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Regulating the Immune system via IL-15 Transpresentation

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Abstract

Transpresentation has emerged as an important mechanism mediating IL-15 responses in a subset of lymphocytes during the steady state. In transpresentation, cell surface IL-15, bound to IL-15R α is delivered to opposing lymphocytes during a cell-cell interaction. The events most dependent on IL-15 include the development and homeostasis of memory CD8 T cells, Natural Killer cells, invariant Natural Killer T cells, and intraepithelial lymphocytes. As lymphocyte development and homeostasis involve multiple steps and mechanisms, IL-15 transpresentation can have diverse roles throughout. Moreover, distinct stages of lymphocyte differentiation require IL-15 transpresented by different cells, which include both hematopoietic and nonhematopoietic cell types. Herein, we will describe the points where IL-15 transpresentation impacts these processes, the specific cells thought to drive IL-15 responses, as well as their role in the course of development and homeostasis.

Keywords

IL-15; IL-15 Receptors; memory CD8 T cells; NK cells; invariant NKT cells; dendritic cells; macrophages; homeostasis; lymphocyte development; transpresentation; cis-presentation; cytokine receptor complexes

1. Introduction

Interleukin 15 (IL-15) is a member of the four α -helix bundle cytokine family, initially described as a T cell growth factor [1, 2]. IL-15 shares many properties with IL-2 [3], including the receptors IL-2/15R β and the common γ chain (γ C) through which both cytokines signal [2, 4]. In addition to these shared receptor components, IL-2 and IL-15 each have their own private binding receptors, IL-2R α and IL-15R α respectively, which have no or little signaling capabilities. Nonetheless, IL-2R α and IL-15R α are important for cytokine responsiveness [5, 6]. Because IL-2 and IL-15 both use IL-2/15R β and γ C, signaling induced by either cytokine is fundamentally the same, although it can vary in duration and

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intensity. Despite these similarities, IL-15 has *in vivo* functions distinct from IL-2, which is largely due to the unique manner in which it uses its IL-15R α receptor.

The most important and well-established functions of IL-15 occur during homeostasis. IL-15 is required for the development and homeostasis of memory CD8 T cells, natural killer (NK) cells, invariant NKT (iNKT) cells, and a subset of intestinal intraepithelial lymphocytes (IEL) as highlighted by their significant deficiency in IL-15-deficient mice[7]. Immune responses in mice deficient in IL-15R α closely resemble those in mice lacking IL-15, revealing that the physiological functions of IL-15 and IL-15R α are intimately linked [8]. Like IL-15, IL-2 acts during the steady state but instead is required in the development and homeostasis of Foxp3+CD4+ regulatory T cells (Tregs)[9]. Tregs are unique among lymphocytes in their constitutive expression of IL-2R α , making them exquisitely IL-2-responsive at steady state. Whereas the roles of IL-15 at homeostasis are clear, its functions during immune activation are not. IL-15 and IL-15R α expression are often upregulated during numerous types of infections, immune activation, and inflammatory diseases[10–15]; however, the importance of IL-15 during early stages of immune activation is often minor or not well described. This is in contrast to IL-2, which promotes T cell proliferation during T cell activation and programs later T cell responsiveness and immunity [16, 17]. Transient upregulation of IL-2R α and IL-2 by T cells upon activation is largely responsible for limiting the activity of IL-2 to the earliest stages of an immune response. Overall, IL-15 and IL-2 regulate the development and homeostasis of different lymphocytes and have different roles during immune activation.

The explanation for how IL-15 mediates such a specific set of responses lies with the unique characteristics of interaction between IL-15 and its receptors. In the absence of any other receptor subunits, IL-15R α has a very high affinity for IL-15 (1.4×10^{-11} M) distinguishing it from IL-2R α which needs to combine with IL-2R β / γ_c to mediate high affinity binding for IL-2[6, 18]. This high affinity along with the co-expression of IL-15R α and IL-15 in the same cell, allow intracellular binding of IL-15 to IL-15R α , which is then shuttled to the cell surface as a complex. Once on the cell surface, the IL-15R α /IL-15 complex can stimulate IL-2R β / γ_c in an opposing cell during a cell-cell interaction [19]. This mechanism of cytokine delivery has been called transpresentation[19]. Since the first description of transpresentation, a number of studies have provided evidence that transpresentation is the major mechanism mediating IL-15 responses during steady state conditions, such as development and homeostasis of memory CD8 T cells, NK cells, and iNKT cells [20–25]. Therefore, this review will primarily focus on the events where a role for IL-15 transpresentation is well established. Within this discussion, we will describe the cells directing these responses and the type of IL-15-mediated responses induced. Nonetheless, alternative mechanisms to transpresentation have been proposed. Therefore, since the role of other IL-15-mediated mechanisms is still being determined, these alternative mechanisms to transpresentation will be briefly discussed.

