein and anti-IL-17-4 mAbs. (A) Production of IL-17-4/IgG1-Fc fusion protein by HEK-293T transfectants. HEK-293T cells were transfected with control plasmids (-) or plasmids expressing IL-17-4/IgG1-Fc fusion protein (+). Supernatants were collected after 48 hours and resolved on reducing SDS-PAGE gel before Western blotting with goat anti-human IgG-HRP. (B) Reactivity of a mouse anti-IL-17-4 mAb (clone114-178) with control IgG1-Fc and IL-17-4/IgG1-Fc proteins, measured by ELISA. (C) Western blot with clone 114-178. Control IgG1-Fc and IL-17-4/IgG1-Fc fusion protein were resolved on reducing SDS-PAGE gel before Western blotting with clone114-178 (1:1000) and goat anti-mouse IgG-HRP.

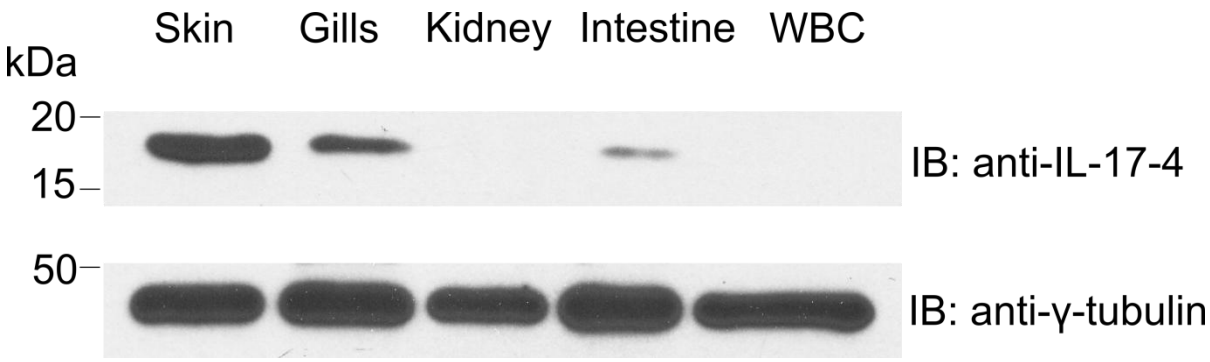

Skin
Gills
Kidney
Intestine
WBC
kDa
20
15
50
IB: anti-IL-17-4
IB: anti-γ-tubulin

Figure 2-6. Tissue distribution of IL-17-4 protein expression. Whole tissue lysates from skin, gills, kidneys, intestine, and white blood cells (WBC) were resolved by reducing SDS-PAGE and blotted with a mouse monoclonal anti-IL-17-4 antibody (clone 114-178). γ-Tubulin served as the loading control.

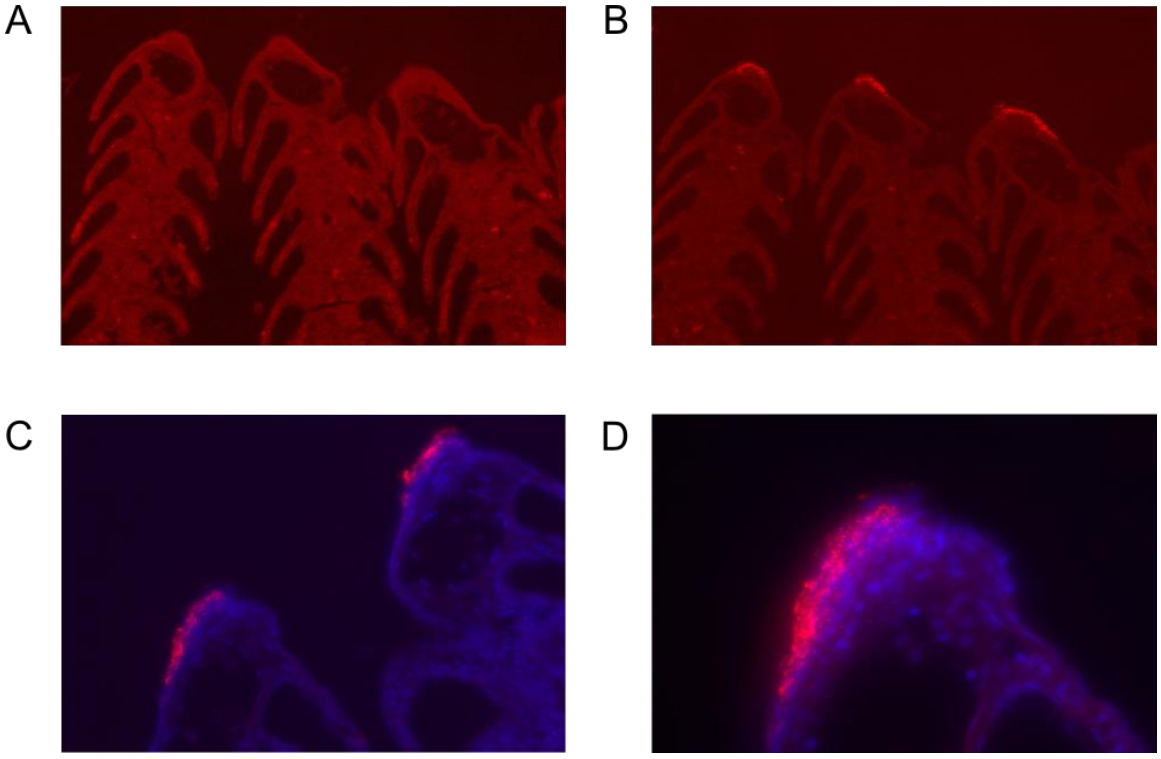

A
B
C
D

Figure 2-7. Immunofluorescence staining of IL-17-4 in gill region of lamprey larvae. (A) Negative control (10x) (B) Immunofluorescence staining (red) by a mouse anti-IL-17-4 mAb clone 114-178 (10x). (C,D) Immunofluorescence staining of IL-17-4 (red) and DAPI counterstaining of nuclei (blue), C, 20x; D,40x.

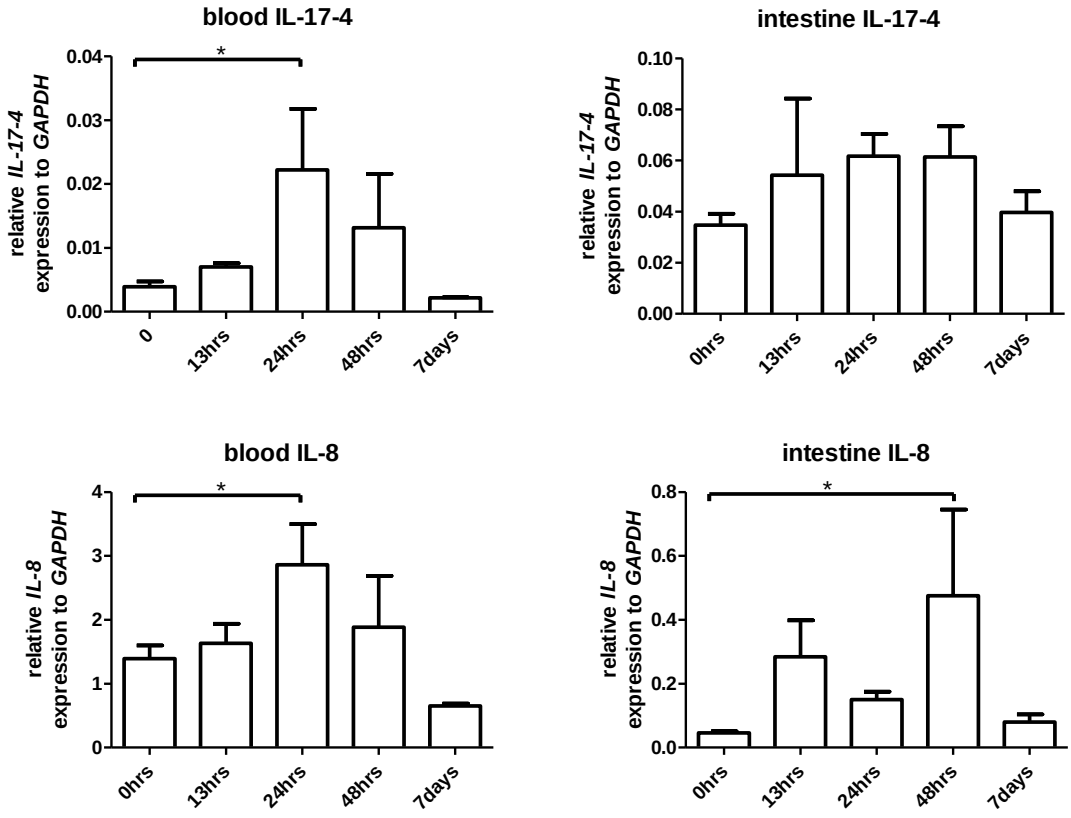

blood IL-17-4
relative IL-17-4 expression to GAPDH
0.04
0.03
0.02
0.01
0.00
*
0
13hrs
24hrs
48hrs
7days
intestine IL-17-4
relative IL-17-4 expression to GAPDH
0.10
0.08
0.06
0.04
0.02
0.00
0hrs
13hrs
24hrs
48hrs
7days
blood IL-8
relative IL-8 expression to GAPDH
4
3
2
1
0
*
0hrs
13hrs
24hrs
48hrs
7days
intestine IL-8
relative IL-8 expression to GAPDH
0.8
0.6
0.4
0.2
0.0
*
0hrs
13hrs
24hrs
48hrs
7days

Figure 2-8. Blood leukocytes and intestine expression of *IL-17-4* and *IL-8* transcripts following bacterial and yeast injection. The expression levels were examined at 13, 24, 48 hours and 7 days (n=3 larvae). mRNA abundance relative to that of GAPDH: $2^{-\Delta Ct}$, $\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{GAPDH.}}$ Error bars indicate SEM. The scales of the y-axis are different on each graph.

Chapter 3

Characterization of lamprey IL-17 receptor family members

This chapter includes work submitted to Journal of Immunology as Han, Q.F., Das, S., Hirano, M., Holland, S., McCurley, N., Guo, P., Boehm, T. and Cooper, M.D.. Characterization of lamprey IL-17 family members and their receptors. The majority of the work was done by Qifeng Han. Sabyasachi Das and Masyuki Hirano helped with experimental design and data analysis. Max D. Cooper and Thomas Boehm edited the manuscript for publication.

3.1 Abstract

IL-17-4 was shown earlier to be expressed preferentially by T-like lymphocytes and epithelial cells of skin, intestine and gills. Here, we have characterized six IL-17 receptor genes (*IL-17R1* to *IL-17R6*) identified in the sea lamprey genome. *IL-17R3* was the most expressed member with its highest expression in the intestine and blood cells including $VLRA^+$ and $VLRC^+$ lymphocytes. *IL-17R1* was found to be expressed preferentially by $VLRB^+$ lymphocytes and monocytes in the blood. A recombinant fusion protein (IL-17-4/IgG1-Fc) was used to demonstrate IL-17-4 binding by B-like cells and monocytes, but not by T-like cells or granulocytes. This recombinant protein stimulated the transcription of some of the B-like cell-expressed genes by blood leukocytes. Our composite findings thus suggest an IL-17 mediated mechanism for coordinating the interaction of T-like cells with cells of both adaptive and innate immune systems in this jawless vertebrate.

3.2 Introduction

The mammalian IL-17R family consists of five members (IL-17RA to IL-17RE) which share limited sequence similarity with other cytokine receptors. Members of the IL-17R family have extracellular fibronectin III-like domains, a single transmembrane domain and a conserved cytoplasmic domain called “similar expression to fibroblast growth factor genes and IL-17R” (SEFIR) that also resembles the Toll/IL-1 receptor (TIR) domain. IL-17RA is the common receptor subunit in that it pairs with most other IL-17R members for the binding of IL-17 family members. In humans, IL-17RA forms a

heterodimer with IL-17RC for IL-17A or IL-17F signaling. IL-17RA has higher affinity for IL-17A, while both IL-17A and IL-17F bind to IL-17RC with similar affinities (Kuestner et al., 2007; Toy et al., 2006; Wright et al., 2008). IL-17RA is expressed on both hematopoietic cells, including B cells and T cells, and non-hematopoietic cells such as fibroblasts, endothelial cells, and epithelial cells (Yao et al., 1995). In *IL-17RA* knockout mice, host defense against infectious *Klebsiella pneumoniae* and *Candida albicans* is compromised, thereby suggesting important roles of IL-17RA and its ligands IL-17A and IL-17F in immune defense (Conti et al., 2009; Huang et al., 2004; Peng Ye et al., 2001). IL-17RB binds IL-17B and can also pair with IL-17RA to bind IL-17E (Gaffen, 2009). The IL-17RA and IL-17RE receptor complex is required for IL-17C signaling (Chang et al., 2011; Ramirez-Carrozzi et al., 2011; Song et al., 2011). The receptor for IL-17D has not yet been identified; IL-17RD is an orphan receptor without an identified ligand. IL-17R orthologues have been found in the invertebrates such as sea urchin, amphioxus and vase tunicate (Hibino et al., 2006; Terajima et al., 2003; Vizzini et al., 2014; Wu et al., 2011b). IL-17RA and IL-17RD orthologues have been identified in the amphioxus genome sequences (Wu et al., 2011b), but the cloning and characterization of these invertebrate *IL-17R* genes have not yet been reported. Several incomplete sequences of IL-17Rs including IL-17RA, IL-17RB, IL-17RC and IL-17RD have been identified in the genome sequence of the cartilaginous fish, elephant shark (Wu et al., 2011b), and IL-17RE (GenBank accession no. XP_007909458) has also been identified in this cartilaginous fish by NCBI Eukaryotic Genome Annotation Pipeline. In most teleost fish, IL-17RA and IL-17RD orthologues have been identified. IL-17RA sequences were reported in fugu, Atlantic salmon, rainbow trout, stickleback, medaka and zebrafish

(Kono et al., 2011; Monte et al., 2013; Wu et al., 2011b). IL-17RD orthologues were found in zebrafish, fugu, green puffer, stickleback and medaka (Kono et al., 2011; Tsang et al., 2002; Wu et al., 2011b). The presence of IL-17RB, IL-17RC and IL-17RE orthologues has been confirmed in the genome sequences of stickleback and zebrafish (Kono et al., 2011). A group of vertebrate IL-17R-like proteins, which lack the intracellular conserved SEFIR domain but resemble the extracellular domain of IL-17RE, have been recently identified in zebrafish, fugu and stickleback (Wu et al., 2011b) and named IL-17RE-like (IL-17REL) proteins.

An *IL-17R* orthologue has also been identified in lampreys and was expressed preferentially by $VLRB^+$ lymphocytes (Guo et al., 2009). Five lamprey IL-17 family members have been characterized in Chapter 2 and IL-17-4 was expressed preferentially by epithelial and T-like lymphocytes. In the present studies, we have defined the members of the lamprey IL-17R family, determined their expression patterns, and examined the potential interaction of IL-17-4 with its receptor and the function of IL-17-4.

3.3 Materials and Methods

Identification of IL-17R sequences from lamprey genome

Known mammalian IL-17R sequences were used as queries for tBLASTn search against sea lamprey and Japanese lamprey genome sequences. Unique ORF collections (Holland *et al*., manuscript in preparation) from European brook lamprey transcriptomes were interrogated using IL-17R sequences of various species. The signal peptide,

transmembrane and putative domains were predicted by SMART software (http://smart.embl-heidelberg.de) and conserved domain database (CDD) of NCBI. The conserved SEFIR domain of IL-17Rs was used in the phylogenetic analysis. Multiple protein sequence alignments and the phylogenetic analysis for IL-17R were performed as described in the Method section of Chapter 2.

Cloning and sequencing sea lamprey (*Petromyzon marinus*) *IL-17Rs*

Sea lamprey larvae were collected and housed as described in Method section of Chapter 2. Total RNA was extracted from lymphoid tissues of lamprey larvae. First-strand cDNA was synthesized with oligo(dT) primer by Superscript III (Invitrogen). The 5' and 3' end of *IL-17R* genes were obtained by SMARTER RACE cDNA Amplification Kit (Clontech). 5' RACE cDNA was synthesized by touchdown PCR using a modified oligo(dT) primer and SMARTer II A oligonucleotides and 3' RACE cDNA synthesis was carried out using a special oligo(dT) primer. The 5' and 3' RACE PCR products were cloned into pBluescript (Stratagene) vector and sequenced. According to the complete sequence information, PCR primers were then designed and nested PCR was performed to clone the *IL-17R* genes. Primers used for RACE and nested PCR are all listed in Table 1.

Real-time PCR

Real-time PCR was performed as described in Method section of Chapter 2. Primers used in this experiment are listed in Table 1.

IL-17R expression in HEK-293T cells

HEK-293T cells were maintained as described in Method section of Chapter 2. The coding sequence of IL-17R1, IL-17R2 or IL-17R3 was subcloned into pDisplay vector (Invotrogen) in-frame with HA tag, myc tag and FLAG tag respectively. The plasmids were transfected into HEK-293T cells and the cell lysates was harvested as described in Method section of Chapter 2.

Production of anti-monocyte and anti-granulocyte mAbs

Anti-monocyte mouse mAb (8A1, IgG2b) and anti-granulocyte mAb (2D4, IgG2a) were produced by immunization of BALB/c mice with fresh peripheral blood leukocytes from the adult lamprey and screened by flow cytometry using FSC/SSC. Two distinct populations of leukocytes were stained by 8A1 and 2D4 antibodies (Figure 3-9). FSC/SSC profile and Wright-Giemsa staining of sorted monocytes (Figure 3-9) and granulocytes (Figure 3-9) are shown.

Immunoblotting

The primary antibodies used in this study include mouse monoclonal anti-HA (HA.11, 1:1000, Covance), mouse monoclonal anti-myc (9B11, 1:1000, Cell signaling), mouse monoclonal anti-FLAG M2 (1:1000, Sigma) and secondary antibodies including HRP conjugated goat anti-mouse Ig (1:10000, Dako). The other procedures are described in Method section of Chapter 2

Flow cytometric analysis and sorting

Leukocytes isolated from blood were stained for flow cytometry as described. Briefly, leukocytes from blood were stained with primary antibodies including anti-VLRA rabbit polyclonal serum (R110), anti-VLRB mouse mAb (4C4), anti-VLRC mouse mAb (3A5), anti-monocyte mouse mAb (8A1) or anti-granulocyte mouse mAb (2D4). The cells were costained with recombinant IL-17-4/IgG1-Fc or control proteins. Secondary antibodies were matched. Staining and washes were in 0.67 × PBS with 1% BSA. Cells were gated as described in Method section of Chapter 2. Flow cytometric analysis was performed on a CyAn ADP (Dako) flow cytometer. $VLRA^+$, $VLRB^+$, $VLRC^+$, VLR triple-negative cells, monocytes, and granulocytes were sorted on BD FACS Aria II (BD Bioscience) for real-time PCR analysis. The purity of the sorted cells was over 90%.

Immunoprecipitation

The IL-17R expressing plasmids were transfected into HEK-293T cells using PEI and the whole cell lysates were used for immunoprecipitation assays. Samples were pre-cleared by incubating with 30 μl of a protein G-agarose suspension for 30 min followed by centrifugation at 12,000*g* for 1 min to remove the beads. The pre-cleared lysates were incubated with 30 μl of the protein G-agarose suspension and IL-17-4/IgG1-Fc or control protein on a rotator for 2 hours at 4 degree. Protein G-agarose beads were centrifuged at 12,000*g* for 12 seconds and washed 4 times with 1% NP-40 lysis buffer. Samples treated with 2-mercaptoethanol were separated on 10% SDS–PAGE gels and then transferred onto nitrocellulose membranes (Millipore) and immunoblotted with corresponding antibodies.

Proliferation assay

Lampreys injected with IL-17-4/IgG1-Fc or control Fc proteins were injected with 5 μg of 5-ethynyl-2′-deoxyuridine (EdU) (Click-iT Plus EdU Alexa Fluor 488 Flow Cytometry Assay Kit, Invitrogen) in 30 μl 0.67× PBS at 24 h before collection of leukocytes for staining. Stained cells were fixed in 4% paraformaldehyde for 15 min at room temperature, centrifuged and then resuspended in 100 μl of the 1× saponin-based permeabilization and wash buffer for 15 min on ice. After centrifugation, cells were incubated with the EdU reaction cocktail, which contains Alexa Fluor 488-azide, Copper protectant and Reaction Buffer Additive, for 30 min at room temperature, washed twice in 1 × saponin-based permeabilization and wash buffer, and analysed by flow cytometry.

Cell culture with IL-17-4 recombinant protein

Buffy coat leukocytes were collected, washed twice with and resuspended in 2/3 IMDM medium (HyClone, GE Healthcare) supplemented with 100 IU/ml of penicillin sulfate and 100 μg/ml of streptomycin. For each lamprey larvae (n=5), blood leukocytes were separated into three groups (saline, IL-17-4/IgG1-Fc, control Fc) and seeded in a sterile 24-well plate at a density of 1.0×10^6 cells/ml in 1.0 ml medium. IL-17-4/IgG1-Fc and control Fc were added to the blood leukocytes at a final concentration of 2μg/ml and equal volume of PBS was also added as the saline control. Cells were cultured at 20 ℃ for 16 h and then collected for RNA extraction and real-time PCR analysis.

