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Unveiling the regulation of NKT17 cell differentiation and function

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ABSTRACT

Invariant natural killer T cells (iNKTs) are distinct from conventional T cells. iNKT cells express a semi-invariant T cell receptor (TCR) that can specifically recognize lipid antigens presented by CD1d, an MHC class I-like antigen-presenting molecule. Currently, iNKT cells are distinguished in three functionally distinct subsets. Each subset is defined by lineage-specifying factors: T-bet shapes the fate of NKT1 subset that mainly secretes IFNγ, Gata3 specifies the NKT2 subset that produces robustly IL-4 whereas RORγt seals the differentiation of NKT17 subset that secretes IL-17. In the present review, the focus is placed on the regulation of NKT17 specification and their function.

1. Introduction

Invariant natural killer T cells (iNKT cells) express an invariant Va14-Ja18 TCRa chain that pairs with a limited amount of Vβ chains (Vβ8, Vβ7, Vβ2) in mice (Bendelac et al., 2007; Engel and Kronenberg, 2012). iNKT cells can recognize self or foreign lipid antigens that are presented to them by cells expressing MHC class I-like CD1d molecules (Bendelac et al., 2007; Wang and Hogquist, 2018). iNKT cells consist a small population of T cells in the thymus (approximately 1% in mice). However, in contrast to conventional T cells, iNKT cells maintain a poised effector state, which allows them to be rapidly activated upon antigen encounter and to potently secrete cytokines (Brennan et al., 2013). Thus, they are considered as first responders with the ability to bridge innate and adaptive immunity (Bendelac et al., 2007; Wang and Hogquist, 2018). As a result, they can recruit macrophages, dendritic cells, they can impact B cell differentiation and also activate natural killer (NK) cells (Brennan et al., 2013). Notably, iNKT cells can impact a variety of immune responses since they reside in various lymphoid and non-lymphoid tissues. iNKT cells can be found in spleen, lymph nodes, lung, liver, adipose tissue and intestine (Brennan et al., 2013; Crosby and Kronenberg, 2018; Lynch et al., 2015; Saez de Guinoa et al., 2018).

2. The role of RORγt in iNKT cell positive selection

iNKT cells diverge from conventional T cells at the CD4+CD8+ double positive (DP) cell stage in the thymus (Egawa et al., 2005). iNKT cells are positively selected upon recognition by their semi-invariant TCR of lipid antigens presented by CD1d molecules expressed by DP thymocytes (Gapin et al., 2001). RORγt is critical for iNKT cell development. It has been shown that RORγt can modulate the expression of Bcl-xL ensuring the survival of DP thymocytes (Fig. 1). Thus, RORγt is required for the optimal rearrangement of Va14-Ja14 TCR chains (Bezbradica et al., 2005).

3. iNKT cell lineage specification and effector function

At this stage, precursor, stage 0 iNKT cells upregulate CD24 (Bendelac et al., 2007) (Fig. 1). The fate of iNKT cells is sealed by the lineage-specifying factor promyelocytic leukemia zinc finger protein (PLZF) (Kovalovsky et al., 2008; Savage et al., 2008). Traditionally, a linear representation has been used to depict the development of iNKT cells in three stages. Stage 1 iNKT cells undergo a wave of proliferation that is controlled by the transcription factor Myc (Carr et al., 2015; Dose et al., 2009). Subsequently, at stage 2 these cells upregulate CD44. Then at stage 3 they also express NK1.1 (Bendelac et al., 2007).

Recently, a new approach that is based on the differential expression of lineage-specifying transcription factors was introduced (Buechel et al., 2015; Constantinides and Bendelac, 2013; Engel and Kronenberg, 2014; Krovi and Gapin, 2018). iNKT cells are subdivided into subsets (Fig. 1): NKT1, NKT2 and NKT17 to mirror the categorization of the T helper lineages: Th1, Th2 and Th17.