2. Mechanisms mediating IL-15 responses

Since the discovery of IL-15 and IL-15R α , the theory of how IL-15 mediates responses has gradually evolved, leading to the emergence of three general mechanisms. The first mechanism is typical for most cytokines and involves soluble IL-15 binding to IL-15R α , which facilitates signaling of adjacent IL-2R β / γ_c on the same cell, similar to IL-2. More recently, this mechanism has been referred to as cis-presentation. This terminology refers to how the cytokine is delivered to the signaling chains and distinguishes itself from transpresentation, which involves cytokine delivery across a cell-cell interface. However, unlike the terms, autocrine or paracrine, cis-presentation does not infer the source of the cytokine. Transpresentation, which was briefly introduced, is the second mechanism and

accounts for both the source and the manner of cytokine delivery. A third potential mechanism has arisen after studies detected soluble IL-15R α /IL-15 complexes in biological samples; these complexes are cleaved from the surface of cells and can act as IL-15 agonists. Presently, most evidence supports transpresentation as the mechanism mediating IL-15 responses *in vivo*.

Transpresentation was first proposed in 2002 after studies found monocytic cell lines expressing cell surface IL-15 and IL-15R α could stimulate proliferation in neighboring T cells [19, 26]. Surprisingly, proliferation required T cell expression of IL-2R β / γ_c , but not IL-15R α [19]. These findings clarified numerous earlier results that were not understood, such as the observation that IL-15 is rarely detected in biological solutions. In fact, the major isoform of IL-15 lacks a signal sequence and is not secreted but associates with intracellular IL-15R α [27]. In addition, it explained why a previous study found that *IL-15 α -/-* T cells respond to IL-15 *in vivo* when transferred into a wildtype recipient [11]. Additional studies, which will be described later, provided further evidence supporting the role of transpresentation *in vivo* by determining the cellular requirements for IL-15 and IL-15R α during CD8 T cell responses, memory CD8 T cell homeostasis, NK cell development and homeostasis, and IEL development [20, 21, 28–30]. In all these cases, IL-15R α expression is not required by IL-15 responding cells but rather by other cells within the environment. Moreover, these *in vivo* responses require co-expression of IL-15 and IL-15R α by the same cells [31, 32]. In general, there is little evidence contradicting transpresentation as a valid model of IL-15 stimulation; however, it is possible there are circumstances when alternative mechanisms are used.

Although multiple studies have shown IL-15R α expression by IL-15-responding lymphocytes is not required for *in vivo* responses, other studies have found instances when IL-15R α expression by responding cells correlates to increased IL-15 responses, supporting cis-presentation as an active mechanism [33–35]. Even though these studies mostly involved the use of *in vitro* model systems and recombinant IL-15, they still demonstrated the potential of cis-presentation. Cis-presentation is feasible from multiple standpoints. For one, IL-15-dependent lymphocytes express some of the highest levels of IL-15R α despite the fact these cells do not require it [14, 22]. Therefore, IL-15R α expression may have been evolutionary preserved for cis-presentation, thus providing an explanation for the function of IL-15R α on IL-15 responding cells. Second, structural analysis by X-ray crystallography, identified a hinge region in the IL-15R α protein just proximal to the cytokine binding region, giving this region the flexibility to either present IL-15 in trans or in cis [36]. Overall, cis-presentation is a mechanism that can mediate IL-15 responses and is likely the dominant mechanism operating when lymphocytes are treated with recombinant IL-15 *in vitro*. Whether cis-presentation is active *in vivo* is not clear.

For cis-presentation to be active *in vivo*, IL-15-responding cells either need to capture free IL-15 or express IL-15, which is shuttled to the surface along with the IL-15R α . This is not feasible for CD8 T cells, NK cells, and iNKT cells as these cells do not express IL-15. It may be possible that IL-15 could come from other cell sources after being released from IL-15R α ; however, this is not likely as the dissociation rate for IL-15 from IL-15R α is very slow [6, 18]. Hence, determining whether cis-presentation is operational *in vivo* requires one to identify circumstances when IL-15 is secreted. Whereas most studies of mouse samples have failed to detect free IL-15, there are multiple reports detecting IL-15 in human serum and tissue-derived samples [37–41]. However, a very recent study, using a new ELISA, reported that all the IL-15 detected in serum of melanoma patients treated with chemotherapy was present as IL-15R α /IL-15 complexes [42]. These results suggest that the commercially-available ELISAs detecting human IL-15 may be unable to distinguish free IL-15 from complexed IL-15R α /IL-15 and opens the possibility that past studies detecting

IL-15 in similar circumstances were not actually detecting free IL-15. Nonetheless, there are a few studies reporting IL-15 in mouse serum [43, 44]; however, these studies used an IL-15 antibody that probably is unable to distinguish free IL-15 from complexed IL-15R α /IL-15 [45]. When this same murine IL-15 antibody was used in another study, it was found that induction of serum IL-15 was dependent on the mice expressing IL-15R α implying all the serum IL-15 was associated with IL-15R α [45]. Therefore, it is currently uncertain whether IL-15 is ever present solely in a soluble form; this needs to be further investigated with ELISAs capable of detecting free IL-15; caveats of ELISA detection will be discussed further below. As monotherapy with recombinant IL-15 is being attempted in immunotherapy for melanoma, cis-presentation might be operational in these therapeutic situations; however, at least in mice, responses to *in vivo* treatment with recombinant IL-15 were completely dependent on host IL-15R α expression suggesting transpresentation was being utilized [21]. In addition, *IL-15 α -/-* and wildtype T cells responded similarly to *in vivo* treatments of recombinant IL-15 [21]. Therefore, until circumstances where IL-15 is in fact secreted are definitively identified, the importance of cis-presentation *in vivo* remains uncertain.