ELISA

For protein ELISA (enzyme-linked immunosorbent assay), plates were coated with IL-17-4/IgG1-Fc fusion protein or control protein for 12h at 4 ℃ and then blocked with 1% BSA (Sigma) in PBS for 2 h at 37 ℃. The supernatants from hybridoma culture were added for 1 h at 37 ℃. After washing, alkaline phosphatase-conjugated goat anti-mouse immunoglobulin antibodies (Southern Biotech, 1:1000) in PBS with 1% BSA were added and incubated for 1 h at 37 ℃ to detect the IL-17-4 reactivity of antibodies. After washing, the plates were developed with phosphatase substrate (Sigma) and read at 405nm (Versamax microplate reader, Molecular Devices, Sunnyvale, CA).

Statistical analysis

For western blotting analysis and flow cytometry staining, data were generated from 3 or more experiments. All data are expressed as the mean ± SEM (n = 3 or more). Real-time PCR data were analyzed using one-way repeated measures ANOVA performed with GraphPad Prism Version 5.0 (GraphPad Software). If a significant difference between multiple means was reported, ANOVAs were followed by Tukey's post-hoc test. A p value of <0.05 was considered significant. The significant difference is denoted with * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.001$).

3.4 Results

Identification of *IL-17R* genes

To identify *IL-17R* family genes in lampreys, we performed tBLASTn searches of the sea lamprey and Japanese lamprey genome sequences using mammalian IL-17R

family members as query sequences. For both lamprey species, we identified six distinct *IL-17R* sequences. Six different *IL-17R* sequences were also identified in the transcriptomes of European brook lamprey (Table 2, Figure 3-1 and Figure 3-2). For all *IL-17R* family genes, we confirmed the presence of the coding region for the characteristic conserved SEFIR domain (Figure 3-2) with the exception of two partial sea lamprey IL-17Rs (IL-17R5 and IL-17R6). We attribute this failure to the incompleteness of the sea lamprey genome sequence. Phylogenetic analysis of these deduced IL-17Rs amino acid sequences indicated that they clustered into six groups and exhibited unambiguous one to one orthologous relationships between the three lamprey species (Figure 3-1 and Figure 3-2). Lamprey IL-17R1, IL-17R2 and IL-17R3 are phylogenetically close to the mammalian IL-17RA sequences, whereas IL-17R5 and IL-17R6 clustered with mammalian IL-17RE and IL-17RD respectively (Figure 3-1 and Figure 3-2). The top blast BLAST hits in the similarity search against the NCBI protein database also supported these conclusions (not shown).

IL-17R gene expression

When expression of the *IL-17R* family members was examined in different tissues by real-time PCR, *IL-17R3* was found to be the predominantly expressed member of this receptor family. It was expressed in the intestine, blood cells, skin, kidneys and gills (Figure 3-3). *IL-17R1* was expressed at highest levels in the intestine and blood cells, with weaker expression being detectable in skin, kidneys and gills, whereas *IL-17R2* expression was most easily detectable in the gills. *IL-17R4* expression was expressed preferentially in the intestine, *IL-17R5* in skin and *IL-17R6* in the kidney. When different

leukocyte populations were isolated from blood samples and examined for expression of the different *IL-17R*s, *IL-17R1* was found to be expressed preferentially by $VLRB^+$ lymphocytes, as noted previously (Guo et al., 2009), and also by monocytes (Figure 3-3). *IL-17R3* expression was detected principally in the $VLRA^+$ and $VLRC^+$ lymphocytes, but not in the monocytes and granulocytes. *IL-17R2* expression was detectable at low levels in all three VLR^+ lymphocyte populations; *IL-17R4* and *IL-17R6* expression was detectable in all blood cell populations examined, albeit at very low levels. Interestingly, *IL-17R5* was preferentially expressed by $VLRA^+$ lymphocytes, whereas the level of IL-17R6 expression was highest in the $VLRC^+$ lymphocytes (Figure 3-3).

Cellular binding and IL-17R interactions of IL-17-4

Since *IL-17-4* proved to be the most highly expressed *IL-17* family member in lampreys, we focused on this cytokine and its potential receptor(s) in the following studies. The chimeric IL-17-4/IgG1-Fc protein that was used to generate monoclonal IL-17-4 antibodies was also used to assess IL-17-4 binding by different types of blood cells. In these experiments, an unrelated recombinant hagfish protein (VLRA) fused with the human IgG1-Fc was used as a control and goat anti-human IgG antibodies as the secondary detection reagent. In flow cytometric analyses, binding of the chimeric IL-17-4/IgG1-Fc protein by both $VLRB^+$ lymphocytes and monocytes was observed, while the control IgG1-Fc fusion protein did not bind to any of the white blood cell types (Figure 3-4). Notably, the IL-17-4/IgG1-Fc protein failed to bind to the $VLRA^+$ lymphocytes, $VLRC^+$ lymphocytes and granulocytes, indicating cell-type specificity of expression of the IL-17-4 receptor.

The full-length cDNAs of three *IL-17R* family members in sea lamprey (*IL-17R1*, *IL-17R2*, and *IL-17R3*) were obtained by RACE and nested PCR. In order to examine which of the receptors could bind IL-17-4, HEK-293T cells were transfected with plasmids expressing IL-17R1 (HA-tagged) or IL-17R2 (myc-tagged) or IL-17R3 (FLAG-tagged) respectively. Flow cytometric analysis of the transfected cells using anti-tag antibodies confirmed that all three IL-17Rs were expressed by the transfectants (data not shown). In order to examine which of these receptors are capable of interacting with IL-17-4, immunoprecipitation analysis of these recombinant IL-17Rs was carried out. The results indicated that IL-17-4 associated with IL-17R1, but not with either IL-17R2 or IL-17R3 (Figure 3-5). These results, together with the findings described above suggest the potential for the $VLRA^+$ and $VLRC^+$ lymphocytes to interact with the $VLRB^+$ lymphocytes and monocytes via IL-17-4 production by the T-like cells and IL-17R1 by the B-like cells and monocytes.

Since IL-17-4 was shown to bind to $VLRB^+$ and monocytes by flow cytometry, we tested the ex vivo effect of IL-17-4 on the transcriptional status of blood leukocytes. In these experiments, blood leukocytes were incubated alone with either IL-17-4/IgG1-Fc or the control protein (extracellular region of IL-17R1/IgG1-Fc). After 16 hours incubation, we examined the expression levels of various candidate genes; in particular, we focused on genes that are preferentially expressed by $VLRB^+$ cells, a presumptive target of IL-17-4 action: the *IL-8* chemokine, *E2A*, *PAX5*, *Blimp1* and *BCL 6* transcription factors, and *Syk* and *BCAP* that are known components of the BCR-mediated signaling pathways in mammals. The results of our real-time PCR analysis indicate that IL-17-4/IgG1-Fc significantly induced the upregulation of *IL-8*, *BCL6* and *BCAP* gene expression, but had

no detectable effect on the expression levels of *E2A*, *PAX5* and *Blimp1* (Figure 3-6). These studies support the notion that recombinant IL-17-4 is biologically active and induces gene expression changes in a cell type that expresses the cognate receptor.

We also checked the proliferative response after injecting the recombinant IL-17-4/IgG1-Fc or control Fc protein into lampreys as a starting point to study the function of IL-17-4 in lampreys. Since sea lamprey larvae fails to initiate VLRB antibody responses when immunized with a soluble protein antigen (Alder et al., 2008), the proliferative response resulting from antibody response to injected proteins is not considered in this experiment. At 3 days or 5 days after injection with IL-17-4/IgG1-Fc protein, lymphocyte populations did not respond to the recombinant IL-17-4 protein with proliferation (Figure 3-7).

Genetic means for depletion of cell populations in lamprey are lacked. To study whether VLRB antibody response needs the help of T-like cells, pilot experiments indicated that anti-VLRA mAb treatment down-modulates the VLRA surface expression (unpublished data). In this experiment, intracoelomic injection of 50μg anti-VLRC mAbs also effectively down-modulates VLRC cell surface expression at day 1 (Figure 3-8). The same staining pattern was seen using a secondary antibody suggesting the decoration of residual surface VLRC by anti-VLRC mAbs. Staining of an untreated animal using the plasma from anti-VLRC mAbs injected larva showed the persistence of anti-VLRC mAb in the blood. When the amount of anti-VLRC monoclonal antibodies injected into lamprey was increased to 100μg, the effect of down-modulation is not significantly increased, suggesting the maximum down-modulation is reached.

3.5 Discussion

In our analysis of the lamprey *IL-17 receptor* gene families, we have identified six members of the IL-17 receptor family (*IL-17R1* through *IL-17R6*). We note that all receptors exhibit the conserved intracellular domain, that is an important functional domain for signal transduction from these receptors. This domain is also found in Act1, which serves an important role in IL-17R signaling (Novatchkova et al., 2003); Act1 is recruited to IL-17R through SEFIR-SEFIR interaction (Qian et al., 2007) and in turn it recruits and ubiquitinates TRAF6 to mediate downstream activation of NF-κB (Gaffen, 2009; Liu et al., 2009). The identification of the lamprey orthologues of mammalian IL-17s, IL-17Rs, Act1, TRAF6 and components of NF-κB signaling (C-Rel; NF-κB p105; IκB -α) suggests that the IL-17R signaling pathway is evolutionarily conserved (Hall and Yermolenko, 2014; Pancer et al., 2004; Smith et al., 2013; Wu et al., 2011a). Three of the lamprey IL-17R orthologues, IL-17R1, IL-17R2 and IL-17R3, are most closely related to mammalian IL-17RA. The sequences of IL-17R5 and IL-17R6 clustered with mammalian IL-17RE and IL-17RD sequences, respectively. The existence of IL-17RA and IL-17RD orthologues in amphioxus, lampreys, cartilaginous fish and teleost supports the idea that amphioxus IL-17RA and IL-17RD are the ancestors of vertebrate IL-17RA and IL-17RD respectively (Wu et al., 2011b). All of these IL-17R family members have also been identified in the shark *Callorhinchus milii,* a cartilaginous fish representative, although their sequences are not yet completely known (Wu et al., 2011b).

IL-17RA is preferentially expressed in hematopoietic tissues of mammals (Ishigame et al., 2009; Yao et al., 1995). Our transcriptional analysis indicated that *IL-17R3* is the most highly expressed member of the six lamprey *IL-17R*s, and its highest

expression levels were observed in skin, kidney, intestine, white blood cells and gills. Lamprey *IL-17R1* is expressed predominantly in the intestine and white blood cells, while *IL-17R2* expression is highest in the gills of lamprey larvae. The expression of *IL-17R4*, *IL-17R5* and *IL-17R6* could be detected in intestine, skin and kidneys respectively, suggesting that they may have differential roles in different tissues. Among the white blood cell types, the $VLRB^+$ B-like lymphocytes and monocytes expressed *IL-17R1* preferentially, whereas $VLRA^+$ and $VLRC^+$ T-like lymphocytes expressed *IL-17R3* preferentially.

When recombinant IL-17-4/IgG1-Fc fusion protein was used to determine which of the IL-17Rs produced by transfected cells could bind IL-17-4, we observed the association of IL-17-4 with IL-17R1, but not with IL-17R2 or IL-17R3. In this context, it is interesting to note that lampreys possess three representatives of IL-17Rs (IL-17R1, IL-17R2 and IL-17R3) that are closely related to mammalian IL-17RA, that serves as a common receptor subunit which participates in the recognition of several IL-17 family cytokines. Our data thus suggest that, despite these similarities, functional differences exist at least between IL-17R1 and IL-17R2 or IL-17R3.

By using the chimeric IL-17-4/IgG1-Fc and appropriate chimeric protein controls, we also found that IL-17-4 binds to $VLRB^+$ lymphocytes and monocytes, but not to $VLRA^+$ lymphocytes, $VLRC^+$ lymphocytes and granulocytes. Transcriptional analysis indicated that $VLRB^+$ B-like lymphocytes and monocytes preferentially express *IL-17R1*. Collectively, these findings indicate that $VLRB^+$ lymphocytes and monocytes express the complementary receptor for IL-17-4 and, by analogy with findings in mammals, imply that the T-like $VLRA^+$ and $VLRC^+$ cells may produce IL-17-4 to regulate B-like $VLRB^+$

cell and monocyte function in lampreys. Regulation of B cell function by IL-17s is not without precendent in the mammalian system. In the BXD2 mouse model of autoimmunity, IL-17A upregulates the B cell expression of regulator of G-protein signaling 13 and 16 (Rgs13 and Rgs16) to reduce the chemotactic response and to promote the formation of autoreactive germinal centers (Hsu et al., 2008). In the K/BxN mouse model of inflammatory arthritis, IL-17A is required for efficient germinal center formation via a direct effect on B cells (Wu et al., 2010). In addition, IL-17A promotes immunoglobulin isotype switching in mice (Mitsdoerffer et al., 2010), and induces monocyte adhesion and differentiation of an anti-inflammatory macrophages subset (Erbel et al., 2014; Zizzo and Cohen, 2013). IL-17A also facilitates monocyte migration through its interaction with IL-17RA and IL-17RC on monocytes in humans (Shahrara et al., 2009).

IL-17-4/IgG1-Fc treatment of lamprey blood leukocytes induced the expression levels of *IL-8*, *BCL6* and *BCAP*, three genes that are preferentially expressed by $VLRB^{+}$ cells. Induction of IL-8 from fibroblasts, endothelial cells, epithelial cells and synoviocytes by IL-17 has been reported in numerous studies (Gaffen, 2011; Hirata et al., 2008; Hwang et al., 2004; Iwakura et al., 2011; Weaver et al., 2007). Previous studies also showed that IL-17 stimulates the expression of *BCL6* in B cells from autoimmune mice to promote germinal center B cell differentiation (Xie et al., 2010a). BCAP has been shown to serve as an adaptor molecule linking CD19 and BCR-associated kinases to the PI3K pathway in B cells (Inabe, 2002; Okada et al., 2000), and as a TIR-domain-containing adaptor in the TLR signaling pathway to regulate inflammatory responses (Troutman et al., 2012). The increase of *BCAP* expression levels by IL-17-4 suggests that

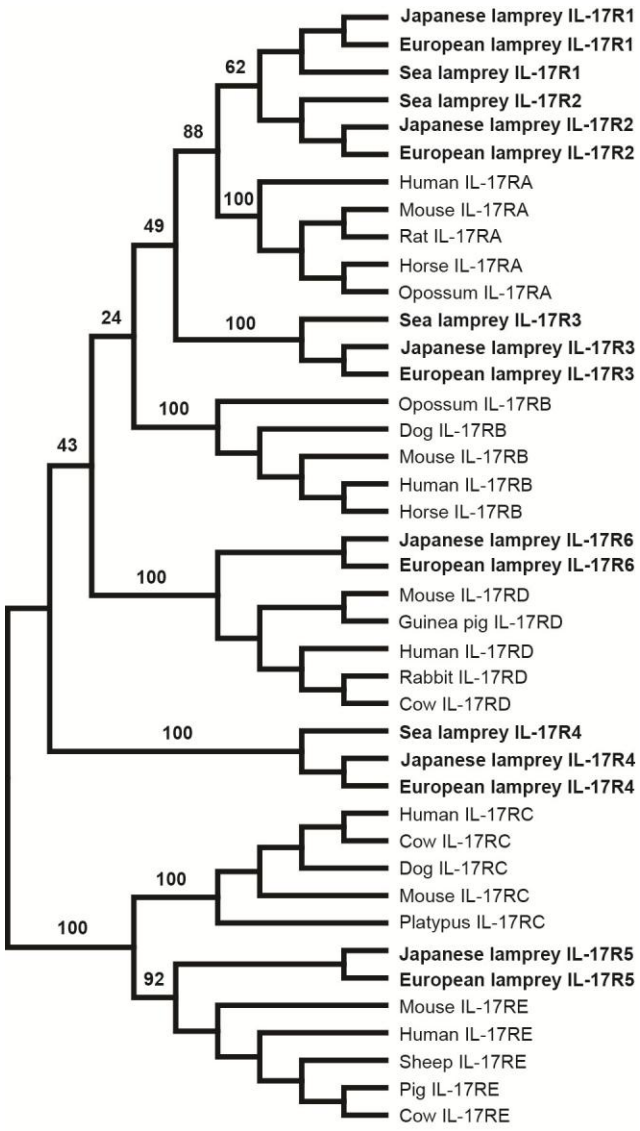
lamprey IL-17-4 may modulate B-like cell signaling and TLR signaling through its effect on *BCAP* expression. In grass carp, recombinant IL-17D promotes *IL-1β*, *TNF-α* and *IL-8* expression in head kidney cells (Du et al., 2014) and in rainbow trout, IL-17A/F2 stimulates splenocyte expression of *IL-6*, *IL-8* and anti-microbial peptide *BD-3*(Monte et al., 2013). Future studies will be required to elucidate the functional consequences of the IL-17-4 and IL-17R1 interactions between innate and adaptive immune system cells in lampreys.

IL-17 has been shown to induce proliferation of human mesenchymal stem cells and fibroblast-like synovial cells (Huang et al., 2009; Saxena et al., 2011), so we started to determine the effect of IL-17-4/IgG1-Fc recombinant protein on proliferation. At 3 days or 5 days after injection, lymphocyte populations did not respond with proliferation. IL-17 synergizes with TNF-α and other cytokines to induce its target genes (Shen and Gaffen, 2008), and the lack of proliferation might be due to the absence of other pro-inflammatory cytokines. Injection of IL-17 proteins into mice has shown multiple effects. Hematopoiesis in the spleen is stimulated by a single injection of IL-17 in mice (Jovcić et al., 2007). It has also been demonstrated that intraperitoneal injection of IL-17 in mice result in a selective recruitment of neutrophils in the peritoneal cavity (Witowski et al., 2000). Skin swelling is induced by intradermal injection of IL-17 in wild-type mice (Cho et al., 2012). IL-17 is also involved in joint inflammation. Single or repeated injections of IL-17 into the knee joint of mice induce joint destruction (Lubberts, 2008; Lubberts et al., 2005). It is interesting to examine whether injection of IL-17-4 have the similar effect of inducing hematopoiesis, neutrophil recruitment and skin inflammation in lampreys.

Genetic manipulation of lampreys is not currently available for selective deletion of lamprey lymphocyte populations. Treatment with VLRC-specific monoclonal antibodies effectively down-modulates VLRC cell surface expression. VLRA cell surface expression has also been demonstrated to be modulated by anti-VLRA mAb injection (data not shown). The down-modulation could be due to the endocytosis of the VLRA or VLRC induced by the ligation of cell surface receptors through anti-VLRA or anti-VLRC mAbs. Another mechanism is that the VLRA or VLRC ligation could induce cell death of immature lymphocytes as in the corresponding situation for immature T and B cells in jawed vertebrates. This anti-VLRA/C treatment could yield a valuable model for further functional studies of these lymphocyte populations. Previous studies have shown that hemagglutinin responses peaked about 19 d after a single intracoelomic injection of 1×10^7 human erythrocytes in lamprey larvae (Alder et al., 2008). So it is interesting to examine whether depletion of VLRA alone, VLRC alone or VLRA plus VLRC could alter the hemagglutinin responses after human erythrocytes injection.

In summary, we have identified six *IL-17R* (*IL-17R1* through *IL-17R6*) genes. *IL-17R3* was the most expressed member with its highest expression in the intestine and blood cells including $VLRA^+$ and $VLRC^+$ lymphocytes. In lamprey lymphocytes, $VLRA^+$ and $VLRC^+$ lymphocytes produced *IL-17-4* while $VLRB^+$ lymphocytes and monocytes expressed *IL-17R1* which bound to IL-17-4. Treatment of lamprey blood cells with recombinant IL-17-4 protein enhanced the transcription of genes expressed by B-like cells. These results suggest functional interaction between $VLRA^+$ and $VLRC^+$ T-like cells and $VLRB^+$ B-like cells.

