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Review Article

Out-sourcing for *Trans*-presentation: Assessing T Cell Intrinsic and Extrinsic IL-15 Expression with *Il15* Gene Reporter Mice

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Conflict of Interest
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Abbreviations
BAC, bacterial artificial chromosome; CFP, cyan fluorescent protein; DC, dendritic cell; EGFP, enhanced green fluorescent protein; EmGFP, emerald green fluorescent protein; IEL, intraepithelial lymphocyte; IL-2Rβ, IL-2 receptor β-chain; *i*NKT, invariant natural killer T; LSP, long signal peptide; NK, natural killer; SSP, short signal peptide; STAT, signal transducer and activator of transcription; γc, common γ-chain

ABSTRACT

IL-15 is a cytokine of the common γ-chain family that is critical for natural killer (NK), invariant natural killer T (*i*NKT), and CD8 memory T cell development and homeostasis. The role of IL-15 in regulating effector T cell subsets, however, remains incompletely understood. IL-15 is mostly expressed by stromal cells, myeloid cells, and dendritic cells (DCs). Whether T cells themselves can express IL-15, and if so, whether such T cell-derived IL-15 could play an autocrine role in T cells are interesting questions that were previously addressed but answered with mixed results. Recently, three independent studies described the generation of IL-15 reporter mice which facilitated the identification of IL-15-producing cells and helped to clarify the role of IL-15 both *in vitro* and *in vivo*. Here, we review the findings of these studies and place them in context of recent reports that examined T cell-intrinsic IL-15 expression during CD4 effector T cell differentiation.

Keywords: Cytokine receptor; Inflammation; Th17 cells; Cytokine signaling

INTRODUCTION

IL-15 was discovered in 1994 as a cytokine that induced IL-2-independent T cell proliferation but in an IL-2 receptor β-chain (IL-2Rβ)-dependent manner (1-4). Cloning and characterization of IL-15 revealed striking structural and functional similarities to IL-2, that include shared usage of the common γ-chain (γc) cytokine receptor, the utilization of identical downstream signaling pathways, and exertion of similar effector functions (1,3,5,6). Moreover, both IL-2 and IL-15 signaling induce phosphorylation of signal transducer and activator of transcription (STAT) 5 and Akt (6-9), upregulate expression of anti-apoptotic factors, such as Bcl-2 and Mcl-1, and promote metabolic activities in signaled cells (10,11). On the other hand, IL-2 and IL-15 can also trigger distinct downstream signaling effects that are specific to each cytokine. For example, IL-2, but not IL-15, induces differentiation of Foxp3$^+$ regulatory T cells (Tregs) (12), whereas IL-15, but not IL-2, signal CD8 lineage specification in the thymus (13,14). Both IL-2 and IL-15 utilize the same cytokine receptors, namely IL-2Rβ and γc, for ligand binding and signaling (6), but how target cells discriminate IL-2 versus IL-15 signaling is unclear (15). To date, the molecular basis for such IL-2 versus IL-15-specific effects remains incompletely understood.

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Author Contributions

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Multiple hypotheses have been put forward to explain why IL-15 signaling results in different cellular outcome compared to IL-2, and why some cells depend on IL-15, but not on IL-2, and *vice versa*. Among others, they include the proposition that differences in the strength or kinetic of IL-2 versus IL-15 signaling can be translated into distinct qualitative or quantitative signals that activate different genes (6,16,17). These differences could be imposed by the distinct usage and contribution of cytokine proprietary receptors, such as the IL-2-specific IL-2Rα and the IL-15-specific IL-15Rα chain (15). Additionally, expression and availability of the ligand can affect cytokine receptor signaling, because the anatomical sites or cellular sources of IL-2 and IL-15 expression differ. IL-2 is primarily expressed by T cells themselves, so that T cells can supply their own IL-2 in any tissue they would migrate into or reside (18). IL-15, on the other hand, is thought to be produced mostly by non-T cells, such as dendritic cells (DCs), macrophages, epithelial cells and skeletal muscle cells (19). If so, IL-15 signaling in T cells would be limited to sites and organs where such IL-15 producers are present.