One reason transpresentation is thought to be such an efficient form of cytokine stimulation is that IL-15 complexed to IL-15R α is approximately 100X more potent than IL-15 alone [46, 47]. Interestingly, soluble IL-15R α /IL-15 complexes have also been detected in media of cultured, TLR-stimulated murine dendritic cells (DC) and in the serum of patients with IBD [48, 49]. Moreover, TNF- α converting enzyme (TACE) has been identified as an enzyme that can cleave IL-15R α from the cell surface [50]. Therefore, generation of soluble IL-15R α /IL-15 complexes through cleavage provides another scenario whereby cells expressing IL-15 and IL-15R α can stimulate nearby lymphocytes. Once again, we are at the point where it is clear that this mechanism is feasible and in fact operates with recombinant molecules. This is fortunate because it provides some novel strategies for stimulating IL-15 responses. Nonetheless, it is still uncertain whether soluble IL-15R α /IL-15 complexes normally mediate IL-15 responses *in vivo*. Even during situations when soluble IL-15R α /IL-15 complexes are detected, Mortier et al [45] showed that membrane-bound IL-15R α /IL-15 complexes mediated NK cell activation rather than soluble IL-15R α /IL-15 complexes. Therefore, cleavage of IL-15R α /IL-15 from the cell surface could be a negative feedback mechanism to reduce the availability of transpresented IL-15. Even if *in vivo*-generated IL-15R α /IL-15 complexes induce stimulatory responses, the responses mediated by the complexes could differ from those mediated by transpresentation because they are not restricted to specific lymphocytes. At least in mice, the amount of soluble IL-15R α /IL-15 complexes present during steady state is near the level of detection suggesting this is not a major mechanism used during steady state. Considering this together with the findings that soluble IL-15R α /IL-15 complexes are produced after immune cell stimulation, we suspect that if soluble IL-15R α /IL-15 complexes do indeed mediate IL-15 responses *in vivo*, they likely function during immune activation.

The discovery that IL-15R α /IL-15 complexes are sometimes present in a soluble form begs the question of whether previous studies detecting IL-15 used methods that could distinguish IL-15 from complexed IL-15, which is dependent on the epitope(s) recognized by the antibodies used in the ELISA. If the antibody recognizes an epitope sequestered by IL-15R α binding, then IL-15 will not be detected when in fact it is present, albeit in a different form. This may have been the case for mouse IL-15 ELISA kits where complexed IL-15 is not detected by IL-15 specific Abs used in the ELISAs. Fortunately, ELISAs are now commercially available that specifically detect the mouse IL-15R α /IL-15 complexes. For analysis of human samples, the reverse may be true whereby the human IL-15-specific Abs used in ELISAs recognize an epitope that is not covered by the IL-15R α binding leading to an inability to distinguish IL-15 from complexed IL-15. Future studies need to validate the

ELISAs used to measure IL-15 and possibly reassess situations of increased IL-15 expression to identify the true form of the expressed IL-15. Once accomplished, there will be better understanding of precise circumstances that generate soluble IL-15R α /IL-15 complexes and the function of these products in the immune system.

Since evidence suggests that transpresentation is a major mechanism mediating IL-15 responses, recent studies have begun elucidating the cell types involved in this process. Unfortunately, identifying cells that transpresent IL-15 cannot be accomplished simply by detecting cell surface IL-15R α /IL-15 as expression of surface IL-15R α /IL-15 is usually negative even in cell types known to transpresent IL-15[22]. Difficulties detecting IL-15 are partially due to numerous post-transcriptional and post-translational regulatory mechanisms that keep IL-15 expression low, and possibly hidden epitopes[27, 51–53]; however, appropriate reagents have also been limited. At the level of transcription, expression of *IL-15* mRNA is much more restricted among cells than *IL-15R α* mRNA[2, 18]. Among various cell types, DCs express some of the most abundant levels of *IL-15* mRNA, which is upregulated upon Toll-like receptor stimulation[14, 54, 55]. Very recently, Lefrançois' group described IL-15 expression using an IL-15 reporter model where GFP expression is driven by the endogenous IL-15 reporter[15]. At steady state, GFP is most evident in CD8+ DCs and CD11b+ CD11c myeloid cells (i.e. monocytes and macrophages) with a lower level of GFP found in CD11b+ DCs. Therefore, DCs and macrophages are likely among the cells expressing the highest levels of IL-15 protein during steady state. To narrow down the cell types that need to express IL-15 and IL-15R α for specific IL-15 responses *in vivo*, mixed bone marrow (BM) chimeras have been useful. While these studies have shown that hematopoietic cells transpresent IL-15, as expected, they also demonstrated that nonhematopoietic cells transpresent IL-15 but to different lymphocytes and under different circumstances. As such, these studies are a good example of how specific cells transpresenting IL-15 indeed restrict IL-15 responses to distinct lymphocytes. Overall, there are numerous cells that transpresent IL-15, but their ability to do so depends on the responding lymphocyte, the tissue of residence, and the stage of lymphocyte differentiation. The fine points of these situations will be discussed in more detail in the next few sections.

