

# Alternative memory in the CD8 T cell lineage

You Jeong Lee, Stephen C. Jameson and Kristin A. Hogquist

The Department of Laboratory Medicine and Pathology, Center for Immunology, University of Minnesota, Minneapolis, MN, USA

**A prominent population of innate CD8<sup>+</sup> T cells develops in the thymus of several gene-deficient mouse strains, including *Itk*, *KLF2*, *CBP* and *Id3*. These cells have the phenotype and function of memory CD8<sup>+</sup> T cells, without previous exposure to antigen. Surprisingly, the cytokine IL-4 plays a key role in their development. As this developmental mechanism was discovered, it came to light that innate CD8<sup>+</sup> T cells exist also in normal mice and in humans. In this review, we discuss how these cells develop, compare and contrast them to other CD8 memory cells, and discuss their potential physiological relevance.**

## Alternative memory in the CD8 lineage

'Innate T cell' is a term loosely used to describe distinct lineages of cells that develop in the thymus, which have a memory phenotype and upon T cell receptor (TCR) stimulation rapidly secrete large amounts of cytokines. These include cells that are restricted by major histocompatibility complex (MHC) class Ib molecules that have limited tissue distribution and polymorphism, such as CD1d, Qa-1, H2-M3 and MR-1 [1–3]. Invariant NKT (iNKT) cells are the prototype of cells belonging to this family. Others include CD8 $\alpha\alpha$  intraepithelial lymphocytes and mucosal-associated invariant T cells (MAIT cells) [4–7]. These innate T cells have several features in common, such as developmental dependence on IL-15, the signaling lymphocyte activation molecule (SLAM)-associated adaptor protein (SAP) signaling pathway, and B7-CD28 interactions for functional maturation [8–10]. All of these subsets have a highly restricted (oligoclonal) TCR repertoire, implicating specific self-antigens (lipid or peptide) in their development [4].

In 2006, a diverse population of polyclonal CD8<sup>+</sup> T cells was described in Tec kinase-deficient mice, which shared functional and phenotypic similarity to innate T cells [11,12]. The ontogeny of these cells had been enigmatic until recently, when it was shown that IL-4 produced by NKT cells drives their development by inducing upregulation of Eomesodermin (Eomes). In this review, we summarize these recent findings and discuss the biological significance of innate CD8<sup>+</sup> T cells.

## Initial discovery of innate CD8<sup>+</sup> T cells in ITK gene-deficient mice

The Tec family of non-receptor tyrosine kinases are important components of the TCR signaling pathway [7,13]. Among the five Tec subtypes, genetic deficiency of inducible

T cell kinase (*Itk*) was found to alter development of CD8<sup>+</sup> T cells in the thymus and the spleen [11,12]. In these mice, the majority of CD8 SP thymocytes had a CD24<sup>lo</sup>CD44<sup>hi</sup>CD122<sup>hi</sup> memory phenotype and rapidly produced IFN $\gamma$  in response to TCR stimulation [11,12]. These cells also expressed the T-box transcription factor Eomes [11], which is associated with expression of CD122 (IL-2 and IL-15 receptor  $\beta$  chain) and secretion of IFN $\gamma$  in memory CD8<sup>+</sup> T cells [14]. However, unlike conventional memory T cells, these cells did not express T-bet. Because such cells resemble innate T cells in terms of their activated/memory phenotype and rapid production of cytokines, they were termed innate CD8<sup>+</sup> T cells [7,15].

Subsequent to these pioneering studies, a number of other mice deficient in T cell signaling molecules or transcription factors were shown to have elevated thymic innate CD8<sup>+</sup> T cells (Table 1). These include the transcription factors Krüppel-like factor 2 (*KLF2*) [16,17], CREB binding protein (*CBP*) [18], and inhibitor of DNA binding 3 (*Id3*) [19]. Mice with a mutation in Src homology 2 domain-containing leukocyte phosphoprotein of 76 kDa (*SLP76:Y145F*) [20] were also found to phenocopy the *Itk*<sup>-/-</sup> mice in terms of thymic CD8<sup>+</sup> T cells. All of the models in Table 1 were shown to have elevated Eomes in CD8<sup>+</sup> T cells in the thymus, arguably a hallmark of innate CD8<sup>+</sup> T cells. In all models, this was associated with a memory phenotype (CD44<sup>hi</sup> and CD122<sup>hi</sup>) and the ability to rapidly produce IFN $\gamma$  when stimulated through the antigen receptor.

To date, it is unclear how all of these mutations result in a similar phenotype in CD8<sup>+</sup> T cells. The Tec family tyrosine kinases are required for full TCR-induced activation of PLC- $\gamma$ , Ca<sup>2+</sup> mobilization and Erk activation. A strong agonistic interaction between T cells and thymic epithelial cells was suggested to program immature thymocytes into T cells that have an innate phenotype and CD8 $\alpha\alpha$  co-receptors [31]. Therefore, it was proposed that reduced signal strength due to the lack of Tec kinase activity promotes the survival of T cells with high-affinity TCRs, resulting in the innate phenotype [7,13]. Indeed, the development of innate T cells in *Itk*-deficient mice was partially rescued by the hyperactive form of Erk [12]. Thus, a reduced TCR signaling model could be consistent with altered CD8 lineage diversification. However, it was less clear how deficiency in the transcription factors *CBP*, *Id3* or *KLF2* would lead to similar phenotypes, particularly for *KLF2*, which is not expressed in DP thymocytes where *Itk*-dependent TCR signaling occurs [32]. Ultimately, clues to the initial discovery of the complex mechanism by which innate CD8<sup>+</sup> T cells arise came from experiments using mice deficient for *KLF2*.

Corresponding author: Hogquist, K.A. (hogqu001@umn.edu).