Additionally, IL-2 signaling upregulates expression of both IL-2Rα and IL-2Rβ, so that IL-2 stimulation results in amplification of IL-2 responsiveness in both autocrine and paracrine manners (20). Whether this is the case for IL-15, however, is not clear. IL-15 stimulation does not induce upregulation of IL-15Rα expression on signaled cells, but when expressed on T cells, IL-15Rα reportedly can contribute to both IL-15 binding and signaling *in cis* (21,22). Moreover, there are reports of cell-intrinsic IL-15 expression in activated CD4 T cells, which was proposed to constrain pathogenic Th17 cell differentiation (23). Curiously, such an effect was found to depend on the *in vivo* tissue environment, and the mechanism underlying this observation has not been fully mapped (23). Also, recent studies with IL-15 reporter mice did not agree with a T cell-derived IL-15 expression (24,25), so that an autocrine role of IL-15 remains questionable. It is thus important to understand the tissue and cellular origin of IL-15 expression, and specifically to re-examine T cells as a potential source of IL-15 production to obtain a complete picture of IL-15's role in T cell differentiation and homeostasis. Recently, three independent groups have generated a series of *Il15* gene reporter mice that helped visualizing the source of *in vivo* IL-15, and thus clarified pending questions on T-cell intrinsic IL-15 and its potential effects on effector T cells. This review aims to summarize and compare the findings of the three IL-15 reporter mice, and to discuss them in the context of recent literature on IL-15's contribution to T cell activation and differentiation.

EXPRESSION AND *TRANS*-PRESENTATION OF IL-15

Ablation or overexpression of IL-15 in animal models has helped to demonstrate the significance of IL-15 *in vivo*. Deficiency in IL-15 results in impaired development of natural killer (NK), invariant natural killer T (*i*NKT), CD8αα intraepithelial T cells and memory CD8 T cells (26). In contrast, overexpression of IL-15 leads to lymphoproliferation and expansion of NK and memory CD8 T cells (27,28). While these reports revealed the identity of cells that require IL-15, identifying the cells that express and supply IL-15 *in vivo* has been difficult. IL-15 is only expressed at low levels *in vivo*, and the complex nature of IL-15 production and secretion made it even more difficult to locate the cellular source of IL-15 using conventional methods.

IL-15 is unique in that it can utilize its proprietary receptor to localize to the cell surface and becomes a membrane-bound molecule that signals target cells in a cell contact dependent manner (29,30). Nascent IL-15 presumably binds to IL-15Rα in the endoplasmic reticulum followed by transport to the cell surface of IL-15-producing cells (29). Surface IL-15/IL-15Rα

complexes then interact with IL-2Rβ/γc receptor on the responder cell *in trans*, a process which is referred to as *trans*-presentation (29). *In vivo* analysis of IL-15 *trans*-presentation mechanisms indicated that IL-15 and IL-15Rα must be synthesized in the same cells for IL-15 to be *trans*-presented (31,32). Accordingly, CD8 T cells and NK cells failed to develop when *Il15ra*$^{-/-}$ mice were reconstituted with either *Il15*$^{-/-}$ donor bone marrow alone or with a mixture of *Il15*$^{-/-}$ and *Il15ra*$^{-/-}$ bone marrow cells (31,32). Mechanistically, these results were explained as the failure of IL-15 *trans*-presentation when IL-15 and IL-15Rα are not produced by the same cell. Notably, IL-15-deficiency did not inhibit IL-15Rα surface expression (31,32), indicating that the formation of IL-15/IL-15Rα complexes are necessary for IL-15 expression and signaling, but not for IL-15Rα expression itself. Thus, detection of surface IL-15Rα does not necessarily indicate that these cells would also express IL-15. In the same vein, IL-15Rα expression alone is not sufficient to indicate functional IL-15 expression.

