Immunogenetics
DOI 10.1007/s00251-014-0781-6

REVIEW

A prominent role for invariant T cells in the amphibian *Xenopus laevis* tadpoles

Jacques Robert · Eva-Stina Edholm

Received: 20 April 2014 / Accepted: 12 May 2014

Abstract Invariant T (iT) cells expressing an invariant or semi-invariant T cell receptor (TCR) repertoire have gained attention in recent years because of their potential as specialized regulators of immune function. These iT cells are typically restricted by nonclassical MHC class I molecules (e.g., CD1d and MR1) and undergo differentiation pathways distinct from conventional T cells. While the benefit of a limited TCR repertoire may appear counterintuitive in regard to the advantage of the diversified repertoire of conventional T cells allowing for exquisite specificity to antigens, the full biological importance and evolutionary conservation of iT cells are just starting to emerge. It is generally considered that iT cells are specialized to recognize conserved antigens equivalent to pathogen-associated molecular pattern. Until recently, little was known about the evolution of iT cells. The identification of class Ib and class I-like genes in nonmammalian vertebrates, despite the heterogeneity and variable numbers of these genes among species, suggests that iT cells are also present in ectothermic vertebrates. Indeed, recent studies in the amphibian *Xenopus* have revealed a drastic overrepresentation of several invariant TCRs in tadpoles and identified a prominent nonclassical MHC class I-restricted iT cell subset critical for tadpole antiviral immunity. This suggests an important and perhaps even dominant role of multiple nonclassical MHC class I-restricted iT cell populations in tadpoles and, by extension, other aquatic vertebrates with rapid external development that are under pressure to produce a functional lymphocyte repertoire with small numbers of cells.

Keywords Developmental immunology · Evolution · iVα6 · Invariant T cells · XNC · Nonclassical MHC

J. Robert (✉) · E.-S. Edholm
Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY 14642, USA
e-mail: Jacques_Robert@urmc.rochester.edu

Introduction

Invariant T (iT) cells expressing a limited or semi-invariant T cell receptor (TCR) repertoire have gained considerable attention in recent years because of their potential as potent and specialized regulators of immune function (Le Bourhis et al. 2013b; Simoni et al. 2013; Treiner 2003; Wilson and Delovitch 2003; Wu et al. 2009). Unlike conventional T cells, these iT cells are typically restricted by nonclassical MHC class I molecules (e.g., CD1d and MR1) and undergo distinct thymic differentiation pathways (Bendelac 1995; Treiner et al. 2003). From an evolutionary standpoint, the existence of adaptive T cells with innate-like characteristics such as a restricted TCR repertoire is somewhat perplexing. Indeed, one of the hallmarks of jawed vertebrate (gnathostome) adaptive immunity is the generation of a vast TCR repertoire by combinatorial post-somatic diversification. This process occurs during T cell development in the thymus, is mediated by recombination-activating gene products RAG1 and RAG2, and relies upon the random recombination of variable (V), diversity (D), and joining (J) gene segments. During development of α/β T cells, the TCRβ chain is first generated by the combinatorial rearrangement of Jβ, Dβ, and Vβ gene segments followed by the subsequent generation of the TCRα chain by rearrangement of Jα and Vα gene segments. This V(D)J recombination results in a α/β TCR repertoire capable of specifically recognizing and distinguishing between a broad array of antigens. Indeed, the resulting TCR combinatorial power is estimated to be in the order of 10^{15} possibilities thus facilitating an evolutionarily advantageous TCR diversity that can specifically recognize a plethora of possible pathogen antigens (Davis and Bjorkman 1988). It is noteworthy that although the mechanisms governing T cell development become more complicated when MHC-mediated positive/negative selection and tolerance are taken into account, the basic underlying mechanisms used to generate the α/β TCR

Published online: 05 June 2014

repertoire (i.e., thymus dependency, RAG-mediated differentiation processes, and MHC restriction) are remarkably similar across all gnathostomes (reviewed in Cooper and Alder 2006; Litman et al. 2010). It is generally thought that the ability to generate a diversified α/β TCR repertoire was one of the fundamental features during vertebrate evolution facilitating the emergence of adaptive immunity. Intuitively, a highly diversified TCR repertoire provides a selective advantage to jawed vertebrates in the arms race with pathogens. As such, T cell subsets with restricted or semi-invariant TCRs have, until recently, been considered as unconventional, specialized effectors primarily confined to endothermic vertebrates. However, we have recently shown that iT cells are present and critically involved in immunity in the amphibian *Xenopus laevis* (Edholm et al. 2013). In addition, the identification of variable numbers of nonclassical MHC genes in all groups of ectothermic vertebrates suggests a more prominent role of nonclassical MHC-restricted unconventional T cells in vertebrate immunity than previously thought. In this review, we will first summarize iT cells in mammals focusing on CD1d and MR1-restricted iT cells and subsequently review what is known about iT cells in *Xenopus*.

Development of conventional and unconventional T cells in mammals

In mammals, intrathymic T cell development is characterized by the successive surface expression of the co-receptors CD4 and CD8, which are used to distinguish three different stages of thymocyte differentiation: (1) an early double negative ($CD4^-CD8^-$; DN) stage; (2) a predominant double positive ($CD4^+CD8^+$; DP) stage; and (3) a $CD4^+$ or $CD8^+$ single positive (SP) stage (reviewed in Rothenberg and Taghon 2005). TCR gene assembly is carried out in two distinct developmental stages and is initiated by *TCR*β locus rearrangement in immature DN thymocytes (Bonnet et al. 2009). Successful surface expression of the TCRβ chain in a pre-receptor complex then drives activation of *TCR*α locus recombination and differentiation into the DP stage (Michie and Zuniga-Pflucker 2002; Krangel et al. 2004). During the DP stage, TCRαβ heterodimers are expressed on the cell surface, and at this point, thymocytes are eligible for both positive and negative selection (Starr et al. 2003). For conventional T cells, these selection processes are dependent on interactions with highly polymorphic MHC molecules presenting self peptides: MHC class II molecules for CD4 T cells and classical MHC class Ia (class Ia) for CD8 T cells. Positive selection is mediated by MHC molecules expressed by the thymic epithelium, while negative selection is facilitated by MHC expressed by hematopoietic cells in the thymic medulla as well as by the thymic medullary epithelium (Anderson et al. 1996; Hogquist et al. 2005; Jameson et al. 1995).

While the education of conventional CD8 T cells with polyclonal TCRαβ repertoires requires interaction with highly polymorphic class Ia molecules, there is now compelling evidence in mammals that several nonclassical molecules, expressed by the immature thymocytes themselves and other hematopoietic cells, selectively mediate the differentiation of various unconventional and iT cell lineages (Bendelac 1995; Jensen et al. 2004; Prince et al. 2009; Treiner et al. 2003; Urdahl et al. 2002).