3.1. RORγt is specifically upregulated in the NKT17 cell subset

NKT1 cells express NK1.1, low levels of PLZF, do not express RORγt and can potently secrete IFNγ. NKT2 and NKT17 both express CD44 and thus cannot be distinguished based on the traditional view of iNKT stages, since both fall in stage 2 (Lee et al., 2013). However, NKT2 are

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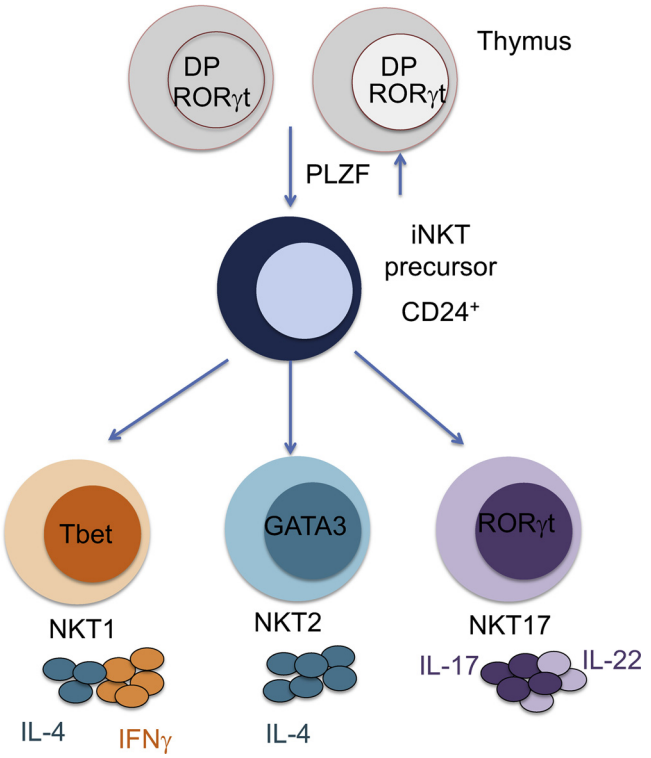

Fig. 1. iNKT cell subset differentiation. DP thymocytes that express RORγt recognize lipid antigens presented by CD1d molecules and give rise to the stage 0 iNKT cell precursor. At this stage the expression of PLZF-the lineage-specifying factor of the iNKT cells- is upregulated. Subsequently, distinct iNKT cell subsets are developed and they can be distinguished based on the transcription factors that govern their fate. NKT1 cells express T-bet and can secrete mainly IFN-γ and in less extent IL-4. NKT2 cells express GATA3 and can potently secrete IL-4. NKT17 cells express high levels of RORγt and can produce IL-17 and IL-22.

$CD4^+$, $PLZF^{high}$, $ROR\gamma t^-$ and secrete IL-4 and can be readily distinquished from the NKT17 cells that express intermediate levels of PLZF, high levels of RORγt and secrete IL-17 (Weinreich et al., 2010).

Notably, these subsets can be further distinguished by differential expression of chemokine receptors and thus can migrate to peripheral organs in a tissue-specific manner (Crosby and Kronenberg, 2018; Lee et al., 2015). Overall, the iNKT subsets exhibit transcriptional heterogeneity as highlighted in independent studies that took advantage of next-generation sequencing technologies (Engel et al., 2016; Georgiev et al., 2016; Lee et al., 2016).

Intriguingly, NKT1 cells are the predominant iNKT subset in C57/Bl6 mice whereas NKT2 cells are more frequent in Balb/c mice (Lee et al., 2013). NKT17 cells are the least frequent among the thymic iNKT cells and their *in vivo* functions are less explored in comparison to the other iNKT subsets. In the next paragraphs, I aim to shed light to the establishment of their identity (Table 1) and their *in vivo* roles in immune response (Table 2).

4. Signaling pathways for NKT17

4.1. TCR signaling strength

TCR signaling strength has been suggested to play a role in the iNKT cell lineage commitment and subsequent diversification. Strong TCR signaling is critical for the initial commitment of stage 0 iNKT cells to the iNKT cell lineage, resulting in upregulation of the transcription factors Egr1 and Egr2 (Seiler et al., 2012). Egr1 and Egr2 regulate the expression of PLZF, the transcription factor that governs the iNKT cell lineage fate (Seiler et al., 2012). iNKT cells share an invariant TCR Va chain and a limited amount of Vβ chains. Interestingly, NKT2 cells express more highly Vβ7 chain, whereas NKT1 cells express in higher frequency Vβ8 chain (Lee et al., 2013). So, it has been postulated that biased TCR Vβ use could result in different TCR signaling strength and preferential differentiation towards subsets (Savage et al., 2008). Increased staining for tetramer loaded α-GalCer and TCRVβ has been observed in NKT2 cells, less in NKT17 and NKT1 cells were the cells with the lowest expression (Georgiev et al., 2016).