A


Japanese lamprey IL-17R1
European lamprey IL-17R1
Sea lamprey IL-17R1
Sea lamprey IL-17R2
Japanese lamprey IL-17R2
European lamprey IL-17R2
Human IL-17RA
Mouse IL-17RA
Rat IL-17RA
Horse IL-17RA
Opossum IL-17RA
Sea lamprey IL-17R3
Japanese lamprey IL-17R3
European lamprey IL-17R3
Opossum IL-17RB
Dog IL-17RB
Mouse IL-17RB
Human IL-17RB
Horse IL-17RB
Japanese lamprey IL-17R6
European lamprey IL-17R6
Mouse IL-17RD
Guinea pig IL-17RD
Human IL-17RD
Rabbit IL-17RD
Cow IL-17RD
Sea lamprey IL-17R4
Japanese lamprey IL-17R4
European lamprey IL-17R4
Human IL-17RC
Cow IL-17RC
Dog IL-17RC
Mouse IL-17RC
Platypus IL-17RC
Japanese lamprey IL-17R5
European lamprey IL-17R5
Mouse IL-17RE
Human IL-17RE
Sheep IL-17RE
Pig IL-17RE
Cow IL-17RE
62
88
100
49
24
100
100
43
100
100
100
100
92

B

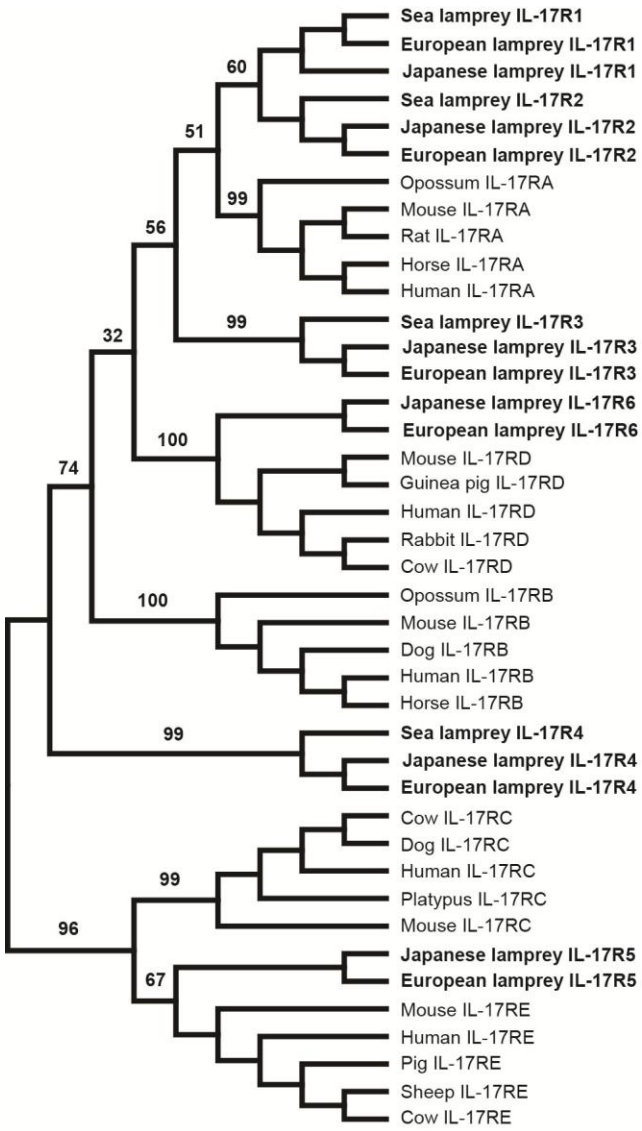

Sea lamprey IL-17R1
European lamprey IL-17R1
Japanese lamprey IL-17R1
Sea lamprey IL-17R2
Japanese lamprey IL-17R2
European lamprey IL-17R2
Opossum IL-17RA
Mouse IL-17RA
Rat IL-17RA
Horse IL-17RA
Human IL-17RA
Sea lamprey IL-17R3
Japanese lamprey IL-17R3
European lamprey IL-17R3
Japanese lamprey IL-17R6
European lamprey IL-17R6
Mouse IL-17RD
Guinea pig IL-17RD
Human IL-17RD
Rabbit IL-17RD
Cow IL-17RD
Opossum IL-17RB
Mouse IL-17RB
Dog IL-17RB
Human IL-17RB
Horse IL-17RB
Sea lamprey IL-17R4
Japanese lamprey IL-17R4
European lamprey IL-17R4
Cow IL-17RC
Dog IL-17RC
Human IL-17RC
Platypus IL-17RC
Mouse IL-17RC
Japanese lamprey IL-17R5
European lamprey IL-17R5
Mouse IL-17RE
Human IL-17RE
Pig IL-17RE
Sheep IL-17RE
Cow IL-17RE
60
51
99
56
99
32
100
74
100
99
99
96
67

Figure 3-1. Phylogenetic comparison of lamprey IL-17Rs with mammalian IL-17Rs. The C-terminal conserved SEFIR domain was used in the analysis. The phylogenetic tree is constructed by NJ method (A) and ML method (B). Due to incompleteness of sea lamprey genome sequence, the two partial IL-17R sequences (IL-17R5 and IL-17R6) were excluded. The numbers indicate bootstrap confidence values obtained for each node after 1000 replications. The accession numbers for sequences used in this analysis were as follows: Mouse IL-17RA, NP 032385; Rat IL-17RA, NP 001101353; Horse IL-17RA, XP 005610881; Opossum IL-17RA, XP 007503896; Human IL-17RA, EAW57738; Mouse IL-17RB, NP 062529; Human IL-17RB, AAF86051; Horse IL-17RB, XP 005600603; Dog IL-17RB, XP 005632474; Opossum IL-17RB, XP 007500672; Mouse IL-17RD, NP 602319; Human IL-17RD, NP 060033; Rabbit IL-17RD, XP 002713305; Cow IL-17RD, NP 001192356; Guinea pig IL-17RD, XP 003480139; Human IL-17RC, NP 703191; Cow IL-17RC, XP 005222441; Dog IL-17RC, XP 005632241; Mouse IL-17RC, NP 849273; Platypus IL-17RC, XP 007657284; Human IL-17RE, NP 705616; Pig IL-17RE, XP 005669819; Mouse IL-17RE, NP 665825; Sheep IL-17RE, XP 004018654; Bos mutus IL-17RE, XP 005907154.

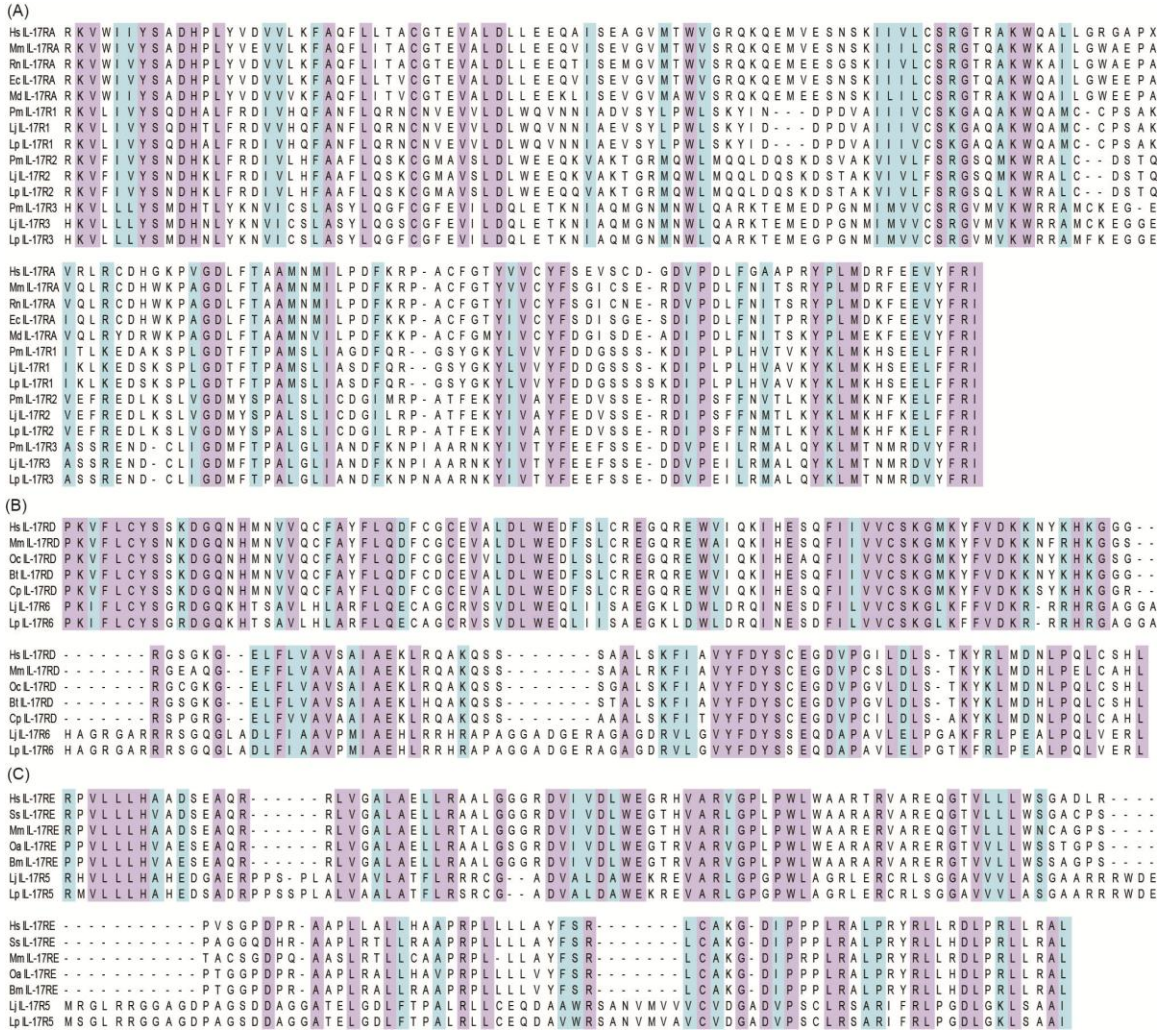
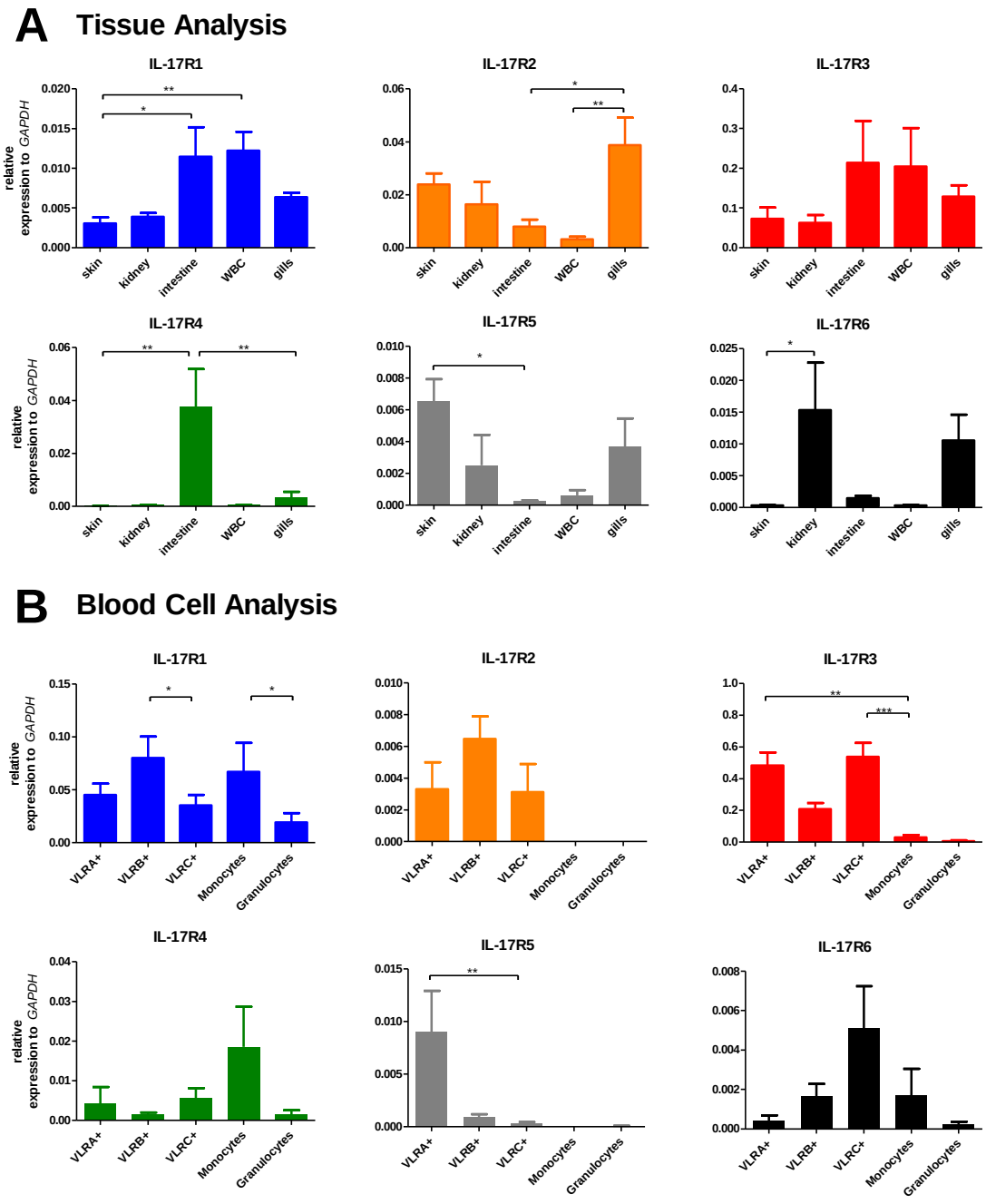
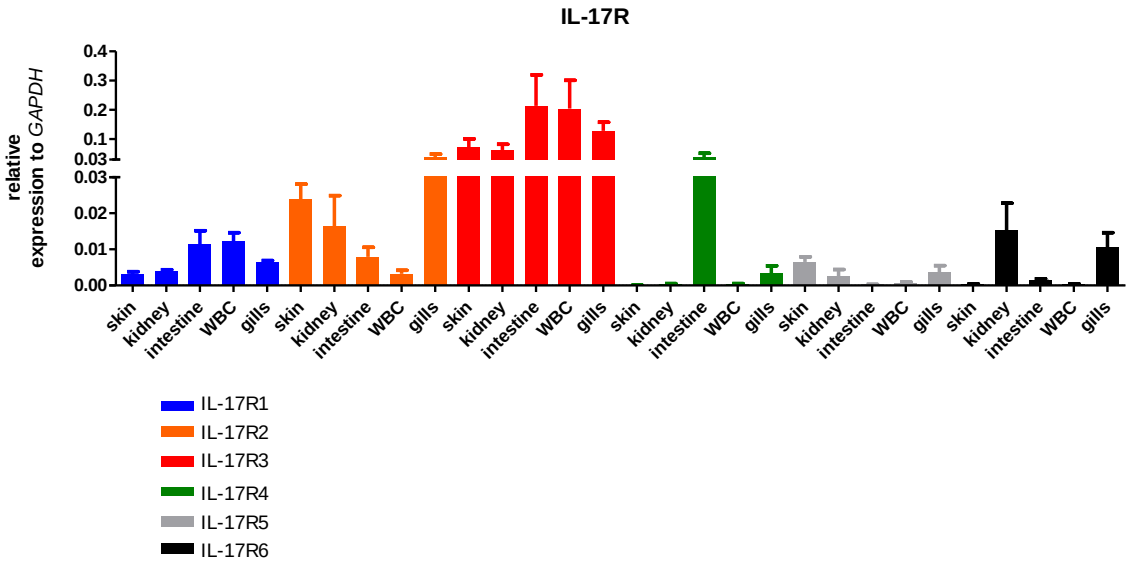

(A)
Hs IL-17RA
Mm IL-17RA
Rn IL-17RA
Ec IL-17RA
Md IL-17RA
Pm IL-17R1
Lj IL-17R1
Lp IL-17R1
Pm IL-17R2
Lj IL-17R2
Lp IL-17R2
Pm IL-17R3
Lj IL-17R3
Lp IL-17R3
(B)
Hs IL-17RD
Mm IL-17RD
Oc IL-17RD
Bt IL-17RD
Cp IL-17RD
Lj IL-17R6
Lp IL-17R6
(C)
Hs IL-17RE
Ss IL-17RE
Mm IL-17RE
Oa IL-17RE
Bm IL-17RE
Lj IL-17R5
Lp IL-17R5

Figure 3-2. Sequence alignment of intracellular SEFIR domains of lamprey IL-17Rs with mammalian IL-17Rs. The alignment was achieved using Clustal W (Larkin et al., 2007). Identical residues are boxed in purple and highly conserved residues in blue. Pm IL-17Rs: sea lamprey IL-17Rs; Lj IL-17Rs: Japanese lamprey IL-17Rs; Lp: European brook lamprey IL-17Rs. Note that lamprey IL-17R4 sequences are distinct and do not group with mammalian IL-17R member. The accession numbers for sequences used in this analysis are listed in Figure 3-1.


A
Tissue Analysis
IL-17R1
IL-17R2
IL-17R3
IL-17R4
IL-17R5
IL-17R6
relative expression to GAPDH
skin
kidney
intestine
WBC
gills
B
Blood Cell Analysis
IL-17R1
IL-17R2
IL-17R3
IL-17R4
IL-17R5
IL-17R6
relative expression to GAPDH
VLRA+
VLRB+
VLRC+
Monocytes
Granulocytes

C


IL-17R
relative expression to GAPDH
0.4
0.3
0.2
0.1
0.03
0.03
0.02
0.01
0.00
skin
kidney
intestine
WBC
gills
IL-17R1
IL-17R2
IL-17R3
IL-17R4
IL-17R5
IL-17R6

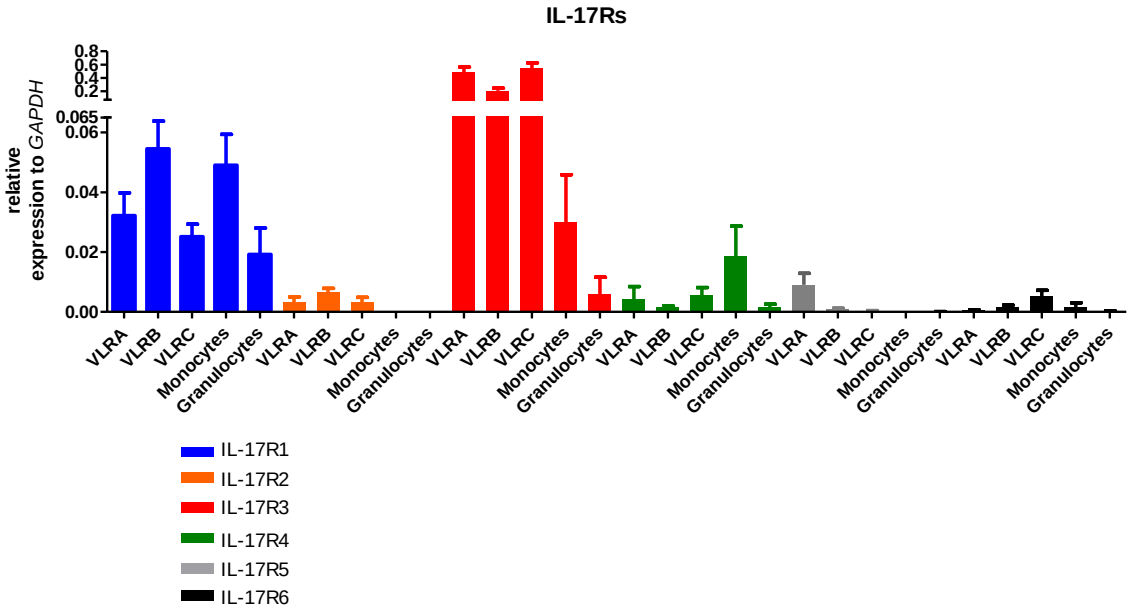

IL-17Rs
relative expression to GAPDH
0.8
0.6
0.4
0.2
0.065
0.06
0.04
0.02
0.00
VLRA
VLRB
VLRC
Monocytes
Granulocytes
IL-17R1
IL-17R2
IL-17R3
IL-17R4
IL-17R5
IL-17R6

Figure 3-3. Sea lamprey expression of *IL-17R* transcripts. (A) Tissue distribution. The relative expression levels of *IL-17R*s were determined by real-time PCR in skin, kidneys, intestine, white blood cells (WBC) and gills (n=6 larvae). (B) Expression of sea lamprey *IL-17R*s by different WBC types. Relative *IL-17R* expression levels were determined by real-time PCR analysis of sorted lymphocyte, monocyte and granulocyte populations (n=3-6 larvae). mRNA abundance relative to that of GAPDH: $2^{-\Delta Ct}$, $\Delta Ct = Ct_{\text{target gene}} - Ct_{GAPDH.}$ Error bars indicate SEM. The scales of the y-axis are different on each graph. The data are plotted on the same scale (fragmented y-axis) in (C).

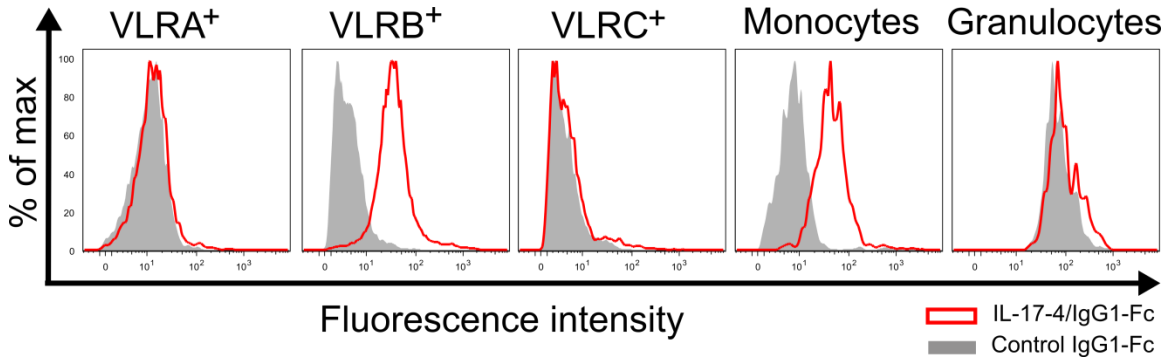

VLRA+
VLRB+
VLRC+
Monocytes
Granulocytes
% of max
100
80
60
40
20
0
0
10^1
10^2
10^3
Fluorescence intensity
IL-17-4/IgG1-Fc
Control IgG1-Fc

Figure 3-4. Analysis of IL-17-4/IgG1-Fc binding by different types of white blood cells. Blood cells were incubated with either control IgG1-Fc (grey area) or IL-17-4/IgG1-Fc (solid line) and stained with a PE-conjugated secondary antibody and co-stained with mAbs specific for VLRA (R110), VLRB (4C4), VLRC (3A5), monocytes (8A1) and granulocytes (2D4). Based on surface staining by these mAbs, the lymphocyte gate excluding dead cells and doublets was further gated. From the gated population, IL-17-4/IgG1-Fc staining was then determined. Fluorescence intensity is shown on a log scale. The selective binding by $VLRB^+$ lymphocytes and monocytes was observed in four independent experiments.