For example, the ontogeny of the two types of iT cells described in mammals—invariant natural killer T (iNKT) cells and Mucosal Associated Invariant T (MAIT) cells—is dependent on selection on CD1d or MR1, respectively, expressed on immature cortical thymocytes (Bendelac 1995; Gapin et al. 2001; Treiner et al. 2003; Gold et al. 2013). Thus, both iNKT and MAIT positive selection are mediated by homotypic interactions between DP thymocytes but are restricted by distinct nonclassical molecules. Following either CD1d- or MR1-mediated positive selection, the differentiation of these two iT cell populations undergoes distinct developmental programs. Differentiation of iNKT cells is further dependent on the interaction between members of the family of signaling lymphocytic activation molecules (SLAM) (Chung et al. 2005), specifically the SLAMF1 and SLAMF6 receptors (Griewank et al. 2007). This interaction leads to downstream recruitment of the SLAM-associated protein SAP and the Scr-family kinase Fyn and then activation of NF-kB providing a signal that drives the cell toward the iNKT cell lineage. Following this initial CD1d/SLAM receptor-mediated positive selection, the iNKT cell precursors undergo a series of well-controlled intrathymic differentiation steps characterized by the sequential expression of various cell surface markers, including CD24, CD44, and the NK cell marker 1.1 (CD161 in humans) (reviewed in Godfrey and Berzins 2007). Most iNKT cells exit the thymus as immature, $NK1.1^-$, cells and reach final maturity, marked by expression of NK1.1 in the periphery. Alternatively, iNKT cells undergo a proliferative burst and remain as mature, long-term residents in the thymus (Bendelac 1995; Coles and Raulet 2000; and reviewed in Godfrey and Berzins 2007). In contrast to iNKT cells, MAIT cell development is not dependent on SLAM receptor-mediated signaling (Martin et al. 2009). Following MR1-mediated thymic selection, the majority of MAIT cells exit the thymus as immature cells (Le Bourhis et al. 2011, 2013b; Gold et al. 2013) and subsequently undergo B cell-dependent clonal expansion and maturation in the periphery (Chun et al. 2003; Martin et al. 2009; Treiner et al. 2003). This peripheral maturation step also appears to be dependent on the commensal flora, as MAIT cells are absent in germ-free mice (Martin et al. 2009). Notably, MAIT cells have been shown to, at least partially, expand after recolonization of the bacterial flora in germ-free mice suggesting a complex relationship between MAIT cells and mucosal microbes. Also, it has been

suggested that microbial products within the gut flora play a role in regulating MR1 surface expression. The main characteristics of iNKT cells and MAIT cells are summarized in Table 1.

Development of conventional T cells in *Xenopus*

The essential role of the thymus in T cell development and education is a hallmark of the adaptive immune system of jawed vertebrates. This is underscored by the conservation of key transcription factors regulating mammalian T cell development (e.g., GATA-3 and Runx3) throughout vertebrates, including agnathans (Du Pasquier et al. 1995; Zon et al. 1991). As in mammals, in *Xenopus*, thymic ontogeny is characterized by successive waves of T cell precursors moving into the thymus where they expand, differentiate, and leave as mature T cells (reviewed in Hansen and Zapata 1998). The thymus anlage appears 3 days after fertilization (stage 40; Nieuwkoop and Faber 1967) and is colonized by embryonic stem cells within a few days (Flajnik et al. 1985; Kau and Turpen 1983; Tochinai 1980). Subsequently, the cortex-medulla architecture is distinguishable at 6–8 days of age (st 48; Du Pasquier and Flajnik 1990). In the absence of monoclonal antibodies (mAb) specific for *X. laevis* CD4, a thymocyte differentiation pathway has been characterized, using *X. laevis*-specific mAbs recognizing CD8, CD5 (a *X. laevis* pan T cell marker, Jurgens et al. 1995), and CD45 (Barritt and Turpen 1995) in conjunction with a *X. laevis* mAb recognizing the cortical thymocyte-specific *Xenopus* (CTX) molecule as a surface marker of immature thymocytes (Chretien et al. 1996; Robert and Cohen 1998). Thymocytes were found to differentiate from an immature DP-like (CTX^{+}, $CD8^{+}$, $CD5^{low}$, $CD45^{low}$) to a more mature SP-like stage (CTX^{neg}, $CD5^{bright}$, $CD45^{bright}$) that could be further subdivided into $CD8^{bright}$ and $CD8^{neg}$ (Robert and Cohen 1999). The first detection of CD8β and CD4 gene expression at the time of thymic organogenesis provides additional evidence of the differentiation of CD8 and CD4 T cells in the tadpole thymus (Chida et al. 2011). However, due to low cell number, the formal demonstration of CD8 and CD4 differential gene expression in T cell subsets (i.e., $CD8^{bright}$ and $CD8^{neg}$) sorted by flow cytometry was only done in adults.

It is noteworthy that this intrathymic differentiation pathway is not only observed in tadpoles but also in young post-metamorphic adults (Fig. 1; Robert et al. 2001; Robert and Cohen 1999). This is because in contrast to mammals, the *Xenopus* immune system undergoes striking additional developmental changes during the transition from tadpole to adult (Du Pasquier et al. 1989; Du Pasquier and Weiss 1973; Flajnik et al. 1987; Rollins-Smith 1998; Rollins-Smith et al. 2000). While intrathymic T cell differentiation appears to be overall similar during embryogenesis and metamorphosis, several distinctive features merit consideration. First, during metamorphosis, the thymus loses more than 90 % of its lymphocytes (Du Pasquier and Weiss 1973). This loss is followed by a second wave of stem cell immigration just after metamorphic completion (Gravenor et al. 1995; Turpen and Smith 1989). The differentiation of these new thymocyte precursors takes place in a different environment, since during metamorphosis, the whole organism is remodeled and many new adult-type proteins are expressed that could potentially be considered antigenic by the larval immune system (Flajnik et al. 1987). It is likely, therefore, that the emerging adult T cells are subjected to a new "adult-type" education including negative selection by the adult "self," resulting in a new balance of self-tolerance. A second important distinction of the post-metamorphic thymic developmental program is reflected by the differential expression of MHC class I and class II molecules between tadpoles and adults. Class II protein is

Table 1 Main characteristics of mammalian and *Xenopus* invariant T cells

	Mammals		*Xenopus*
	MAIT	iNKT	XNC10-iT
TCRα	Vα7.2/19-Jα33	Vα14/24-Jα18	Vα6-Jα1.43
Phenotype	DN, $CD8^{+}$	Type I DN/CD4 Type II $CD8^{dim}$	Type I DN Type II $CD8^{dim}$
Restriction	MR1	CD1d	XNC10
Ligand(s)	Vitamin B metabolites	Lipids	?
Conservation	Mammals (chicken)	Mammals and chicken	*Xenopodinea*
Development	Thymus/gut	Thymus	Thymus
Prominence	Rare (mouse) Abundant (human)	Abundant (mouse) Rare (human)	Abundant
Location	Mucosa gut, lung	Spleen, liver	Spleen
Cytokine	IFN-γ, TGF-β	IFN-γ, IL-4	?
Function	Antimicrobial immunity	Tumor immunity, viral immunity, and autoimmunity	Viral immunity, tumor immunity