3. Regulating CD8 T cell responses and the generation of memory CD8 T cells via IL-15 transpresentation

One obvious phenotype of *IL-15*^{-/-} and *IL-15R α* ^{-/-} mice is a 50% deficiency in total CD8 T cells that preferentially affects CD44^{hi} memory phenotype CD8 T cells [7, 8]. This is not surprising as among naïve and memory CD4 and CD8 T cells, memory CD8 T cells are the most responsive to IL-15[14]. In the response to a number of pathogens, *IL-15*^{-/-} mice have a deficiency in Ag-specific memory CD8 T cells that becomes apparent during the transition of effector T cells into memory CD8 T cells, i.e. during the contraction phase [56–60]. During this time, the death of many of those effector CD8 T cells is a normal part of the contraction process; however, the contraction of CD8 T cells is more dramatic in the absence of IL-15 and IL-15R α (Figure 1). As proliferation has ceased during contraction[61], the loss of CD8 T cells is due to a more pronounced T cell death. Indeed, expression of the survival protein Bcl-2, which normally increases as memory CD8 T cell differentiation proceeds, is decreased in CD8 T cells in the absence of IL-15 or IL-15R α [62]. IL-15 is not the only factor that facilitates survival of effectors as IL-7 also has a role[63–65].

In addition to promoting the generation of memory CD8 T cells, IL-15 is important for maintaining memory CD8 T cell numbers[56, 57]. Once memory CD8 T cells are generated, their numbers are relatively stable over a long period of time. This maintenance of memory CD8 T cells is not merely mediated by enhanced survival of the memory T cells, an attribute

acquired during differentiation, but more uniquely by an ability to undergo self-renewal or homeostatic proliferation[66]. Homeostatic proliferation of memory CD8 T cells is an unusual form of T cell proliferation as it does not involve signals mediated through the TCR[67, 68]. As such, homeostatic proliferation together with enhanced survival are the underlying mechanisms conferring long-lived T cell-mediated immunity in the absence of reinfection. Very recently, memory CD8 T cells were shown to use a different metabolic pathway (oxidative phosphorylation rather than glycolysis) than naïve and effector CD8 T cells that contributes to their enhanced stability [69]. Interestingly, IL-15 promotes conversion to this altered metabolic state[69]. Like transitioning effector CD8 T cells, survival of memory CD8 T cells is regulated by IL-15 along with other factors, such as IL-7, CD27, and 4-1BBL[70–72]. In contrast, IL-15 is the only factor, to date, shown to mediate homeostatic proliferation of memory CD8 T cells in the steady state.

It is clear that IL-15 is important for CD8 T cells but does transpresentation mediate all IL-15 responses in CD8 T cells and if so, which cells transpresent IL-15 to CD8 T cells? The evidence supporting transpresentation as the responsible mechanism begins with the observation that an absence of IL-15R α by cells in the environment, not the responding cells, leads to a decrease in the number of memory CD8 T cells generated and deficient homeostatic proliferation [21, 28]. In studies analyzing IL-15R α BM chimeras, naïve CD8 T cells require IL-15R α expression by both radiation-sensitive and radiation-resistant cells (i.e. hematopoietic cells and non-hematopoietic cells respectively) [21, 28] (Figure 1). Conversely, the generation and homeostasis of memory CD8 T cells is highly dependent on hematopoietic cells expressing IL-15R α [21, 28].

To further define the hematopoietic cells transpresenting IL-15, our group used cellrestricted IL-15R α expression to study the role of DCs in transpresenting IL-15 to CD8 T cells. By genetically engineering IL-15R α expression under the control of the *Cd11c* promoter in an *IL-15 α* ^{-/-} background, we generated a transgenic mouse model whereby CD11c⁺ cells (i.e. DCs) are the only cells expressing IL-15R α and thus capable of transpresenting IL-15. The role of DCs transpresenting IL-15 was also addressed by Averil Ma's groups using conditional deletion of IL-15R α in CD11c⁺ cells [25]. In both model systems, IL-15R α expression by DCs is shown to be important for the presence of memory phenotype CD8 T cells, preferentially the CD62L⁺ subset[22, 25] (Figure 1). During Ag-specific CD8 T cell responses, we found that contraction of VSV-specific CD8 T cells is less profound in mice with IL-15R α expression restricted to DCs than *IL-15 α* ^{-/-} mice but still more pronounced than in wildtype mice, suggesting that DCs can support survival of effectors during the contraction but alone are not sufficient. In contrast, evidence that DCs support survival during the contraction is not observed in mice conditionally deleted of IL-15R α in CD11c⁺ cells[25]. This discrepancy regarding the role of DCs during the contraction could be related to differences in the immune responses studied (VSV infection versus adoptive transfer of TCR Tg CD8 T cells plus peptide vaccination) or the transgenic (Tg) expression of IL-15R α extending beyond that of the promoter. For example, CD11c^{low} myeloid cells would express IL-15R α in our Tg model but may not completely delete IL-15R α in a conditional knockout model. Nonetheless, DCs are clearly important for homeostasis of established memory CD8 T cells, as the number of memory CD8 T cells declined when DCs are deficient in IL-15R α but normal when DCs are the only cells expressing IL-15R α [22, 25]. In addition, homeostatic proliferation is almost completely restored when DCs are the only cells expressing IL-15R α [22]. Despite the findings that DCs preferentially affect the CD62L⁺ memory phenotype CD8 T cells, this was not evident among virus-specific memory CD8 T cells in our studies [22]. Altogether, these studies suggest that DCs predominately transpresent IL-15 to established memory CD8 T cells for their maintenance.