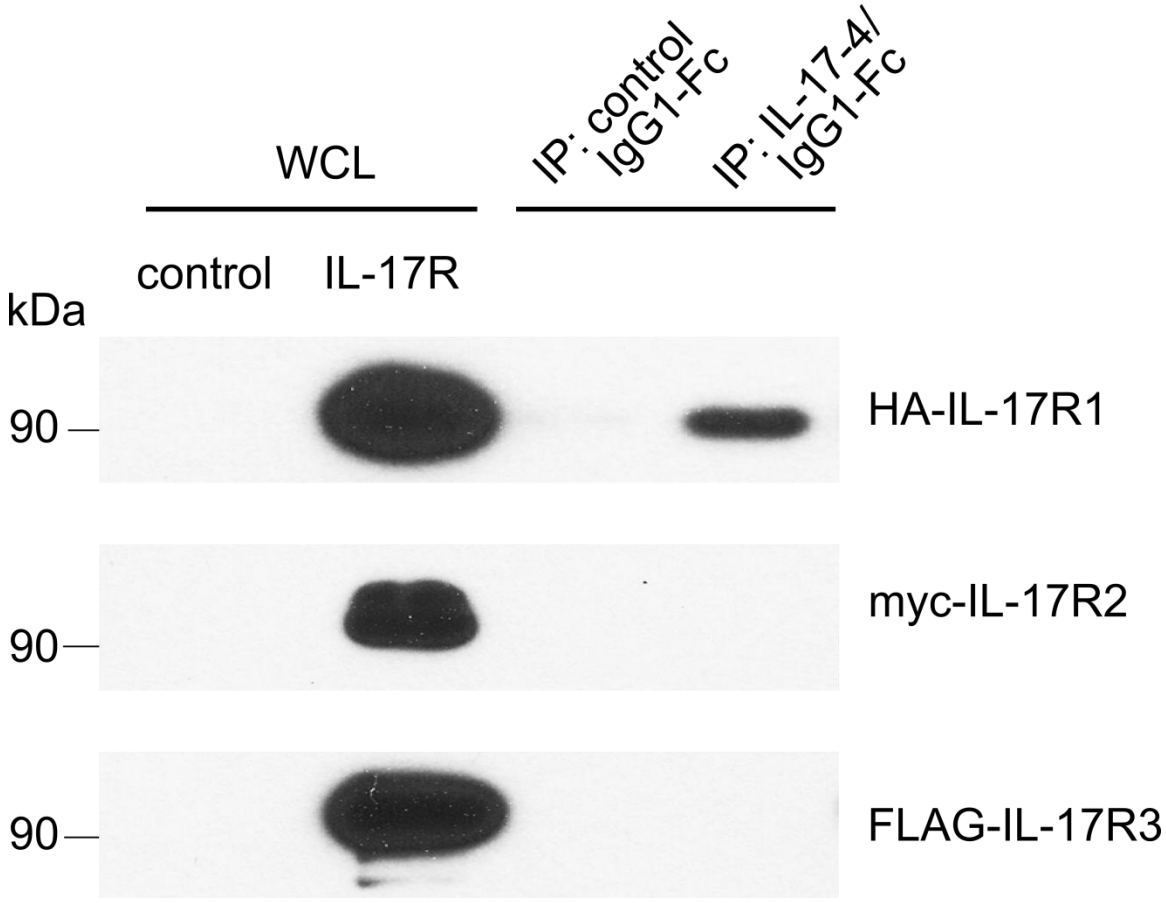

WCL
IP: control IgG1-Fc
IP: IL-17-4/ IgG1-Fc
control
IL-17R
kDa
90
HA-IL-17R1
90
myc-IL-17R2
90
FLAG-IL-17R3

Figure 3-5. Association of recombinant IL-17-4 and IL-17R1 proteins. HEK-293T cells were transfected with control vector, HA-IL-17R1, myc-IL-17R2 or FLAG-IL-17R3 vector. After 48 hours, whole cell lysates (WCL) were immunoprecipitated (IP) with IL-17-4/IgG1-Fc or control IgG1-Fc proteins followed by protein G beads. Cell lysates and immunoprecipitated proteins were resolved on reducing SDS-PAGE before Western blotting with anti-HA, anti-myc or anti-FLAG antibodies.

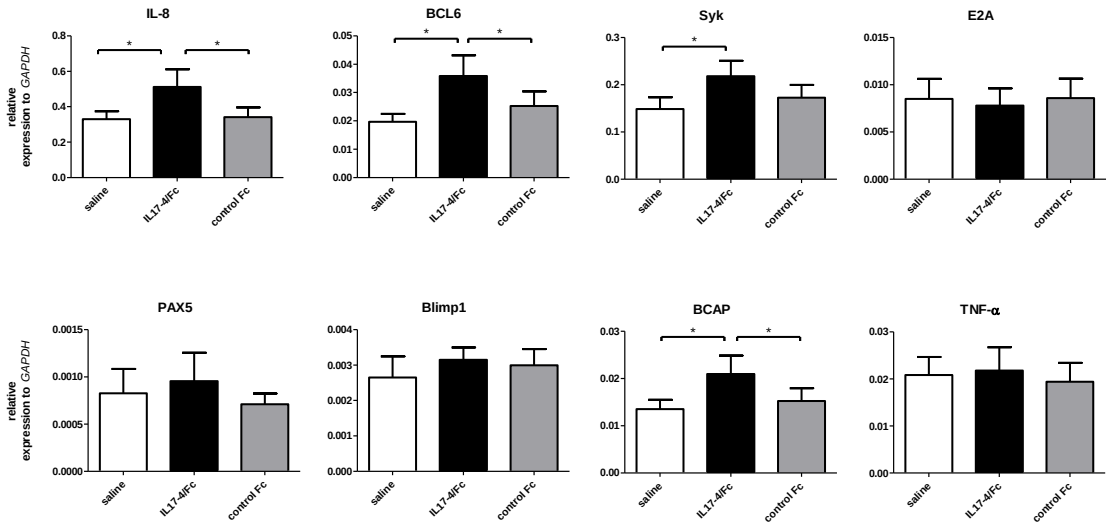

IL-8
BCL6
Syk
E2A
PAX5
Blimp1
BCAP
TNF-α
relative expression to GAPDH
saline
IL17-4/Fc
control Fc
*

Figure 3-6 Induction of gene expression in lamprey blood leukocytes by IL-17-4.

Blood leukocytes were incubated with saline, 2μg/ml IL-17-4/IgG1-Fc, or 2μg/ml control Fc for 16 hours. The expression levels of various genes were examined by real-time PCR after incubation (n=5 larvae). mRNA abundance relative to that of GAPDH: $2^{-\Delta Ct}$, $\Delta Ct = Ct_{target\ gene} - Ct_{GAPDH.}$ Error bars indicate SEM. The scales of the y-axis are different on each graph.

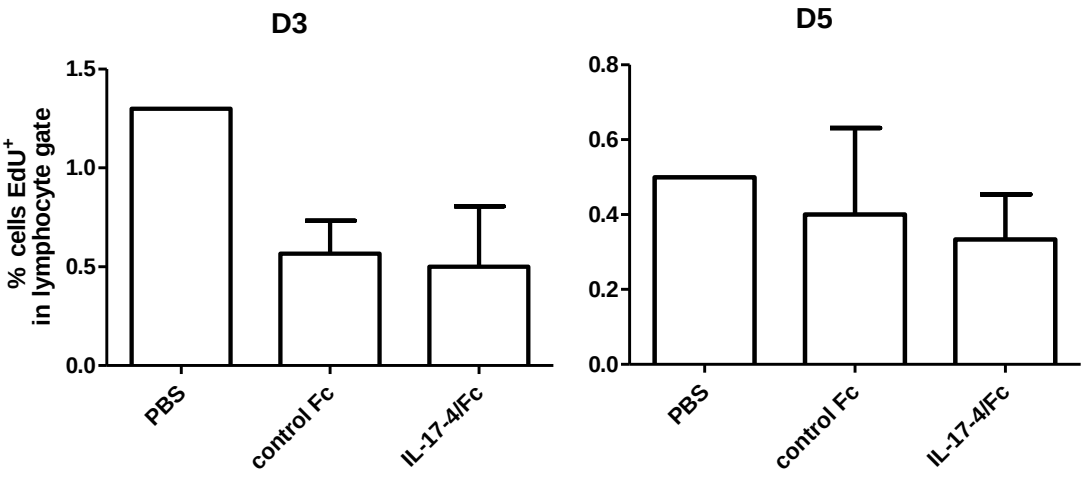

D3
D5
% cells EdU+ in lymphocyte gate
1.5
1.0
0.5
0.0
0.8
0.6
0.4
0.2
0.0
PBS
control Fc
IL-17-4/Fc
PBS
control Fc
IL-17-4/Fc

Figure 3-7. No proliferative response to recombinant IL-17-4/IgG1-Fc protein injection. (A) The percentage of proliferating cells in lymphocyte gate at 3 days (D3) after PBS, IL-17-4/IgG1-Fc or control Fc protein injection. (B) The percentage of proliferating cells in lymphocyte gate at 5 days (D5) after injection. EdU, 5-ethynyl-2'-deoxyuridine. n = 3. Error bars indicate SEM. The scales of the y-axis are different on each graph.

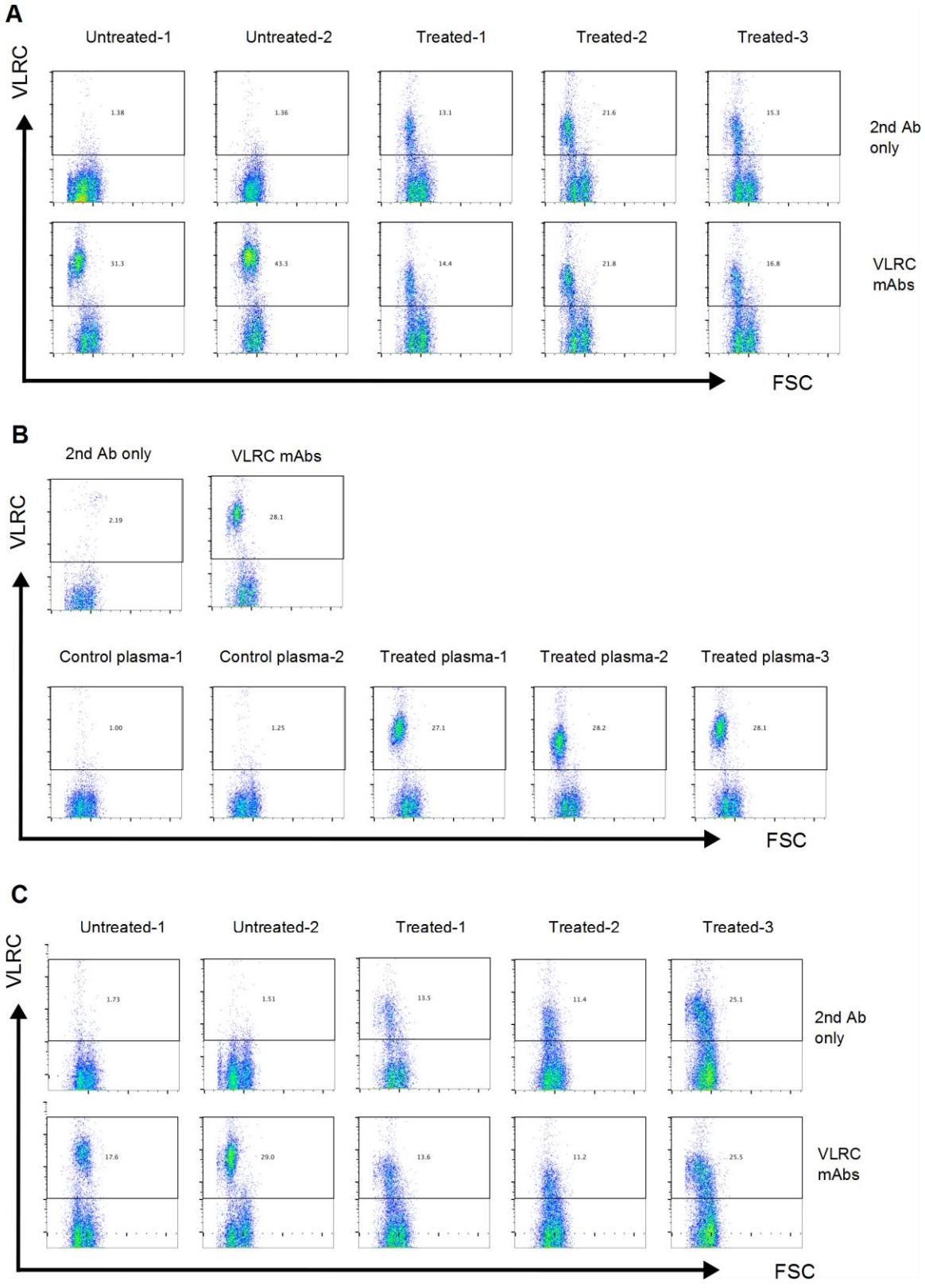
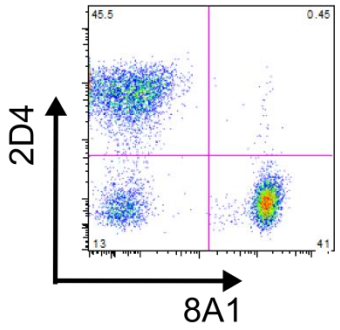

A
Untreated-1
Untreated-2
Treated-1
Treated-2
Treated-3
VLRC
1.38
1.36
13.1
21.6
15.3
2nd Ab only
31.3
43.3
14.4
21.8
16.8
VLRC mAbs
FSC
B
2nd Ab only
VLRC mAbs
VLRC
2.19
28.1
Control plasma-1
Control plasma-2
Treated plasma-1
Treated plasma-2
Treated plasma-3
1.00
1.25
27.1
28.2
28.1
FSC
C
Untreated-1
Untreated-2
Treated-1
Treated-2
Treated-3
VLRC
1.73
1.51
13.5
11.4
25.1
2nd Ab only
17.6
29.0
13.6
11.2
25.5
VLRC mAbs
FSC

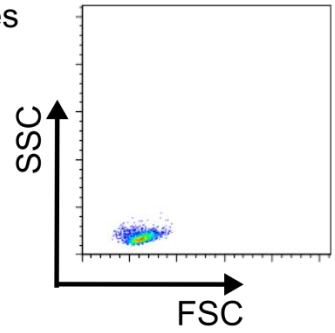
Figure 3-8. Down-modulation of VLRC cell surface expression by treatment with VLRC-specific mAbs injection. (A) Cell surface VLRC staining of blood cells on day 1 after injection of anti-VLRC mAb (50 μg) in three lamprey larva. The staining for two untreated larva was also shown. Blood cells were stained with the mAb (upper panel) or a secondary goat-anti-mouse Ig antibody (lower panel). (B) Persistence of anti-VLRC mAb in the treated larva. staining of blood leukocytes from an untreated animal using plasma(1:20 dilution) from untreated larva (control plasma) or anti-VLRC mAb injected (at day 1) larva. Staining using anti-VLRC mAb serves as a positive control. Results shown for cells in the lymphocyte gate. (C) Cell surface VLRC staining of blood cells on day 1 after injection of anti-VLRC mAb (100 μg) in three lamprey larva.

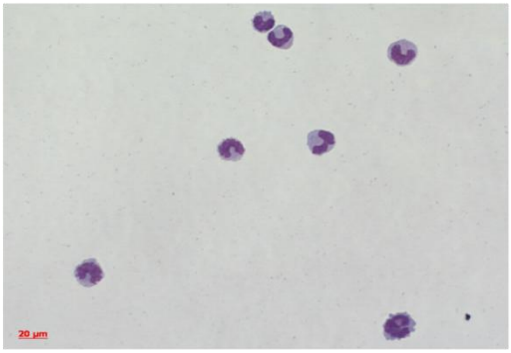
A



B

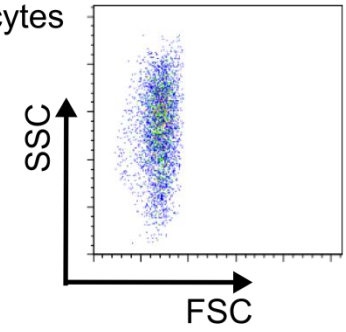
Monocytes
(8A1^{+})





C

Granulocytes
(2D4^{+})



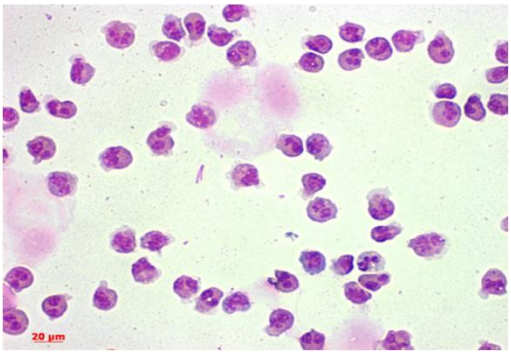


Figure 3-9. Characterization of monocytes and granulocytes by flow cytometry and Wright-Giemsa stain. (A) Cell surface staining of adult lamprey blood leukocytes with anti-monocyte and anti-granulocyte antibodies. Adult lamprey blood leukocytes were co-stained with anti-monocyte (8A1) antibodies (X-axis) and anti-granulocyte (2D4) antibodies (Y-axis) and analyzed by flow cytometry. Cells were gated using FSC-A vs FSC-H for singlets, and negative propidium iodide staining for live cells. (B) Analysis of $8A1^+$ monocytes by FSC/SSC and Wright-Giemsa stain (scale bar, 20 μm). (C) Analysis of $2D4^+$ granulocytes by FSC/SSC and Wright-Giemsa stain (scale bar, 20 μm).

Chapter 4

Conclusions and Future directions

4.1 Summary and conclusions

In this study, five lamprey orthologues of mammalian *IL-17* genes (*IL-17-1* through *IL-17-5*) and six lamprey orthologues of mammalian *IL-17R* genes (*IL-17R1* through *IL-17R6*) were identified in the sea lamprey genome. Lamprey IL-17-4 and IL-17-1 sequences are close to mammalian IL-17D, whereas IL-17-2, IL-17-3 and IL-17-5 sequences are similar to mammalian IL-17B, IL-17E and IL-17C sequences, respectively. No IL-17A or IL-17F orthologues have been identified in the lamprey. In cartilaginous fish, IL-17A-like sequences are present. In teleosts, IL-17A and IL-17F orthologues have been found, indicating the common ancestor of cartilaginous fish and bony fish has IL-17A. IL-17B and IL-17E orthologues have not been found in cartilaginous fish and bony fish. The missing of IL-17B and IL-17E orthologues in fish could be explained by several possibilities: (1) they were lost through evolution from lampreys to fish; (2) they exist in fish and have not been identified yet; (3) IL-17B orthologue indentified in lamprey could function similarly to both mammalian IL-17B and IL-17D according to the close relationship in the phylogenetic tree. Meanwhile, IL-17E orthologue could function similarly to both mammalian IL-17E and IL-17C. The identification of IL-17C and IL-17D orthologues in lampreys and teleosts suggest that the common ancestor of vertebrates have IL-17C and IL-17D. In cartilaginous fish, IL-17D orthologue has not been reported yet. This might be due to the incompleteness of the genome sequences.

Three of the lamprey IL-17R orthologues, IL-17R1, IL-17R2 and IL-17R3, are most closely related to mammalian IL-17RA. The sequences of IL-17R5 and IL-17R6 clustered with mammalian IL-17RE and IL-17RD sequences, respectively. According to the

phylogenic tree, IL-17RA and IL-17RD are closely related. The existence of IL-17RA and IL-17RD orthologues in amphioxus, lampreys, cartilaginous fish and teleost fish supports the idea that amphioxus IL-17RA and IL-17RD are the ancestors of vertebrate IL-17RA and IL-17RD respectively (Wu et al., 2011b). According to the phylogenic tree, IL-17RC and IL-17RE are closely related. Their syntenic relationship suggests that IL-17RC and IL-17RE could be derived from a common gene ancestor (Wu et al., 2011b). All of these IL-17R family members (IL-17RA through IL-17RE) have been identified in the shark *Callorhinchus milii* and teleost fish (Kono et al., 2011; Wu et al., 2011b). Lamprey IL-17R5 is most similar to IL-17RE, indicating IL-17RE is present in the common ancestor of all vertebrates. A possible scenario for the evolution of IL-17Rs could be proposed: IL-17RA and IL-17RD are present in the common ancestor of chordates. Whole-genome duplications might have led to the expansion of the two IL-17Rs into multiple vertebrate IL-17Rs during the evolution of early vertebrates (Holland et al., 1994; Ohno, 1970). After duplications, the multiple *IL-17R* genes have evolved unique functions by adopting a new function (neofunctionalization) or partitioning old functions (subfunctionalization).

IL-17-1, *IL-17-2*, *IL-17-3* and *IL-17-5* were expressed primarily in the intestine and gills at much lower levels than *IL-17-4* which was the most expressed member with its highest expression in skin and gills. In agreement with the real-time PCR analysis, IL-17-4 protein was also detected in skin, gills and intestine by Western blot analysis with an IL-17-4-specific mAb. Epithelial cells (skin, gill filaments and intestine), $VLRA^+$ lymphocytes and $VLRC^+$ lymphocytes were found to express *IL-17-4* transcripts. Injection of bacteria and yeast upregulates *IL-17-4* transcripts in blood leukocytes at day 2.