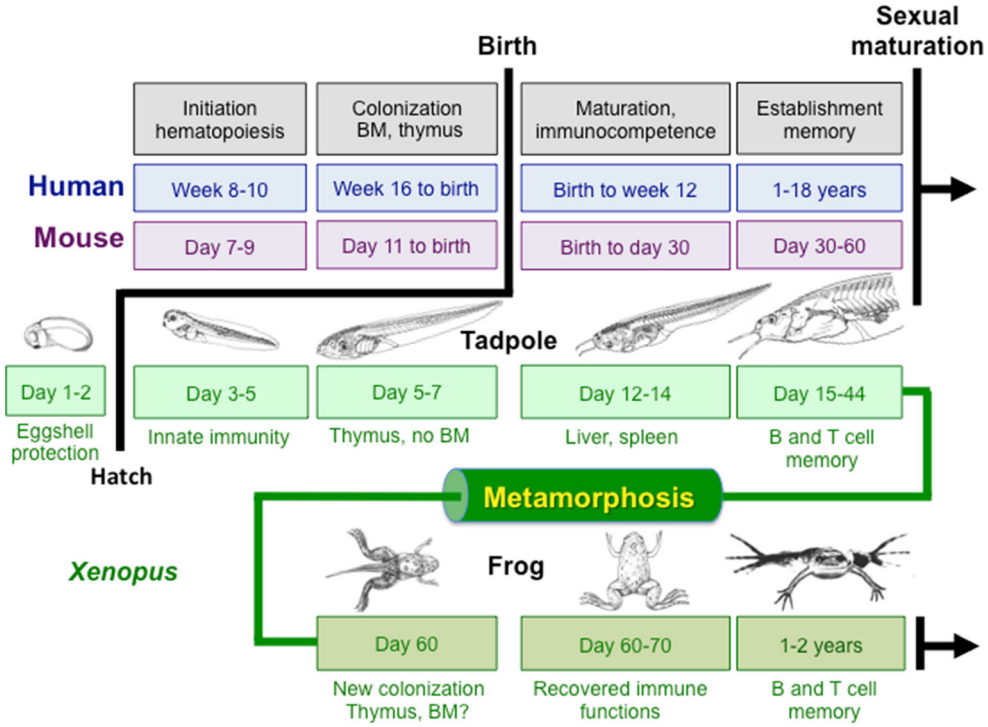

Fig. 1 Schematic comparison of the main developmental stages of the immune system of human, mouse, and *Xenopus*. Compared to mammals, the *Xenopus* embryonic development is external and leads to a free-swimming organism (hatch) 2 days post-fertilization. *Xenopus* thymus colonization by precursor cells and T cell differentiation already occurs from 5 to 7 days of age, compared to 11 days in mouse and 16 weeks in human. Furthermore, in contrast to mammals, a second major developmental program takes place in *Xenopus* during metamorphosis wherein most thymocytes die and are replaced by a new wave of stem cells colonizing the thymus at the end of metamorphosis, which differentiate into new T cells expressing a TCR repertoire distinct from that of tadpoles. Intriguingly, B and T cell memory established in the tadpole can persist in adults. Also, in contrast to mammals, B cell differentiation occurs in the liver and spleen of tadpoles and adults, whereas bone marrow (BM) appears to be mainly involved in later stages of macrophage differentiation and as an accumulation site of other leukocytes

expressed by cells located in the thymic medulla, B cells, and leukocytes of both tadpoles and adults. Comparably, thymocytes and T cells are class II negative in tadpoles, whereas they are positive in adults (Du Pasquier and Flajnik 1990).

The case of classical MHC class Ia expression is even more intriguing in *Xenopus*, since class Ia molecules are not expressed at the surface of most cells until metamorphosis (Flajnik et al. 1986). More specifically, surface class Ia protein expression is first detected by anti-class I pAbs and mAbs on erythrocytes and splenocytes at metamorphic stages (Rollins-Smith et al. 1997a, b). Indeed, the tadpole thymus not only lacks significant class Ia protein but also LMP7 expression until metamorphosis (Salter-Cid et al. 1998). However, despite the lack of class Ia protein detection by Abs, some class Ia mRNA is detected in the tadpole's thymus (Goyos et al. 2007). Thus, even though low levels of class Ia protein expression cannot be ruled out, this suggests a deficient or suboptimal class Ia-restricted thymic education during tadpole life. Whereas in mammals, experimental impairment of class Ia expression results in severe immunodeficiency and/or death, *Xenopus* pre-metamorphic tadpoles are immunocompetent and have circulating CD8 T cells even without optimal class Ia expression and function. One possible mechanism that may explain these observations would be the utilization of nonclassical MHC molecules in tadpole thymic T cell education and an overall more limited TCR repertoire in tadpoles. The main characteristics of the *Xenopus* immune system during development are summarized in Table 2.

Nonclassical MHC and unconventional T cells in *Xenopus*

Interestingly, while *X. laevis* possesses only one polymorphic class Ia gene per genome, it possesses a large family of nonclassical MHC genes located outside the MHC proper in the telomeric region of the same chromosome (Flajnik et al. 1993). From the recent whole genome sequencing and annotation of *X. laevis*, a total of 23 *Xenopus* nonclassical MHC genes (*XNCs*) have been identified (Edholm et al 2014; Goyos et al. 2011). As in mammals, these genes are oligomorphic and have a more limited tissue expression than class Ia genes. Unlike the unsteadiness of mammalian nonclassical genes, most *XNC* genes present an unusual degree of evolutionary conservation within the *Xenopus* subfamily *Xenopodinae* (Goyos et al. 2011; Edholm et al 2014). Indeed, the *Xenopodinae* subfamily has an evolutionary history of at least

Table 2 Main characteristics of the *Xenopus* immune system during development

	Tadpoles	Metamorphosis	Adults
B cell response	Poor class switch (IgM to IgY[a])	?	Class switch (IgM to IgY)
MLR	Weak MLR[b]	No MLR	Strong MLR
T cell cytotoxicity	Weak or absent	Weak or absent	Strong
NK cells	Not detected	Some	Yes (spleen, liver)
NK cytotoxicity	Not detected	Not detected	Yes
iT cells	Yes (spleen)	?	Yes (spleen)
Viral immunity	Delayed and poor innate immunity iT cells critical	?	Efficient innate, CD8 T and B cell responses

[a] IgG functional analog in *Xenopus*

[b] Mixed lymphocyte reaction

65 million years, which is as long a period of time as that separating primates and rodents from a common ancestor (Evans 2008; Evans et al. 2004). This is intriguing, as nonclassical genes in general are known to have a rapid rate of evolution. With the exception of MR1 genes that are highly conserved in mammals (Tsukamoto et al. 2013) and possibly chickens (Hee et al. 2010) and CD1 genes that are conserved in mammals (Dascher 2007) and avians (Salomonsen et al. 2005; Miller et al. 2005), no clear phylogeny can be established for most other nonclassical genes. In contrast, the detailed comparison of *X. laevis* XNC genes with nonclassical genes identified from a divergent *Xenopus* species belonging to a different genus, *Xenopus* (*Siluriana*) *tropicalis*, revealed a remarkably high degree of homology among multiple nonclassical gene families (Goyos et al. 2011; Edholm et al. 2014). Furthermore, for some XNCs, this degree of conservation extends to many other *Xenopodinae* species. This is the case for the monogenic lineage represented by XNC10 that is conserved in all ten *Xenopodinae* species tested so far (Edholm et al. 2014). The remarkable degree of conservation of these XNC genes implies that they have been selected and maintained during the evolution of this taxon to fulfill important physiological functions.

To explore this possibility, we have focused on XNC10 that is one of the most conserved XNC genes. In *X. laevis* and *X. tropicalis*, XNC10 (or SNC10) is mainly expressed in the thymus from early thymic organogenesis (Goyos et al. 2009, 2011). In fact, XNC/SNC10 is preferentially expressed by the cortical thymocytes rather than the thymic epithelium. Low expression of XNC10 is also detected in the spleen. Given the preferential expression of XNC10 by thymocytes from early thymic organogenesis (when class Ia is suboptimal), we hypothesized that XNC10 was involved in the differentiation of an unconventional T cell subset. To identify cells interacting with XNC10, we generated XNC10 tetramers (XNC10-T) by employing a methodology similar to that used for CD1d tetramers (Sidobre and Kronenberg 2002) consisting of fusing the *b2m* with the XNC10 heavy chain. Using this approach, we successfully generated XNC10 tetramers that were utilized to identify two XNC10-restricted iT cell subsets termed type I and type II (Edholm et al. 2013; Table 1). Specifically, the type I iT cell subset is CD8/CD4 DN and expresses an invariant TCRα rearrangement (Vα6-Jα1.43) without n-nucleotide diversity. Comparably, the type II subset displays low CD8α/α surface expression and a more diverse TCRα repertoire, however with a clear predominance of the Vα6-Jα1.43 rearrangement. Both type I and II subsets expressed, in contrast to conventional CD8 T cells, a limited TCRβ diversity (Edholm et al. 2013). To demonstrate the requirement of XNC10 for the development of these iT cell subsets, we took advantage of a reverse genetic loss-of-function method that combines RNA interference with transgenesis (Nedelkovska and Robert 2012). Silencing XNC10 gene expression in transgenic animals resulted in the loss of the invariant TCRα rearrangement and both iT cell subsets (Edholm et al. 2013).