In this regard, it is curious that IL-15Rα expression is not a requirement for productive IL-15 signaling *in vitro* conditions. In fact, recombinant IL-15 alone is sufficient to induce downstream STAT5 phosphorylation on cells that express IL-2Rβ/γc even without *trans*-presentation (23,25,33). Also, overexpression of IL-15 alone was sufficient to promote NK cell, CD8 memory, and γδ T cell generation that was associated with an increase in circulating soluble IL-15 (28,34,35). Thus, a role of soluble IL-15 proteins that does not require IL-15Rα still needs to be considered. Altogether, mapping the source of IL-15 expression is integral to understanding the regulatory mechanisms of IL-15 signaling. However, the scarcity of its expression and difficulties in detecting IL-15 in tissues required the development of new tools, which are now available in the form of IL-15 reporter mice.

GENERATION AND CHARACTERIZATION OF IL-15 REPORTER MICE

The *Il15* gene is located in a 34-kb region on chromosome 4q31 in humans and on chromosome 8 in mice (36). In mice, the *Il15* gene comprises 8 exons and 7 introns, wherein the mature IL-15 protein is encoded in exons 5–8. Depending on the tissue origin and the activation status of IL-15-producing cells, the mature IL-15 protein is generated either from a precursor protein that has a 48 amino acids (aa) long signal peptide (LSP) or a 21 aa short signal peptide (SSP). Both precursor proteins are produced from the same pre-mRNA, but through alternative mRNA splicing (37). Because the LSP impairs intracellular trafficking and secretion of IL-15 proteins, distinct utilization of long or short signal peptides controls the efficiency of mature IL-15 protein production (37,38). Therefore, alternative mRNA splicing provides an additional layer of controlling IL-15 expression. Notably, both LSP and SSP transcripts contain both exon 3 and 4, but the SSP isoform contains an additional exon 4A which harbors the translational start site for the SSP. Consequently, expression of exon 4A is specific to SSP, but exons 3 and 4 are common to both the LSP and SSP isoforms. It would be interesting to examine how the expression of LSP versus SSP transcripts differs between distinct IL-15 producing cells or between activation and differentiation. Unfortunately, the IL-15 reporter mice that are currently available cannot distinguish between these splice isoforms.

The first IL-15 reporter mouse was reported in 2012 by Lefrancois's group (**Table 1**) (39). In these animals, a bacterial artificial chromosome (BAC) reporter construct was engineered to express an emerald green fluorescent protein (EmGFP) under the control of *Il15* regulatory

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Table 1. IL-15 reporter mice

Methodology	Origin of clone	Source	Reporter gene	Detection	References
IL-15 BAC transgene	RP24-275P1	NA	EmGFP	Direct fluorescence	Colpitts et al. (39)
IL-15 BAC transgene	RP23-331F16	Children's Hospital Oakland Research Institute	EGFP (via 2A peptide)	Direct fluorescence	Sosinowski et al. (40)
Reporter knock-in	NA	NA	CFP	Anti-GFP antibody	Cui et al. (44)

NA, not available.

elements by inserting EmGFP into exon 3 of the *Il15* gene. To ensure that all regulatory elements were preserved, the BAC construct contained the entire *Il15* gene, including 42 kb of upstream genomic sequences. The construct was further designed so that the EmGFP insertion disrupted the IL-15 translational start site in exon 3. Consequently, no functional IL-15 protein is produced from the BAC transgene. Utilizing this reporter mouse, the authors reported that IL-15 reporter activity was distinct among different DC populations, and that CD8α$^+$ DCs contained the highest level of IL-15 reporter expression. This study also documented that IL-15 reporter activity was upregulated upon viral infection in DCs and monocytes, a process that is dependent on interferon (IFN)-α receptor expression (39). Thus, these reporter mice revealed previously unappreciated regulatory pathways of IL-15 expression during viral infection and a role for type I IFN signaling (39).

