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mTOR and its tight regulation for iNKT cell development and effector function

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ABSTRACT

Invariant NKT (iNKT) cells, which express the invariant Vα14Jα18 TCR that recognizes lipid antigens, have the ability to rapidly respond to agonist stimulation, producing a variety of cytokines that can shape both innate and adaptive immunity. iNKT cells have been implicated in host defense against microbial infection, in anti-tumor immunity, and a multitude of diseases such as allergies, asthma, graft versus host disease, and obesity. Emerging evidence has demonstrated crucial role for mammalian target of rapamycin (mTOR) in immune cells, including iNKT. In this review we will discuss current understanding of how mTOR and its tight regulation control iNKT cell development, effector lineage differentiation, and function.

1. Introduction

Invariant natural killer T (iNKT) cells harbor the invariant Vα14-Jα18 (iVα14) TCRα chain in mice and the invariant Vα24-Jα18 TCR in humans paired with restricted Vβ chains (Bendelac et al., 2007; Godfrey et al., 2010; Salio et al., 2014). Unlike conventional TCRαβ T (cαβ T) cells that recognize the major histocompatibility complex (MHC)–peptide complex, iNKT cells selectively recognize lipid-antigens such as endogenous, microbial derived, and synthetic ligands presented by MHC class I-like CD1d molecules via the iVα14 TCR (Kawano et al., 1997; Gapin et al., 2001; Mendiratta et al., 1997). Engagement of iVα14TCR with endogenous ligand–CD1d complexes presented by $CD4^+CD8^+$ double positive (DP) thymocytes in the thymic cortex leads to positive selection and generation of iNKT cells. Following TCR stimulation with a synthetic ligand α-galactosylceramide (α-GalCer), mature iNKT cells rapidly release various cytokines such as IL-4, IL-17, IL-10, IL-13, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α (Brennan et al., 2013; Coquet et al., 2008; Milpied et al., 2011), which enable them to play important roles in both innate and adaptive immune responses. Although iNKT cells only comprise a small portion of T cells, their roles have been described in various immune responses and diseases, including tumor surveillance, defense against microbial infection, as well as pathogenesis of autoimmune diseases, graft-versus-host disease, and obesity (Van Kaer et al., 2013; Berzins and Ritchie, 2014; Terashima et al., 2008; Osman et al., 2000).

Traditionally, thymic development of *i*NKT cells from $CD4^+CD8^+$ DP precursor has been defined into four stages based on surface levels of CD24, CD44, and NK1.1 (Fig. 1): stage 0 ($CD24^+CD44^-NK1.1^-$), stage 1 ($CD24^-CD44^-NK1.1^-$), stage 2 ($CD24^-CD44^+NK1.1^-$), and

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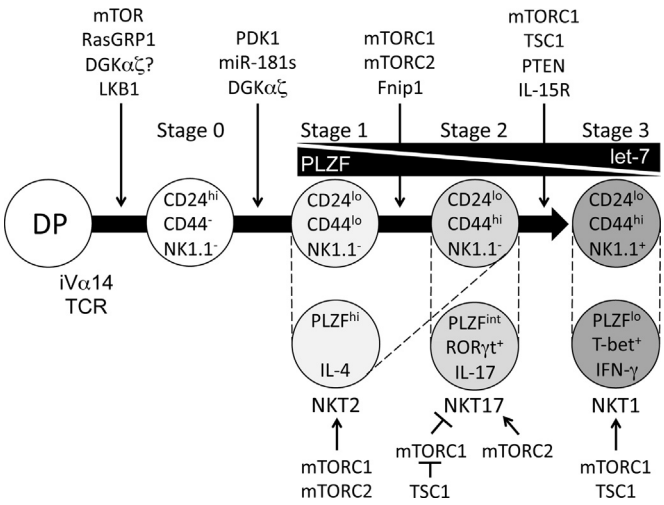

Fig. 1. iNKT cell development and effector lineages. iNKT cells are originated from $CD4^+CD8^+$ DP thymocyte precursors that express the iVα14TCR. Engagement of the iVα14TCR with CD1d bearing self-lipid ligand expressed by DP thymocytes ensures the generation of iNKT cell lineages. Immature iNKT cells from DP thymocytes undergo four maturation stages. Each stage is characterized by different expression of CD24, CD44, and NK1.1 on their surface. Based on their distinctive expression of transcription factors and cytokines, iNKT cell effector subsets can be defined into iNKT1, iNKT2, and iNKT17 cells. These two categories are closely correlated as marked with dotted lines. Various receptors, signaling molecules, miRNAs, and transcription factors dictating iNKT maturation are depicted.

terminally matured stage 3 ($CD24^-CD44^+NK1.1^+$) (Bendelac et al., 2007; Godfrey et al., 2010). More recently, iNKT cells have been classified into multiple terminally differentiated effector lineages that include IFN-γ-producing iNKT1, IL-4-producing iNKT2, and IL-17-producing iNKT17 lineage (Michel et al., 2007, 2008; Matsuda et al., 2006). In addition, IL-10-producing iNKT10, T follicular helper (Tfh)- and regulatory T cell (Treg)-like iNKT cells ($iNKT_{FH}$) have also been recently reported (Chang et al., 2012; Rampuria and Lang, 2015; Tonti et al., 2012; Lynch et al., 2015; Sag et al., 2014). Similar to Th lineages, iNKT effector lineages are governed by critical transcription factors including RORγt, T-bet, Gata3, and PLZF (Godfrey et al., 2010; Michel et al., 2008; Lee et al., 2013; Wu et al., 2014a). iNKT1 cells express low levels of promyelocytic leukemia zinc-finger (PLZF) but high levels of T-bet ($PLZF^{low}T\text{-}bet^+$); iNKT2 cells are $PLZF^{high}$; while iNKT17 are $PLZF^{int}ROR\gamma t^+$. iNKT1 cells mostly reside in the $CD44^+NK1.1^+$ stage 3 population, iNKT2 cells reside in both stage 1 and stage 2 populations, and iNKT17 cells are restricted to the $CD44^+NK1.1^-ICOS^+$ population (Lee et al., 2013; Wu et al., 2014a; Constantinides and Bendelac, 2013; Watarai et al., 2012).