These results provided the first evidence that nonclassical MHC-restricted iT cells exist outside endothermic vertebrates. Perhaps more intriguingly, this suggests that other XNCs might play similar roles by providing alternative ways to mediate the differentiation and function of unconventional T cell subsets in class Ia-deficient tadpoles. Consistent with this idea, several other XNCs are expressed by thymocytes themselves including XNC1, 4, 5, 9, 11, and 14 (Edholm and Robert, unpublished observations). Furthermore, using deep sequencing, we have identified six unique TCRα rearrangements, including the canonical invariant Vα6-Jα1.43, that are markedly overrepresented in the $CD8^{neg}$ and $CD8^{dim}$ T cell pools obtained from the spleen of pre-metamorphic tadpoles (Edholm et al. 2013). In contrast, repertoire analysis revealed that the TCRα repertoire obtained from the $CD8^{bright}$ T cell subset exhibited a wide variety of distinct Vα and Jα segments indicative of a diversified TCRα repertoire. Thus, although $CD8^{bright}$ T cells represent a minor fraction of the total T cell pool, their differentiation appears to not involve nonclassical MHC, which suggests that the suboptimal class Ia protein expression (below antibody detection) may be sufficient to drive the differentiation of a subset of $CD8^{+}$ T cells. Alternatively, these CD8 T cells may be selected by

MHC class II molecules as originally proposed by Flajnik et al. (1987).

Nevertheless, in addition to these $CD8^+$ T cells, it remains that a large fraction of tadpole T cells expresses a TCRα repertoire that is limited to six distinct rearrangements (Fig. 2). Although the TCRβ repertoire remains to be determined, and even if it is more diversified, it is probable that T cells in tadpoles overall have a more limited TCR repertoire than *Xenopus* adults. While no deep sequencing data have been performed in adults, characterization of TCRα and β has been reported and does not indicate obvious restrictions (Chretien et al. 1997; Haire et al. 2002). Based on these limited data, we would like to propose that the restricted TCRα repertoire observed in tadpoles is an adaptation related to this developmental stage. The differentiation of invariant T cells able to recognize distinct conserved pathogen determinants would allow the tadpole to maximize the use of the small number of T cells they can differentiate. The differentiation of iT cells with limited, germline encoded rearrangements would also minimize the risk of autoimmune recognition in the absence of a strong class Ia-mediated negative selection.

Function of invariant T cells in mammals and *Xenopus*

While the functions of iT cells are still not fully elucidated, it is becoming increasingly evident that these cells are critically and specifically involved in several aspects of the mammalian immune response. Human MAIT cells have been shown to have antimicrobial properties in vitro, suggesting a role of these cells during bacterial infections (Le Bourhis et al. 2010). This was further supported in murine models where MR1-dependent MAIT cell activation was demonstrated to a number of different microbes, including gram-positive and gram-negative bacteria and yeast (Le Bourhis et al. 2010). Furthermore, a lack of MAIT cells correlated with increased bacterial loads following infection with *Escherichia*, *Mycobacteria*, and *Klebsiella* in several murine infection models. However, MAIT cells are not universally activated, as several bacterial species including Streptococci and Listeria as well as viruses were unable to activate these cells indicating a level of specificity in recognition of these cells (Gold et al. 2010; Le Bourhis et al. 2010). Nonetheless, the ability of MAIT cells to respond to a wide variety of microbes suggests that they recognize a conserved antigenic structure. Recently, the ligand of MR1 was identified as petrin analogs derived from vitamin B metabolism (Kjer-Nielsen et al. 2012). As many vitamin biosynthetic pathways are unique to and present in most (but not all) bacteria and yeast, this provides a way for MAIT cells to sense a microbial infection (Kjer-Nielsen et al. 2012; Patel et al. 2013). To date, how MAIT cells mediate their antimicrobial activities is not fully elucidated. Upon stimulation, MAIT cells rapidly produce cytokines, mostly INF-γ and TNF-α and, under certain circumstances, IL-17 (Dusseaux et al. 2011), which most likely contribute to their antimicrobial properties (Le Bourhis et al. 2010). Recently, it was also shown that MAIT cells are capable of lysing epithelial cells infected with *Shigella flexneri* demonstrating a direct cytotoxic effect of these cells (Le Bourhis et al. 2013a). In addition to their antimicrobial properties, it has also been suggested that MAIT cells are critically involved in regulating the

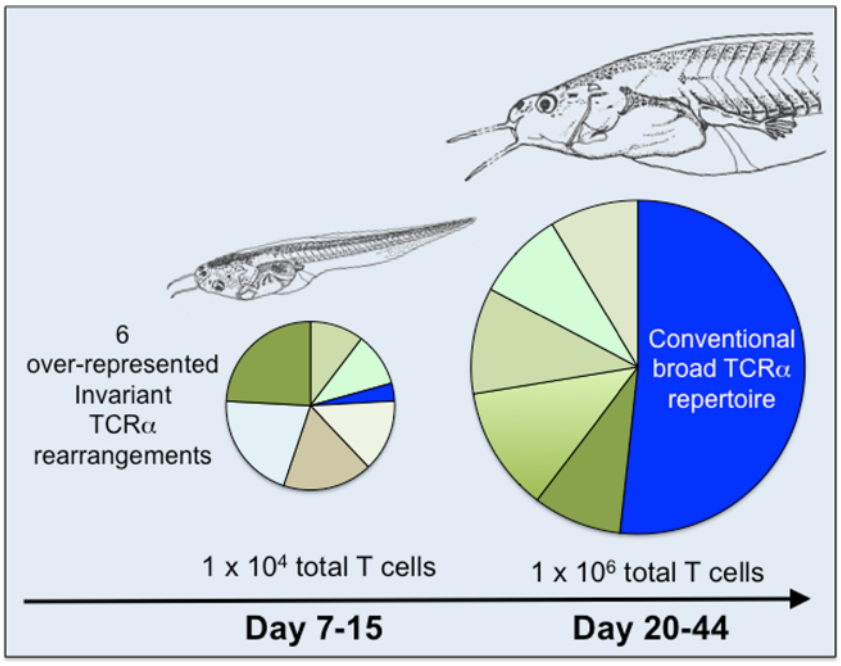

Fig. 2 Proposed invariant T cell-based immunity during early ontogeny in *Xenopus*. During early development, a wave of nonclassical MHC class I-mediated T cell differentiation is predominant resulting in a limited number of T cells (15–20,000 T cells) expressing six unique overrepresented TCRα rearrangements. Over time, as development continues, an increase in the peripheral T cell compartment progressively replaces this limited invariant TCRα repertoire by a conventional and diversified TCRα repertoire either selected alternatively by classical MHC class II molecules or supported by a low level of classical MHC class Ia protein expression

commensal microbial balance of the gut, thus maintaining immune homeostasis (Le Bourhis et al. 2013b).

iNKT cells have been implicated in immunity to a variety of different bacteria as well as in parasitic infections, viral infections, and fungal disease (Kinjo and Kronenberg 2005; Skold and Behar 2003). Similar to MAIT cells, CD1d-restricted iNKT cells are capable of rapidly producing large amounts of cytokines following stimulation through their TCR. Notably, following antigen recognition, iNKT cells can produce both TH1 (INF-γ and TNF-α) and TH2 (IL-4, IL-10, IL-3, and IL-5)-type cytokines and thus influence a number of different cells including NK cells, conventional T cells as well as macrophages, dendritic cells, and neutrophils (reviewed in Wilson and Delovitch 2003). This dual ability to either promote or suppress the immune response highlights the immunoregulatory abilities of iNKT cells, which can recognize a range of different lipids, both synthetic and natural, presented in the context of CD1d, presumably through the recognition of a conserved structural motif. Unlike conventional TCR-peptide-class Ia recognition, the interaction between the semi-invariant TCR on iNKT cells and the lipid-CD1d complex is primarily mediated by the invariant CDR3α loop and to a lesser extent the CDR2β loop (Burrows et al. 2010; reviewed in Rossjohn et al. 2012; Rudolph et al. 2006). Furthermore, the pattern of TCR-antigen-CD1d complex interaction is similar regardless of the bound antigen, i.e., the same iTCR is capable of recognizing different antigens.