IL-17R3 was the most expressed member with its highest expression in the intestine and blood cells including $VLRA^+$ and $VLRC^+$ lymphocytes. *IL-17R1* was found to be expressed preferentially by $VLRB^+$ lymphocytes and monocytes in the blood. These two populations were shown to bind the recombinant IL-17-4 protein by flow cytometry. In agreement with these observations, the association of recombinant IL-17-4 with IL-17R1 was demonstrated by immunoprecipitation analysis. Functionally, treatment of recombinant IL-17-4 protein significantly upregulates the transcription of *IL-8*, *BCL6* and *BCAP* in blood leukocytes.

This study shows the T-like $VLRA^+$ and $VLRC^+$ lymphocytes in lampreys preferentially produced *IL-17-4*, whereas the B-like $VLRB^+$ lymphocytes and monocytes express *IL-17R1* which binds to IL-17-4, thereby suggesting functional interaction between the T-like cells and cells of the adaptive and innate immune systems in lampreys.

4.2 Remaining questions and Future directions

Cloning of other IL-17s and other IL-17Rs

Five IL-17 ligands and six IL-17Rs have been identified in sea lamprey and full-length *IL-17-4*, *IL-17R1*, *IL-17R2* and *IL-17R3* have been cloned. Cloning of other members of the ligand and receptor family would facilitate the expression of their recombinant proteins, which would help construct a big picture of ligand-ligand, ligand-receptor and receptor-receptor interactions. The availability of the transcriptome database of sea lamprey tissues and lymphocytes populations will also facilitate the RT-PCR based cloning. In addition, genome database of Japanese lamprey and transcriptomes of

European brook lamprey (*Lampetra planeri*) would also help design the primers for cloning the full-length cDNAs. It is important to identify and optimize a condition which could induce the transcription of lamprey *IL-17*s and *IL-17R*s. This would increase the transcripts abundance and help the RT-PCR cloning. Bacterial and yeast injection into lamprey larvae upregulates the transcription of *IL-17-4* after 48 hours in blood leukocytes. It is interesting to see whether other family members in specific tissues and at a specific time point could be induced using the same injection. cDNAs could be generated from transcripts in different tissues, cell types with or without stimulation. If low expression of some genes pose difficulty for successful RT-PCR based cloning, conventional cDNA library screening could also be used. However, the library construction step of cDNA library screening could suffer from the under-representation of some cDNAs due to low expression levels, transcript instability, restricted temporal and tissue expression patterns, and therefore conventional cDNA library screening is less sensitive than RT–PCR. If the effort of cloning fails using RT-PCR, it is still worthwhile to use conventional cDNA library screen which could complement RT-PCR approach with its own advantages.

Ligand-receptor and receptor-receptor interactions

Six IL-17Rs have been identified in the lamprey genome. It is interesting to examine whether lamprey IL-17Rs form pre-associated, multisubunit complexes as tumor necrosis factor receptors do. To test the interactions of different lamprey IL-17Rs, HEK-293T cells will be transfected with IL-17Rs with various protein tags and immunoprecipitation analysis could be used by pulling-down one receptor and examining the presence of other receptors. This study will only demonstrate the composition of

receptor complex without ligand binding. Homotypic or heterotypic receptor complex may be pre-assembled when overexpressed by cell lines. It could be still possible that endogenous receptors, however, have little preassociation on the lamprey cell surface. Upon ligand binding, conformation of IL-17R could be altered to favor a different interaction. To study the ligand-receptor interactions, a straightforward way is to express individual receptor or combinations of receptors on the cell surface of HEK-293T cells or fish cell lines (lamprey cell lines are not available), incubate the recombinant ligand protein with the transfected cell lines, and use flow cytometry analysis to test if any positive binding could be demonstrated. The positive binding would suggest the ligand-receptor interaction. Another way to study is to use recombinant ligand protein to immunoprecipitate the lysates of lamprey tissues or cells and mass spectrometry can be applied to determine the identity of the immunoprecipitated proteins. One advantage of this method is that no prior knowledge of the interacting receptors is required. However, the abundance of the receptor and the affinity of ligand-receptor interactions will determine the success of identifying the receptor(s) for the ligand. Our immunoprecipitation analysis showed that IL-17-4 is associated with recombinant IL-17R1 expressed by HEK-293T cells, so it is probably that IL-17R1 is one component of the receptor complex for IL-17-4.

IL-17 signaling in lamprey

Recombinant IL-17-4 protein stimulates the transcription of *IL-8*, *BCL6* and *BCAP* in lamprey blood leukocytes. The signaling pathways leading to the upregulation of these genes are unknown. Dissecting the signaling pathway would offer insights into

how lamprey IL-17 signaling is conserved compared to mammals. Upon IL-17 stimulation, Act1 is recruited to IL-17R through SEFIR-SEFIR interaction (Qian et al., 2007) and in turn recruits and ubiquitinates TRAF6 to mediate downstream activation of NF-κB (Gaffen, 2009; Liu et al., 2009). Lamprey orthologues of the mammalian signaling molecules of IL-17 pathway including Act1, TRAF6 and components of NF-κB signaling (C-Rel; NF-κB p105; IκBα) have been identified before (Hall and Yermolenko, 2014; Pancer et al., 2004; Smith et al., 2013; Wu et al., 2011a). Since the sequences of signaling molecules are largely conserved across species, mAbs recognizing mammalian Act1, TRAF6 and NF-κB signaling components will be tested to see whether they also recognize lamprey orthologues. mAb to lamprey IL-17R1 is also needed to check the Act1 recruitment to the receptor at different time points (such as 5min, 10min and 1 hour) after IL-17-4 stimulation by immunoprecipitating IL-17R1. To examine the ubiquitination of TRAF6, TRAF6 needs to be immunoprecipitated from blood cell lysates and subjected to Western blot with anti-ubiquitin antibody after IL-17-4 treatment. For NF-κB signaling components, Western blot analysis of phosphorylated IκBα could be performed after IL-17-4 treatment. Since IL-17-4 immunoprecipitates IL-17R1 expressed by HEK-293T cells, NF-κB reporter assay could be applied based on the ectopic expression system. HEK-293T cells will be transfected with a plasmid containing a NF-κB responsive promoter, which activates luciferase transcription. NF-κB activity will be measured using the luciferase reporter assay after treatment by IL-17-4.

Study of IL-17 function by inhibiting IL-17 signaling

After immunization with *E. coli* or *S. pneumoniae*, the $VLRA^+$ and $VLRB^+$ cells proliferate and $VLRB^+$ cells differentiate into plasmacytes that secrete multimeric VLRB antibodies (Guo et al., 2009). Immunization with mixture of *E. coli*, *S. pneumoniae* and yeast upregulates *IL-17-4* transcription by blood leukocytes. Reciprocal expression of IL-17 and IL-17R in $VLRA^+$ and $VLRB^+$ cells, respectively, suggests the functional crosstalk between these two populations. So it is important to determine whether IL-17 is involved in the specific immune response to the antigens. Blocking the IL-17 and IL-17R crosstalk would be a reasonable way to study the function of IL-17 in lampreys. One straightforward strategy is to use an IL-17 member (such as IL-17-4) or IL-17R specific antibody. However, this depends on the availability of a mAb which could target the region where IL-17 binds to IL-17R. The screening of such antibodies could be based on the ability of inhibiting the IL-17-4 binding to $VLRB^+$ cells and monocytes. Dissecting the receptor complex would also provide valuable information for effective blocking of the ligand-receptor interaction. Another strategy is to use soluble IL-17R without transmembrane and intracellular domain. This soluble IL-17R could also be fused to VLR stalk region considering the possible effector function mediated by VLR stalk region. Blocking of IL-17 signaling might be incomplete and thus the residual IL-17 may still function. Therefore, the optimal concentration of blocking mAbs or soluble receptors and the time frame for the depletion of IL-17 should be determined. After blocking IL-17 signaling by above strategies, lampreys will then be immunized with those antigens. The proliferation of VLR^+ populations could be analyzed by EdU assay while the percentage of antigen-specific VLR^+ cells will be determined by lymphocytes antigen-binding assay Fluorescence labeled bacteria will be incubated with lamprey leukocytes and then stained

with anti-VLRA, anti-VLRB or anti-VLRC antibodies. The percentage of the antigen binding cells will be analyzed by flow cytometry. The level of plasma antigen-specific VLRB antibodies could be examined by ELISA. The possible outcome after blocking the IL-17 signaling is that the proliferation of VLR^+ cells is compromised when the percentage of EdU^+ population and antigen-specific VLR^+ cells is decreased while the VLRB secretion is affected when the plasma VLRB level is decreased.

In summary, lamprey T- and B-like lymphocyte lineages resemble the adaptive immune system of jawed vertebrates. Our study characterized the IL-17 cytokine family and their receptors in the lamprey and their potential involvement in the crosstalk between T-like and cells of both adaptive and innate immune systems. This implies the cooperative interactions between T-like and B-like cells, and the function of IL-17 on innate cells during an immune response. In mammals, interaction between antigen-specific $CD4^+$ T helper cells and B cells is required for generation of memory B cells and long-lived plasma cells secreting high-affinity antibodies. The study of lamprey T-like and B-like cells interactions indicates an evolutionarily conserved form of cell-cell interactions during an immune response.

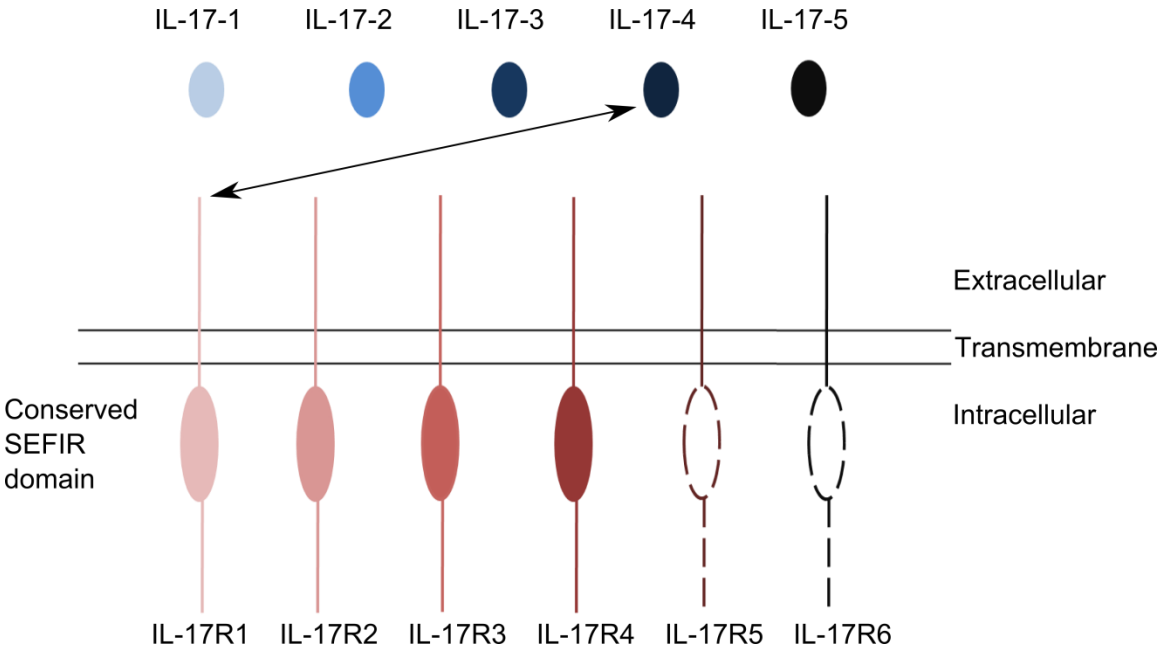

IL-17-1
IL-17-2
IL-17-3
IL-17-4
IL-17-5
Extracellular
Transmembrane
Intracellular
Conserved
SEFIR
domain
IL-17R1
IL-17R2
IL-17R3
IL-17R4
IL-17R5
IL-17R6

Figure 4-1. IL-17 and IL-17R family members in sea lamprey. Five members of the sea lamprey *IL-17* family (*IL-17-1* to *IL-17-5*) and six *IL-17 receptor* genes (*IL-17R1* to *IL-17R6*) have been identified. The binding of IL-17-4 to recombinant IL-17R1 is shown as arrow in the figure. The SEFIR domains of IL-17R5 and IL-17R6 are absent in the sea lamprey genome database and shown in dashed line. SEFIR, similar expression to fibroblast growth factor genes and IL-17R.

References

Adema, C.M., Hertel, L.A., Miller, R.D., Loker, E.S., 1997. A family of fibrinogen-related proteins that precipitates parasite-derived molecules is produced by an invertebrate after infection. Proc. Natl. Acad. Sci. U. S. A. 94, 8691–6.

Alder, M.N., Herrin, B.R., Sadlonova, A., Stockard, C.R., Grizzle, W.E., Gartland, L.A., Gartland, G.L., Boydston, J.A., Turnbough, C.L., Cooper, M.D., 2008. Antibody responses of variable lymphocyte receptors in the lamprey. Nat. Immunol. 9, 319–27. doi:10.1038/ni1562

Angkasekwinai, P., Park, H., Wang, Y.-H., Wang, Y.-H., Chang, S.H., Corry, D.B., Liu, Y.-J., Zhu, Z., Dong, C., 2007. Interleukin 25 promotes the initiation of proallergic type 2 responses. J. Exp. Med. 204, 1509–17. doi:10.1084/jem.20061675

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, 90–4. doi:10.1038/nature09655

Beklen, A., Ainola, M., Hukkanen, M., Gürgan, C., Sorsa, T., Konttinen, Y.T., 2007. MMPs, IL-1, and TNF are regulated by IL-17 in periodontitis. J. Dent. Res. 86, 347–51.

Bermejo, D.A., Jackson, S.W., Gorosito-Serran, M., Acosta-Rodriguez, E. V, Amezcua-Vesely, M.C., Sather, B.D., Singh, A.K., Khim, S., Mucci, J., Liggitt, D., Campetella, O., Oukka, M., Gruppi, A., Rawlings, D.J., 2013. Trypanosoma cruzi trans-sialidase initiates a program independent of the transcription factors RORγt and Ahr that leads to IL-17 production by activated B cells. Nat. Immunol. 14, 514–22. doi:10.1038/ni.2569

Brüstle, A., Heink, S., Huber, M., Rosenplänter, C., Stadelmann, C., Yu, P., Arpaia, E., Mak, T.W., Kamradt, T., Lohoff, M., 2007. The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4. Nat. Immunol. 8, 958–66. doi:10.1038/ni1500

Bulek, K., Liu, C., Swaidani, S., Wang, L., Page, R.C., Gulen, M.F., Herjan, T., Abbadi, A., Qian, W., Sun, D., Lauer, M., Hascall, V., Misra, S., Chance, M.R., Aronica, M., Hamilton, T., Li, X., 2011. The inducible kinase IKKi is required for IL-17-dependent signaling associated with neutrophilia and pulmonary inflammation. Nat. Immunol. 12, 844–52. doi:10.1038/ni.2080

Cai, X.Y., Gommoll, C.P., Justice, L., Narula, S.K., Fine, J.S., 1998. Regulation of granulocyte colony-stimulating factor gene expression by interleukin-17. Immunol. Lett. 62, 51–8.

Cannon, J., Haire, R., Litman, G., 2002. Identification of diversified genes that contain immunoglobulin-like variable regions in a protochordate. Nat. Immunol. 3, 1200–1207. doi:citeulike-article-id:7058290

Chang, S.H., Park, H., Dong, C., 2006. Act1 adaptor protein is an immediate and essential signaling component of interleukin-17 receptor. J. Biol. Chem. 281, 35603–7. doi:10.1074/jbc.C600256200

Chang, S.H., Reynolds, J.M., Pappu, B.P., Chen, G., Martinez, G.J., Dong, C., 2011. Interleukin-17C Promotes Th17 Cell Responses and Autoimmune Disease via Interleukin-17 Receptor E. Immunity 35, 611–621. doi:10.1016/j.immuni.2011.09.010

Cho, J.S., Pietras, E.M., Garcia, N.C., Ramos, R.I., Farzam, D.M., Monroe, H.R., Magorien, J.E., Blauvelt, A., Kolls, J.K., Cheung, A.L., Cheng, G., Modlin, R.L., Miller, L.S., 2010. IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice. J. Clin. Invest. 120, 1762–73. doi:10.1172/JCI40891

Cho, K.-A., Suh, J.W., Lee, K.H., Kang, J.L., Woo, S.-Y., 2012. IL-17 and IL-22 enhance skin inflammation by stimulating the secretion of IL-1β by keratinocytes via the ROS-NLRP3-caspase-1 pathway. Int. Immunol. 24, 147–58. doi:10.1093/intimm/dxr110

Ciric, B., El-behi, M., Cabrera, R., Zhang, G.-X., Rostami, A., 2009. IL-23 drives pathogenic IL-17-producing CD8+ T cells. J. Immunol. 182, 5296–305. doi:10.4049/jimmunol.0900036

Claudio, E., Sonder, S.U., Saret, S., Carvalho, G., Ramalingam, T.R., Wynn, T.A., Chariot, A., Garcia-Perganeda, A., Leonardi, A., Paun, A., Chen, A., Ren, N.Y., Wang, H., Siebenlist, U., 2009. The Adaptor Protein CIKS/Act1 Is Essential for IL-25-Mediated Allergic Airway Inflammation. J. Immunol. 182, 1617–1630. doi:10.4049/jimmunol.182.3.1617

Conti, H.R., Shen, F., Nayyar, N., Stocum, E., Sun, J.N., Lindemann, M.J., Ho, A.W., Hai, J.H., Yu, J.J., Jung, J.W., Filler, S.G., Masso-Welch, P., Edgerton, M., Gaffen, S.L., 2009. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. J. Exp. Med. 206, 299–311. doi:10.1084/jem.20081463

Cortez, D.M., Feldman, M.D., Mummidi, S., Valente, A.J., Steffensen, B., Vincenti, M., Barnes, J.L., Chandrasekar, B., 2007. IL-17 stimulates MMP-1 expression in primary human cardiac fibroblasts via p38 MAPK- and ERK1/2-dependent C/EBP-beta , NF-kappaB, and AP-1 activation. Am. J. Physiol. Heart Circ. Physiol. 293, H3356–65. doi:10.1152/ajpheart.00928.2007

Cua, D.J., Tato, C.M., 2010. Innate IL-17-producing cells: the sentinels of the immune system. Nat. Rev. Immunol. 10, 479–89. doi:10.1038/nri2800

Da Matta Guedes, P.M., Gutierrez, F.R.S., Maia, F.L., Milanezi, C.M., Silva, G.K., Pavanelli, W.R., Silva, J.S., 2010. IL-17 produced during Trypanosoma cruzi infection plays a central role in regulating parasite-induced myocarditis. PLoS Negl. Trop. Dis. 4, e604. doi:10.1371/journal.pntd.0000604

Du, L., Qin, L., Wang, X., Zhang, A., Wei, H., Zhou, H., 2014. Characterization of grass carp (Ctenopharyngodon idella) IL-17D: molecular cloning, functional implication and signal transduction. Dev. Comp. Immunol. 42, 220–8. doi:10.1016/j.dci.2013.09.015

Ely, L.K., Fischer, S., Garcia, K.C., 2009. Structural basis of receptor sharing by interleukin 17 cytokines. Nat. Immunol. 10, 1245–51. doi:10.1038/ni.1813

Erbel, C., Akhavanpoor, M., Okuyucu, D., Wangler, S., Dietz, A., Zhao, L., Stellos, K., Little, K.M., Lasitschka, F., Doesch, A., Hakimi, M., Dengler, T.J., Giese, T., Blessing, E., Katus, H. a, Gleissner, C. a, 2014. IL-17A Influences Essential Functions of the Monocyte/Macrophage Lineage and Is Involved in Advanced Murine and Human Atherosclerosis. J. Immunol. doi:10.4049/jimmunol.1400181

Fallon, P.G., Ballantyne, S.J., Mangan, N.E., Barlow, J.L., Dasvarma, A., Hewett, D.R., McIlgorm, A., Jolin, H.E., McKenzie, A.N.J., 2006. Identification of an interleukin (IL)-25–dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. J. Exp. Med. 203, 1105–1116. doi:10.1084/jem.20051615

Fort, M.M., Cheung, J., Yen, D., Li, J., Zurawski, S.M., Lo, S., Menon, S., Clifford, T., Hunte, B., Lesley, R., Muchamuel, T., Hurst, S.D., Zurawski, G., Leach, M.W., Gorman, D.M., Rennick, D.M., 2001. IL-25 Induces IL-4, IL-5, and IL-13 and Th2-Associated Pathologies In Vivo 15, 985–995.

Gaffen, S.L., 2009. Structure and signalling in the IL-17 receptor family. Nat Rev Immunol 9, 556–567.