In an alternative approach, Kedl's group (40) generated a BAC transgene (IL-15TE), where an enhanced green fluorescent protein (EGFP) reporter was linked downstream to the IL-15 coding region via a *Thosea asigna* 2A peptide sequence (**Table 1**). The self-cleaving 2A peptide permits expression of two independent proteins, in this case IL-15 and EGFP, from a single open reading frame (41-43). To achieve this, exon 8 of the BAC *Il15* gene was modified to destroy the *Il15* stop codon and to include an 2A peptide sequence followed by EGFP and a stop codon (40). Because the IL-15 coding region remains intact, this reporter construct also overexpresses IL-15. Consequently, this engineered mouse is both an IL-15 transgene and an IL-15 reporter. In their original study, however, the effect of IL-15 overexpression on lymphocyte homeostasis was not addressed. Instead, the primary aim of this reporter mouse was to identify the peripheral source of IL-15 that would induce generation of virtual memory (VM) CD8 T cells (40). Reporter protein expression revealed that CD8α$^+$ and CD103$^+$ DCs were among the highest expressers of the IL-15 reporter, and thus they concluded that IL-15 production from CD8α$^+$ DCs was associated with the generation of VM CD8 T cells.

More recently, Ikuta's group (44) generated an IL-15 reporter mouse using gene knock-in technology (**Table 1**). By directly inserting the reporter construct into the *Il15* gene, all endogenous regulatory elements are preserved, and epigenetic control mechanisms remain intact. Thus, this mouse model increases the likelihood of identifying all sources of IL-15 *in vivo*. Of note, knocking-in the reporter construct into the *Il15* gene disrupted IL-15 expression from the same *Il15* allele. Consequently, the IL-15 reporter allele is defective in IL-15 expression and only reports *Il15* gene transcription. Here, IL-15 transcriptional activity was reported by enhanced cyan fluorescent protein (CFP), which was inserted into the ATG codon located in exon 3 of the *Il15* gene. Using anti-GFP antibodies and immunohistochemistry, the authors then visualized reporter protein expression in various tissues and provided new clues as to how IL-15 is expressed *in situ* in both primary and secondary lymphoid organs. Most prominently, medullary epithelial cells in the thymus and C-X-C motif chemokine ligand (CXCL) 12-abundant reticular (CAR) cells in the bone marrow (45) turned out to be major producers of IL-15 (44). Additionally, IL-15 reporter activity was found in blood, but not in lymphatic endothelial cells, demonstrating the importance of stromal components in IL-15 production and its regulation.

Collectively, these new strains of IL-15 reporter mice enrich the tool kit to assess IL-15 expression in immune cells and tissues, and thus allow us to gain new insights on the role of IL-15-producing cells in controlling both adaptive and innate immunity.

IL-15 EXPRESSION IN NON-LYMPHOID STROMAL AND EPITHELIAL CELLS

T cell development and homeostasis depend on cytokines and major histocompatibility complex (MHC) molecules in the tissue microenvironment, which contains non-lymphoid stromal cells and epithelial cells. Depending on the anatomical site, different cytokines are expressed. Among these, IL-15 was found to be quite selective in its tissue distribution.

In the thymus, IL-15 expression contributes to the development and cytotoxic features of CD8 T cells (13,14), and it is also critical for *i*NKT cell development and differentiation (46-48). Based on studies of thymic *i*NKT cell development, it was assumed that medullary epithelial cells would be the source of intrathymic IL-15, since the medulla is the site of final maturation of *i*NKT cells which requires IL-15 (49,50). In fact, *Relb*-deficient mice which lack the thymic medulla also fail to generate *i*NKT cells. Interestingly, *i*NKT cell development can be restored in *Relb*$^{-/-}$ mice by injection of soluble IL-15/IL-15Rα complexes, indicating that the major function of medullary epithelial cells in *i*NKT cell development is the supply of IL-15 (51).

The compartmentalization of thymic IL-15 expression was directly visualized by Ikuta's group (44), where they found IL-15 reporter expression to be clustered in confined areas of the thymus. Specifically, IL-15 reporter activity was primarily observed in MHC II^{high} medullary thymic epithelial cells (TECs), but not in cortical TECs (44). These results demonstrated that IL-15 expression is highly regulated, and that it correlates with the function of specific anatomical niches within tissues.