Indeed, iNKT cells with compromised ζ chain associated protein kinase 70 (ZAP70) have less efficient early TCR signaling. There have been reports of mice strains with compromised ZAP70 activity; SKG mice have a hypomorphic allele for *Zap70* due to a spontaneous mutation in the SH2 domain (Sakaguchi et al., 2003). Further analysis of these mice revealed increased representation of NKT1 cells in the thymus accompanied by reduction in the NKT2 and NKT17 subsets (Zhao et al., 2018).

4.2. mTOR signaling pathway

The mammalian target of rapamycin (mTOR) signaling consists of two components, mTORC1 and mTORC2 that are defined respectively by Raptor and Rictor. mTORC1 and mTORC2 exert distinct functions in the process of T cell activation and differentiation (Chi, 2012). mTORC2 signaling was shown to play a key role in NKT17 cell lineage differentiation. Specifically, loss of Rictor significantly reduces NKT17 cells and compromises IL-17 production (Wei et al., 2014). Thus, Rictor is critical for the NKT17 cell development and function (Sklarz et al., 2017; Verykokakis and Kee, 2017). Moreover, deletion of *Pten*, a suppressor of mTOR signaling, in the DP thymocytes resulted in a striking increase of NKT17 cells (Wei et al., 2014).

In addition, mTORC1 activation can be inhibited by a complex formed by tuberous sclerosis 1 (TSC1) and 2 (TSC2). Moreover, TSC1 can promote mTORC2 activation. T- cell specific deletion of TSC1 results in an increase of NKT17 cells and a simultaneous decrease of NKT1 cells. This is attributed to aberrant activation of mTORC1, which suppresses T-bet expression and results in skewing to NKT17 lineage (Wu et al., 2014). An additional feature of TSC1 deficient iNKT cells was the upregulation of ICOS that is linked to NKT17 selective differentiation (Wu et al., 2014).

Another key player of the mTOR signaling is Akt that can activate mTORC1 and is activated by mTORC2 (Chi, 2012). There are three isoforms of the Akt family of proteins; Akt1, Akt2 and Akt3. Importantly, Akt can suppress FoxO-1. This is achieved *via* phosphorylation of FoxO-1 and its subsequent migration from the nucleus to the cytoplasm where after Lys48 ubiquitination the factor is degraded (Hedrick et al., 2012).

Germline deletion of Akt2 resulted in reduction of NKT17 cells as well as IL-17 secretion (Niu et al., 2018). Further analysis showed that Akt2 can mediate the upregulation of ICOS that promotes NKT17 differentiation (Wu et al., 2014).

4.3. Notch signaling pathway

The Notch signaling pathway is highly conserved. Four Notch receptors have been described in mammals (Notch1-4). These receptors can be bound by ligands [Delta-like (Dll) 1, 2, 3 as well as Jagged 1 and 2) (Bray, 2006). The intracellular domain of Notch receptors (NICD) can migrate to the nucleus where it heterodimerizes with the transcription factor CBF1, Suppressor of Hairless, Lag1 (CSL). CSL is also known as Recombination Signal Binding Protein for Immunoglobulin kappa *J* region (RBP-*J*) in mice. Notch signaling is critical for various aspects of embryonic development as well as hematopoiesis and immune function (Radtke et al., 2010).

T cell-specific simultaneous deletion of both Notch1 and Notch2, using CD4-cre mice, resulted in increased percentage and number of stage 2 iNKT cells (Oh et al., 2015). Further analysis revealed an

Table 1
Summary of phenotypes in mice with altered NKT17 development.