To investigate the role of macrophages transpresenting IL-15, Ma's group performed parallel studies of mice with conditional deletion of IL-15R α in LysM $^{+}$ cells [25]. While LysM deletion should also affect all granulocytes, IL-15R α expression is normally absent from neutrophils, the most abundant type of granulocyte [25]. As with the conditional deletion of IL-15R α in DCs, deletion of IL-15R α in LysM $^{+}$ cells also leads to a decrease in the number of memory phenotype CD8 T cells but instead, more dramatically affecting the CD62L $^{-}$ CD8 T cells. Within the secondary lymphoid tissues (spleen and lymph nodes), the deficiency in CD44 hi CD8 T cells with IL-15R α deletion in LysM $^{+}$ cells is not as severe as that seen with IL-15R α deletion in CD11c $^{+}$ cells; however, within the BM, the deficiency in CD44 hi CD8 T cells is more pronounced than that observed with IL-15R α deletion in CD11c $^{+}$ cells. While this could reflect the abilities of DCs and macrophages to preferentially affect one memory CD8 T cell subset over another, the LN and BM are both enriched with Tcm compared to the spleen. As such, these findings are evidence that distinct cell types in different tissues mediate IL-15 transpresentation. This is most evident in the BM as deletion of IL-15R α by DCs did not affect the number of memory CD8 T cells, regardless of subset, which is not surprising considering CD11c high cells are rare in the BM. In an Ag-specific response, deletion of IL-15R α in LysM $^{+}$ cells did enhance the contraction of CD8 T cells thus identifying macrophages as one cell type transpresenting IL-15 during the contraction (Figure 1). Furthermore, the maintenance of those memory CD8 T cells is defective in the absence of IL-15R α expression by macrophages suggesting that macrophages act together with DCs to regulate memory CD8 T cell homeostasis. However, as expression of IL-15R α by macrophages plays a role prior to the generation of memory CD8 T cells, there lies the potential that programming of memory CD8 T cells is altered. Presently, it is not clear if macrophages and DCs transpresenting IL-15 differentially impact Tem and Tcm differentiation or just preferentially promotes the homeostasis of the respective memory CD8 T cell subset.

Overall, the various cellular requirements for IL-15R α by CD8 T cells are an interesting example of how the cell type transpresenting IL-15 changes as differentiation proceeds (Figure 1). We suspect this change in cellular requirements is partially because the specific niches of CD8 T cells change with differentiation. For instance, DCs could be the dominant cell type transpresenting IL-15 to memory CD8 T cells because those are the IL-15-expressing cells most likely to be encountered by memory CD8 T cells during their migratory pathway. Moreover, this extends to the different memory T cell subsets that each have different migratory properties. In addition to their differential preference for lymphoid and non-lymphoid tissues, memory T cells subsets also reside in different regions of the tissues. Within the spleen, Tcm are preferentially found in the T cell zone of the white pulp where DCs are present while Tem are found in the red pulp, an area enriched in macrophages [73]. Hence, this is one explanation for how macrophages and DCs transpresenting IL-15 differentially influences the memory CD8 T cells. Another possibility is that the IL-15 signal induced by macrophages and DC is somewhat different. This could be due to differences in cell surface molecules between macrophages and DCs; however, this has not yet been determined. Another possibility is that the strength of the IL-15 signal delivered by these two cells is different. This is an interesting idea in light of a recent report by Malek's group showing that a weak CD122 signal favored Tcm by promoting Bcl-2 and survival while Tem were unaffected [74]. Future studies will be needed to clarify why distinct cell types transpresent IL-15 to CD8 T cells and whether those respective IL-15 responses differ.

4. The role of IL-15 transpresentation in iNKT cell biology

iNKT cells are an unusual subset of T cells that express an invariant TCR recognizing glycolipid antigens and possess functional activities upon completing development rather

than requiring priming by antigen stimulation. In many regards, iNKT cells are similar to memory CD8 T cells in their immediate effector functions, general phenotype, and dependence on IL-15. In the absence of IL-15 or any of its receptors, iNKT cell numbers are severely deficient in the thymus and in the periphery, resulting from defective development and homeostasis [7, 8, 75–77]. Interestingly, the decrease in iNKT cell numbers in the spleen of *IL-15*^{−/−} and *IL-15Rα*^{−/−} mice is not as striking as that observed in the thymus and liver, indicating other factors regulate iNKT cell numbers in the spleen [24, 77]. The generation of the iNKT cell compartment is a transitional process that is demarcated by phenotypic and functional changes. These phenotypic changes begin with iNKT cells appearing as CD44^{low} NK1.1[−] (stage 1) then transition to CD44^{high} NK1.1[−] (stage 2) and then finally into a CD44^{high} NK1.1⁺ (stage 3) (Figure 2). Earlier stages are restricted to the thymus, whereas later stages can occur in both the thymus and periphery, such as in the liver and spleen, the predominant sites where peripheral iNKT cells reside. All the iNKT cells differentiation stages occur in the absence of IL-15 or IL-15Rα; however, the CD44^{high} NK1.1⁺ (stage 3) numbers are the most dramatically reduced. This is likely because CD44^{high} NK1.1⁺ cells express higher levels of CD122 and are more responsive to IL-15 compared to the more immature populations [77, 78]. Overall, the similar defects in iNKT cells in both IL-15- and IL-15Rα-deficient mice demonstrate that IL-15 responses are heavily dependent on IL-15Rα.