Unlike IL-15 expression in the thymus, IL-15 expression in epithelial and stromal cells of other organs is not clearly defined. The gut is another major organ that expresses IL-15 to drive the differentiation of T cells. Specifically, the generation and maintenance of small intestine CD8αα^{+} intraepithelial lymphocytes (IELs) requires IL-15, so that IL-15- or IL-15Rα-deficient mice lack these cells (26,52,53). While the precursors of CD8αα^{+} IELs develop in the thymus, it is also established that an extrathymic source of IL-15 drives their differentiation and survival (54,55). Notably, removing IL-15Rα from intestinal epithelial cells resulted in a dramatic decrease in CD8αα^{+} IEL numbers, indicating that intestinal epithelial cells are a non-redundant source of IL-15 for CD8αα^{+} IELs (55). Along these lines, restricting IL-15Rα expression to epithelial cells of the intestine was sufficient to restore CD8αα^{+} IEL numbers in *Il15ra*$^{-/-}$ mice (55), suggesting that IL-15 expression and *trans*-presentation by gut epithelial cells is not only necessary but also sufficient for CD8αα^{+} IELs differentiation. Together with previous reports that identified IL-15 mRNA transcripts in gut epithelial cells (56,57), these results documented intestinal epithelial cells as a major source of IL-15 *in vivo*.

Thus, it was surprising when no major IL-15 reporter activity was detected in the intestine, but only found in a few select intestinal epithelial cells (44). These findings indicated that IL-15 expression is not common to all gut epithelial cells, but presumably a feature associated with a specific subset of intestinal cells. It remains an important issue to identify these IL-15-producing epithelial cells. The availability of IL-15 reporter mice makes this task feasible

as they can provide single cell resolution of IL-15-expressing cells in tissues. Initial analysis indicated that endothelial cells in the gut lamina propria were robust expressers of IL-15 reporter proteins (44). However, it is unclear whether these cells are the direct suppliers of IL-15 to CD8αα$^+$ IELs or whether they only secrete IL-15 that could be picked up by IL-15Rα expressing cells for *trans*-presentation to IELs. Of note, DCs or other hematopoietic origin cells that are in the IEL compartment are not likely involved in IL-15 *trans*-presentation, because IL-15Rα expression in hematopoietic cells was found to be obsolete for CD8αα$^+$ IEL generation (58). Therefore, the expression and *trans*-presentation of IL-15 in the gut seems to be mediated by a small population of epithelial cells that suffice to provide the cytokine signals for IL-15-dependent T cell differentiation.

Because the current literature identifies the gut as a major site of IL-15-expression, the scarcity of IL-15 producers in the gut was unexpected. Nonetheless, these results reconcile previous observations where IL-15 overexpression in intestinal epithelial cells led to severe inflammation and autoimmunity in the small intestine (59). Altogether, these data suggest that IL-15 expression in stromal cells is tightly regulated, and that dysregulation or ectopic expression of IL-15 in specific cell types can be deleterious.

IL-15 EXPRESSION BY DENDRITIC CELLS

DCs play an integral part in controlling the adaptive immune response. Among others, DCs activate T cells and direct their cellular differentiation by secreting cytokines and chemokines, a role that is in part linked to their production of IL-15 (60,61). The importance of DC-mediated IL-15 *trans*-presentation is illustrated in defects of NK and memory CD8 T cell differentiation and survival, when IL-15Rα was specifically deleted in DCs by CD11c-Cre recombinases in IL-15Rα floxed mice (62). A similar effect was observed for antigen-specific response to ovalbumin, where CD11c$^+$-specific deletion of IL-15Rα resulted in a reduction of ovalbumin-specific memory T cell generation (62). In further support of a role for DCs to supply IL-15 *in vivo*, constraining IL-15Rα expression to DCs was sufficient to support homeostasis of both NK and memory CD8 T cells (49,63,64). These studies illustrate that DCs play critical roles in maintaining the IL-15 niche, and they further highlight DCs as an essential source of IL-15, not only under steady state conditions, but also during immune activation.