Genetically modified mouse	Target	Impact on NKT17 cells	Reference
Akt2-/-	Germline deletion of Akt2	Increased number of NKT17 cells	Niu et al., Frontiers in Immunology, 2018 PMID:30258434
SKG	Mutation in SH2 domain of ZAP70 resulting in hypomorphic allele	Increased number of NKT17 cells	Zhao et al., Nature Communication 2018 PMID: 29980684
Rictor $^{flx/flx}$ CD4-cre	Deletion of Rictor, the signature factor of mTORC2, in DP thymocytes	Decreased number of NKT17 cells	Wei et al., Journal of Immunology, 2014 PMID: 25261481
Pten $^{flx/flx}$ CD4-cre	Deletion of *Pten*, a suppressor of mTORC, in DP thymocytes	Increased number of NKT17 cells	Wei et al., Journal of Immunology, 2014 PMID: 25261481
Tsc1$^{flx/flx}$ CD4 -cre	Deletion of Tsc1 at the DP thymocytes	Predominance of the NKT17 subset	Wu et al., JCI 2014 PMID: 24,614,103
Notch1$^{flx/flx}$ *Notch2* $^{flx/flx}$ CD4-cre	Deletion of *Notch1* and *Notch2* at the DP stage	Increased NKT17 cells	Oh SJ et al., JLB 2015 PMID: 26188077
Bcl11b $^{flx/flx}$ PLZF-cre	Deletion of *Bcl11b* at the stage 0 of iNKT cell development	Increased representation of NKT17 cells	Uddin et al., PNAS 2016 PMID: 27,330,109
Th-POK $^{hd/hd}$	Spontaneous mutation in the zinc finger domain of Th-POK abrogates the DNA binding activity	Increased number of NKT17 cells	Engel et al., Blood 2012 PMID: 23034280
Runx1$^{flx/flx}$ PLZF-cre	Deletion of *Runx1* at the stage 0 of iNKT cell development	Decrease of NKT17 cells	Thapa et al., Scientific reports, 2017 PMID: 28765611
Nkap $^{flx/flx}$ PLZF-cre	Deletion of NKAP at the developmental stage 0 iNKTs	Reduction of NKT17 subset	Thapa et al. Scientific reports, 2016 PMID : 27183586
Med23$^{flx/flx}$ CD4-cre	Deletion of *Med23* at the DP thymocytes	Dramatic loss of NKT17 cells	Xu et al. Nature Communications 2018 PMID: 30,250,136
Tet3 $^{flx/flx}$ CD4-cre	Deletion of *Tet3* in DP thymocytes	Increase of NKT17 cells	Tsagaratou et al. Nature Immunology, 2017 PMID: 27869820
Tet2 $^{-/-}$ *Tet3*$^{flx/flx}$ CD4-cre Or *Tet2*$^{flx/flx}$ *Tet3*$^{flx/flx}$ CD4-cre	Simultaneous deletion of *Tet2* (germline or in DP thymocytes) and *Tet3* (DP thymocytes)	-Increase of NKT17 cells -TCR mediated expansion of NKT17 cells	Tsagaratou et al., Nature Immunology, 2017 PMID: 27869820
Gcn5 $^{flx/flx}$ *Lck-cre*	Deletion of *Gcn5* in DN3 stage	Impaired NKT17 cell development	Wang et al., Cell reports, 2017 PMID: 28,723,564
Hdac3 $^{flx/flx}$ *PLZF-cre*	Deletion of *Hdac3* in PLZF-expressing cells	NKT17 cells that lack HDAC3 secrete less IL-17	Thapa et al. Scientific reports, 2017 PMID: 28724935

increase in the percentage of NKT17 cell lineage in the thymus of Notch1 and Notch2 double-deficient mice. The increase of the NKT17 cells was also observed in lymph nodes of the mutant mice when compared to wild-type mice (Oh et al., 2015). Collectively, these data suggest a key role of both Notch1 and Notch2 in regulating NKT17 development.

5. Transcriptional regulation of NKT17 lineage

RORγt drives the NKT17 lineage specification. However, research in the field has recently shed light on the complexity of the transcriptional networks that regulate the fate of the NKT17 subset. Along these lines, transcription factors and transcription regulatory complexes act in concert to instruct cell fate choice.