Gaffen, S.L., 2011. Recent advances in the IL-17 cytokine family. Curr. Opin. Immunol. 23, 613–9. doi:10.1016/j.coi.2011.07.006

Garg, A. V, Ahmed, M., Vallejo, A.N., Ma, A., Gaffen, S.L., 2013. The deubiquitinase A20 mediates feedback inhibition of interleukin-17 receptor signaling. Sci. Signal. 6, ra44. doi:10.1126/scisignal.2003699

Gerhardt, S., Abbott, W.M., Hargreaves, D., Pauptit, R.A., Davies, R.A., Needham, M.R.C., Langham, C., Barker, W., Aziz, A., Snow, M.J., Dawson, S., Welsh, F., Wilkinson, T., Vaugan, T., Beste, G., Bishop, S., Popovic, B., Rees, G., Sleeman, M., Tuske, S.J., Coales, S.J., Hamuro, Y., Russell, C., 2009. Structure of IL-17A in

complex with a potent, fully human neutralizing antibody. J. Mol. Biol. 394, 905–21. doi:10.1016/j.jmb.2009.10.008

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., Regev, A., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29, 644–52. doi:10.1038/nbt.1883

Gu, C., Wu, L., Li, X., 2013. IL-17 family: cytokines, receptors and signaling. Cytokine 64, 477–85. doi:10.1016/j.cyto.2013.07.022

Gunimaladevi, I., Savan, R., Sakai, M., 2006. Identification, cloning and characterization of interleukin-17 and its family from zebrafish. Fish Shellfish Immunol. 21, 393–403. doi:10.1016/j.fsi.2006.01.004

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. doi:http://www.nature.com/nature/journal/v459/n7248/suppinfo/nature08068_S1.html

Hall, S., Yermolenko, Z.I., 2014. Transcriptome Analysis of Sea Lamprey Embryogenesis Transcriptome Analysis of Sea Lamprey Embryogenesis.

Hamada, S., Umemura, M., Shiono, T., Tanaka, K., Yahagi, A., Begum, M.D., Oshiro, K., Okamoto, Y., Watanabe, H., Kawakami, K., Roark, C., Born, W.K., O'Brien, R., Ikuta, K., Ishikawa, H., Nakae, S., Iwakura, Y., Ohta, T., Matsuzaki, G., 2008. IL-17A produced by gammadelta T cells plays a critical role in innate immunity against listeria monocytogenes infection in the liver. J. Immunol. 181, 3456–63.

Hartupee, J., Liu, C., Novotny, M., Li, X., Hamilton, T., 2007. IL-17 Enhances Chemokine Gene Expression through mRNA Stabilization. J. Immunol. 179, 4135–4141. doi:10.4049/jimmunol.179.6.4135

Hibino, T., Loza-Coll, M., Messier, C., Majeske, A.J., Cohen, A.H., Terwilliger, D.P., Buckley, K.M., Brockton, V., Nair, S. V, Berney, K., Fugmann, S.D., Anderson, M.K., Pancer, Z., Cameron, R.A., Smith, L.C., Rast, J.P., 2006. The immune gene repertoire encoded in the purple sea urchin genome. Dev. Biol. 300, 349–65. doi:10.1016/j.ydbio.2006.08.065

Hijnen, D., Knol, E.F., Gent, Y.Y., Giovannone, B., Beijn, S.J.P., Kupper, T.S., Bruijnzeel-Koomen, C.A.F.M., Clark, R.A., 2013. CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-γ, IL-13, IL-17, and IL-22. J. Invest. Dermatol. 133, 973–9. doi:10.1038/jid.2012.456

Hirano, M., Das, S., Guo, P., Cooper, M.D., 2011. Chapter 4 - The Evolution of Adaptive Immunity in Vertebrates, in: Frederick, W.A. (Ed.), Advances in Immunology. Academic Press, pp. 125–157. doi:10.1016/B978-0-12-387664-5.00004-2

Hirano, M., Guo, P., McCurley, N., Schorpp, M., Das, S., Boehm, T., Cooper, M.D., 2013. Evolutionary implications of a third lymphocyte lineage in lampreys. Nature 501, 435–8. doi:10.1038/nature12467

Hirata, T., Osuga, Y., Hamasaki, K., Yoshino, O., Ito, M., Hasegawa, A., Takemura, Y., Hirota, Y., Nose, E., Morimoto, C., Harada, M., Koga, K., Tajima, T., Saito, S., Yano, T., Taketani, Y., 2008. Interleukin (IL)-17A stimulates IL-8 secretion, cyclooxygensase-2 expression, and cell proliferation of endometriotic stromal cells. Endocrinology 149, 1260–7. doi:10.1210/en.2007-0749

Ho, A.W., Shen, F., Conti, H.R., Patel, N., Childs, E.E., Peterson, A.C., Hernández-Santos, N., Kolls, J.K., Kane, L.P., Ouyang, W., Gaffen, S.L., 2010. IL-17RC is required for immune signaling via an extended SEF/IL-17R signaling domain in the cytoplasmic tail. J. Immunol. 185, 1063–70. doi:10.4049/jimmunol.0903739

Holland, P.W.H., Garcia-Fernández, J., Williams, N.A., Sidow, A., 1994. Gene duplication and the origins of vertebrate development.

Hsu, H.-C., Yang, P., Wang, J., Wu, Q., Myers, R., Chen, J., Yi, J., Guentert, T., Tousson, A., Stanus, A.L., Le, T.L., Lorenz, R.G., Xu, H., Kolls, J.K., Carter, R.H., Chaplin, D.D., Williams, R.W., Mountz, J.D., 2008. Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. Nat. Immunol. 9, 166–75. doi:10.1038/ni1552

Hu, Y., Ota, N., Peng, I., Refino, C.J., Danilenko, D.M., Caplazi, P., Ouyang, W., 2010. IL-17RC is required for IL-17A- and IL-17F-dependent signaling and the pathogenesis of experimental autoimmune encephalomyelitis. J. Immunol. 184, 4307–16. doi:10.4049/jimmunol.0903614

Huang, C.-K., Yang, C.-Y., Jeng, Y.-M., Chen, C.-L., Wu, H.-H., Chang, Y.-C., Ma, C., Kuo, W.-H., Chang, K.-J., Shew, J.-Y., Lee, W.-H., 2014. Autocrine/paracrine mechanism of interleukin-17B receptor promotes breast tumorigenesis through NF-κB-mediated antiapoptotic pathway. Oncogene 33, 2968–77. doi:10.1038/onc.2013.268

Huang, H., Kim, H.J., Chang, E.-J., Lee, Z.H., Hwang, S.J., Kim, H.-M., Lee, Y., Kim, H.-H., 2009. IL-17 stimulates the proliferation and differentiation of human mesenchymal stem cells: implications for bone remodeling. Cell Death Differ. 16, 1332–43. doi:10.1038/cdd.2009.74

Huang, S., Yuan, S., Guo, L., Yu, Y.Y., Li, J., Wu, T., Liu, T., Yang, M., Wu, K., Liu, H., Ge, J., Yu, Y.Y., Huang, H., Dong, M., Yu, C., Chen, S., Xu, A., 2008. Genomic

analysis of the immune gene repertoire of amphioxus reveals extraordinary innate complexity and diversity. Genome Res. 18, 1112–1126. doi:10.1101/gr.069674.107

Huang, W., Na, L., Fidel, P.L., Schwarzenberger, P., 2004. Requirement of Interleukin-17A for Systemic Anti-Candida albicans Host Defense in Mice. J. Infect. Dis. 190, 624–631. doi:10.1086/422329

Huber, M., Heink, S., Pagenstecher, A., Reinhard, K., Ritter, J., Visekruna, A., Guralnik, A., Bollig, N., Jeltsch, K., Heinemann, C., Wittmann, E., Buch, T., Prazeres da Costa, O., Brüstle, A., Brenner, D., Mak, T.W., Mittrücker, H.-W., Tackenberg, B., Kamradt, T., Lohoff, M., 2013. IL-17A secretion by CD8+ T cells supports Th17-mediated autoimmune encephalomyelitis. J. Clin. Invest. 123, 247–60. doi:10.1172/JCI63681

Hurst, S.D., Muchamuel, T., Gorman, D.M., Gilbert, J.M., Clifford, T., Kwan, S., Menon, S., Seymour, B., Jackson, C., Kung, T.T., Brieland, J.K., Zurawski, S.M., Chapman, R.W., Zurawski, G., Coffman, R.L., 2002. New IL-17 Family Members Promote Th1 or Th2 Responses in the Lung: In Vivo Function of the Novel Cytokine IL-25. J. Immunol. 169, 443–453. doi:10.4049/jimmunol.169.1.443

Hwang, S.-Y., Kim, J.-Y., Kim, K.-W., Park, M.-K., Moon, Y., Kim, W.-U., Kim, H.-Y., 2004. IL-17 induces production of IL-6 and IL-8 in rheumatoid arthritis synovial fibroblasts via NF-kappaB- and PI3-kinase/Akt-dependent pathways. Arthritis Res. Ther. 6, R120–8. doi:10.1186/ar1038

Hymowitz, S.G., Filvaroff, E.H., Yin, J., Lee, J., Cai, L., Risser, P., Maruoka, M., Mao, W., Foster, J., Kelley, R.F., Pan, G., Gurney, A.L., de Vos, A.M., Starovasnik, M.A., 2001. IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding. EMBO J 20, 5332–5341.

Inabe, K., 2002. Tyrosine phosphorylation of B-cell adaptor for phosphoinositide 3-kinase is required for Akt activation in response to CD19 engagement. Blood 99, 584–589. doi:10.1182/blood.V99.2.584

Ishigame, H., Kakuta, S., Nagai, T., Kadoki, M., Nambu, A., Komiyama, Y., Fujikado, N., Tanahashi, Y., Akitsu, A., Kotaki, H., Sudo, K., Nakae, S., Sasakawa, C., Iwakura, Y., 2009. Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity 30, 108–19. doi:10.1016/j.immuni.2008.11.009

Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K.C., Santee, C.A., Lynch, S. V, Tanoue, T., Imaoka, A., Itoh, K., Takeda, K., Umesaki, Y., Honda, K., Littman, D.R., 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139, 485–98. doi:10.1016/j.cell.2009.09.033

Ivanov, I.I., Frutos, R. de L., Manel, N., Yoshinaga, K., Rifkin, D.B., Sartor, R.B., Finlay, B.B., Littman, D.R., 2008. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe 4, 337–49. doi:10.1016/j.chom.2008.09.009

Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., Littman, D.R., 2006. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126, 1121–33. doi:10.1016/j.cell.2006.07.035

Iwakura, Y., Ishigame, H., Saijo, S., Nakae, S., 2011. Functional specialization of interleukin-17 family members. Immunity 34, 149–62. doi:10.1016/j.immuni.2011.02.012

Johansen, C., Usher, P.A., Kjellerup, R.B., Lundsgaard, D., Iversen, L., Kragballe, K., 2009. Characterization of the interleukin-17 isoforms and receptors in lesional psoriatic skin. Br. J. Dermatol. 160, 319–24. doi:10.1111/j.1365-2133.2008.08902.x

Johnston, A., Fritz, Y., Dawes, S.M., Diaconu, D., Al-Attar, P.M., Guzman, A.M., Chen, C.S., Fu, W., Gudjonsson, J.E., McCormick, T.S., Ward, N.L., 2013. Keratinocyte overexpression of IL-17C promotes psoriasiform skin inflammation. J. Immunol. 190, 2252–62. doi:10.4049/jimmunol.1201505

Jones, C.E., Chan, K., 2002. Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene-alpha, and granulocyte-colony-stimulating factor by human airway epithelial cells. Am. J. Respir. Cell Mol. Biol. 26, 748–53.

Jones, D.T., Taylor, W.R., Thornton, J.M., 1992. The rapid generation of mutation data matrices from protein sequences. Comput. Appl. Biosci. 8, 275–82.

Jovanovic, D. V, Di Battista, J.A., Martel-Pelletier, J., Jolicoeur, F.C., He, Y., Zhang, M., Mineau, F., Pelletier, J.P., 1998. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J. Immunol. 160, 3513–21.

Jovcić, G., Bugarski, D., Krstić, A., Vlaski, M., Petakov, M., Mojsilović, S., Stojanović, N., Milenković, P., 2007. The effect of interleukin-17 on hematopoietic cells and cytokine release in mouse spleen. Physiol. Res. 56, 331–9.

Kao, C.-Y., Chen, Y., Thai, P., Wachi, S., Huang, F., Kim, C., Harper, R.W., Wu, R., 2004. IL-17 markedly up-regulates beta-defensin-2 expression in human airway epithelium via JAK and NF-kappaB signaling pathways. J. Immunol. 173, 3482–91.

Karlsen, J.R., Borregaard, N., Cowland, J.B., 2010. Induction of neutrophil gelatinase-associated lipocalin expression by co-stimulation with interleukin-17 and tumor

necrosis factor-alpha is controlled by IkappaB-zeta but neither by C/EBP-beta nor C/EBP-delta. J. Biol. Chem. 285, 14088–100. doi:10.1074/jbc.M109.017129

Kasamatsu, J., Oshiumi, H., Matsumoto, M., Kasahara, M., Seya, T., 2010a. Phylogenetic and expression analysis of lamprey toll-like receptors. Dev. Comp. Immunol. 34, 855–65. doi:10.1016/j.dci.2010.03.004

Kasamatsu, J., Sutoh, Y., Fugo, K., Otsuka, N., Iwabuchi, K., Kasahara, M., 2010b. Identification of a third variable lymphocyte receptor in the lamprey. Proc. Natl. Acad. Sci. U. S. A. 107, 14304–8. doi:10.1073/pnas.1001910107

Kawaguchi, M., Kokubu, F., Kuga, H., Matsukura, S., Hoshino, H., Ieki, K., Imai, T., Adachi, M., Huang, S.K., 2001. Modulation of bronchial epithelial cells by IL-17. J. Allergy Clin. Immunol. 108, 804–9. doi:10.1067/mai.2001.119027

Kawaguchi, M., Kokubu, F., Kuga, H., Tomita, T., Matsukura, S., Hoshino, H., Imai, T., Adachi, M., 1999. [Effect of IL-17 on ICAM-1 expression of human bronchial epithelial cells, NCI-H 292]. Arerugi 48, 1184–7.

Kim, M.R., Manoukian, R., Yeh, R., Silbiger, S.M., Danilenko, D.M., Scully, S., Sun, J., DeRose, M.L., Stolina, M., Chang, D., Van, G.Y., Clarkin, K., Nguyen, H.Q., Yu, Y. Bin, Jing, S., Senaldi, G., Elliott, G., Medlock, E.S., 2002. Transgenic overexpression of human IL-17E results in eosinophilia, B-lymphocyte hyperplasia, and altered antibody production. Blood 100, 2330–40. doi:10.1182/blood-2002-01-0012

Kono, T., Korenaga, H., Sakai, M., 2011. Genomics of fish IL-17 ligand and receptors: a review. Fish Shellfish Immunol. 31, 635–43. doi:10.1016/j.fsi.2010.11.028

Korenaga, H., Kono, T., Sakai, M., 2010. Isolation of seven IL-17 family genes from the Japanese pufferfish Takifugu rubripes. Fish Shellfish Immunol. 28, 809–18. doi:10.1016/j.fsi.2010.01.016

Korn, T., Bettelli, E., Oukka, M., Kuchroo, V.K., 2009. IL-17 and Th17 Cells. Annu. Rev. Immunol. 27, 485–517. doi:10.1146/annurev.immunol.021908.132710

Kotake, S., Udagawa, N., Takahashi, N., Matsuzaki, K., Itoh, K., Ishiyama, S., Saito, S., Inoue, K., Kamatani, N., Gillespie, M.T., Martin, T.J., Suda, T., 1999. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J. Clin. Invest. 103, 1345–52. doi:10.1172/JCI5703

Kovalenko, D., Yang, X., Nadeau, R.J., Harkins, L.K., Friesel, R., 2003. Sef Inhibits Fibroblast Growth Factor Signaling by Inhibiting FGFR1 Tyrosine Phosphorylation and Subsequent ERK Activation. J. Biol. Chem. 278, 14087–14091. doi:10.1074/jbc.C200606200

Kuestner, R.E., Taft, D.W., Haran, A., Brandt, C.S., Brender, T., Lum, K., Harder, B., Okada, S., Ostrander, C.D., Kreindler, J.L., Aujla, S.J., Reardon, B., Moore, M., Shea, P., Schreckhise, R., Bukowski, T.R., Presnell, S., Guerra-Lewis, P., Parrish-Novak, J., Ellsworth, J.L., Jaspers, S., Lewis, K.E., Appleby, M., Kolls, J.K., Rixon, M., West, J.W., Gao, Z., Levin, S.D., 2007. Identification of the IL-17 Receptor Related Molecule IL-17RC as the Receptor for IL-17F. J. Immunol. 179, 5462–5473.

Kumari, J., Larsen, A.N., Bogwald, J., Dalmo, R.A., 2009. Interleukin-17D in Atlantic salmon (Salmo salar): molecular characterization, 3D modelling and promoter analysis. Fish Shellfish Immunol. 27, 647–59. doi:10.1016/j.fsi.2009.08.009

Laan, M., Cui, Z.H., Hoshino, H., Lötvall, J., Sjöstrand, M., Gruenert, D.C., Skoogh, B.E., Lindén, A., 1999. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. J. Immunol. 162, 2347–52.

Laan, M., Lötvall, J., Chung, K.F., Lindén, A., 2001. IL-17-induced cytokine release in human bronchial epithelial cells in vitro: role of mitogen-activated protein (MAP) kinases. Br. J. Pharmacol. 133, 200–6. doi:10.1038/sj.bjp.0704063

Laan, M., Prause, O., Miyamoto, M., Sjöstrand, M., Hytönen, A.M., Kaneko, T., Lötvall, J., Lindén, A., 2003. A role of GM-CSF in the accumulation of neutrophils in the airways caused by IL-17 and TNF-alpha. Eur. Respir. J. 21, 387–93.

Larkin, M. a, Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P. a, McWilliam, H., Valentin, F., Wallace, I.M., Wilm, a, Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–8. doi:10.1093/bioinformatics/btm404

Lee, J., Ho, W.H., Maruoka, M., Corpuz, R.T., Baldwin, D.T., Foster, J.S., Goddard, a D., Yansura, D.G., Vandlen, R.L., Wood, W.I., Gurney, a L., 2001. IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1. J. Biol. Chem. 276, 1660–4. doi:10.1074/jbc.M008289200

LeGrand, A., Fermor, B., Fink, C., Pisetsky, D.S., Weinberg, J.B., Vail, T.P., Guilak, F., 2001. Interleukin-1, tumor necrosis factor alpha, and interleukin-17 synergistically up-regulate nitric oxide and prostaglandin E2 production in explants of human osteoarthritic knee menisci. Arthritis Rheum. 44, 2078–83. doi:10.1002/1529-0131(200109)44:9<2078::AID-ART358>3.0.CO;2-J

Léonard, P.M., Adema, C.M., Zhang, S.M., Loker, E.S., 2001. Structure of two FREP genes that combine IgSF and fibrinogen domains, with comments on diversity of the FREP gene family in the snail Biomphalaria glabrata. Gene 269, 155–65.

Leonardi, A., Chariot, A., Claudio, E., Cunningham, K., Siebenlist, U., 2000. CIKS, a connection to Ikappa B kinase and stress-activated protein kinase. Proc. Natl. Acad. Sci. 97, 10494–10499. doi:10.1073/pnas.190245697

Li, H., Chen, J., Huang, A., Stinson, J., Heldens, S., Foster, J., Dowd, P., Gurney, A.L., Wood, W.I., 2000. Cloning and characterization of IL-17B and IL-17C, two new members of the IL-17 cytokine family. Proc. Natl. Acad. Sci. 97, 773–778.

Li, J., Zhang, Y., Zhang, Y., Xiang, Z., Tong, Y., Qu, F., Yu, Z., 2014. Genomic characterization and expression analysis of five novel IL-17 genes in the Pacific oyster, Crassostrea gigas. Fish Shellfish Immunol. 40, 455–65. doi:10.1016/j.fsi.2014.07.026

Li, L., Huang, L., Vergis, A.L., Ye, H., Bajwa, A., Narayan, V., Strieter, R.M., Rosin, D.L., Okusa, M.D., 2010. IL-17 produced by neutrophils regulates IFN-gamma-mediated neutrophil migration in mouse kidney ischemia-reperfusion injury. J. Clin. Invest. 120, 331–42. doi:10.1172/JCI38702

Liang, S.C., Tan, X.-Y., Luxenberg, D.P., Karim, R., Dunussi-Joannopoulos, K., Collins, M., Fouser, L.A., 2006. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J. Exp. Med. 203, 2271–9. doi:10.1084/jem.20061308

Liu, C., Qian, W., Qian, Y., Giltiay, N. V, Lu, Y., Swaidani, S., Misra, S., Deng, L., Chen, Z.J., Li, X., 2009. Act1, a U-box E3 ubiquitin ligase for IL-17 signaling. Sci. Signal. 2, ra63. doi:10.1126/scisignal.2000382

Liu, L., Okada, S., Kong, X.-F., Kreins, A.Y., Cypowyj, S., Abhyankar, A., Toubiana, J., Itan, Y., Audry, M., Nitschke, P., Masson, C., Toth, B., Flatot, J., Migaud, M., Chrabieh, M., Kochetkov, T., Bolze, A., Borghesi, A., Toulon, A., Hiller, J., Eyerich, S., Eyerich, K., Gulácsy, V., Chernyshova, L., Chernyshov, V., Bondarenko, A., Grimaldo, R.M.C., Blancas-Galicia, L., Beas, I.M.M., Roesler, J., Magdorf, K., Engelhard, D., Thumerelle, C., Burgel, P.-R., Hoernes, M., Drexel, B., Seger, R., Kusuma, T., Jansson, A.F., Sawalle-Belohradsky, J., Belohradsky, B., Jouanguy, E., Bustamante, J., Bué, M., Karin, N., Wildbaum, G., Bodemer, C., Lortholary, O., Fischer, A., Blanche, S., Al-Muhsen, S., Reichenbach, J., Kobayashi, M., Rosales, F.E., Lozano, C.T., Kilic, S.S., Oleastro, M., Etzioni, A., Traidl-Hoffmann, C., Renner, E.D., Abel, L., Picard, C., Marodi, L., Boisson-Dupuis, S., Puel, A., Casanova, J.-L., 2011. Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. J. Exp. Med. 208, 1635–48. doi:10.1084/jem.20110958

Liu, S., Song, X., Chrunyk, B.A., Shanker, S., Hoth, L.R., Marr, E.S., Griffor, M.C., 2013. Crystal structures of interleukin 17A and its complex with IL-17 receptor A. Nat. Commun. 4, 1888. doi:10.1038/ncomms2880

Liu, S.-J., Tsai, J.-P., Shen, C.-R., Sher, Y.-P., Hsieh, C.-L., Yeh, Y.-C., Chou, A.-H., Chang, S.-R., Hsiao, K.-N., Yu, F.-W., Chen, H.-W., 2007. Induction of a distinct CD8 Tnc17 subset by transforming growth factor-beta and interleukin-6. J. Leukoc. Biol. 82, 354–60. doi:10.1189/jlb.0207111

Lockhart, E., Green, A.M., Flynn, J.L., 2006. IL-17 production is dominated by gammadelta T cells rather than CD4 T cells during Mycobacterium tuberculosis infection. J. Immunol. 177, 4662–9.