Given the heterogeneity of DCs, however, it has not been clear whether IL-15 expression is a common feature to all DC subpopulations or limited to specific DC subsets. DCs are generally subdivided into plasmacytoid dendritic cell (pDC) and classical dendritic cell (cDC) (65). The cDC subpopulation can be further divided into CD8α$^-$ and CD8α$^+$ cells, with the latter playing a critical role in antigen cross-presentation (66). A previous study reported low level IL-15 mRNA expression in both splenic CD8α$^-$ and CD8α$^+$ DCs (67), which was confirmed by cell surface staining with anti-IL-15 antibodies on both DC subsets (64). However, single cell analysis of IL-15 reporter mice indicated that IL-15 expression may not be uniform, but may differ among DC subpopulations. In this regard, both the Lefrancois's and the Kedl's groups (39,40) found that IL-15 reporter activity was highly induced in splenic CD8α$^+$ DCs, but minimally expressed in the CD11b$^+$ and the CD8α$^-$ DC subsets. Notably, pDCs also lacked IL-15 reporter expression (24,44), suggesting that IL-15 expression is not a common feature for all DCs but limited to CD8α$^+$ DCs. Altogether, these data revealed that IL-15 expression is distinct across DC populations, and they also put forward the question

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whether such characteristic would be developmentally set or can be altered depending on their activation or maturation.

Pro-inflammatory agents, such as poly I:C and LPS, were found to upregulate IL-15 mRNA expression in DCs so that inflammatory signals were considered IL-15 inducers for all CD11c$^+$ DCs (67). Contrary to such views, vesicular stomatitis virus infection (39) or LPS injection only upregulated IL-15 reporter activity in CD8α$^+$ DCs (44), and IL-15 reporter expression was minimally increased in CD8α$^-$ DCs (39,44). These findings favor a model where IL-15 expression can be induced by pro-inflammatory signals but remain limited to the CD8α$^+$ DC subset, and the ability to produce IL-15 is lost in CD8α$^-$ DCs. In fact, adoptive transfer studies of pre-DCs, which are the precursors of both CD8α$^-$ and CD8α$^+$ DCs, showed that IL-15 expression is developmentally controlled. Pre-DCs actively expressed IL-15 reporter proteins, but only the CD8α$^+$ DC progenies remained IL-15 reporter positive and CD8α$^-$ DCs had terminated the expression. These findings suggested that IL-15 transcription is actively silenced in the latter population (24). Collectively, the use of IL-15 reporter mice revealed the surprising finding of a developmentally and subset specifically controlled pathway of IL-15 expression, reaffirming the complex regulatory mechanisms of IL-15 expression in immune cells.

IL-15 EXPRESSION BY T CELLS

Because T cells are major targets of IL-15, there is a keen interest to understand whether T cells themselves can produce IL-15. Most cytokines of the γc family are expressed by T cells. For example, IL-2, which is the prototypic cytokine of the γc cytokine family, is a major cytokine produced by T cells (20). However, whether IL-15 is expressed by T cells has remained controversial. Autocrine IL-15 signaling would constitute a significant benefit for T cells, because IL-15 upregulates expression of anti-apoptotic molecule in T cells (68,69), and would thus provide survival benefits to T cells independently of their tissue environment. However, uncontrolled and unlimited access to survival cytokines would complicate the homeostatic control of the size and diversity of the peripheral T cell pool, and could be detrimental for maintaining an immunocompetent T cell repertoire (10,70,71).

Evidence supporting autocrine IL-15 expression in T cells has been previously put forward by studies that detected IL-15 mRNA transcripts in both human and mouse T cells (23,68,72). IL-15 proteins were also detected in freshly isolated human CD3$^+$ cells by intracellular staining (61). In fact, it was even proposed that explanted human T cells could survive and proliferate by an autocrine loop of IL-15 expression and signaling *in vitro* (73). Contrary to this idea, however, there is also a large body of evidence that T cells fail to produce their own IL-15. In this regard, IL-15 proteins were not found in T cell supernatants, and they were not detected as membrane-bound forms on T cells either (60,68). Collectively, these studies suggested that IL-15 may be produced from T cells, but even if so that it would be limited to specific circumstances or that it is expressed at too low levels to be clearly discernible.