Importantly, Runx1 has been described as a key transcription factor that orchestrates NKT17 lineage specification, ensuring the upregulation of genes that promote NKT17 lineage (Thapa et al., 2017). *Runx1* deficient iNKT cells show reduced expression of fundamental genes of NKT17 lineage such as IL-7Ra, BATF and c-Maf (Thapa et al., 2017). Bcl11b has been shown to suppress the NKT17 lineage fate (Uddin et al., 2016). Similarly, Th-POK (encoded by *Zbtb7b*) has been found to suppress RORγt expression in iNKT cells, acting thus as a negative regulator of the NKT17 lineage (Engel et al., 2012). T-bet has also been described to partially suppress RORγt expression in iNKT cells (Tsagaratou et al., 2017a). In addition, the transcriptional repressor NKAP has been shown to contribute in NKT17 cell differentiation. Mice that lack NKAP after the iNKT stage 0 exhibit a dramatic reduction of NKT17 cells and impaired capacity to produce IL-17 (Thapa et al., 2016).

Transcription can be regulated by large complexes that consist of many subunits. Mediator is a large transcriptional co-activator that consists of multiple subunits (Jeronimo and Robert, 2017). In humans, Mediator complex comprises 30 subunits. This complex can interact with transcription factors that are bound at enhancers as well as with the RNA polymerase II machinery at promoters (Jeronimo and Robert, 2017). Beyond its effects in transcriptional regulation the Mediator

Table 2
In vivo functions of NKT17 cells.

Tissue	Pathology	Role of IL-17	Reference
Lung	Pulmonary infection *Streptococcus pneumoniae*	IL-17 secretion exerts potential positive role by mediating neutrophil recruitment	Ivanov et al., Journal of Infectious Disease, 2012 PMID: 22723642 Wu et al., JCI PMID: 24,614,103
Lung	-Ozone Induced Asthma -Aerosolized methacholine	IL-17 secretion results in airway hyperreactivity	Pichavant et al., JEM 2008 PMID: 18250191 Wu et al., JCI PMID: 24,614,103
Liver	iNKT mediated acute Liver Failure	Increased secretion of IL-17 results in hepatocyte necrosis	Milosavljevic et al., Liver Transplantation, 2017 PMID: 28481005
Prostate	Prostate Cancer	Increased number of NKT1 cells and reduction of NKT17 cells is associated with impaired prostate cancer progression	Cortesi F. et al, Cell reports 2018 PMID: 29539427

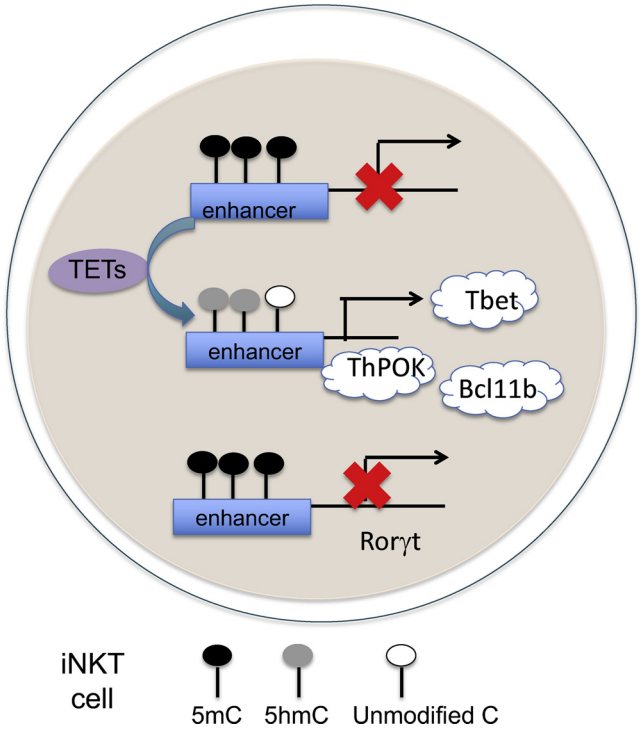

Fig. 2. Crosstalk of the epigenome and the transcription factor machinery regulates iNKT subset differentiation. The interplay of epigenetic regulators (TET proteins) and transcription factors (Th-POK, T-bet, Bcl11b) results in suppression of RORγt and decreased levels of IL-17 secretion in iNKT cells.

complex has also been implicated in DNA repair as well as in class switch recombination (Jeronimo and Robert, 2017). Mediator complex is critical for development (Yin and Wang, 2014). Mutations related to components of the complex resulting in aberrant expression have been linked to developmental disorders and cancer emergence (Yin and Wang, 2014).