In *Xenopus*, the availability of transgenic animals with XNC10 loss-of-function provided a powerful way to investigate the immunological role of XNC10-restricted iT cells. An additional advantage was the establishment of *Xenopus* as a model system to study amphibian immune responses to the devastating ranavirus infections that affect amphibian, fish, and reptiles worldwide (reviewed in Robert and Gregory Chinchar 2012). Ranavirus such as Frog Virus 3 (FV3) are pox-like large double strand DNA viruses that cause extensive disease and mortalities of wild and cultured amphibian species. Typically, the adult *Xenopus* are able to successfully clear the infection within a month post-FV3 inoculation. Furthermore, this viral clearance has been shown to be dependent on CD8 T cells and antibody responses (Maniero et al. 2006; Morales and Robert 2007). By comparison, the tadpoles are more susceptible and typically fail to clear the FV3 and, thus, succumb to the infection within a month. Nevertheless, following FV3 infection, tadpoles do mount an immune response against this virus including the upregulation of inflammatory cytokines (TNF-α, IFN-γ) and antiviral type I IFN (De Jesus et al. 2012; Grayfer et al. 2012). We used this system to investigate the role of iT cells in tadpoles. Transgenic tadpoles with XNC10 deficiency were more susceptible to FV3 (Edholm et al. 2013). Notably, in these transgenic tadpoles, FV3 infection resulted in a marked increased mortality at the early stage of infection (3–7 days) accompanied with a sharp increase in viral loads in tissue of infected tadpoles and global viral dissemination (Edholm and Robert, unpublished observations). Therefore, XNC10-iT cells are critical for tadpole resistance to viral infection, in particular during early viral immunity. More recently, we have also observed that XNC10-restricted iT cells accumulate and become a prominent fraction of immune cells (up to 20 %) in the peritoneal cavity of LG-15 cloned tadpoles transplanted with a *X. laevis* thymic lymphoid tumor (15/0), which contrast the virtual absence of these iT cells in the peritoneal exudates of naïve tadpoles (Haynes-Gilmore et al. 2014). This suggests that, as their mammalian counterpart, XNC10-iT cells play a multifaceted role in immunity including antitumor immunity.

Can we explain differential MHC expression in tadpoles and adult frogs by relative levels of iT and conventional T cells?

The identification of iT cells restricted by nonclassical MHC molecules in a species as genetically and evolutionarily distant from mouse and human as *Xenopus* constitutes a strong argument for the functional relevance of these cells. Moreover, it is possible that *Xenopus* rely more extensively on iT cells during early developmental stage when tadpoles have a limited number of T cells. Unlike mammalian embryos, which remain in a relatively antigen-free uterus, *Xenopus* tadpoles (like other ectothermic vertebrates) hatch in the surrounding antigen-rich water (Fig. 1). As such, within 2 weeks (developmental stage 50), the *Xenopus* immune system is under pressure to develop quickly and to produce a lymphocyte receptor repertoire with very small numbers of lymphocytes (5,000 B cells and 15–20,000 T cells; Fig. 2). Since the potential BCR and TCR repertoire in *Xenopus* far exceed the number of lymphocytes, it is likely that additional mechanisms have evolved to produce a functional but more limited lymphocyte repertoire during early ontogeny. For B cells, some evidence has been reported (e.g., lack of n-nucleotide diversity and a temporal stepwise utilization of VH genes; Du Pasquier and Schwager 1991; Mussmann et al. 1998; Schwager et al. 1989; Schwager et al. 1991). In the case of TCRα, we have shown that the vast majority of $CD8^{neg}$ and $CD8^{dim}$ T cells utilize one out of six predominant TCRα rearrangements (Fig. 2). The preferential expression of several XNC genes by thymocytes provides likely mechanisms by which T cells expressing these dominant rearrangements can undergo thymic selection. Indeed, in the absence of suboptimal class Ia protein expression in tadpoles, it is quite possible that XNC genes drive the differentiation of T cells through a developmental program similar to that of mammalian nonclassical restricted iT cells.

Whether and how iT cell differentiates/occurs in the post-metamorphic animals remains to be elucidated. XNC10-iT cells are present in adults and, similar to the situation in

tadpoles, also appear to be involved in the adult anti-FV3 immune response. However, at this stage, it is likely that other immune effector cells, including conventional $CD8^+$ T cells, are involved.

How widespread are iT cells in phylogeny?

Given the variety in numbers and structure of nonclassical MHC genes in jawed vertebrates, it seems plausible that some of these genes are involved in immunity during early development. Indeed, some species including *Xenopus*, the urodel amphibian *Ambystoma mexicanium* (Sammut et al. 1997, 1999), and the teleost fish Atlantic cod (*Gadus morhua*) (Star et al. 2011) display a very large number of nonclassical MHC genes suggesting a further diversification and possible neofunctionalization of these genes. Although not as extreme, expansions and diversification of class I genes have also occurred in other teleost species such as Atlantic salmon (Lukacs et al. 2010) and zebrafish (Dijkstra et al. 2007), where a number of divergent class I genes have been grouped into distinct lineages (U-, Z/ZE-, L-, and S-lineage) based on evolutionary relationships. The U-lineage contains both putative class Ia and nonclassical MHC class I genes and is broadly represented among divergent species, whereas the L-lineage, which consists of highly divergent nonclassical MHC class I genes, appears so far limited to salmonids and cyprinids (Dijkstra et al. 2007). Variable numbers of highly divergent species-specific nonclassical genes have also been described in elasmobranchs (Bartl et al. 1997; Wang et al. 2003). In chicken, two divergent CD1d gene homologs have been identified (Miller et al. 2005; Salomonsen et al. 2005) as well as an MHC class I-like molecule, YF1*7.1, that shares 38 % sequence identity with, and is structurally similar to, human MR1 (Hee et al. 2010; Kjer-Nielsen et al. 2012). Thus, one can infer that mammalian type CD1d-restricted iNKT and MR1-restricted MAIT are present in birds.

More generally, based on the wide diversity of nonclassical MHC class I genes among species, it is tempting to speculate that iT cells are widely present in jawed vertebrates. For aquatic species with external development, larval stage exposed to pathogens would likely benefit from a TCR repertoire based on a small number of T cells. However, a specialized role of iT cells during early ontogeny in mammals is also conceivable. Indeed, it is known that class Ia function is suboptimal in human and murine newborns, while various nonclassical MHC class I genes are strongly expressed (David-Watine et al. 1987; Cheroutre et al. 1991; Houlihan et al. 1992; Hunt et al. 1994). The possible involvement of nonclassical MHC class I-mediated iT cells during early ontogeny clearly merits further exploration. At these early developmental stages, when a mature diversified TCR repertoire is not achieved, we can envision a role of these iT cells as sentinels able to rapidly activate innate-like immune responses through production of cytokines and chemokines or alternatively through direct cytolytic effects.