In an alternative approach, recent studies re-addressed the question of T cell-intrinsic IL-15 expression in functional assays. Interestingly, some of these studies reported that IL-15-deficient T cells displayed distinct effector functions compared to wildtype control T cells. In brief, exogenous IL-2 suppresses the generation of IL-17-producing Th17 cells *in vitro* through a STAT5-dependent mechanism (74), and recombinant IL-15 reportedly had the same effect on activated CD4 T cells (23). Notably, when IL-15-deficient CD4 T cells were induced to

differentiate under Th17 skewing conditions, *Il15*$^{-/-}$ naïve CD4 T cells were significantly more effective in differentiating into IL-17-producing cells than wildtype CD4 T cells. These results implied that activated wildtype CD4 T cells normally do express IL-15, and that T cell-derived IL-15 would dampen the differentiation of Th17 cells (23).

Because IL-15 expression is not static but dynamically regulated by activation and inflammatory signals (75), we recently revisited the question of T cell-intrinsic IL-15 expression, with the aim to delineate the contribution of autocrine IL-15 to CD4 effector T cell differentiation (11). Surprisingly, we failed to detect meaningful amounts of IL-15 mRNA in either resting CD4 T cells or *in vitro* generated effector T cells, including Th1 and Th17 cells (25). Because our results were discrepant from previous reports, we made use of the IL-15 EmGFP reporter mice to assess IL-15 expression at single cell level (39). We confirmed robust IL-15 reporter expression in a series of non-T cells, such as NK, *i*NKT and CD11c^{+} DC, as previously reported (24). Among freshly isolated T cells, we observed clearly discernible IL-15 reporter protein expression in a subset of CD8 T cells. The identity of the IL-15 reporter expressing CD8 T cells remains unclear, but it was not associated with their memory/activation status as determined by CD44, IL-2Rβ expression (25). Importantly, we did not detect IL-15 reporter activities in resting CD4 T cells, and did not find IL-15 reporter proteins in CD4 effector T cells either. Thus, in contrast to some previous studies (23,76), we did not find evidence for T cell-intrinsic IL-15 expression in CD4 T cells. Moreover, we failed to observe any significant differences in Th17 cell generation between *Il15*$^{-/-}$ and wildtype CD4 T cells (25). Because we also did not detect IL-15Rα expression on Th17 cells (25), we considered it unlikely that T cell-derived IL-15 could be *trans*-presented to other CD4 T cells. Thus, these results do not favor a T cell-autocrine effect of IL-15 in effector T cell generation and differentiation (**Fig. 1**).