Specific deletion of the subunit Med23 in DP thymocytes revealed a crucial role in iNKT cell development (Xu et al., 2018). Both the frequency and the absolute number of iNKT cells was significantly decreased in mice that lacked Med23 specifically in DP thymocytes. More detailed analysis revealed an increase of stage 2 iNKTs and a dramatic reduction of stage 3 iNKT cells, suggesting a block in iNKT cell terminal maturation. The observed increase at the developmental stage 2 was due to increased generation of NKT2 cells, whereas interestingly the NKT17 cells were significantly reduced (Xu et al., 2018). It is suggested that Med23 can up-regulate transcription factors that are important for the transition from stage 2 to stage 3 iNKT cells. Reduced expression of c-Jun was shown to be instrumental in the entrapment of *Med23-/-* iNKT cells primordially in NKT2 cell lineage. Ectopic expression of c-Jun could partially restore the differentiation and maturation of *Med23-/-* iNKT cells (Xu et al., 2018).

Collectively, these results highlight the context-specific functions of transcription factors that act as activators or repressors of gene expression to ultimately license the lineage specific gene expression program.

6. Epigenetic regulation of NKT17 lineage

Even though transcription factors drive gene expression, their binding to their genomic targets is defined by the epigenetic landscape. Briefly, epigenetic mechanisms result in modification in chromatin or in DNA bases but without changing the sequence of the DNA (Allis and Jenuwein, 2016). They must meet one of the following criteria: 1) propagation of the signal by cell division, 2) inheritance to daughter cells and 3) impact gene expression (Bonasio et al., 2010). Chromatin modifications (Zhou et al., 2011) as well as DNA modifications (Pastor et al., 2013; Smith and Meissner, 2013) constitute the dynamic epigenome. Epigenetic modifications can specifically recruit: writers that deposit these modifications, readers that can specifically recognize and bind to these modifications as well as erasers, which can remove these modifications.

Recent studies have started to shed light on the epigenetic regulation of iNKT cells (Tsagaratou, 2018; Verykokakis and Kee, 2018; Wang and Hogquist, 2018). In the present study, emphasis is placed on epigenetic regulators that have been shown to play a role in shaping NKT17 cell lineage.

The Ten Eleven Translocation (TET) family of proteins are 2-oxoglutarate and Fe(II) dependent dioxygenases and can catalyze the oxidization of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) (Tahiliani et al., 2009) as well as further downstream oxidized cytosines (oxi-mCs); 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) (He et al., 2011; Ito et al., 2011). TET proteins not only can mediate active DNA demethylation through their enzymatic activity but, in addition, can form complexes with suppressive or promoting impact on gene expression (Pastor et al., 2013; Tsagaratou et al., 2017b; Wu and Zhang, 2017).

Among the three members of the TET family of proteins, TET2 and TET3 are more highly expressed in T cells whereas TET1 is much less expressed (Tsagaratou and Rao, 2013). Increased levels of 5hmC in T cells have been observed in the gene body of very highly expressed genes as well as in active enhancers (Ichiyama et al., 2015; Tsagaratou et al., 2014).

It has been shown that *Tet3*-deficient iNKT cells are skewed towards NKT17 lineage due to decreased expression of NKT17 suppressive genes such as *Zbtb7b*, which encodes Th-POK (Tsagaratou et al., 2017a). Interestingly, iNKT cells concomitantly lacking Tet2 and Tet3 show even more pronounced downregulation of transcription factors that suppress NKT17 lineage, such as T-bet, Th-POK as well as Bcl11b. The genes encoding for these factors (*Tbx21*, *Zbtb7b* and *Bcl11b* respectively) show increased hydroxymethylation, suggesting that TET proteins can directly regulate their expression in iNKT cells (Fig. 2) (Tsagaratou et al., 2017a). The *Tet2/3 DKO* iNKT cells express increased levels of RORγt and as a result can potently secrete IL-17 (Tsagaratou et al., 2017a).