Many past studies have focused on identifying the various roles of IL-15 in the development and homeostasis of iNKT cells. Although this topic is more thoroughly described elsewhere, a brief description will be covered herein. In general, IL-15 regulates iNKT cell numbers by controlling both cell survival and proliferation throughout development and during homeostasis. The major role of IL-15 in the thymus is to enhance survival of developing iNKT cells, as studies have shown that BrdU incorporation in thymic iNKT cells is not affected by the absence of IL-15Rα [24, 79, 80]. IL-15 is a known survival factor affecting both anti- and pro-apoptotic factors. Specifically, anti-apoptotic factors, such as Bcl-2, Bcl-xL and Mcl-1 are increased by IL-15 in iNKT cells whereas pro-apoptotic factors like Bim are decreased [24, 79–81]. Conversely, in the periphery, IL-15 regulates both proliferation and survival of iNKT cells. IL-15-mediated survival and proliferation are mechanisms controlling cell numbers in both iNKT cell development and homeostasis, as these activities are evident in both developing iNKT cells as well as mature iNKT cells. More recently, a new function of IL-15 has been identified; IL-15 regulates the differentiation, functional maturation and activation of iNKT cells. Our group and others have recently revealed that IL-15 regulates T-bet and T-bet-regulated genes. Specifically, T-bet is important for upregulating CD122 on CD44^{high} NK1.1⁺ cells [77, 78]. We found that the level of T-bet and the ability to produce substantial amounts of IFN-γ in response to α-galactosylceramide is deficient in *IL-15Rα*^{−/−} mice, but could be restored by treatment with IL-15/IL-15Rα complexes. These results were further validated by Chang et al [79] and Gordy et al [80] who revealed that IL-15 is not only critical for IFN-γ production but also crucial for the acquisition of both activating and inhibitory Ly49 and NKG2 receptors. This defect is not merely due to a preferential loss of Ly49⁺/NKG2⁺ IFN-γ producing iNKT cells as restoration of iNKT cell numbers in *IL-15*^{−/−} mice by overexpressing Bcl-xL failed to generate iNKT cells that could produce high quantities of IFN-γ upon stimulation [80]. So it appears that IL-15 not only maintains iNKT cells, it also conditions them to acquire functional capabilities. These recent findings align with the downstream signals mediated by the receptors for IL-15. The γC subunit utilizes the Jak3/STAT5 pathway, which has been shown to grant T-bet chromatin access to the *ifn-γ* promoter in Th1 cells during IL-2 signaling [82].

Identifying the cells that mediate IL-15 functions and presumably transpresent IL-15 to iNKT cells has not been as thoroughly studied as other IL-15-dependent cell populations.

The first hint of the IL-15 source for iNKT cells in the thymus came from studies of mice with genetic defects in the NF- κ B family member, RelB. RelB-deficient mice lack thymic DCs and medullary thymic epithelial cells (mTEC) and have a severe deficiency in iNKT cells [83–85]. In these *Relb*^{-/-} mice, *IL-15* mRNA levels are decreased in the thymic stroma suggesting non-hematopoietic cells are a source of IL-15. Studies utilizing BM chimeras, where IL-15R α is expressed by either the hematopoietic or non-hematopoietic compartment, revealed that IL-15R α expression by only non-hematopoietic cells is completely sufficient for thymic iNKT development [24, 79]. Additionally, these studies also demonstrated that IL-15R α expression by hematopoietic cells is not required, which also demonstrates cis-presentation is not crucial for development [20, 24, 79]. Liao's group [79] nicely demonstrated the importance of non-hematopoietic cells expressing IL-15R α specifically within the thymus. By using thymectomized mice, they showed that complete thymic iNKT cell maturation required that the engrafted fetal thymus must express IL-15R α regardless of whether IL-15R α was expressed by the recipient or BM [79]. mTECs are a logical cell type transpresenting IL-15 considering CD44^{high} NK1.1⁺ T cells come to reside in the thymus medulla as long-term residents due to CXCR3 expression by iNKT cells and CXCL10 expression by mTECs [86]. The role of DCs in transpresenting IL-15 in the thymus was further ruled out in our studies where we demonstrated that IL-15R α expressed solely on DCs had no impact on thymic iNKT development [24]. Altogether, these studies provide strong evidence that mTECs are the main cells transpresenting IL-15 in the thymus, which primarily enhance survival of developing iNKT cells and promote their functional maturation [24] (Figure 2).