**Table 1. Multiple gene deficiency models give rise to innate CD8<sup>+</sup> T cells.**

Gene deficiency or transgenic model	Eomes expression in CD8 SP thymocytes	Cell extrinsic effect	Role of IL-4	Role of NKT lineage cells	References
<i>Itk</i> <sup>-/-</sup>	Elevated	Yes	Increased IL-4R Hyper IgE Dependent on IL-4	Increased $\gamma\delta$ NKT Dependent on PLZF and SAP	[10–12,16,21]
<i>Klf2</i> <sup>fl/fl</sup> <i>Cd4</i> <sup>Cre</sup>	Elevated	Yes	Increased IL-4R Hyper IgE Dependent on IL-4	Increased $\alpha\beta$ and $\gamma\delta$ NKT Dependent on PLZF	[16,17]
<i>Cbp</i> <sup>fl/fl</sup> <i>Lck</i> <sup>Cre</sup>	Elevated	Yes	Increased IL-4R	n.d.	[16,18]
SLP76:Y145F	Elevated	n.d. <sup>a</sup>	n.d.	n.d.	[20,22]
<i>Id3</i> <sup>-/-</sup>	Elevated	Yes	Increased IL-4R Hyper IgE Dependent on IL-4	Increased $\alpha\beta$ and $\gamma\delta$ NKT Dependent on PLZF and SAP	[19,22–25]
CIITA <sup>tg</sup>	n.d. <sup>a</sup>	Yes	Dependent on STAT6	Increased PLZF expression in polyclonal CD4 <sup>+</sup> T cells Dependent on SAP	[1,26–30]

<sup>a</sup>Not determined.

### Development of innate CD8<sup>+</sup> T cells is non-autonomous and requires IL-4

As described, CD8 single positive (SP) thymocytes from KLF2-deficient mice display a marked innate CD8 phenotype, with high levels of CD44, CD122, CXCR3 and Eomes, and rapid production of IFN $\gamma$ . KLF2-deficient mice also have profound peripheral T cell lymphopenia, as this transcription factor is required for T cell trafficking, and in its absence progenitors fail to emigrate from the thymus [33]. To distinguish autonomous and non-autonomous effects in KLF2-deficient mice, unequal mixed bone marrow chimeras were generated (Figure 1a) [16]. When KLF2-deficient bone marrow cells were transferred into irradiated hosts, together with a minority of WT cells, the WT ‘bystander’ CD8 SP thymocytes adopted an innate CD8<sup>+</sup> T cell phenotype, similar to the KLF2-deficient thymocytes (Figure 1a, center panel). By contrast, when WT cells were in the majority, neither population showed an innate CD8 phenotype (Figure 1a, right panel). These data demonstrate that innate CD8<sup>+</sup> T cell development in the KLF2-deficient mice is caused by extrinsic factors. Using the same strategy, the generation of Eomes-expressing innate CD8<sup>+</sup> T cells in the absence of *Itk*-, *CBP*- and *Id3*-mice was also found to be due to cell extrinsic effects [16,19].

The cytokine IL-4 is the extrinsic factor that causes innate CD8 development, because bystander cells that lack IL-4R do not upregulate Eomes, or show any other aspect of the innate phenotype, and they fail to produce IFN $\gamma$  [16,19]. Furthermore, when KLF2-, *Itk*-, or *Id3*-deficient mice were crossed with IL-4R-deficient mice, innate CD8<sup>+</sup> T cells did not develop [16,19]. IL-4 is known to induce Eomes in antigen-stimulated CD8<sup>+</sup> T cells [34], and Eomes was required for the innate CD8 phenotype in KLF2-deficient mice, indicating that IL-4 exerts its effects via Eomes upregulation (Figure 1b) [17].

In the periphery, IL-4 stimulates naïve and memory CD8<sup>+</sup> T cells to proliferate in antigen-induced responses [35]. However, the bystander process occurs in the thymus, where IL-4 presumably acts on naïve CD8 progenitors. Therefore, *Klf2*, *Itk* and *Id3* gene deficiency models clearly share a common mechanism whereby thymic overproduc-

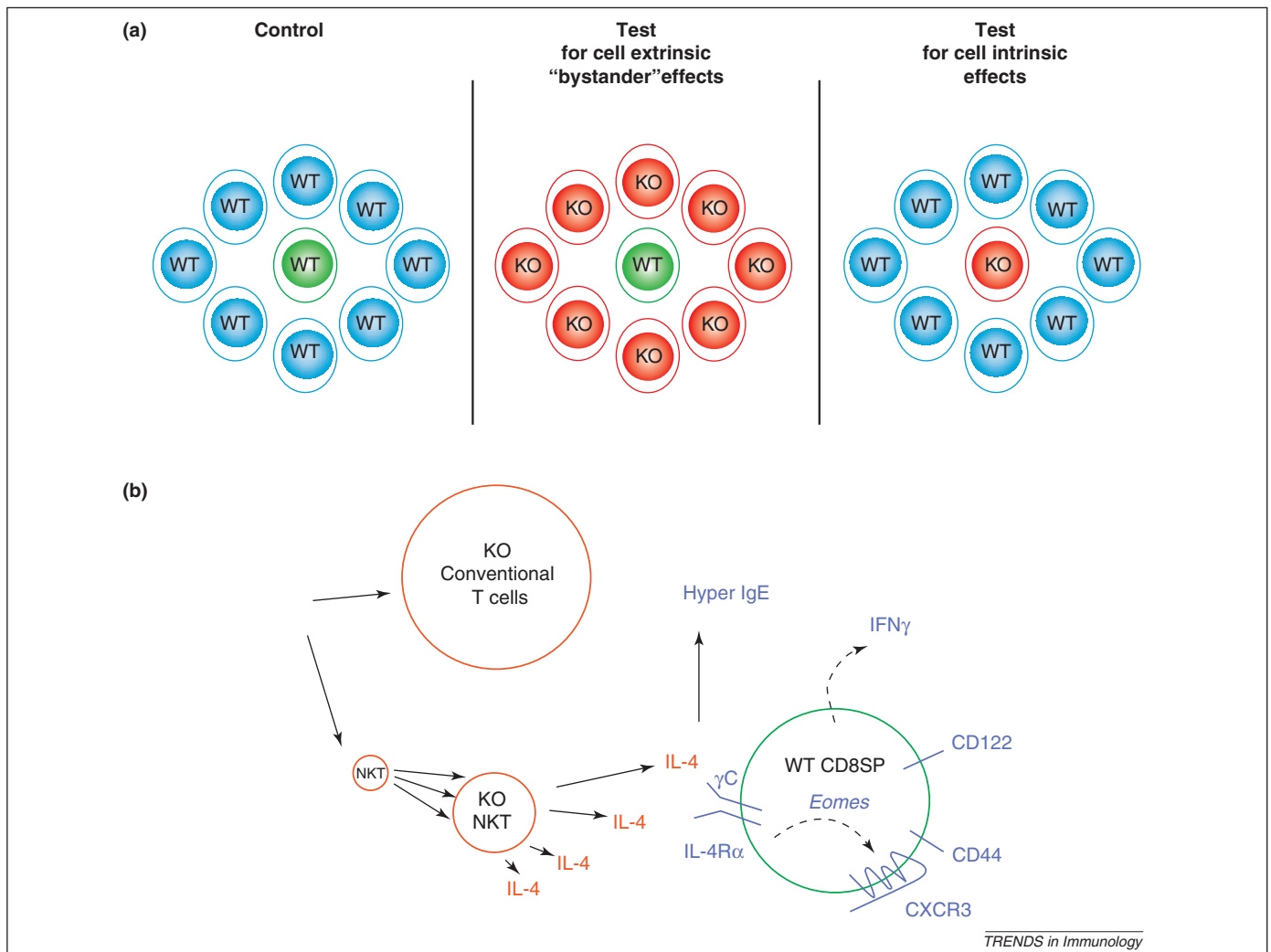
tion of IL-4 results in the generation of innate CD8<sup>+</sup> T cells (Figure 1b and Table 1).