Lu, Y.-J., Gross, J., Bogaert, D., Finn, A., Bagrade, L., Zhang, Q., Kolls, J.K., Srivastava, A., Lundgren, A., Forte, S., Thompson, C.M., Harney, K.F., Anderson, P.W., Lipsitch, M., Malley, R., 2008. Interleukin-17A mediates acquired immunity to pneumococcal colonization. PLoS Pathog. 4, e1000159. doi:10.1371/journal.ppat.1000159

Lubberts, E., 2008. IL-17/Th17 targeting: on the road to prevent chronic destructive arthritis? Cytokine 41, 84–91. doi:10.1016/j.cyto.2007.09.014

Lubberts, E., Koenders, M.I., van den Berg, W.B., 2005. The role of T-cell interleukin-17 in conducting destructive arthritis: lessons from animal models. Arthritis Res. Ther. 7, 29–37. doi:10.1186/ar1478

Lubberts, E., van den Bersselaar, L., Oppers-Walgreen, B., Schwarzenberger, P., Coenen-de Roo, C.J.J., Kolls, J.K., Joosten, L.A.B., van den Berg, W.B., 2003. IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-kappa B ligand/osteoprotegerin balance. J. Immunol. 170, 2655–62.

Maezawa, Y., Nakajima, H., Suzuki, K., Tamachi, T., Ikeda, K., Inoue, J. -i., Saito, Y., Iwamoto, I., 2006. Involvement of TNF Receptor-Associated Factor 6 in IL-25 Receptor Signaling. J. Immunol. 176, 1013–1018. doi:10.4049/jimmunol.176.2.1013

Martin, B., Hirota, K., Cua, D.J., Stockinger, B., Veldhoen, M., 2009. Interleukin-17-Producing γδ T Cells Selectively Expand in Response to Pathogen Products and Environmental Signals. Immunity 31, 321–330. doi:10.1016/j.immuni.2009.06.020

McDonald, N.Q., Lapatto, R., Murray-Rust, J., Gunning, J., Wlodawer, A., Blundell, T.L., 1991. New protein fold revealed by a 2.3-A resolution crystal structure of nerve growth factor. Nature 354, 411–4. doi:10.1038/354411a0

Mellett, M., Atzei, P., Horgan, A., Hams, E., Floss, T., Wurst, W., Fallon, P.G., Moynagh, P.N., 2012. Orphan receptor IL-17RD tunes IL-17A signalling and is required for neutrophilia. Nat. Commun. 3, 1119. doi:10.1038/ncomms2127

Michel, M.-L., Keller, A.C., Paget, C., Fujio, M., Trottein, F., Savage, P.B., Wong, C.-H., Schneider, E., Dy, M., Leite-de-Moraes, M.C., 2007. Identification of an IL-17-producing NK1.1neg iNKT cell population involved in airway neutrophilia. J. Exp. Med. 204, 995–1001. doi:10.1084/jem.20061551

Milner, J.D., Brenchley, J.M., Laurence, A., Freeman, A.F., Hill, B.J., Elias, K.M., Kanno, Y., Spalding, C., Elloumi, H.Z., Paulson, M.L., Davis, J., Hsu, A., Asher,

A.I., O'Shea, J., Holland, S.M., Paul, W.E., Douek, D.C., 2008. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature 452, 773–6. doi:10.1038/nature06764

Miossec, P., Korn, T., Kuchroo, V.K., 2009. Interleukin-17 and type 17 helper T cells. N. Engl. J. Med. 361, 888–98. doi:10.1056/NEJMra0707449

Mitsdoerffer, M., Lee, Y., Jäger, A., Kim, H.-J., Korn, T., Kolls, J.K., Cantor, H., Bettelli, E., Kuchroo, V.K., 2010. Proinflammatory T helper type 17 cells are effective B-cell helpers. Proc. Natl. Acad. Sci. U. S. A. 107, 14292–7. doi:10.1073/pnas.1009234107

Miyazaki, Y., Hamano, S., Wang, S., Shimanoe, Y., Iwakura, Y., Yoshida, H., 2010. IL-17 is necessary for host protection against acute-phase Trypanosoma cruzi infection. J. Immunol. 185, 1150–7. doi:10.4049/jimmunol.0900047

Monte, M.M., Wang, T., Holland, J.W., Zou, J., Secombes, C.J., 2013. Cloning and characterization of rainbow trout interleukin-17A/F2 (IL-17A/F2) and IL-17 receptor A: expression during infection and bioactivity of recombinant IL-17A/F2. Infect. Immun. 81, 340–53. doi:10.1128/IAI.00599-12

Moore, E.E., Presnell, S., Garrigues, U., Guilbot, A., LeGuern, E., Smith, D., Yao, L., Whitmore, T.E., Gilbert, T., Palmer, T.D., Horner, P.J., Kuestner, R.E., 2002. Expression of IL-17B in neurons and evaluation of its possible role in the chromosome 5q-linked form of Charcot–Marie–Tooth disease. Neuromuscul. Disord. 12, 141–150. doi:10.1016/S0960-8966(01)00250-4

Mosmann, T.R., Li, L., Sad, S., 1997. Functions of CD8 T-cell subsets secreting different cytokine patterns. Semin. Immunol. 9, 87–92. doi:10.1006/smim.1997.0065

Mudter, J., Yu, J., Zufferey, C., Brüstle, A., Wirtz, S., Weigmann, B., Hoffman, A., Schenk, M., Galle, P.R., Lehr, H. a, Mueller, C., Lohoff, M., Neurath, M.F., 2011. IRF4 regulates IL-17A promoter activity and controls RORγt-dependent Th17 colitis in vivo. Inflamm. Bowel Dis. 17, 1343–58. doi:10.1002/ibd.21476

Nigam, P., Kwa, S., Velu, V., Amara, R.R., 2011. Loss of IL-17-producing CD8 T cells during late chronic stage of pathogenic simian immunodeficiency virus infection. J. Immunol. 186, 745–53. doi:10.4049/jimmunol.1002807

Novatchkova, M., Leibbrandt, A., Werzowa, J., Neubüser, A., Eisenhaber, F., 2003. The STIR-domain superfamily in signal transduction, development and immunity. Trends Biochem. Sci. 28, 226–9. doi:10.1016/S0968-0004(03)00067-7

Numasaki, M., Takahashi, H., Tomioka, Y., Sasaki, H., 2004. Regulatory roles of IL-17 and IL-17F in G-CSF production by lung microvascular endothelial cells stimulated with IL-1beta and/or TNF-alpha. Immunol. Lett. 95, 97–104. doi:10.1016/j.imlet.2004.06.010

O'Connor, W., Kamanaka, M., Booth, C.J., Town, T., Nakae, S., Iwakura, Y., Kolls, J.K., Flavell, R.A., 2009. A protective function for interleukin 17A in T cell-mediated intestinal inflammation. Nat. Immunol. 10, 603–9. doi:10.1038/ni.1736

O'Sullivan, T., Saddawi-Konefka, R., Gross, E., Tran, M., Mayfield, S.P., Ikeda, H., Bui, J.D., 2014. Interleukin-17D mediates tumor rejection through recruitment of natural killer cells. Cell Rep. 7, 989–98. doi:10.1016/j.celrep.2014.03.073

Ohno, S., 1970. Evolution by gene duplication.

Okada, T., Maeda, A., Iwamatsu, A., Gotoh, K., Kurosaki, T., 2000. BCAP: the tyrosine kinase substrate that connects B cell receptor to phosphoinositide 3-kinase activation. Immunity 13, 817–27.

Okamoto, K., Iwai, Y., Oh-Hora, M., Yamamoto, M., Morio, T., Aoki, K., Ohya, K., Jetten, A.M., Akira, S., Muta, T., Takayanagi, H., 2010. IkappaBzeta regulates T(H)17 development by cooperating with ROR nuclear receptors. Nature 464, 1381–5. doi:10.1038/nature08922

Onishi, R.M., Park, S.J., Hanel, W., Ho, A.W., Maitra, A., Gaffen, S.L., 2010. SEF/IL-17R (SEFIR) is not enough: an extended SEFIR domain is required for il-17RA-mediated signal transduction. J. Biol. Chem. 285, 32751–9. doi:10.1074/jbc.M110.121418

Owyang, A.M., Zaph, C., Wilson, E.H., Guild, K.J., McClanahan, T., Miller, H.R.P., Cua, D.J., Goldschmidt, M., Hunter, C.A., Kastelein, R.A., Artis, D., 2006. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. J. Exp. Med. 203, 843–9. doi:10.1084/jem.20051496

Pan, G., French, D., Mao, W., Maruoka, M., Risser, P., Lee, J., Foster, J., Aggarwal, S., Nicholes, K., Guillet, S., Schow, P., Gurney, A.L., 2001. Forced Expression of Murine IL-17E Induces Growth Retardation, Jaundice, a Th2-Biased Response, and Multiorgan Inflammation in Mice. J. Immunol. 167, 6559–6567. doi:10.4049/jimmunol.167.11.6559

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 22–27.

Pappu, R., Rutz, S., Ouyang, W., 2012. Regulation of epithelial immunity by IL-17 family cytokines. Trends Immunol. 33, 343–9. doi:10.1016/j.it.2012.02.008

Park, H., Li, Z., Yang, X.O., Chang, S.H., Nurieva, R., Wang, Y.-H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., Dong, C., 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat. Immunol. 6, 1133–41. doi:10.1038/ni1261

Passos, S.T., Silver, J.S., O'Hara, A.C., Sehy, D., Stumhofer, J.S., Hunter, C.A., 2010. IL-6 promotes NK cell production of IL-17 during toxoplasmosis. J. Immunol. 184, 1776–83. doi:10.4049/jimmunol.0901843

Pettinello, R., Dooley, H., 2014. The immunoglobulins of cold-blooded vertebrates. Biomolecules 4, 1045–69. doi:10.3390/biom4041045

Prause, O., 2004. Increased matrix metalloproteinase-9 concentration and activity after stimulation with interleukin-17 in mouse airways. Thorax 59, 313–317. doi:10.1136/thx.2003.008854

Preger, E., Ziv, I., Shabtay, A., Sher, I., Tsang, M., Dawid, I.B., Altuvia, Y., Ron, D., 2004. Alternative splicing generates an isoform of the human Sef gene with altered subcellular localization and specificity. Proc. Natl. Acad. Sci. U. S. A. 101, 1229–34. doi:10.1073/pnas.0307952100

Puel, A., Cypowyj, S., Bustamante, J., Wright, J.F., Liu, L., Lim, H.K., Migaud, M., Israel, L., Chrabieh, M., Audry, M., Gumbleton, M., Toulon, A., Bodemer, C., El-Baghdadi, J., Whitters, M., Paradis, T., Brooks, J., Collins, M., Wolfman, N.M., Al-Muhsen, S., Galicchio, M., Abel, L., Picard, C., Casanova, J.-L., 2011. Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. Science 332, 65–8. doi:10.1126/science.1200439

Qian, Y., Liu, C., Hartupee, J., Altuntas, C.Z., Gulen, M.F., Jane-Wit, D., Xiao, J., Lu, Y., Giltiay, N., Liu, J., Kordula, T., Zhang, Q.-W., Vallance, B., Swaidani, S., Aronica, M., Tuohy, V.K., Hamilton, T., Li, X., 2007. The adaptor Act1 is required for interleukin 17-dependent signaling associated with autoimmune and inflammatory disease. Nat. Immunol. 8, 247–56. doi:10.1038/ni1439

Raffatellu, M., Santos, R.L., Verhoeven, D.E., George, M.D., Wilson, R.P., Winter, S.E., Godinez, I., Sankaran, S., Paixao, T.A., Gordon, M.A., Kolls, J.K., Dandekar, S., Bäumler, A.J., 2008. Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes Salmonella dissemination from the gut. Nat. Med. 14, 421–8. doi:10.1038/nm1743

Ramirez-Carrozzi, V., Sambandam, A., Luis, E., Lin, Z., Jeet, S., Lesch, J., Hackney, J., Kim, J., Zhou, M., Lai, J., Modrusan, Z., Sai, T., Lee, W., Xu, M., Caplazi, P., Diehl, L., de Voss, J., Balazs, M., Gonzalez, L., Singh, H., Ouyang, W., Pappu, R., 2011. IL-17C regulates the innate immune function of epithelial cells in an autocrine manner. Nat. Immunol. 12, 1159–66. doi:10.1038/ni.2156

Reynolds, J.M., Martinez, G.J., Nallaparaju, K.C., Chang, S.H., Wang, Y.-H., Dong, C., 2012. Cutting edge: regulation of intestinal inflammation and barrier function by IL-17C. J. Immunol. 189, 4226–30. doi:10.4049/jimmunol.1103014

Roberts, S., Gueguen, Y., de Lorgeril, J., Goetz, F., 2008. Rapid accumulation of an interleukin 17 homolog transcript in Crassostrea gigas hemocytes following bacterial exposure. Dev. & Comp. Immunol. 32, 1099–1104. doi:10.1016/j.dci.2008.02.006

Rong, Z., Wang, A., Li, Z., Ren, Y., Cheng, L., Li, Y., Wang, Y., Ren, F., Zhang, X., Hu, J., Chang, Z., 2009. IL-17RD (Sef or IL-17RLM) interacts with IL-17 receptor and mediates IL-17 signaling. Cell Res. 19, 208–15. doi:10.1038/cr.2008.320

Round, J.L., Lee, S.M., Li, J., Tran, G., Jabri, B., Chatila, T.A., Mazmanian, S.K., 2011. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science 332, 974–7. doi:10.1126/science.1206095

Rouvier, E., Luciani, M.F., Mattei, M.G., Denizot, F., Golstein, P., 1993. CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene. J. Immunol. 150, 5445–5456.

Rudner, X.L., Happel, K.I., Young, E.A., Shellito, J.E., 2007. Interleukin-23 (IL-23)-IL-17 cytokine axis in murine Pneumocystis carinii infection. Infect. Immun. 75, 3055–61. doi:10.1128/IAI.01329-06

Rumfelt, L.L., McKinney, E.C., Taylor, E., Flajnik, M.F., 2002. The development of primary and secondary lymphoid tissues in the nurse shark Ginglymostoma cirratum: B-cell zones precede dendritic cell immigration and T-cell zone formation during ontogeny of the spleen. Scand. J. Immunol. 56, 130–48.

Sad, S., Marcotte, R., Mosmann, T.R., 1995. Cytokine-induced differentiation of precursor mouse CD8+ T cells into cytotoxic CD8+ T cells secreting Th1 or Th2 cytokines. Immunity 2, 271–279. doi:10.1016/1074-7613(95)90051-9

Saijo, S., Ikeda, S., Yamabe, K., Kakuta, S., Ishigame, H., Akitsu, A., Fujikado, N., Kusaka, T., Kubo, S., Chung, S., Komatsu, R., Miura, N., Adachi, Y., Ohno, N., Shibuya, K., Yamamoto, N., Kawakami, K., Yamasaki, S., Saito, T., Akira, S., Iwakura, Y., 2010. Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against Candida albicans. Immunity 32, 681–91. doi:10.1016/j.immuni.2010.05.001

Sanders, A.J., Guo, X., Mason, M.D., Jiang, W.G., 2010. IL-17B Can Impact on Endothelial Cellular Traits Linked to Tumour Angiogenesis. J. Oncol. 2010, 817375. doi:10.1155/2010/817375

Saunders, H.L., Oko, A.L., Scott, A.N., Fan, C.W., Magor, B.G., 2010. The cellular context of AID expressing cells in fish lymphoid tissues. Dev. Comp. Immunol. 34, 669–76. doi:10.1016/j.dci.2010.01.013

Saxena, A., Raychaudhuri, S.K., Raychaudhuri, S.P., 2011. Interleukin-17-induced proliferation of fibroblast-like synovial cells is mTOR dependent. Arthritis Rheum. 63, 1465–6. doi:10.1002/art.30278

Schraml, B.U., Hildner, K., Ise, W., Lee, W.-L., Smith, W.A.-E., Solomon, B., Sahota, G., Sim, J., Mukasa, R., Cemerski, S., Hatton, R.D., Stormo, G.D., Weaver, C.T., Russell, J.H., Murphy, T.L., Murphy, K.M., 2009. The AP-1 transcription factor Batf controls T(H)17 differentiation. Nature 460, 405–9. doi:10.1038/nature08114

Schwarzenberger, P., Huang, W., Ye, P., Oliver, P., Manuel, M., Zhang, Z., Bagby, G., Nelson, S., Kolls, J.K., 2000. Requirement of endogenous stem cell factor and granulocyte-colony-stimulating factor for IL-17-mediated granulopoiesis. J. Immunol. 164, 4783–9.

Shahrara, S., Pickens, S.R., Dorfleutner, A., Pope, R.M., 2009. IL-17 induces monocyte migration in rheumatoid arthritis. J. Immunol. 182, 3884–91. doi:10.4049/jimmunol.0802246

Shalom-Barak, T., Quach, J., Lotz, M., 1998. Interleukin-17-induced gene expression in articular chondrocytes is associated with activation of mitogen-activated protein kinases and NF-kappaB. J. Biol. Chem. 273, 27467–73.

Shen, F., Gaffen, S.L., 2008. Structure-function relationships in the IL-17 receptor: implications for signal transduction and therapy. Cytokine 41, 92–104. doi:10.1016/j.cyto.2007.11.013

Shen, F., Li, N., Gade, P., Kalvakolanu, D. V, Weibley, T., Doble, B., Woodgett, J.R., Wood, T.D., Gaffen, S.L., 2009. IL-17 receptor signaling inhibits C/EBPbeta by sequential phosphorylation of the regulatory 2 domain. Sci. Signal. 2, ra8. doi:10.1126/scisignal.2000066

Shi, P., Zhu, S., Lin, Y., Liu, Y., Liu, Y., Chen, Z., Shi, Y., Qian, Y., 2011. Persistent stimulation with interleukin-17 desensitizes cells through SCFβ-TrCP-mediated degradation of Act1. Sci. Signal. 4, ra73. doi:10.1126/scisignal.2001653

Shi, Y., Ullrich, S.J., Zhang, J., Connolly, K., Grzegorzewski, K.J., Barber, M.C., Wang, W., Wathen, K., Hodge, V., Fisher, C.L., Olsen, H., Ruben, S.M., Knyazev, I., Cho, Y.H., Kao, V., Wilkinson, K. a, Carrell, J. a, Ebner, R., 2000. A novel cytokine receptor-ligand pair. Identification, molecular characterization, and in vivo immunomodulatory activity. J. Biol. Chem. 275, 19167–76. doi:10.1074/jbc.M910228199

Shibata, M., Shintaku, Y., Matsuzaki, K., Uematsu, S., 2014. The effect of IL-17 on the production of proinflammatory cytokines and matrix metalloproteinase-1 by human periodontal ligament fibroblasts. Orthod. Craniofac. Res. 17, 60–8. doi:10.1111/ocr.12033

Smith, J.J., Kuraku, S., Holt, C., Sauka-Spengler, T., Jiang, N., Campbell, M.S., Yandell, M.D., Manousaki, T., Meyer, A., Bloom, O.E., Morgan, J.R., Buxbaum, J.D., Sachidanandam, R., Sims, C., Garruss, A.S., Cook, M., Krumlauf, R., Wiedemann, L.M., Sower, S. a, Decatur, W. a, Hall, J. a, Amemiya, C.T., Saha, N.R., Buckley, K.M., Rast, J.P., Das, S., Hirano, M., McCurley, N., Guo, P., Rohner, N., Tabin, C.J., Piccinelli, P., Elgar, G., Ruffier, M., Aken, B.L., Searle, S.M.J., Muffato, M., Pignatelli, M., Herrero, J., Jones, M., Brown, C.T., Chung-Davidson, Y.-W., Nanlohy, K.G., Libants, S. V, Yeh, C.-Y., McCauley, D.W., Langeland, J. a, Pancer, Z., Fritzsch, B., de Jong, P.J., Zhu, B., Fulton, L.L., Theising, B., Flicek, P., Bronner, M.E., Warren, W.C., Clifton, S.W., Wilson, R.K., Li, W., 2013. Sequencing of the sea lamprey (Petromyzon marinus) genome provides insights into vertebrate evolution. Nat. Genet. 45, 415–21, 421e1–2. doi:10.1038/ng.2568

Song, X., Zhu, S., Shi, P., Liu, Y., Shi, Y., Levin, S.D., Qian, Y., 2011. IL-17RE is the functional receptor for IL-17C and mediates mucosal immunity to infection with intestinal pathogens. Nat. Immunol. 12, 1151–8. doi:10.1038/ni.2155

Starnes, T., Broxmeyer, H.E., Robertson, M.J., Hromas, R., 2002. Cutting Edge: IL-17D, a Novel Member of the IL-17 Family, Stimulates Cytokine Production and Inhibits Hemopoiesis.