Acknowledgments We would like to thank Drs. Leon Grayfer and Nikesha Haynes-Gilmore for helpful discussions and critical reading of the manuscript. This research was supported by grants R24-AI-059830 from the National Institute of Allergy and Infectious Diseases (NIH/NIAID).

References

Anderson G, Moore NC, Owen JJ, Jenkinson EJ (1996) Cellular interactions in thymocyte development. Annu Rev Immunol 14:73–99

Barritt LC, Turpen JB (1995) Characterization of lineage restricted forms of a Xenopus CD45 homologue. Dev Comp Immunol 19:525–536

Bartl S, Baish MA, Flajnik MF, Ohta Y (1997) Identification of class I genes in cartilaginous fish, the most ancient group of vertebrates displaying an adaptive immune response. J Immunol 159: 6097–6104

Bendelac A (1995) Positive selection of mouse NK1+ T cells by CD1-expressing cortical thymocytes. J Exp Med 182:2091–2096

Bonnet M, Ferrier P, Spicuglia S (2009) Molecular genetics at the T-cell receptor beta locus: insights into the regulation of V(D)J recombination. Adv Exp Med Biol 650:116–132

Burrows SR, Chen Z, Archbold JK, Tynan FE, Beddoe T, Kjer-Nielsen L, Miles JJ, Khanna R, Moss DJ, Liu YC, Gras S, Kostenko L, Brennan RM, Clements CS, Brooks AG, Purcell AW, McCluskey J, Rossjohn J (2010) Hard wiring of T cell receptor specificity for the major histocompatibility complex is underpinned by TCR adaptability. Proc Natl Acad Sci U S A 107:10608–10613

Cheroutre H, Kronenberg M, Brorson K, Hunt SW 3rd, Eghtesady P, Hood L, Nickerson DA (1991) Analysis of MHC class I gene expression in adult bone marrow and fetal liver of the BALB/c mouse. J Immunol 146:3263–3272

Chida AS, Goyos A, Robert J (2011) Phylogenetic and developmental study of CD4, CD8 alpha and beta T cell co-receptor homologs in two amphibian species, Xenopus tropicalis and Xenopus laevis. Dev Comp Immunol 35:366–377

Chretien I, Robert J, Marcuz A, Garcia-Sanz JA, Courtet M, Du Pasquier L (1996) CTX, a novel molecule specifically expressed on the surface of cortical thymocytes in Xenopus. Eur J Immunol 26: 780–791

Chretien I, Marcuz A, Fellah J, Charlemagne J, Du Pasquier L (1997) The T cell receptor beta genes of Xenopus. Eur J Immunol 27:763–771

Chun T, Page MJ, Gapin L, Matsuda JL, Xu H, Nguyen H, Kang HS, Stanic AK, Joyce S, Koltun WA, Chorney MJ, Kronenberg M, Wang CR (2003) CD1d-expressing dendritic cells but not thymic epithelial cells can mediate negative selection of NKT cells. J Exp Med 197:907–918

Chung B, Aoukaty A, Dutz J, Terhorst C, Tan R (2005) Signaling lymphocytic activation molecule-associated protein controls NKT cell functions. J Immunol 174:3153–3157

Coles MC, Raulet DH (2000) NK1.1+ T cells in the liver arise in the thymus and are selected by interactions with class I molecules on CD4 + CD8+ cells. J Immunol 164:2412–2418

Cooper MD, Alder MN (2006) The evolution of adaptive immune systems. Cell 124:815–822

Dascher CC (2007) Evolutionary biology of CD1. Curr Top Microbiol Immunol 314:3–26

David-Watine B, Transy C, Gachelin G, Kourilsky P (1987) Tissue-specific expression of the mouse Q10 H-2 class-I gene during embryogenesis. Gene 61:145–154

Davis MM, Bjorkman PJ (1988) T-cell antigen receptor genes and T-cell recognition. Nature 334:395–402

De Jesus AF, Chen G, Li Z, Grayfer L, Robert J (2012) Susceptibility of Xenopus laevis tadpoles to infection by the ranavirus Frog-Virus 3 correlates with a reduced and delayed innate immune response in comparison with adult frogs. Virology 432:435–443

Dijkstra JM, Katagiri T, Hosomichi K, Yanagiya K, Inoko H, Ototake M, Aoki T, Hashimoto K, Shiina T (2007) A third broad lineage of major histocompatibility complex (MHC) class I in teleost fish; MHC class II linkage and processed genes. Immunogenetics 59: 305–321

Du Pasquier L, Flajnik MF (1990) Expression of MHC class II antigens during Xenopus development. Dev Immunol 1:85–95

Du Pasquier L, Schwager J (1991) Immunoglobulin genes and B cell development in amphibians. Adv Exp Med Biol 292:1–9

Du Pasquier L, Weiss N (1973) The thymus during the ontogeny of the toad Xenopus laevis: growth, membrane-bound immunoglobulins and mixed lymphocyte reaction. Eur J Immunol 3:773–777

Du Pasquier L, Schwager J, Flajnik MF (1989) The immune system of Xenopus. Annu Rev Immunol 7:251–275

Du Pasquier L, Courtet M, Robert J (1995) A Xenopus lymphoid tumor cell line with complete Ig genes rearrangements and T-cell characteristics. Mol Immunol 32:583–593

Dusseaux M, Martin E, Serriari N, Peguillet I, Premel V, Louis D, Milder M, Le Bourhis L, Soudais C, Treiner E, Lantz O (2011) Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. Blood 117:1250–1259

Edholm ES, Albertorio Saez LM, Gill AL, Gill SR, Grayfer L, Haynes N, Myers JR, Robert J (2013) Nonclassical MHC class I-dependent invariant T cells are evolutionarily conserved and prominent from early development in amphibians. Proc Natl Acad Sci U S A 110: 14342–14347

Edholm ES, Goyos A, Taran J, De JEsus AF, Ohta Y, Robert J (2014) Unusual evolutionary conservation and further species-specific adaptations of a large family of nonclassical MHC class Ib genes across different degrees of genome ploidy in the amphibian subfamily Xenopodinae. Immunogenetics. doi:10.1007/s0025101407745

Evans BJ (2008) Genome evolution and speciation genetics of clawed frogs (Xenopus and Silurana). Front Biosci 13:4687–4706

Evans BJ, Kelley DB, Tinsley RC, Melnick DJ, Cannatella DC (2004) A mitochondrial DNA phylogeny of African clawed frogs: phylogeography and implications for polyploid evolution. Mol Phylogenet Evol 33:197–213

Flajnik MF, Du Pasquier L, Cohen N (1985) Immune responses of thymus/lymphocyte embryonic chimeras: studies on tolerance and major histocompatibility complex restriction in Xenopus. Eur J Immunol 15:540–547

Flajnik MF, Kaufman JF, Hsu E, Manes M, Parisot R, Du Pasquier L (1986) Major histocompatibility complex-encoded class I molecules are absent in immunologically competent Xenopus before metamorphosis. J Immunol 137:3891–3899

Flajnik MF, Hsu E, Kaufman JF, Dupasquier L (1987) Changes in the immune-system during metamorphosis of Xenopus. Immunol Today 8:58–64

Flajnik MF, Kasahara M, Shum BP, Salter-Cid L, Taylor E, Du Pasquier L (1993) A novel type of class I gene organization in vertebrates: a large family of non-MHC-linked class I genes is expressed at the RNA level in the amphibian Xenopus. EMBO J 12:4385–4396

Gapin L, Matsuda JL, Surh CD, Kronenberg M (2001) NKT cells derive from double-positive thymocytes that are positively selected by CD1d. Nat Immunol 2:971–978

Godfrey DI, Berzins SP (2007) Control points in NKT-cell development. Nat Rev Immunol 7:505–518