### PLZF<sup>+</sup> NKT cells are the source of IL-4 that drives innate CD8 development

Because the innate CD8 phenotype was dependent on IL-4, it was of interest to determine why the *Klf2*, *Itk* and *Id3* deficiency models overproduce IL-4. As NKT cells can produce IL-4, it was intriguing that thymocytes in KLF2-deficient mice show an increased expression of promyelocytic leukemia zinc finger protein (PLZF), a key transcription factor involved in NKT cell development [36]. PLZF is a member of the BTB-zinc finger (BTB-ZF) protein family that controls a wide variety of cellular responses [37], including the proper development of  $\alpha\beta$  iNKT cells and  $\gamma\delta$  lineage cells expressing NK1.1, called  $\gamma\delta$  NKT cells [21,38–40]. In the absence of PLZF, most thymic iNKT cells fail to fully differentiate, and remain in the CD24<sup>hi</sup> immature state. The reduced numbers of peripheral PLZF-deficient iNKT cells express high level of CD62L and lack specific functions, including preferential residence in non-lymphoid organs and rapid secretion of cytokines, especially IL-4 [39,40]. By contrast, transgenic overexpression of PLZF in conventional  $\alpha\beta$  T cells induced homing to non-lymphoid tissue and rapid cytokine production [40,41].

Considering the crucial role of IL-4 for the generation of innate CD8<sup>+</sup> T cells in KLF2-, *Itk*- and *Id3*-deficient mice, and the fact that PLZF-deficient iNKT cells lose their ability to secrete IL-4, it was hypothesized that PLZF<sup>+</sup> cells could be the major source of IL-4 in the thymus [16]. Indeed, mice deficient for KLF2, *Itk* or *Id3* have an expanded population of PLZF<sup>+</sup> cells, which are mostly  $\alpha\beta$  iNKT in KLF2-deficient mice [16] and  $\gamma\delta$  NKT cells in *Itk*- [21,42] and *Id3*-deficient mice (Table 2) [19,22,23]. Furthermore, double gene deficiency of PLZF together with KLF2, *Itk* or *Id3* led to the failure of innate CD8<sup>+</sup> T cell development [16,19].

The accumulation of PLZF<sup>+</sup> cells in the thymus could be through enhanced differentiation, survival or proliferation (Figure 2). The positive role of *Itk* and *Id3* in TCR signaling cascades suggested that *Itk*- or *Id3*-deficient  $\gamma\delta$  T cells might have differentiated to PLZF-expressing cells instead



**Figure 1.** Innate CD8<sup>+</sup> T cells develop via a cell-extrinsic mechanism. (a) Schematic of an 'unequal mixed bone marrow chimera' approach wherein bone marrow cells are mixed at skewed ratios and used to reconstitute irradiated host animals. If a cell extrinsic bystander effect exists, WT progenitors in the minority would show a phenotypic change (middle). If the effect is cell intrinsic, gene-deficient (KO) progenitors in the minority would show a phenotypic change (right). These experiments showed innate CD8<sup>+</sup> T cells develop via a cell-extrinsic mechanism. (b) NKT cells expand and secrete IL-4 in various gene-deficient mice. This induces wild-type 'bystander' CD8 SP T cells to adopt memory phenotype and function. This effect of IL-4 on CD8<sup>+</sup> T cells is dependent on the transcription factor Eomes.

of being negatively selected, due to reduced signaling strength [15]. CBP-deficiency or SLP76:Y145F mutation might lead to a similar process, as they have common defects in Itk-dependent genes after TCR stimulation [18,20]. As mentioned, however, KLF2 is unlikely to act at the stage where NKTs are being selected, as it is not expressed in DP progenitors. KLF2 is known to control T cell migration by modulating cell surface receptor S1P<sub>1</sub> and CD62L [33,43]. Altered cellular migration in KLF2-deficient mice is not likely to be responsible for the accumulation of PLZF<sup>+</sup> cells because S1P<sub>1</sub> deficiency does not induce an innate CD8<sup>+</sup> T cell phenotype [16]. Instead, a more likely hypothesis is that KLF2 deficiency facilitate the survival or proliferation of PLZF<sup>+</sup> cells after selection. This model is consistent with previous findings that KLF2 is