Whereas non-hematopoietic cells have a crucial and unconditional role transpresenting IL-15 to thymic iNKT cells, this is not the case in the periphery. Our studies using Tg model (IL-15R α +DCs) and IL-15R α BM chimeras revealed two essential points concerning the identity of cells transpresenting IL-15 to peripheral iNKT cells [24]. The first is that IL-15R α expression by both hematopoietic and non-hematopoietic are equally important [24]. The BM chimeras revealed neither compartment could completely recover hepatic iNKT cells while acting mutually exclusive. The second point is that a defect in the thymus did not equate to a defect in peripheral iNKT cell development. When hematopoietic cells or DCs are the only cell type expressing IL-15R α , thymic iNKT development is defective while differentiation and expansion of hepatic iNKT cells is still apparent demonstrating a role for IL-15-mediated extrathymic iNKT cell development [24] (Figure 2). In the liver, the microenvironment contains IL-15-producing DCs and Kupffer cells (liver-resident macrophages [87]). In addition, hepatic stellate cells (non-hematopoietic origin) were recently shown to be a source of IL-15 and can maintain hepatic iNKT cells [88]. In our studies examining the role of DCs, we also found that DC-mediated IL-15 transpresentation generated functionally mature iNKT cells [24] (Figure 2). We demonstrated that treatment with soluble IL-15R α /IL-15 complexes could stimulate IFN- γ production in iNKT cells in *IL-15 α* ^{-/-} mice, suggesting IL-15 signals are completely sufficient for functional maturation of iNKT cells [24]. As such, other IL-15 transpresenting cells such as Kupffer cells or stellate cells should also be able to induce functional maturation of iNKT cells, although this has yet to be determined. Future studies could discern the specific roles of DCs and macrophage-related cells in iNKT cell maturation by analyzing IL-15R α -conditional knockout models, as this has not yet been examined. In conclusion, the cell types regulating the development and homeostasis of iNKT cells via IL-15 trans-presentation is very much dependent on the tissue microenvironments where iNKT cells reside.

5. IL-15 transpresentation in NK cell biology

NK cells are lymphocytes with the innate ability to recognize and kill virus-infected cells and transformed cells. Like iNKT cells, this function is acquired during development and

does not require priming. Development of NK cells is a complex and continuous event that occurs in both the BM and peripheral tissues, such as the spleen and liver; however, the earliest stages are confined to the BM (Figure 3). The first stage of NK cell commitment involves the transition of common lymphoid progenitors into NK precursors (NKp)[21]. NKp express IL-15R β (CD122) and are completely negative for all other known NK cell markers[89, 90]. In mice, transition from NKp to immature NK cells (immNK) is marked by the expression of NKG2D, NK1.1 and the CD94/NKG2 heterodimeric complex. This is subsequently followed by the indiscriminate expression of both activating and inhibitory Ly49 receptors (Ly49R); yet, these cells are still considered immature[89]. The conversion from immature to mature NK cell is distinguished by the capacity to kill and produce pro-inflammatory cytokines, which is marked by the expression of CD49b (DX5) and identified as the M1 maturation stage [91]. These functional attributes become enhanced with the up-regulation of CD11b and CD43, identifying the M2 stage [89]. Recently, the expression CD27 and CD11b have also been used to further define late maturation with immature NK cells expressing CD27^{high}CD11b^{low} and progressing through a CD27^{high} CD11b^{high} and then to a more mature stage, CD27^{low}CD11b^{high} [92]. Differentiation of NK cells is driven in part by the tandem expression of multiple transcription factors (e.g., Id2, Id3, Ikaros, Runx3, E4bp4, Gata-3, T-bet and Eomesodermin) that regulate various events such as expression of CD122 and differentiation and functional maturation of NK cells [93–101]. In addition, IL-15 is crucial for NK cell development.

Beginning with NKp and continuing throughout the life span of NK cells, NK cells express CD122 and subsequently depend on IL-15 for both their development and maintenance. Consequently, the absence of IL-15 or any of its receptor subunits results in a deficiency in NK cells [7, 8, 75, 102]. Throughout development, NK cell numbers are regulated by expansion and survival. IL-15 induces survival of NK cells by maintaining the anti-apoptotic molecules Bcl-2 and Mcl-1 as well as preventing the accumulation of the pro-apoptotic molecules Bim and Noxa[81, 103, 104]. Interestingly, while IL-15 is critical for survival of both immature and mature NK cells in the BM and periphery, it affects proliferation of NK cells only in the BM and not the periphery[23]. Once development is complete, mature NK cell numbers are maintained by IL-15-mediated proliferation and survival [105, 106]. In lymphopenic hosts, NK cells undergo a more robust level of proliferation that is dependent on IL-15 [106–108]. IL-15 is not only important for differentiation, survival, and proliferation of developing NK cells but is also crucial for NK cell activation. In all these events, there is strong evidence that transpresentation is involved.

As with the other IL-15-dependent lymphocytes, evidence that transpresentation regulates IL-15 responses in NK cells began with studies using IL-15R α BM chimeras and adoptive transfers. For example, IL-15R α expression by NK cells is not required for survival of NK cells after transfer into wildtype recipients or for the proliferation in lymphopenic recipients [29, 30]. Conversely, these responses require IL-15R α expression by the recipients [29]. In addition, NK cells develop in BM chimeras lacking IL-15R α expression by hematopoietic cells, albeit at a decreased level [20, 29, 31]. Nonetheless, normal NK cell numbers are also only partially achieved in BM chimeras lacking IL-15R α expression by non-hematopoietic cells. Altogether, these findings suggest that Cis-presentation is not essential and that IL-15 provided via IL-15R α by opposing cells consist of both hematopoietic and non-hematopoietic cells.