An additional layer of epigenetic regulation is histone modification. Several histone modifications have been described. Among the first to be reported was histone acetylation at lysine residues of histone tails. Histone acetylation is a dynamic process. Histone acetyltransferases (HATs) acetylate lysine residues ensuring an open accessible chromatin conformation, which is considered permissive for gene expression (Kuo and Allis, 1998). On the other hand, histone deacetylases (HDACs) remove the acetyl group resulting in a closed chromatin conformation, exerting a suppressive role in gene expression (Kuo and Allis, 1998). It is now established that lysine (K) acetylation is a post-translational modification that extends beyond the modification of histones and can include both nuclear as well as cytoplasmic proteins (Choudhary et al., 2014). Thus, lysine acetyltransferases (KATs) and lysine deacetyltransferases (KDACs) can determine the outcome of various biological processes (Choudhary et al., 2014).

Lysine acetyltransferase general control non-derepressible 5 (GCN5) was identified as the first histone acetyltransferase (Brownell et al., 1996) and is a component of the transcriptional co-activator complex Spt-Ada-Gcn5 Acetyl transferase (SAGA) (Helmlinger and Tora, 2017). GCN5 plays important role in various biological processes such as development, cell growth, DNA repair (Wang and Dent, 2014; Zhao et al., 2017). Specific deletion of GCN5 at the double negative stage of T cell development using the Lck-cre mice revealed a role in regulating T cell development (Wang et al., 2017). Strikingly, a significant reduction in iNKT cells was observed. Further analysis demonstrated that NKT2 cells were increased whereas differentiation of NKT17 and NKT1 subsets was impaired. Egr2, a transcription factor that is upregulated early on iNKT

cell development and drives PLZF expression (Seiler et al., 2012), was identified as a direct substrate of GCN5 (Wang et al., 2017).

Histone deacetyltransferase 3 (HDAC3) can mediate the removal of acetyl groups from proteins. Specific deletion of HDAC3 in PLZF expressing cells leads to a dramatic reduction in NKT1 cell numbers (Thapa et al., 2017). NKT2 and NKT17 cells develop but their ability to produce IL-4 and IL-17 respectively is compromised (Thapa et al., 2017).

7. microRNAs and NKT17 lineage

MicroRNAs are small non-coding single-strand (ss) RNAs that influence the stability of messenger RNAs and deregulation or loss of individual microRNAs in mouse models has established a critical role in hematopoietic development and immune function (Xiao and Rajewsky, 2009). Notably, Let-7 microRNAs are highly expressed in NKT1 cells where they target *Zbtb16* mRNAS and thus result in downregulation of PLZF (Pobezinsky et al., 2015). LIN28, an RNA binding protein, was shown to suppress Let-7. As a result, Lin28 transgenic mice show a striking increase in the NKT2 and NKT17 lineages since they maintain high expression levels of PLZF (Pobezinsky et al., 2015).

8. *In vivo* functions of NKT17 cells

It is well established that NKT17 cells are developed in the thymus and they can potently secrete IL-17. As they migrate in the periphery they are abundant in the lymph nodes (Lee et al., 2015) and the lung (Michel et al., 2007). Interestingly, while NKT1 and NKT2 cells are mostly present in the vasculature, NKT17 cells are predominant within the lung parenchyma (Crosby and Kronenberg, 2018; Scanlon et al., 2011; Trottein and Paget, 2018). In the following paragraphs the *in vivo* functions of these cells will be explored (Table 2).

8.1. Bacterial lung infection

Streptococcus pneumoniae is a Gram positive bacterium that induces community acquired pneumonia in western countries (Trottein and Paget, 2018). iNKT cells have been shown to play a role in host defense against pulmonary bacterial infection (Crosby and Kronenberg, 2016). Indeed, Ja18-/- mice that lack iNKT cells are more susceptible to *Streptococcus pneumoniae* infection (Kawakami et al., 2003). The glycolipids of the cell wall of various *Streptococcus pneumoniae* strains can be presented by Cd1d molecules to mediate iNKT activation (Kinjo et al., 2011). Exogenous activation of iNKT cells can exert protective effect to *Streptococcus pneumoniae* infection (Kinjo et al., 2005). This is due to secretion of both IFN-γ as well as IL-17 that result in neutrophil recruitment in the lungs (Ivanov et al., 2012). Interestingly, mice that lack TSC1 specifically in T cells exhibit skewing towards the NKT17 cell lineage. Upon challenge with *S. pneumoniae* these mice exhibit increased infiltration of neutrophils. This observation was linked to increased IL-17 secretion by iNKT cells (Wu et al., 2014).