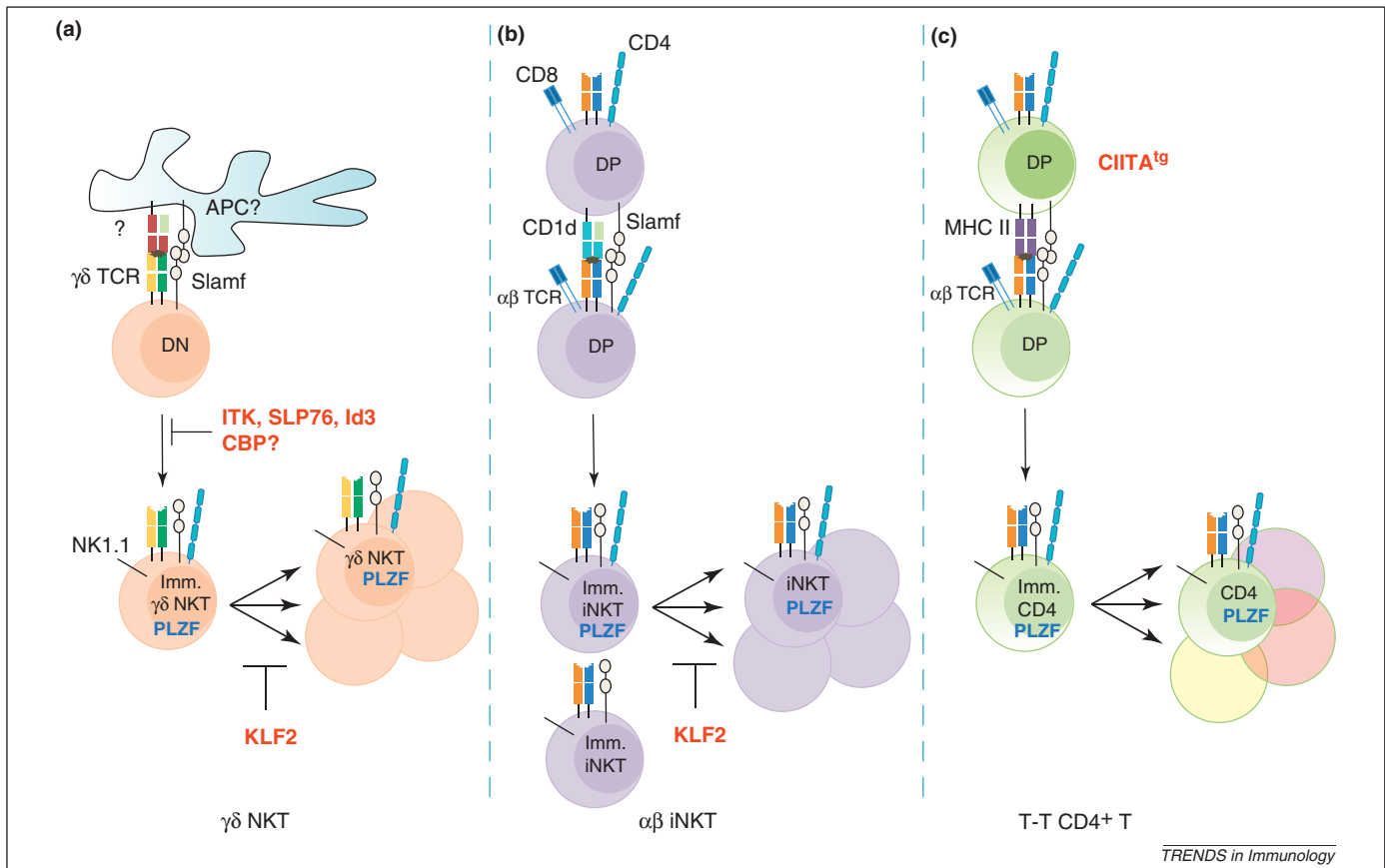
sufficient to program a quiescent phenotype in T cells by suppressing c-Myc expression [32], given that c-Myc is required for iNKT cell proliferation [44]. Therefore, it is possible that Itk, CBP and Id3 act at the selection stage of NKT cells from DP thymocytes and KLF2 regulates the proliferation of NKT cells after this stage (Figure 2).

#### Innate CD8<sup>+</sup> T cells develop in BALB/c but not C57BL/6 mice

In the various gene-deficient mice discussed, expanded PLZF<sup>+</sup> αβ or γδ NKT cells regulate the development of innate CD8<sup>+</sup> T cells in the thymus via production of IL-4. But, does a similar pathway regulate CD8<sup>+</sup> T cells in normal mice? Interestingly, inbred strains of mice were shown to vary in their frequency of iNKT cells, with BALB/

**Table 2.** Different types of memory phenotype CD8<sup>+</sup> T cells.

	Signals involved in generation		T-box transcription factor	Recall response	Innate function	
	TCR	Cytokines				
Conventional (primed)	Foreign antigens	IL-2, IL-12, IFNα	T-bet Eomes	Yes	Yes	
Non-conventional (unprimed)	Homeostatic memory	Self antigens	T-bet (?) Eomes (?)	No	Yes	
	IL-4 memory (innate CD8s <sup>+</sup> )	Self antigens?	IL-4 (others?)	Eomes	No	Yes



**Figure 2.** Various genes act at distinct stages to expand three types of PLZF<sup>+</sup> cells. The genes indicated in red regulate the number of PLZF<sup>+</sup>  $\gamma\delta$  NKT (a),  $\alpha\beta$  iNKT (b), or T-T CD4<sup>+</sup> T cells (c) in mice. ITK, SLP76, Id3 and CBP probably act downstream of the selection step that initiates  $\gamma\delta$  NKT development. KLF2 is more likely to regulate the later expansion of NKT, and affects both  $\gamma\delta$  NKT and  $\alpha\beta$  iNKT. The CIITA<sup>tg</sup> creates MHC class II dependent thymocyte–thymocyte (T–T) interactions that generate polyclonal CD4<sup>+</sup> T cells expressing PLZF.

c mice on the high end of the spectrum and C57BL/6 mice on the low end [45,46]. BALB/c mice have three to five times greater numbers of PLZF<sup>+</sup> cells in the thymus compared to C57BL/6 mice, with the majority of them being iNKT in BALB/c mice. Interestingly, CD8 SP thymocytes from BALB/c (but not C57BL/6) mice contain a distinct subpopulation of Eomes<sup>hi</sup>T-bet<sup>lo</sup>CD44<sup>hi</sup>CD122<sup>hi</sup> innate phenotype CD8<sup>+</sup> T cells that produce IFN $\gamma$ . As in the various gene-deficient models, this innate phenotype is dependent on IL-4 produced by NKT cells, because it is eliminated in BALB/c *I4r*<sup>-/-</sup> and BALB/c *Cd1d*<sup>-/-</sup> mice [16]. Therefore, the developmental regulation of innate CD8<sup>+</sup> T cells by PLZF<sup>+</sup> population is a phenotype of some gene-deficient mice and a physiological process in inbred mouse strains.