Sun, D., Novotny, M., Bulek, K., Liu, C., Li, X., Hamilton, T., 2011. Treatment with IL-17 prolongs the half-life of chemokine CXCL1 mRNA via the adaptor TRAF5 and the splicing-regulatory factor SF2 (ASF). Nat. Immunol. 12, 853–60. doi:10.1038/ni.2081

Suryawanshi, A., Veiga-Parga, T., Rajasagi, N.K., Reddy, P.B.J., Sehrawat, S., Sharma, S., Rouse, B.T., 2011. Role of IL-17 and Th17 cells in herpes simplex virus-induced corneal immunopathology. J. Immunol. 187, 1919–30. doi:10.4049/jimmunol.1100736

Suzukawa, M., Morita, H., Nambu, A., Arae, K., Shimura, E., Shibui, A., Yamaguchi, S., Suzukawa, K., Nakanishi, W., Oboki, K., Kajiwara, N., Ohno, T., Ishii, A., Körner, H., Cua, D.J., Suto, H., Yoshimoto, T., Iwakura, Y., Yamasoba, T., Ohta, K., Sudo, K., Saito, H., Okumura, K., Broide, D.H., Matsumoto, K., Nakae, S., 2012. Epithelial cell-derived IL-25, but not Th17 cell-derived IL-17 or IL-17F, is crucial for murine asthma. J. Immunol. 189, 3641–52. doi:10.4049/jimmunol.1200461

Swaidani, S., Bulek, K., Kang, Z., Gulen, M.F., Liu, C., Yin, W., Abbadi, A., Aronica, M., Li, X., 2011. T cell-derived Act1 is necessary for IL-25-mediated Th2 responses and allergic airway inflammation. J. Immunol. 187, 3155–64. doi:10.4049/jimmunol.1002790

Swaidani, S., Bulek, K., Kang, Z., Liu, C., Lu, Y., Yin, W., Aronica, M., Li, X., 2009. The Critical Role of Epithelial-Derived Act1 in IL-17- and IL-25-Mediated

Pulmonary Inflammation. J. Immunol. 182, 1631–1640. doi:10.4049/jimmunol.182.3.1631

Takahashi, N., Vanlaere, I., de Rycke, R., Cauwels, A., Joosten, L.A.B., Lubberts, E., van den Berg, W.B., Libert, C., 2008. IL-17 produced by Paneth cells drives TNF-induced shock. J. Exp. Med. 205, 1755–61. doi:10.1084/jem.20080588

Tamachi, T., Maezawa, Y., Ikeda, K., Kagami, S.-I., Hatano, M., Seto, Y., Suto, A., Suzuki, K., Watanabe, N., Saito, Y., Tokuhisa, T., Iwamoto, I., Nakajima, H., 2006. IL-25 enhances allergic airway inflammation by amplifying a TH2 cell-dependent pathway in mice. J. Allergy Clin. Immunol. 118, 606–14. doi:10.1016/j.jaci.2006.04.051

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–9. doi:10.1093/molbev/msr121

Terajima, D., Yamada, S., Uchino, R., Ikawa, S., Ikeda, M., Shida, K., Arai, Y., Wang, H.-G., Satoh, N., Satake, M., 2003. Identification and Sequence of Seventy-nine New Transcripts Expressed in Hemocytes of Ciona intestinalis, Three of Which May Be Involved in Characteristic Cell-cell Communication. DNA Res. 10, 203–212. doi:10.1093/dnares/10.5.203

Teunissen, M.B., Koomen, C.W., de Waal Malefyt, R., Wierenga, E.A., Bos, J.D., 1998. Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. J. Invest. Dermatol. 111, 645–9. doi:10.1046/j.1523-1747.1998.00347.x

Toy, D., Kugler, D., Wolfson, M., Bos, T. Vanden, Gurgel, J., Derry, J., Tocker, J., Peschon, J., 2006. Cutting Edge: Interleukin 17 Signals through a Heteromeric Receptor Complex. J. Immunol. 177, 36–39.

Trajkovic, V., Stosic-Grujicic, S., Samardzic, T., Markovic, M., Miljkovic, D., Ramic, Z., Mostarica Stojkovic, M., 2001. Interleukin-17 stimulates inducible nitric oxide synthase activation in rodent astrocytes. J. Neuroimmunol. 119, 183–91.

Troutman, T.D., Hu, W., Fulenchek, S., Yamazaki, T., Kurosaki, T., Bazan, J.F., Pasare, C., 2012. Role for B-cell adapter for PI3K (BCAP) as a signaling adapter linking Toll-like receptors (TLRs) to serine/threonine kinases PI3K/Akt. Proc. Natl. Acad. Sci. U. S. A. 109, 273–8. doi:10.1073/pnas.1118579109

Tsang, M., Friesel, R., Kudoh, T., Dawid, I.B., 2002. Identification of Sef, a novel modulator of FGF signalling. Nat. Cell Biol. 4, 165–9. doi:10.1038/ncb749

Tsutsui, S., Nakamura, O., Watanabe, T., 2007. Lamprey (Lethenteron japonicum) IL-17 upregulated by LPS-stimulation in the skin cells. Immunogenetics 59, 873–82. doi:10.1007/s00251-007-0254-2

Umemura, M., Yahagi, A., Hamada, S., Begum, M.D., Watanabe, H., Kawakami, K., Suda, T., Sudo, K., Nakae, S., Iwakura, Y., Matsuzaki, G., 2007. IL-17-mediated regulation of innate and acquired immune response against pulmonary Mycobacterium bovis bacille Calmette-Guerin infection. J. Immunol. 178, 3786–96.

Valenzuela-Muñoz, V., Gallardo-Escárate, C., 2014. Molecular cloning and expression of IRAK-4, IL-17 and I-κB genes in Haliotis rufescens challenged with Vibrio anguillarum. Fish Shellfish Immunol. 36, 503–9. doi:10.1016/j.fsi.2013.12.015

Vizzini, A., Falco, F. Di, Parrinello, D., Sanfratello, M.A., Mazzarella, C., Parrinello, N., Cammarata, M., 2014. Ciona intestinalis interleukin 17-like genes expression is upregulated by LPS challenge. Dev. Comp. Immunol. 48, 129–137. doi:10.1016/j.dci.2014.09.014

Wang, T., Martin, S. a M., Secombes, C.J., 2010. Two interleukin-17C-like genes exist in rainbow trout Oncorhynchus mykiss that are differentially expressed and modulated. Dev. Comp. Immunol. 34, 491–500. doi:10.1016/j.dci.2009.11.011

Wang, X., Li, C., Thongda, W., Luo, Y., Beck, B., Peatman, E., 2014. Characterization and mucosal responses of interleukin 17 family ligand and receptor genes in channel catfish Ictalurus punctatus. Fish Shellfish Immunol. 38, 47–55. doi:10.1016/j.fsi.2014.02.020

Wang, Y.-H., Angkasekwinai, P., Lu, N., Voo, K.S., Arima, K., Hanabuchi, S., Hippe, A., Corrigan, C.J., Dong, C., Homey, B., Yao, Z., Ying, S., Huston, D.P., Liu, Y.-J., 2007. IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. J. Exp. Med. 204, 1837–47. doi:10.1084/jem.20070406

Watson, F.L., Püttmann-Holgado, R., Thomas, F., Lamar, D.L., Hughes, M., Kondo, M., Rebel, V.I., Schmucker, D., 2005. Extensive Diversity of Ig-Superfamily Proteins in the Immune System of Insects. Science (80-.). 309, 1874–1878.

Weaver, C.T., Hatton, R.D., Mangan, P.R., Harrington, L.E., 2007. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu. Rev. Immunol. 25, 821–52. doi:10.1146/annurev.immunol.25.022106.141557

Wells, J.M., Rossi, O., Meijerink, M., van Baarlen, P., 2011. Epithelial crosstalk at the microbiota-mucosal interface. Proc. Natl. Acad. Sci. U. S. A. 108 Suppl , 4607–14. doi:10.1073/pnas.1000092107

Witowski, J., Pawlaczyk, K., Breborowicz, A., Scheuren, A., Kuzlan-Pawlaczyk, M., Wisniewska, J., Polubinska, A., Friess, H., Gahl, G.M., Frei, U., Jorres, A., 2000. IL-17 Stimulates Intraperitoneal Neutrophil Infiltration Through the Release of GRO Chemokine from Mesothelial Cells. J. Immunol. 165, 5814–5821. doi:10.4049/jimmunol.165.10.5814

Wright, J.F., Bennett, F., Li, B., Brooks, J., Luxenberg, D.P., Whitters, M.J., Tomkinson, K.N., Fitz, L.J., Wolfman, N.M., Collins, M., Dunussi-Joannopoulos, K., Chatterjee-Kishore, M., Carreno, B.M., 2008. The Human IL-17F/IL-17A Heterodimeric Cytokine Signals through the IL-17RA/IL-17RC Receptor Complex. J. Immunol. 181, 2799–2805. doi:10.4049/jimmunol.181.4.2799

Wright, J.F., Guo, Y., Quazi, A., Luxenberg, D.P., Bennett, F., Ross, J.F., Qiu, Y., Whitters, M.J., Tomkinson, K.N., Dunussi-Joannopoulos, K., Carreno, B.M., Collins, M., Wolfman, N.M., 2007. Identification of an Interleukin 17F/17A Heterodimer in Activated Human CD4+ T Cells. J. Biol. Chem. 282, 13447–13455. doi:10.1074/jbc.M700499200

Wu, B., Jin, M., Gong, J., Du, X., Bai, Z., 2011a. Dynamic evolution of CIKS (TRAF3IP2/Act1) in metazoans. Dev. Comp. Immunol. 35, 1186–92. doi:10.1016/j.dci.2011.03.027

Wu, B., Jin, M., Zhang, Y., Wei, T., Bai, Z., 2011b. Evolution of the IL17 receptor family in chordates: a new subfamily IL17REL. Immunogenetics 63, 835–45. doi:10.1007/s00251-011-0554-4

Wu, F., Chen, L., Liu, X., Wang, H., Su, P., Han, Y., Feng, B., Qiao, X., Zhao, J., Ma, N., Liu, H., Zheng, Z., Li, Q., 2013. Lamprey variable lymphocyte receptors mediate complement-dependent cytotoxicity. J. Immunol. 190, 922–30. doi:10.4049/jimmunol.1200876

Wu, H.-H., Hwang-Verslues, W.W., Lee, W.-H., Huang, C.-K., Wei, P.-C., Chen, C.-L., Shew, J.-Y., Lee, E.Y.-H.P., Jeng, Y.-M., Tien, Y.-W., Ma, C., Lee, W.-H., 2015. Targeting IL-17B-IL-17RB signaling with an anti-IL-17RB antibody blocks pancreatic cancer metastasis by silencing multiple chemokines. J. Exp. Med. jem.20141702–. doi:10.1084/jem.20141702

Wu, H.-J., Ivanov, I.I., Darce, J., Hattori, K., Shima, T., Umesaki, Y., Littman, D.R., Benoist, C., Mathis, D., 2010. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity 32, 815–27. doi:10.1016/j.immuni.2010.06.001

Wu, S.-Z., Huang, X.-D., Li, Q., He, M.-X., 2013. Interleukin-17 in pearl oyster (Pinctada fucata): molecular cloning and functional characterization. Fish Shellfish Immunol. 34, 1050–6. doi:10.1016/j.fsi.2013.01.005

Xie, S., Hsu, H.-C., Li, J., Wu, Q., Yang, P., Mountz, J.D., 2010a. IL-17 Downregulates NF-B1/P50 And Upregulates BCL6 To Promote Germinal Center B Cell Differentiation In Autoimmune BXD2 Mice. [WWW Document]. Arthritis Rheum. URL http://www.blackwellpublishing.com/acrmeeting/abstract.asp?MeetingID=774&id=90059 (accessed 2.22.15).

Xie, S., Li, J., Wang, J.H., Wu, Q., Yang, P., Hsu, H.-C., Smythies, L.E., Mountz, J.D., 2010b. IL-17 activates the canonical NF-kappaB signaling pathway in autoimmune B cells of BXD2 mice to upregulate the expression of regulators of G-protein signaling 16. J. Immunol. 184, 2289–96. doi:10.4049/jimmunol.0903133

Xiong, S., Zhao, Q., Rong, Z., Huang, G., Huang, Y., Chen, P., Zhang, S., Liu, L., Chang, Z., 2003. hSef inhibits PC-12 cell differentiation by interfering with Ras-mitogen-activated protein kinase MAPK signaling. J. Biol. Chem. 278, 50273–82. doi:10.1074/jbc.M306936200

Xu, S., Han, Y., Xu, X., Bao, Y., Zhang, M., Cao, X., 2010. IL-17A-producing gammadeltaT cells promote CTL responses against Listeria monocytogenes infection by enhancing dendritic cell cross-presentation. J. Immunol. 185, 5879–87. doi:10.4049/jimmunol.1001763

Yamaguchi, Y., Fujio, K., Shoda, H., Okamoto, A., Tsuno, N.H., Takahashi, K., Yamamoto, K., 2007. IL-17B and IL-17C are associated with TNF-alpha production and contribute to the exacerbation of inflammatory arthritis. J. Immunol. 179, 7128–36.

Yang, R.-B., Ng, C.K.D., Wasserman, S.M., Kömüves, L.G., Gerritsen, M.E., Topper, J.N., 2003. A novel interleukin-17 receptor-like protein identified in human umbilical vein endothelial cells antagonizes basic fibroblast growth factor-induced signaling. J. Biol. Chem. 278, 33232–8. doi:10.1074/jbc.M305022200

Yang, X., Kovalenko, D., Nadeau, R.J., Harkins, L.K., Mitchell, J., Zubanova, O., Chen, P.-Y., Friesel, R., 2004. Sef interacts with TAK1 and mediates JNK activation and apoptosis. J. Biol. Chem. 279, 38099–102. doi:10.1074/jbc.C400318200

Yang, X.O., Panopoulos, A.D., Nurieva, R., Chang, S.H., Wang, D., Watowich, S.S., Dong, C., 2007. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J. Biol. Chem. 282, 9358–63. doi:10.1074/jbc.C600321200

Yang, X.O., Pappu, B.P., Nurieva, R., Akimzhanov, A., Kang, H.S., Chung, Y., Ma, L., Shah, B., Panopoulos, A.D., Schluns, K.S., Watowich, S.S., Tian, Q., Jetten, A.M., Dong, C., 2008. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. Immunity 28, 29–39. doi:10.1016/j.immuni.2007.11.016

Yao, Z., Fanslow, W.C., Seldin, M.F., Rousseau, A.-M., Painter, S.L., Comeau, M.R., Cohen, J.I., Spriggs, M.K., 1995. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity 3, 811–821. doi:10.1016/1074-7613(95)90070-5

Ye, P., Garvey, P.B., Zhang, P., Nelson, S., Bagby, G., Summer, W.R., Schwarzenberger, P., Shellito, J.E., Kolls, J.K., 2001. Interleukin-17 and lung host defense against Klebsiella pneumoniae infection. Am. J. Respir. Cell Mol. Biol. 25, 335–40. doi:10.1165/ajrcmb.25.3.4424

Ye, P., Rodriguez, F.H., Kanaly, S., Stocking, K.L., Schurr, J., Schwarzenberger, P., Oliver, P., Huang, W., Zhang, P., Zhang, J., Shellito, J.E., Bagby, G.J., Nelson, S., Charrier, K., Peschon, J.J., Kolls, J.K., 2001. Requirement of Interleukin 17 Receptor Signaling for Lung Cxc Chemokine and Granulocyte Colony-Stimulating Factor Expression, Neutrophil Recruitment, and Host Defense. J. Exp. Med. 194, 519–528. doi:10.1084/jem.194.4.519

Yen, H.-R., Harris, T.J., Wada, S., Grosso, J.F., Getnet, D., Goldberg, M. V, Liang, K.-L., Bruno, T.C., Pyle, K.J., Chan, S.-L., Anders, R.A., Trimble, C.L., Adler, A.J., Lin, T.-Y., Pardoll, D.M., Huang, C.-T., Drake, C.G., 2009. Tc17 CD8 T cells: functional plasticity and subset diversity. J. Immunol. 183, 7161–8. doi:10.4049/jimmunol.0900368

You, Z., DuRaine, G., Tien, J.Y.L., Lee, C., Moseley, T.A., Reddi, A.H., 2005. Expression of interleukin-17B in mouse embryonic limb buds and regulation by BMP-7 and bFGF. Biochem. Biophys. Res. Commun. 326, 624–31. doi:10.1016/j.bbrc.2004.11.087

Zaph, C., Du, Y., Saenz, S.A., Nair, M.G., Perrigoue, J.G., Taylor, B.C., Troy, A.E., Kobuley, D.E., Kastelein, R.A., Cua, D.J., Yu, Y., Artis, D., 2008. Commensal-dependent expression of IL-25 regulates the IL-23-IL-17 axis in the intestine. J. Exp. Med. 205, 2191–8. doi:10.1084/jem.20080720

Zepp, J.A., Liu, C., Qian, W., Wu, L., Gulen, M.F., Kang, Z., Li, X., 2012. Cutting edge: TNF receptor-associated factor 4 restricts IL-17-mediated pathology and signaling processes. J. Immunol. 189, 33–7. doi:10.4049/jimmunol.1200470

Zhang, B., Liu, C., Qian, W., Han, Y., Li, X., Deng, J., 2013. Crystal structure of IL-17 receptor B SEFIR domain. J. Immunol. 190, 2320–6. doi:10.4049/jimmunol.1202922

Zhang, B., Liu, C., Qian, W., Han, Y., Li, X., Deng, J., 2014. Structure of the unique SEFIR domain from human interleukin 17 receptor A reveals a composite ligand-binding site containing a conserved α-helix for Act1 binding and IL-17 signaling. Acta Crystallogr. D. Biol. Crystallogr. 70, 1476–83. doi:10.1107/S1399004714005227

Zhang, F., Meng, G., Strober, W., 2008. Interactions among the transcription factors Runx1, RORgammat and Foxp3 regulate the differentiation of interleukin 17-producing T cells. Nat. Immunol. 9, 1297–306. doi:10.1038/ni.1663

Zhang, F., Tanaka, H., Kawato, T., Kitami, S., Nakai, K., Motohashi, M., Suzuki, N., Wang, C.-L., Ochiai, K., Isokawa, K., Maeno, M., 2011. Interleukin-17A induces cathepsin K and MMP-9 expression in osteoclasts via celecoxib-blocked prostaglandin E2 in osteoblasts. Biochimie 93, 296–305. doi:10.1016/j.biochi.2010.10.001

Zhang, S.-M., Adema, C.M., Kepler, T.B., Loker, E.S., 2004. Diversification of Ig Superfamily Genes in an Invertebrate. Science (80-.). 305, 251–254.

Zhang, S.M., L éonard, P.M., Adema, C.M., Loker, E.S., 2001. Parasite-responsive IgSF members in the snail Biomphalaria glabrata: characterization of novel genes with tandemly arranged IgSF domains and a fibrinogen domain. Immunogenetics 53, 684–94. doi:10.1007/s00251-001-0386-8

Zhao, A., Urban, J.F., Sun, R., Stiltz, J., Morimoto, M., Notari, L., Madden, K.B., Yang, Z., Grinchuk, V., Ramalingam, T.R., Wynn, T.A., Shea-Donohue, T., 2010. Critical Role of IL-25 in Nematode Infection-Induced Alterations in Intestinal Function. J. Immunol. 185, 6921–6929. doi:10.4049/jimmunol.1000450

Zhong, B., Liu, X., Wang, X., Chang, S.H., Liu, X., Wang, A., Reynolds, J.M., Dong, C., 2012. Negative regulation of IL-17-mediated signaling and inflammation by the ubiquitin-specific protease USP25. Nat. Immunol. 13, 1110–7. doi:10.1038/ni.2427

Zhu, S., Pan, W., Shi, P., Gao, H., Zhao, F., Song, X., Liu, Y., Zhao, L., Li, X., Shi, Y., Qian, Y., 2010. Modulation of experimental autoimmune encephalomyelitis through TRAF3-mediated suppression of interleukin 17 receptor signaling. J. Exp. Med. 207, 2647–62. doi:10.1084/jem.20100703

Zhu, S., Pan, W., Song, X., Liu, Y., Shao, X., Tang, Y., Liang, D., He, D., Wang, H., Liu, W., Shi, Y., Harley, J.B., Shen, N., Qian, Y., 2012. The microRNA miR-23b suppresses IL-17-associated autoimmune inflammation by targeting TAB2, TAB3 and IKK-α. Nat. Med. 18, 1077–86. doi:10.1038/nm.2815

Zimmerman, L.M., Vogel, L.A., Bowden, R.M., 2010. Understanding the vertebrate immune system: insights from the reptilian perspective. J. Exp. Biol. 213, 661–71. doi:10.1242/jeb.038315

Zizzo, G., Cohen, P.L., 2013. IL-17 stimulates differentiation of human anti-inflammatory macrophages and phagocytosis of apoptotic neutrophils in response to IL-10 and glucocorticoids. J. Immunol. 190, 5237–46. doi:10.4049/jimmunol.1203017