Gold MC, Cerri S, Smyk-Pearson S, Cansler ME, Vogt TM, Delepine J, Winata E, Swarbrick GM, Chua WJ, Yu YY, Lantz O, Cook MS, Null MD, Jacoby DB, Harriff MJ, Lewinsohn DA, Hansen TH, Lewinsohn DM (2010) Human mucosal associated invariant T cells detect bacterially infected cells. PLoS Biol 8:e1000407

Gold MC, Eid T, Smyk-Pearson S, Eberling Y, Swarbrick GM, Langley SM, Streeter PR, Lewinsohn DA, Lewinsohn DM (2013) Human thymic MR1-restricted MAIT cells are innate pathogen-reactive effectors that adapt following thymic egress. Mucosal Immunol 6:35–44

Goyos A, Guselnikov S, Chida AS, Sniderhan LF, Maggirwar SB, Nedelkovska H, Robert J (2007) Involvement of nonclassical MHC class Ib molecules in heat shock protein-mediated anti-tumor responses. Eur J Immunol 37:1494–1501

Goyos A, Ohta Y, Guselnikov S, Robert J (2009) Novel nonclassical MHC class Ib genes associated with CD8 T cell development and thymic tumors. Mol Immunol 46:1775–1786

Goyos A, Sowa J, Ohta Y, Robert J (2011) Remarkable conservation of distinct nonclassical MHC class I lineages in divergent amphibian species. J Immunol 186:372–381

Gravenor I, Horton TL, Ritchie P, Flint E, Horton JD (1995) Ontogeny and thymus-dependence of T cell surface antigens in Xenopus: flow cytometric studies on monoclonal antibody-stained thymus and spleen. Dev Comp Immunol 19:507–523

Grayfer L, Andino Fde J, Chen G, Chinchar GV, Robert J (2012) Immune evasion strategies of ranaviruses and innate immune responses to these emerging pathogens. Viruses 4:1075–1092

Griewank K, Borowski C, Rietdijk S, Wang N, Julien A, Wei DG, Mamchak AA, Terhorst C, Bendelac A (2007) Homotypic interactions mediated by Slamf1 and Slamf6 receptors control NKT cell lineage development. Immunity 27:751–762

Haire RN, Kitzan Haindfield MK, Turpen JB, Litman GW (2002) Structure and diversity of T-lymphocyte antigen receptors alpha and gamma in Xenopus. Immunogenetics 54:431–438

Hansen JD, Zapata AG (1998) Lymphocyte development in fish and amphibians. Immunol Rev 166:199–220

Haynes-Gilmore N, Banach M, Edholm ES, Lord E, Robert J (2014) A critical role of nonclassical MHC in tumor immune evasion in the amphibian Xenopus model. Carcinogenesis. doi:10.1093/carcin/bgu100

Hee CS, Gao S, Loll B, Miller MM, Uchanska-Ziegler B, Daumke O, Ziegler A (2010) Structure of a classical MHC class I molecule that binds "non-classical" ligands. PLoS Biol 8:e1000557

Hogquist KA, Baldwin TA, Jameson SC (2005) Central tolerance: learning self-control in the thymus. Nat Rev Immunol 5:772–782

Houlihan JM, Biro PA, Fergar-Payne A, Simpson KL, Holmes CH (1992) Evidence for the expression of non-HLA-A, -B, -C class I genes in the human fetal liver. J Immunol 149:668–675

Hunt DW, Huppertz HI, Jiang HJ, Petty RE (1994) Studies of human cord blood dendritic cells: evidence for functional immaturity. Blood 84: 4333–4343

Jameson SC, Hogquist KA, Bevan MJ (1995) Positive selection of thymocytes. Annu Rev Immunol 13:93–126

Jensen PE, Sullivan BA, Reed-Loisel LM, Weber DA (2004) Qa-1, a nonclassical class I histocompatibility molecule with roles in innate and adaptive immunity. Immunol Res 29:81–92

Jurgens JB, Gartland LA, Du Pasquier L, Horton JD, Gobel TW, Cooper MD (1995) Identification of a candidate CD5 homologue in the amphibian Xenopus laevis. J Immunol 155:4218–4223

Kau CL, Turpen JB (1983) Dual contribution of embryonic ventral blood island and dorsal lateral plate mesoderm during ontogeny of hemopoietic cells in Xenopus laevis. J Immunol 131:2262–2266

Kinjo Y, Kronenberg M (2005) V alpha14 i NKT cells are innate lymphocytes that participate in the immune response to diverse microbes. J Clin Immunol 25:522–533

Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, Bhati M, Chen Z, Kostenko L, Reantragoon R, Williamson NA, Purcell

AW, Dudek NL, McConville MJ, O'Hair RA, Khairallah GN, Godfrey DI, Fairlie DP, Rossjohn J, McCluskey J (2012) MR1 presents microbial vitamin B metabolites to MAIT cells. Nature 491:717–723

Krangel MS, Carabana J, Abbarategui I, Schlimgen R, Hawwari A (2004) Enforcing order within a complex locus: current perspectives on the control of V(D)J recombination at the murine T-cell receptor alpha/delta locus. Immunol Rev 200:224–232

Le Bourhis L, Martin E, Peguillet I, Guihot A, Froux N, Core M, Levy E, Dusseaux M, Meyssonnier V, Premel V, Ngo C, Riteau B, Duban L, Robert D, Huang S, Rottman M, Soudais C, Lantz O (2010) Antimicrobial activity of mucosal-associated invariant T cells. Nat Immunol 11:701–708

Le Bourhis L, Guerri L, Dusseaux M, Martin E, Soudais C, Lantz O (2011) Mucosal-associated invariant T cells: unconventional development and function. Trends Immunol 32:212–218

Le Bourhis L, Dusseaux M, Bohineust A, Bessoles S, Martin E, Premel V, Core M, Sleurs D, Serriari NE, Treiner E, Hivroz C, Sansonetti P, Gougeon ML, Soudais C, Lantz O (2013a) MAIT cells detect and efficiently lyse bacterially-infected epithelial cells. PLoS Pathog 9: e1003681

Le Bourhis L, Mburu YK, Lantz O (2013b) MAIT cells, surveyors of a new class of antigen: development and functions. Curr Opin Immunol 25:174–180

Litman GW, Rast JP, Fugmann SD (2010) The origins of vertebrate adaptive immunity. Nat Rev Immunol 10:543–553

Lukacs MF, Harstad H, Bakke HG, Beetz-Sargent M, McKinnel L, Lubieniecki KP, Koop BF, Grimholt U (2010) Comprehensive analysis of MHC class I genes from the U-, S-, and Z-lineages in Atlantic salmon. BMC Genomics 11:154

Maniero GD, Morales H, Gantress J, Robert J (2006) Generation of a long-lasting, protective, and neutralizing antibody response to the ranavirus FV3 by the frog Xenopus. Dev Comp Immunol 30: 649–657

Martin E, Treiner E, Duban L, Guerri L, Laude H, Toly C, Premel V, Devys A, Moura IC, Tilloy F, Cherif S, Vera G, Latour S, Soudais C, Lantz O (2009) Stepwise development of MAIT cells in mouse and human. PLoS Biol 7:e54

Michie AM, Zuniga-Pflucker JC (2002) Regulation of thymocyte differentiation: pre-TCR signals and beta-selection. Semin Immunol 14: 311–323

Miller MM, Wang C, Parisini E, Coletta RD, Goto RM, Lee SY, Barral DC, Townes M, Roura-Mir C, Ford HL, Brenner MB, Dascher CC (2005) Characterization of two avian MHC-like genes reveals an ancient origin of the CD1 family. Proc Natl Acad Sci U S A 102: 8674–8679