#### Innate CD8<sup>+</sup> T cells in CIITA transgenic mice and an analogous pathway in humans

CIITA transgenic mice also produce an enlarged population of PLZF<sup>+</sup> CD4<sup>+</sup> T cells in the thymus [1,26]. CIITA is a transcriptional activator of MHC class II expression, and in CIITA<sup>tg</sup> mice thymocytes express MHC class II. This model system [27,28] was first designed to investigate the functional significance of CD4<sup>+</sup> T cells that could potentially develop by thymocyte–thymocyte interactions (T–T CD4<sup>+</sup> T cells) because human thymocytes, unlike mouse thymocytes, express MHC class II molecules on their surface [26,47]. Thus, it was hypothesized that CD4<sup>+</sup> T

cells are positively selected by MHC II expressed on other thymocytes, which was confirmed in an *in vitro* reaggregate culture system [48]. In subsequent experiments using CIITA<sup>tg</sup> mice, T–T CD4<sup>+</sup> (or T-CD4) T cells were found to have a striking similarity to iNKT cells, including IL-4 secretion upon activation, acquisition of memory markers [26] and developmental dependence on the SLAM-SAP-mediated signaling pathway [30]. Remarkably, about 30–40% of polyclonal CD4 SP thymocytes developed by T–T interaction expressed PLZF (Figure 2), suggesting that T–T interactions during selection are crucial for specifying an ‘NKT-like’ T cell lineage, even when the restricting element was conventional MHC class II.

Interestingly, in CIITA<sup>tg</sup> mice, almost all TCR $\beta$ <sup>hi</sup> CD8 SP thymocytes had CD24<sup>lo</sup>CD44<sup>hi</sup>CD122<sup>hi</sup> memory phenotype. Similar to the described gene-deficient mice, and wild type (WT) BALB/c mice, increased numbers of CD8<sup>+</sup> T cells in CIITA<sup>tg</sup> mice were normalized in CIITA<sup>tg</sup> *I4r*<sup>-/-</sup> and CIITA<sup>tg</sup> *Stat6*<sup>-/-</sup> mice, demonstrating that IL-4 and its signaling pathway are essential [27].

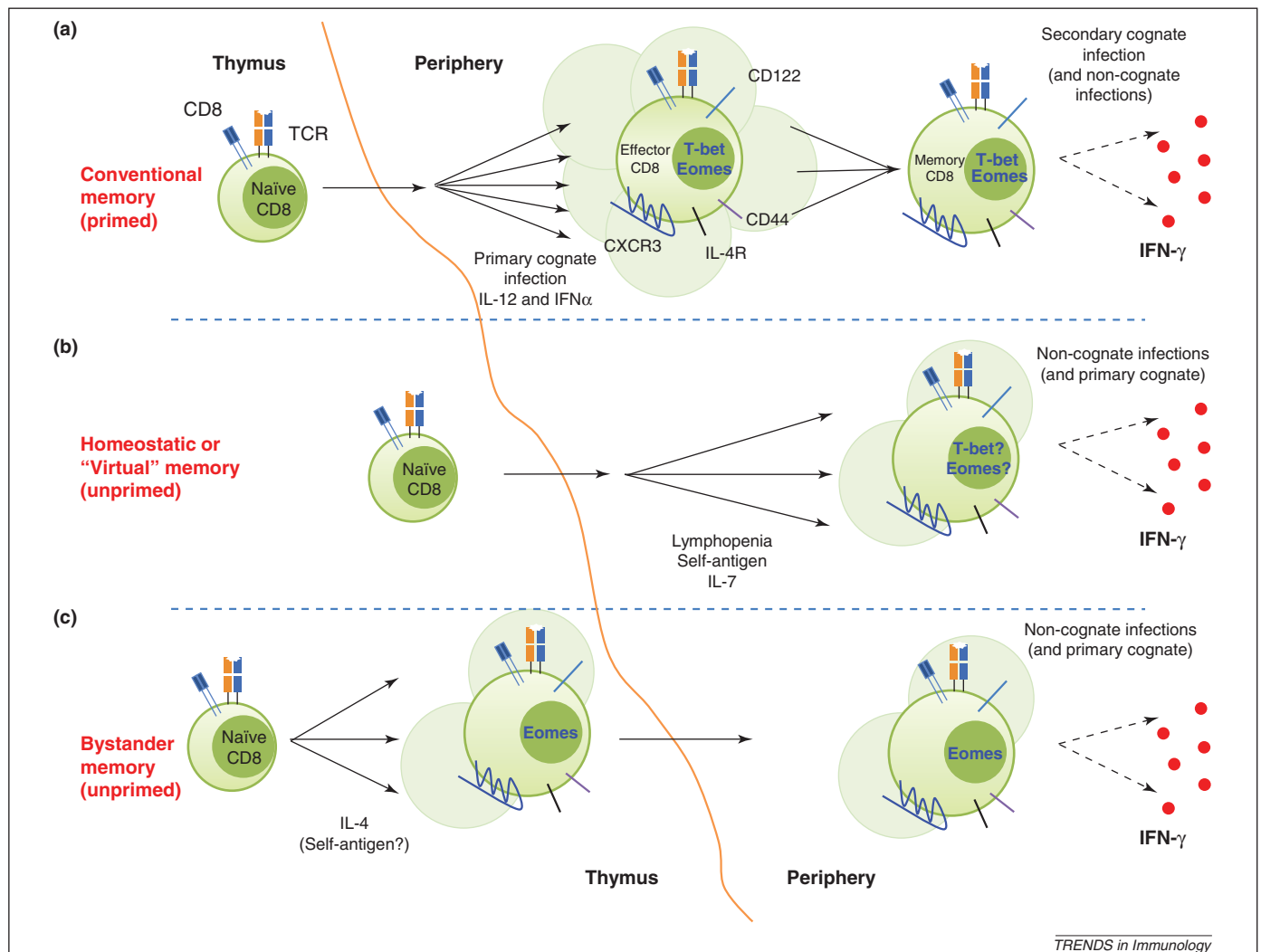
The expression of HLA-DR in human thymocytes peaks during the fetal to perinatal stage and gradually decreases in postnatal thymocytes, and becomes virtually absent from 3 to ~4 years old onwards [47]. The phenotype in CIITA<sup>tg</sup> mice reflected that of human fetal thymocytes, in which up to 8% of CD4 SP thymocytes and 15% of splenic CD4<sup>+</sup> T cells expressed PLZF [26]. Although human thymocytes express MHC class II and SLAM molecules in the

neonatal period, the newborn human thymus or cord blood contains few PLZF<sup>+</sup> CD4<sup>+</sup> T cells, the reason for which needs further investigation [26]. Nonetheless, it is interesting that MHC class II- and CD1d-dependent T–T interactions share a common thymic ontogeny in humans and mice, respectively, and this suggests that IL-4 might be involved in generating Eomes-expressing CD8<sup>+</sup> T cells in humans as well.