2. Roles of intracellular signaling pathways in iNKT development and function

Multiple receptors, including the iVα14TCR, co-stimulatory molecules, IL-15R, IL-7R, and intracellularly located Vitamin D receptor (VDR) transduce signals that are important for iNKT cell development and/or function. Engagement of the iVα14TCR triggers the activation of proximal tyrosine kinases Lck and Zap70 and subsequently activates phospholipase Cγ1 (PLCγ1), which hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) to diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3) as second messengers (Godfrey et al., 2009; Gorentla and Zhong, 2012; Krishna and Zhong, 2013) (Fig. 2). Diacylglycerol (DAG) is an essential second messenger downstream of the TCR that activates several signaling pathways. The membrane-bound DAG induces activation of the Ras guanyl nucleotide-releasing protein 1 (RasGRP1)-Ras-extracellular signal regulated kinase 1/2 (Erk1/2) pathways, which is critical for proper iNKT cell development (Shen et al., 2011a; Hu et al., 2011). IP_3 induces calcium release from the endoplasmic reticulum and subsequent extracellular calcium influx into the cytosol, leading to calcineurin-mediated dephosphorylation and nuclear translocation of nuclear factor of activated T cells (NFAT). NFAT induces maximal gene expression of both early growth response 1 and 2 (Egr1 and 2). This pathway is critical for early iNKT cell development (Lazarevic et al., 2009; Li et al., 2012). Additionally, Egr2 directly binds to the *Zbtb16* promoter and activates PLZF expression (Lazarevic et al., 2009; Seiler et al., 2012). PLZF-deficient iNKT cells in mice show developmental blockage at stage 1 and fail to differentiate to cytokine-producing cells, highlighting the importance of this molecule for iNKT cells to acquire effector function (Kovalovsky et al., 2008; Savage et al., 2008).

Signaling through the signaling lymphocytic-activation molecule (SLAM) family is also required for early iNKT cell maturation. Homotypic interactions of SLAM molecules such as SLAMSF1 and SLAMSF6 on iNKT cells and thymocytes activate the downstream SLAM adaptor protein (SAP)-FynT pathway, which is critical for iNKT cell development and function in both human and mice (Chung et al., 2005; Nichols et al., 2005; Pasquier et al., 2005; Griewank et al., 2007). The SLAM-SAP-FynT pathway, together with DAG, activates NF-κB signaling cascade via protein kinase θ (PKCθ) and the Bcl10 adaptor protein. The PKCθ-Bcl10-IKK-NFκB pathway plays essential roles in the ontogeny of functional iNKT cells at least in part by increasing expression of anti-apoptotic proteins such as Bcl-xL (Stanic et al., 2004a, 2004b; Schmidt-Supprian et al., 2004; Sivakumar et al., 2003). Interestingly, although CARMA1 and Malt1 (mucosa-associated lymphoid tissue lymphoma translocation protein 1) are crucial for TCR induced NFκB activation, they are dispensable for iNKT cell development or survival (Medoff et al., 2009), suggesting that SLAM-SAP-FynT axis activates NFκB via PKCθ-Bcl10 but bypassing CARMA1 and Malt1 to promote iNKT cell development.

Homeostasis and terminal differentiation of iNKT cells are highly dependent on IL-15R signal, which induces the expression of pro-survival molecules Bcl-xl and Bcl-2 and T-bet. Mice deficient of either IL-15 or IL-15R display iNKT cell terminal maturation defect and have severely decreased stage 3 iNKT cells (Matsuda et al., 2000; Ranson et al., 2003; Townsend et al., 2004; Gordy et al., 2011). T-bet directly induces CD122 (IL-15Rβ) transcription and subsequently promotes iNKT cell survival (Lazarevic et al., 2013). T-bet deficiency also results in defective terminal maturation of iNKT cells (Townsend et al., 2004).

Vitamin D binds to the intracellular VDR, a member of the steroid thyroid super family of nuclear receptors (Cantorna et al., 2012). VDR signals to regulate T cell responses, but not T cell development. TCR induced PLCγ1 expression is dependent on Vitamin D and VDR, which is critical for T cell activation (von Essen et al., 2010). VDR deficient mice display normal T cell development but have diminished iNKT numbers in thymus and periphery. VDR deficient iNKT cells display defective terminal maturation as observed in T-bet deficient mice. Intriguingly, VDR deficient iNKT cells express normal levels of CD122 even though lack of T-bet expression (Yu and Cantorna, 2008). The exact mechanisms by which VDR control iNKT development and function remain unclear.

Finally, IL-7 regulates T cell homeostasis by enhancing survival and proliferation of naive and memory T cells. Similarly, it has been documented that IL-7 also play roles in the expansion and/or survival of iNKT cells (Matsuda et al., 2002). A recent report demonstrated that the survival requirements are distinct among effector NKT subsets. Tissue derived iNKT-17 cells are maintained in the absence of IL-15. However, in the absence of IL-7, their survival has been dramatically impaired compared to conventional iNKT cells. This strict dependence on IL-7 does not affect intracellular STAT or TCR signaling pathways, but significantly modulates the PI3K/Akt/mTOR pathway, suggesting that IL-7 controls tissue