Morales HD, Robert J (2007) Characterization of primary and memory CD8 T-cell responses against ranavirus (FV3) in Xenopus laevis. J Virol 81:2240–2248

Mussmann R, Courtet M, Du Pasquier L (1998) Development of the early B cell population in Xenopus. Eur J Immunol 28:2947–2959

Nedelkovska H, Robert J (2012) Optimized transgenesis in Xenopus laevis/gilli isogenetic clones for immunological studies. Genesis 50:300–306

Nieuwkoop PD, Faber J (1967) Normal tables of Xenopus laevis (Daudin). In: Nieuwkoop PD (ed) A systematic and chronological survey of the development from the fertilized egg till the end of metamorphosis, 2nd edn. North-Holland, Amsterdam, pp 162–188

Patel O, Kjer-Nielsen L, Le Nours J, Eckle SB, Birkinshaw R, Beddoe T, Corbett AJ, Liu L, Miles JJ, Meehan B, Reantragoon R, Sandoval-Romero ML, Sullivan LC, Brooks AG, Chen Z, Fairlie DP, McCluskey J, Rossjohn J (2013) Recognition of vitamin B metabolites by mucosal-associated invariant T cells. Nat Commun 4:2142

Prince AL, Yin CC, Enos ME, Felices M, Berg LJ (2009) The Tec kinases Itk and Rlk regulate conventional versus innate T-cell development. Immunol Rev 228:115–131

Robert J, Cohen N (1998) Ontogeny of CTX expression in Xenopus. Dev Comp Immunol 22:605–612

Robert J, Cohen N (1999) In vitro differentiation of a CD4/CD8 double-positive equivalent thymocyte subset in adult Xenopus. Int Immunol 11:499–508

Robert J, Gregory Chinchar V (2012) "Ranaviruses: an emerging threat to ectothermic vertebrates" report of the First International Symposium on Ranaviruses, Minneapolis MN July 8, 2011. Dev Comp Immunol 36:259–261

Robert J, Sung M, Cohen N (2001) In vitro thymocyte differentiation in MHC class I-negative Xenopus larvae. Dev Comp Immunol 25: 323–336

Rollins-Smith LA (1998) Metamorphosis and the amphibian immune system. Immunol Rev 166:221–230

Rollins-Smith LA, Barker KS, Davis AT (1997a) Involvement of glucocorticoids in the reorganization of the amphibian immune system at metamorphosis. Dev Immunol 5:145–152

Rollins-Smith LA, Flajnik MF, Blair PJ, Davis AT, Green WF (1997b) Involvement of thyroid hormones in the expression of MHC class I antigens during ontogeny in Xenopus. Dev Immunol 5:133–144

Rollins-Smith LA, Davis AT, Reinert LK (2000) Pituitary involvement in T cell renewal during development and metamorphosis of Xenopus laevis. Brain Behav Immun 14:185–197

Rossjohn J, Pellicci DG, Patel O, Gapin L, Godfrey DI (2012) Recognition of CD1d-restricted antigens by natural killer T cells. Nat Rev Immunol 12:845–857

Rothenberg EV, Taghon T (2005) Molecular genetics of T cell development. Annu Rev Immunol 23:601–649

Rudolph MG, Stanfield RL, Wilson IA (2006) How TCRs bind MHCs, peptides, and coreceptors. Annu Rev Immunol 24:419–466

Salomonsen J, Sorensen MR, Marston DA, Rogers SL, Collen T, van Hateren A, Smith AL, Beal RK, Skjodt K, Kaufman J (2005) Two CD1 genes map to the chicken MHC, indicating that CD1 genes are ancient and likely to have been present in the primordial MHC. Proc Natl Acad Sci U S A 102:8668–8673

Salter-Cid L, Nonaka M, Flajnik MF (1998) Expression of MHC class Ia and class Ib during ontogeny: high expression in epithelia and coregulation of class Ia and lmp7 genes. J Immunol 160:2853–2861

Sammut B, Laurens V, Tournefier A (1997) Isolation of MHC class I cDNAs from the axolotl Ambystoma mexicanum. Immunogenetics 45:285–294

Sammut B, Du Pasquier L, Ducoroy P, Laurens V, Marcuz A, Tournefier A (1999) Axolotl MHC architecture and polymorphism. Eur J Immunol 29:2897–2907

Schwager J, Burckert N, Courtet M, Du Pasquier L (1989) Genetic basis of the antibody repertoire in Xenopus: analysis of the Vh diversity. EMBO J 8:2989–3001

Schwager J, Burckert N, Courtet M, Du Pasquier L (1991) The ontogeny of diversification at the immunoglobulin heavy chain locus in Xenopus. EMBO J 10:2461–2470

Sidobre S, Kronenberg M (2002) CD1 tetramers: a powerful tool for the analysis of glycolipid-reactive T cells. J Immunol Methods 268: 107–121

Simoni Y, Diana J, Ghazarian L, Beaudoin L, Lehuen A (2013) Therapeutic manipulation of natural killer (NK) T cells in autoimmunity: are we close to reality? Clin Exp Immunol 171:8–19

Skold M, Behar SM (2003) Role of CD1d-restricted NKT cells in microbial immunity. Infect Immun 71:5447–5455

Star B, Nederbragt AJ, Jentoft S, Grimholt U, Malmstrom M, Gregers TF, Rounge TB, Paulsen J, Solbakken MH, Sharma A, Wetten OF, Lanzen A, Winer R, Knight J, Vogel JH, Aken B, Andersen O, Lagesen K, Tooming-Klunderud A, Edvardsen RB, Tina KG, Espelund M, Nepal C, Previti C, Karlsen BO, Moum T, Skage M, Berg PR, Gjoen T, Kuhl H, Thorsen J, Malde K, Reinhardt

R, Du L, Johansen SD, Searle S, Lien S, Nilsen F, Jonassen I, Omholt SW, Stenseth NC, Jakobsen KS (2011) The genome sequence of Atlantic cod reveals a unique immune system. Nature 477:207–210

Starr TK, Jameson SC, Hogquist KA (2003) Positive and negative selection of T cells. Annu Rev Immunol 21:139–176

Tochinai S (1980) Direct observation of cell migration into Xenopus thymus rudiments through mesenchyme. Dev Comp Immunol 4: 273–282

Treiner E (2003) MAIT lymphocytes, regulators of intestinal immunity? Press Med 32:1636–1637

Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, Affaticati P, Gilfillan S, Lantz O (2003) Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature 422:164–169

Tsukamoto K, Deakin JE, Graves JA, Hashimoto K (2013) Exceptionally high conservation of the MHC class I-related gene, MR1, among mammals. Immunogenetics 65:115–124

Turpen JB, Smith PB (1989) Precursor immigration and thymocyte succession during larval development and metamorphosis in Xenopus. J Immunol 142:41–47

Urdahl KB, Sun JC, Bevan MJ (2002) Positive selection of MHC class Ib-restricted CD8(+) T cells on hematopoietic cells. Nat Immunol 3: 772–779

Wang C, Perera TV, Ford HL, Dascher CC (2003) Characterization of a divergent non-classical MHC class I gene in sharks. Immunogenetics 55:57–61

Wilson SB, Delovitch TL (2003) Janus-like role of regulatory iNKT cells in autoimmune disease and tumour immunity. Nat Rev Immunol 3: 211–222

Wu L, Gabriel CL, Parekh VV, Van Kaer L (2009) Invariant natural killer T cells: innate-like T cells with potent immunomodulatory activities. Tissue Antigens 73:535–545

Zon LI, Mather C, Burgess S, Bolce ME, Harland RM, Orkin SH (1991) Expression of GATA-binding proteins during embryonic development in Xenopus laevis. Proc Natl Acad Sci U S A 88:10642–10646