### What is the function of innate CD8<sup>+</sup> T cells?

Conventional memory T cells develop as naïve T cells in the thymus and become activated in the periphery by recognition of foreign antigen in an inflammatory context (i.e. infection) (Figure 3 and Table 2). Innate CD8<sup>+</sup> T cells phenotypically resemble memory T cells, yet do not require antigen experience to obtain this status, as demonstrated by the fact that OT-I *Rag*<sup>-/-</sup> cells adopt a memory phenotype and function when present as bystander cells in

KLF2-deficient mixed bone marrow chimeras [16]. In this regard, innate CD8<sup>+</sup> T cells resemble homeostatic (or virtual) memory T cells [50], which are generated in peripheral lymphoid organs in lymphopenic animals, in response to IL-7, IL-15 and self MHC-peptide [51,52] (Figure 3 and Table 2). By contrast, innate CD8<sup>+</sup> T cells develop in the thymus in an IL-4-dependent manner (and presumably in response to self MHC-peptide). Are these three subsets of memory cells functionally equivalent? Certainly, the fact that homeostatic and innate memory CD8<sup>+</sup> T cells do not require foreign antigen recognition for their generation means that they are unlikely to play a crucially important role in secondary infections, unlike conventional memory CD8<sup>+</sup> T cells that were clonally expanded during a primary response (Figure 3). However, there is growing evidence for an ‘innate’ role of memory CD8<sup>+</sup> T cells in primary infections, as sensors of an inflammatory environment [53]. For example, conventional mem-



**Figure 3.** Multiple pathways to becoming memory-phenotype cells. (a) Conventional memory cells are generated when naïve T cells become primed during infection via recognition of cognate antigens and inflammatory cytokines like IL-12 and IFN $\gamma$ . Such cells upregulate T-bet and Eomes and facilitate the rapid clearance of pathogens during secondary infection, via cytotoxicity and production of IFN $\gamma$ . (b) Homeostatic or virtual (unprimed) memory cells are generated when naïve T cells are in lymphopenic conditions and respond to self-antigens and homeostatic cytokines like IL-7. Lymphopenic conditions can be induced by artificial radiation and acute or chronic infection. Such cells produce IFN $\gamma$  in response to inflammatory cytokines IL-12 and IL-18, and thus could provide non-cognate or innate protection early on in infections. (c) Bystander or unprimed memory CD8<sup>+</sup> T cells are generated when developing thymocytes respond to elevated IL-4, presumably together with self-antigen. Like homeostatic memory cells, bystander memory cells produce IFN $\gamma$  in response to inflammatory cytokines and could provide non-cognate or innate protection early during infections. Bystander memory CD8<sup>+</sup> T cells upregulate Eomes, but not T-bet, although the functional implications of this are not yet clear.

ory OT-I T cells can produce IFN $\gamma$  early in the course of infection with pathogens that do not encode the ovalbumin antigen [54]. This response could be induced by IL-12 and IL-18 produced by activated myeloid cells [53,55,56]. Furthermore, this response is protective, at least when no other cells can produce IFN $\gamma$  [54]. Both homeostatic and innate CD8 $^{+}$  memory cells also produce IFN $\gamma$  in response to IL-12 and IL-18 [17,50]. Therefore, it would seem most likely that homeostatic memory and innate CD8 $^{+}$  T cells play roles early during infection, via production of IFN $\gamma$ . In the human immune system, these types of non-conventional or unprimed CD8 $^{+}$  T cells could be important because they are able to participate in host defense during the neonatal and early childhood period before conventional memory networks are established [1,16,26].

Whereas conventional memory CD8 $^{+}$  T cells are composed of heterogeneous subsets expressing T-bet and Eomes [57], innate CD8 $^{+}$  T cells selectively express Eomes. There is a complex interplay between Eomes and T-bet in the generation of central and effector memory responses, with expression of T-bet being generally associated with good effector responses [14] and Eomes with long-lived memory responses. Therefore, the lifespan and role of innate CD8 $^{+}$  T cells in the development of protective immunity remains to be investigated.

IL-4 is not typically associated with the generation of protective CD8 $^{+}$  T cell responses. The ability of IL-4 to drive expression of Eomes, CXCR3 and IFN $\gamma$  production in CD8 $^{+}$  T cells is counterintuitive, given its role in CD4 helper responses, where it promotes a Th2 response and suppresses a T-bet-mediated Th1 response [58]. However, the effect of IL-4 on CD8 $^{+}$  T cells is not without precedent in the literature. Complexes of IL-4–anti-IL-4 drive the proliferation of CD8 $^{+}$  T cells [35,59]. In fact, IL-4 is a potent growth factor for memory CD8 $^{+}$  T cells at doses produced during normal immune responses [35]. In addition, IL-4 supports the proliferation [60] and conversion of naïve CD8 $^{+}$  T cells into memory phenotype CD8 $^{+}$  T cells in lymphopenic mice [61].

There is an important role for IL-4 in the development of CD8 $^{+}$  T cell protective anti-malaria immunity, in which IL-4R deficient CD8 $^{+}$  T cells specific for the circumsporozoite protein of *Plasmodium yoelii* fail to develop into tissue-residing memory cells [62,63]. Interestingly, WT BALB/c mice are much more effective than C57BL/6 strains at controlling malaria pathogens after immunization with radiation-inactivated forms of *Plasmodium berghei* or *P. yoelii* sporozoites [64], which might be related to the high frequency of innate CD8 $^{+}$  T cells in the BALB/c strain.

### Concluding remarks

Until now, three types of PLZF $^{+}$  cells,  $\alpha\beta$  iNKT,  $\gamma\delta$  NKT and T-T CD4 $^{+}$  T cells, have been identified in the thymus. Under certain conditions, each of these can facilitate the development of innate CD8 $^{+}$  T cells in mice and humans. Genetic evidence has proved that IL-4 is required for this developmental effect. To date, it is not clear what stimuli cause NKT cells to produce IL-4 in the steady state. Interestingly, TLR signaling was recently shown to cause antigen-presenting cells (APCs) to present stimulatory self-lipids, through inhibition of  $\alpha$ -galactosidase activity

[65]. Perhaps endogenous signals can cause  $\alpha$ -galactosidase inhibition in thymic APCs, and these differ between inbred strains of mice. Regardless of the source, these findings highlight IL-4 as an important cytokine for the biology of memory CD8 $^{+}$  T cells. Future work should focus on potential differences in the function of memory cells that are generated in an IL-4-dependent fashion, particularly as they are known to have skewed expression of T-box transcription factors. Since many pathogens, such as parasites, induce a strong IL-4 response, it will be interesting to determine how the CD8 response is qualitatively different in these infections compared to bacterial and viral infections that induce a primarily Th1 response.