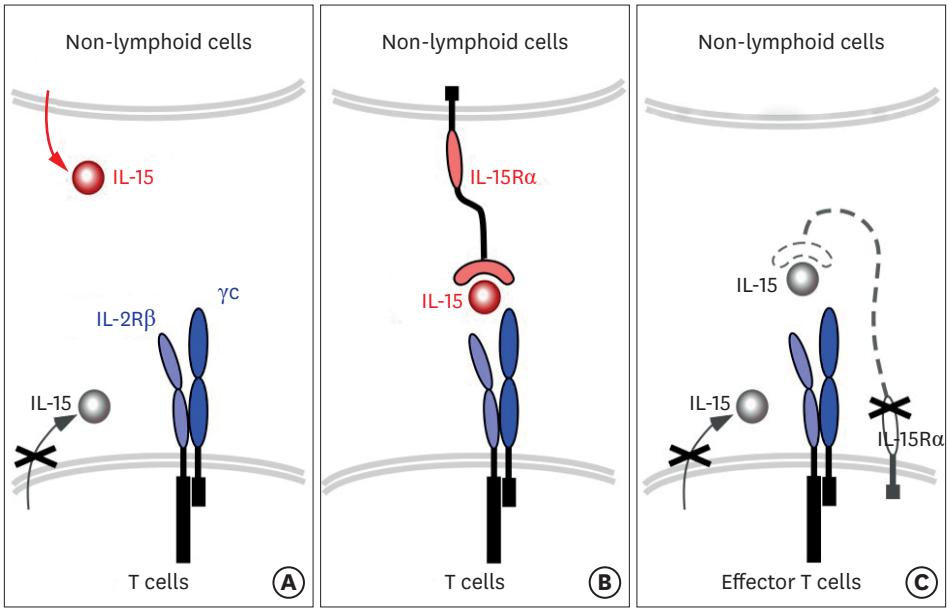

Figure 1. IL-15 expression and *trans*-presentation. (A) IL-15 is mostly expressed by non-lymphoid cells, such as stromal cells, myeloid cells, and DCs. T cells, on the other hand, are an unlikely source of IL-15. (B) IL-15 is *trans*-presented by IL-15Rα and signals through the IL-2Rβ/γc heterodimeric cytokine receptor that is expressed on T cells. (C) IL-15 signaling in T cells depend on IL-15 that is expressed and presented *in trans*, because effector T cells do not express IL-15 and cannot signal *in cis*.

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It is currently not clear to us why experiments using the same IL-15-deficient CD4 T cells would show different efficiencies of Th17 cell differentiation (23,25). As a potential explanation, we consider that *Il15*$^{-/-}$ CD4 T cells are generated and maintained in an IL-15-deficient environment that lack pro-inflammatory NK and *i*NKT cells (26,48,77). Consequently, the activation status and phenotype of IL-15-deficient naïve CD4 T cells might differ from those of wildtype naïve CD4 T cells depending on the housing environment of *Il15*$^{-/-}$ mice. Additionally, any difference in the sorting strategy or the purity of sorted naïve CD4 T cells could result in variable experimental outcomes. Differences in the microbiota of the mouse colony cannot be excluded either. Nonetheless, the lack of IL-15 reporter expression in effector T cell is more in line with the current view that IL-15 signaling is primarily, if not exclusively, triggered by IL-15 *trans*-presentation, and that T cells are not a major source of IL-15.

In fact, the ability to produce IL-15 is presumably lost during early T lineage commitment in thymus. Early lymphoid progenitor cells in the thymus express high levels of IL-15 reporter proteins, which is downregulated upon DN2 to DN3 thymocyte progression and then completely lost in DP thymocytes (24). *In vitro* OP9-DL1 culture of bone marrow stem cells from IL-15 reporter mice further demonstrated a role for Notch signaling in terminating IL-15 expression, thus linking T lineage fate decision to loss of IL-15 expression. Altogether, these results suggest that commitment into T lineage cells renders them dependent on exogenous IL-15, so that IL-15 availability would control T cell homeostasis and effector function by T cell-extrinsic regulatory mechanisms.

CONCLUSION AND PERSPECTIVE

While the full regulatory mechanisms of IL-15 expression remain to be uncovered, IL-15 reporter analysis have provided insights into the identity of cells that actively transcribe IL-15, and thus supplied new clues as to how the IL-15 niche is shaped *in vivo*. Notably, IL-15 reporter mice revealed discrepancies to cells and tissues that were previously proposed to express IL-15 mRNA or protein, and further showed that IL-15 reporter activity is limited to the CD8α^{+} subset among DCs (39,40). Also, there is a curious paucity of IL-15 reporter expressing cells in the intestinal epithelium which was proposed to be a IL-15-rich environment (44,55). Importantly, IL-15 reporter expression also conclusively demonstrated that activated CD4 T cells fail to transcribe IL-15, thus excluding an autocrine role for IL-15 in CD4 effector T cell differentiation (25). While these reporter mice were useful to highlight the absence of IL-15 transcriptional activities in specific immune cell subsets, it also needs to be emphasized that the IL-15 reporter protein expression does not necessarily indicate IL-15 protein expression. Multiple post-transcriptional mechanisms, such alternative mRNA splicing or differential usage of signal peptides, represent additional layers of regulation for IL-15 expression (19). Therefore, tracking the actual source of a biologically active IL-15 requires further investigations and probably the generation of additional tools. Nonetheless, we anticipate that these IL-15 reporter mice will continue to provide novel insights into the biology of IL-15 and its regulatory role in T cell immunity.

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