8.2. Asthma

Airway hyperreactivity consists a hallmark of asthma. A role for iNKT cells has been reported in the case of asthma induced specifically by exposure to ozone but not to allergens (Pichavant et al., 2008). Ozone exposure resulted in airway hyperreactivity associated with NKT cells secreting IL-17 and was not observed in IL-17 -/- mice or wild-type mice treated with anti-IL-17. Collectively, these results highlight a key role of IL-17 in this type of asthma (Pichavant et al., 2008). In addition, T cell-specific TSC1 deficient mice show skewing towards the NKT17 lineage and demonstrate increased airway hyperreactivity when challenged with α-GalCer (Wu et al., 2014).

8.3. Liver failure

Interestingly, in a model of iNKT-cell mediated acute liver failure induced by α-galactoceramide increased secretion of IL-17 is considered to play a major role. The source of increased levels of IL-17 are both $CD4^+$ T cells as well as NKT17 cells. Overproduction of IL-17 leads to hepatocyte necrosis (Milosavljevic et al., 2017).

8.4. Cancer

Data support a significant role of iNKT cells in mediating protective immune response against tumors. Increased frequency of peripheral blood type I NKT cells and numbers of tumor-infiltrating NKTs in cancer patients correlate with better prognosis and clinical outcome (Metelitsa, 2011; Metelitsa et al., 2004). Since tumor-associated macrophages (TAMs) provide a critical stromal support for tumor cell growth in many types of cancer, iNKT cell-mediated killing or inhibition of TAMs explains how iNKTs may indirectly affect tumor growth. It is postulated that IFN-γ secretion either directly by iNKT cells or indirectly due to the influence of iNKT cells to macrophages, NK cells, dendritic cells or $CD8^+$ T cells can result in tumor regression (Brennan et al., 2013).

However, the precise role of distinct iNKT subsets in the tumor microenvironment remains elusive. Notably, an increase of NKT1 subset infiltration and a simultaneous reduction of NKT17 cells has been reported recently in a mouse model of prostate cancer to result in cancer regression (Cortesi et al., 2018). The proposed mechanism is that increased NKT1 cells modulate tumor associated macrophages and promote the selective killing of proangiogenic, cancer promoting M2 macrophages (Cortesi et al., 2018).

9. Conclusions

Invariant NKT cells are a T cell subset with unique properties. They constitute a very small subset of the thymocytes but yet can potently secrete cytokines and thus rapidly and efficiently modulate the immune response. They can act both in a protective or immune compromising way. The current knowledge that iNKT cells can be subdivided in subsets, based on the transcription factors that they express, has helped us to better understand their function and explain previously contradictory observations. Indeed, iNKT cells are a heterogeneous population that consists of distinct subsets with unique functions.

Importantly, as these subsets migrate in the periphery they can be found in most tissues. However, the frequency of iNKT subsets is variable (Lee et al., 2015). So, the question is what are the environmental cues and the iNKT cell-instrinsic factors that impact their homing to different organs? As mentioned above, NKT17 cells can be found in lung and lymph nodes. Interestingly, in many pathological conditions can be found together with NKT1 cells. How stable are these cells? Is there plasticity and interconversion as observed for Th1, Th17 and regulatory T cells (Murphy and Stockinger, 2010) or each lineage is sealed? What is the role of epigenetic modifications in initiating the expression and maintaining the stability of lineage specifying factors?

NKT17 cells are the least studied of the thymic iNKT subsets but gradually we start to gain more knowledge of their *in vivo* functions. It is critical to study in further detail how they are recruited to the tissues of action, how they are activated and how they interact with other cell types in place. Moreover, analyzing the molecular identity of these cells in their residing tissues both in steady-state conditions as well as upon infection/ disease will shed light on tissue-specific and/ or disease-specific regulatory elements. Ultimately, this deeper knowledge will allow us to understand how NKT17 cells can modulate the immune response in different tissues and different immune challenges.

Conflict of interest

The author declares no conflict of interest.

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