### Acknowledgments

This work was supported by grants to K.A.H. (AI39560) and S.C.J. (AI38903).

### References

- Lee, Y.J. *et al.* (2009) MHC class II-dependent T–T interactions create a diverse, functional and immunoregulatory reaction circle. *Immunol. Cell Biol.* 87, 65–71
- Urdahl, K.B. *et al.* (2002) Positive selection of MHC class Ib-restricted CD8 $^{+}$  T cells on hematopoietic cells. *Nat. Immunol.* 3, 772–779
- Kumanovics, A. *et al.* (2003) Genomic organization of the mammalian MHC. *Annu. Rev. Immunol.* 21, 629–657
- Treiner, E. and Lantz, O. (2006) CD1d- and MR1-restricted invariant T cells: of mice and men. *Curr. Opin. Immunol.* 18, 519–526
- Johansson-Lindbom, B. and Agace, W.W. (2007) Generation of gut-homing T cells and their localization to the small intestinal mucosa. *Immunol. Rev.* 215, 226–242
- Lambalez, F. *et al.* (2007) Thymic differentiation of TCR  $\alpha\beta^{+}$  CD8  $\alpha\alpha^{+}$  IELs. *Immunol. Rev.* 215, 178–188
- Prince, A.L. *et al.* (2009) The Tec kinases Itk and Rlk regulate conventional versus innate T-cell development. *Immunol. Rev.* 228, 115–131
- Veillette, A. *et al.* (2007) Consequence of the SLAM-SAP signaling pathway in innate-like and conventional lymphocytes. *Immunity* 27, 698–710
- Zheng, X. *et al.* (2008) Modulation of NKT cell development by B7-CD28 interaction: an expanding horizon for costimulation. *PLoS One* 3, e2703
- Horai, R. *et al.* (2007) Requirements for selection of conventional and innate T lymphocyte lineages. *Immunity* 27, 775–785
- Atherly, L.O. *et al.* (2006) The Tec family tyrosine kinases Itk and Rlk regulate the development of conventional CD8 $^{+}$  T cells. *Immunity* 25, 79–91
- Broussard, C. *et al.* (2006) Altered development of CD8 $^{+}$  T cell lineages in mice deficient for the Tec kinases Itk and Rlk. *Immunity* 25, 93–104
- Readinger, J.A. *et al.* (2009) Tec kinases regulate T-lymphocyte development and function: new insights into the roles of Itk and Rlk/Txk. *Immunol. Rev.* 228, 93–114
- Intlekofer, A.M. *et al.* (2005) Effector and memory CD8 $^{+}$  T cell fate coupled by T-bet and eomesodermin. *Nat. Immunol.* 6, 1236–1244
- Berg, L.J. (2007) Signalling through TEC kinases regulates conventional versus innate CD8 $^{+}$  T-cell development. *Nat. Rev. Immunol.* 7, 479–485
- Weinreich, M.A. *et al.* (2010) T cells expressing the transcription factor PLZF regulate the development of memory-like CD8 $^{+}$  T cells. *Nat. Immunol.* 11, 709–716
- Weinreich, M.A. *et al.* (2009) KLF2 transcription-factor deficiency in T cells results in unrestrained cytokine production and upregulation of bystander chemokine receptors. *Immunity* 31, 122–130
- Fukuyama, T. *et al.* (2009) Histone acetyltransferase CBP is vital to demarcate conventional and innate CD8 $^{+}$  T-cell development. *Mol. Cell Biol.* 29, 3894–3904
- Verykokakis, M. *et al.* (2010) SAP protein-dependent natural killer T-like cells regulate the development of CD8 $^{+}$  T cells with innate lymphocyte characteristics. *Immunity* 33, 203–215

- 20 Jordan, M.S. *et al.* (2008) Complementation in trans of altered thymocyte development in mice expressing mutant forms of the adaptor molecule SLP76. *Immunity* 28, 359–369
- 21 Felices, M. *et al.* (2009) Tec kinase Itk in gammadeltaT cells is pivotal for controlling IgE production in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8308–8313
- 22 Alonzo, E.S. *et al.* (2010) Development of promyelocytic zinc finger and ThPOK-expressing innate  $\gamma\delta$  T cells is controlled by strength of TCR signaling and Id3. *J. Immunol.* 184, 1268–1279
- 23 Lauritsen, J.P. *et al.* (2009) Marked induction of the helix-loop-helix protein Id3 promotes the  $\gamma\delta$  T cell fate and renders their functional maturation Notch independent. *Immunity* 31, 565–575
- 24 Ueda-Hayakawa, I. *et al.* (2009) Id3 restricts the developmental potential of gamma delta lineage during thymopoiesis. *J. Immunol.* 182, 5306–5316
- 25 Vergykokakis, M. *et al.* (2010) Inhibitor of DNA binding 3 limits development of murine Slam-associated adaptor protein-dependent “innate”  $\gamma\delta$ T cells. *PLoS One* 5, e9303
- 26 Lee, Y.J. *et al.* (2010) Generation of PLZF<sup>+</sup> CD4<sup>+</sup> T cells via MHC class II-dependent thymocyte-thymocyte interaction is a physiological process in humans. *J. Exp. Med.* 207, 237–246 S231-237
- 27 Li, W. *et al.* (2005) An alternate pathway for CD4 T cell development: thymocyte-expressed MHC class II selects a distinct T cell population. *Immunity* 23, 375–386
- 28 Choi, E.Y. *et al.* (2005) Thymocyte-thymocyte interaction for efficient positive selection and maturation of CD4 T cells. *Immunity* 23, 387–396
- 29 Patel, D.R. *et al.* (2005) Constitutive expression of CIITA directs CD4 T cells to produce Th2 cytokines in the thymus. *Cell Immunol.* 233, 30–40
- 30 Li, W. *et al.* (2007) The SLAM-associated protein signaling pathway is required for development of CD4<sup>+</sup> T cells selected by homotypic thymocyte interaction. *Immunity* 27, 763–774
- 31 Yamagata, T. *et al.* (2004) Self-reactivity in thymic double-positive cells commits cells to a CD8 $\alpha$  lineage with characteristics of innate immune cells. *Nat. Immunol.* 5, 597–605
- 32 Buckley, A.F. *et al.* (2001) Transcription factor LKLF is sufficient to program T cell quiescence via a c-Myc-dependent pathway. *Nat. Immunol.* 2, 698–704
- 33 Carlson, C.M. *et al.* (2006) Kruppel-like factor 2 regulates thymocyte and T-cell migration. *Nature* 442, 299–302
- 34 Takemoto, N. *et al.* (2006) Cutting edge: IL-12 inversely regulates T-bet and eomesodermin expression during pathogen-induced CD8<sup>+</sup> T cell differentiation. *J. Immunol.* 177, 7515–7519
- 35 Morris, S.C. *et al.* (2009) Endogenously produced IL-4 nonredundantly stimulates CD8<sup>+</sup> T cell proliferation. *J. Immunol.* 182, 1429–1438
- 36 D’Cruz, L.M. *et al.* (2010) Transcriptional regulation of NKT cell development and homeostasis. *Curr. Opin. Immunol.* 22, 199–205
- 37 Kelly, K.F. and Daniel, J.M. (2006) POZ for effect – POZ-ZF transcription factors in cancer and development. *Trends Cell Biol.* 16, 578–587
- 38 Kreslavsky, T. *et al.* (2009) TCR-inducible PLZF transcription factor required for innate phenotype of a subset of  $\gamma\delta$  T cells with restricted TCR diversity. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12453–12458
- 39 Kovalovsky, D. *et al.* (2008) The BTB-zinc finger transcriptional regulator PLZF controls the development of invariant natural killer T cell effector functions. *Nat. Immunol.* 9, 1055–1064
- 40 Savage, A.K. *et al.* (2008) The transcription factor PLZF directs the effector program of the NKT cell lineage. *Immunity* 29, 391–403
- 41 Raberger, J. *et al.* (2008) The transcriptional regulator PLZF induces the development of CD44 high memory phenotype T cells. *Proc. Natl. Acad. Sci. U.S.A.* 105, 17919–17924
- 42 Qi, Q. *et al.* (2009) Enhanced development of CD4<sup>+</sup>  $\gamma\delta$  T cells in the absence of Itk results in elevated IgE production. *Blood* 114, 564–571
- 43 Bai, A. *et al.* (2007) Kruppel-like factor 2 controls T cell trafficking by activating L-selectin (CD62L) and sphingosine-1-phosphate receptor 1 transcription. *J. Immunol.* 178, 7632–7639
- 44 Dose, M. *et al.* (2009) Intrathymic proliferation wave essential for V $\alpha$ 14<sup>+</sup> natural killer T cell development depends on c-Myc. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8641–8646
- 45 Rymarchyk, S.L. *et al.* (2008) Widespread natural variation in murine natural killer T-cell number and function. *Immunology* 125, 331–343
- 46 Hammond, K.J. *et al.* (2001) CD1d-restricted NKT cells: an interstrain comparison. *J. Immunol.* 167, 1164–1173
- 47 Park, S.H. *et al.* (1992) HLA-DR expression in human fetal thymocytes. *Hum. Immunol.* 33, 294–298
- 48 Choi, E.Y. *et al.* (1997) Thymocytes positively select thymocytes in human system. *Hum. Immunol.* 54, 15–20
- 50 Haluszczak, C. *et al.* (2009) The antigen-specific CD8<sup>+</sup> T cell repertoire in unimmunized mice includes memory phenotype cells bearing markers of homeostatic expansion. *J. Exp. Med.* 206, 435–448
- 51 Surh, C.D. and Sprent, J. (2008) Homeostasis of naive and memory T cells. *Immunity* 29, 848–862
- 52 Takada, K. and Jameson, S.C. (2009) Naive T cell homeostasis: from awareness of space to a sense of place. *Nat. Rev. Immunol.* 9, 823–832
- 53 Berg, R.E. and Forman, J. (2006) The role of CD8 T cells in innate immunity and in antigen non-specific protection. *Curr. Opin. Immunol.* 18, 338–343
- 54 Berg, R.E. *et al.* (2003) Memory CD8<sup>+</sup> T cells provide innate immune protection against *Listeria monocytogenes* in the absence of cognate antigen. *J. Exp. Med.* 198, 1583–1593
- 55 Berg, R.E. *et al.* (2005) Relative contributions of NK and CD8 T cells to IFN-gamma mediated innate immune protection against *Listeria monocytogenes*. *J. Immunol.* 175, 1751–1757
- 56 Berg, R.E. *et al.* (2002) Contribution of CD8<sup>+</sup> T cells to innate immunity: IFN- $\gamma$  secretion induced by IL-12 and IL-18. *Eur. J. Immunol.* 32, 2807–2816
- 57 Jameson, S.C. and Masopust, D. (2009) Diversity in T cell memory: an embarrassment of riches. *Immunity* 31, 859–871
- 58 Murphy, K.M. and Reiner, S.L. (2002) The lineage decisions of helper T cells. *Nat. Rev. Immunol.* 2, 933–944
- 59 Boyman, O. *et al.* (2006) Selective stimulation of T cell subsets with antibody-cytokine immune complexes. *Science* 311, 1924–1927
- 60 Tan, J.T. *et al.* (2001) IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc. Natl. Acad. Sci. U.S.A.* 98, 8732–8737
- 61 Ueda, N. *et al.* (2006) CD1d-restricted NKT cell activation enhanced homeostatic proliferation of CD8<sup>+</sup> T cells in a manner dependent on IL-4. *Int. Immunol.* 18, 1397–1404
- 62 Carvalho, L.H. *et al.* (2002) IL-4-secreting CD4<sup>+</sup> T cells are crucial to the development of CD8<sup>+</sup> T-cell responses against malaria liver stages. *Nat. Med.* 8, 166–170
- 63 Morrot, A. *et al.* (2005) IL-4 receptor expression on CD8<sup>+</sup> T cells is required for the development of protective memory responses against liver stages of malaria parasites. *J. Exp. Med.* 202, 551–560
- 64 Schmidt, N.W. *et al.* (2010) Extreme CD8 T cell requirements for anti-malarial liver-stage immunity following immunization with radiation attenuated sporozoites. *PLoS Pathog.* 6, e1000998
- 65 Darmoise, A. *et al.* (2010) Lysosomal  $\alpha$ -galactosidase controls the generation of self lipid antigens for natural killer T cells. *Immunity* 33, 216–228

#### Further reading

- 49 Blumer, N. *et al.* (2007) Development of mucosal immune function in the intrauterine and early postnatal environment. *Curr. Opin. Gastroenterol.* 23, 655–660