During NK cell development, both hematopoietic and non-hematopoietic cells provide IL-15; however, they have somewhat different but overlapping roles in the developmental process. In general, IL-15 transpresentation by hematopoietic cells is more efficient than by non-hematopoietic cells, as limiting IL-15R α expression to hematopoietic cells is sufficient to generate normal NK cell numbers in the BM and only slightly deficient numbers in the

periphery [20, 23]. In contrast, restricting IL-15R α to the non-hematopoietic compartment is less able to generate NK cell numbers albeit the differentiation process is still intact. This ability of non-hematopoietic cells to generate NK cells is most evident in the BM and virtually non-existent in the spleen or liver [23]. This is not surprising since BM stromal cells have long been considered a source of IL-15 [2, 109]. The role of these supposed BM stromal cells expressing IL-15R α appears to be fairly specific within NK cell development as non-hematopoietic cells expressing IL-15R α are completely sufficient for supporting immature NK cells, but are less capable as maturation progresses [23]. We suspect the specific roles of non-hematopoietic cells and hematopoietic cells reflect the different niches within the BM that NK cells inhabit while they undergo differentiation. These studies indicate that both compartments can drive differentiation with hematopoietic cells acting as the primary cells regulating the late stages of maturation and subsequent maintenance.

The fact that IL-15R α expression by hematopoietic cells is important prompted the examination of DCs and macrophages in transpresenting IL-15 to NK cells. By using the CD11c/IL-15R α Tg(*IL-15R α -/-*) mouse model, we showed that CD11c⁺ cells (i.e. DCs) indeed contribute to the development of NK cells, as observed in the partial recovery of NK cells in all tissues. Ma and colleagues confirmed a role for CD11c⁺ cells in transpresenting IL-15 to NK cells using the conditional-knockout model (IL-15R α ^{fl/-} CD11c-Cre) demonstrating that absence of IL-15R α in DCs lead to decreased NK cell numbers in the spleen, liver, and lung but not BM [25]. The discrepancy in the role of DCs in BM between the conditional knockout and Tg studies could be due to caveats in the models systems. Nonetheless, DCs expressing IL-15R α did not account for all the IL-15 provided by hematopoietic cells [23]. When IL-15R α is eliminated in macrophages (IL-15R α ^{fl/-} LysM-Cre), similar defects in NK cell numbers are observed as in the IL-15R α ^{fl/-} CD11c-Cre mice [25], including the absence of a role for LysM⁺ cells in the BM. The findings that IL-15R α expression by CD11c⁺ and LysM⁺ cells is not important for NK cells in BM while IL-15R α expression by hematopoietic cells indicates that unidentified, CD11c-LysM-hematopoietic cells transpresent IL-15 in the BM. Altogether, the present studies suggest that BM stromal cells drive the early stages of NK cell development in the BM while some unidentified hematopoietic cells drive the late stages of NK cell maturation (Figure 3).

Once developing NK cells leave the BM, DCs and macrophages have a major role of transpresenting IL-15 to NK cells (Figure 3). Interestingly, simultaneous deletion of IL-15R α in both CD11c⁺ and LysM⁺ cells did not further amplify the NK cell deficiency suggesting that macrophages and DCs fulfill the same role in transpresenting IL-15 to NK cells. In addition, the NK cell deficiency observed with IL-15R α deletion by DCs and macrophages is not as dramatic as that observed in *IL-15R α -/-* mice confirming that additional cell types provide IL-15 to NK cells [25]. So what is the role of IL-15 transpresentation by DCs and macrophages on NK cells? While our studies using the Tg model showed DCs help support survival of mature NK cells, the conditional knockout models did not see evidence that either DCs or macrophages support NK cell survival. This discrepancy may be related to the differences in time points analyzed as Mortier et al [25] analyzed the recovery of NK cells at 4 hour, 2 and 7 days, while our studies analyzed NK cell recovery 21 days post-transfer [23]. Alternatively, these differences could be due to unidentified CD11^{low} cells transpresenting IL-15 in our Tg model but did not completely delete IL-15R α in the conditional knockout model. Interestingly, Sonderquest et al [110] recently revealed that monocytes could drive NK cell differentiation in an IL-15/IL-15R α manner. As mentioned earlier, the late stages of NK cell maturation have been further delineated by CD27 expression, where CD11b⁺CD27⁺ NK cells differentiate into the more mature CD11b⁺CD27⁻ NK cells. Upon investigating the different maturation stages, it was found that absence of IL-15R α by either DCs or macrophages leads to a specific loss in the most mature CD11b⁺CD27⁻ NK cells [25] which could be restored by IL-15R α .

expressing monocytes [110]. Additionally, proliferation of CD11b+CD27+ NK cells but not CD11b+CD27- NK cells is also impaired by the loss of IL-15R α [25]. As proliferation is active in CD11b+CD27+ NK cells and is thought to accompany the differentiation into CD11b+CD27- NK cells, the deficiency in CD11b+CD27- NK cells observed in the conditional mice is likely due to the inability of DCs and macrophages to stimulate proliferation of CD11b+CD27+ NK cells. Overall, these reports reveal that DCs, macrophages and monocytes are the major hematopoietic cells capable of transpresenting IL-15 during NK cell development but alone are not crucial for the generation of mature NK cells.

As mentioned earlier, IL-15 is also important for NK cell activation as IFN- γ and Granzyme B expression in NK cells is induced by IL-15 and deficient in the absence of IL-15 [103, 111] (Figure 3). IL-15 likely promotes Granzyme B expression as IL-15 induces Blimp-1, a transcription factor important for Granzyme B expression, but not other cytokines [112]. On the other hand, IFN- γ expression could be regulated via IL-15 mediated T-bet expression. Notably, multiple studies have shown that IL-15 transpresentation by DCs promotes NK cell activation [45, 111, 113]. Not surprisingly, NK cells in mice with IL-15R α deleted by DCs had a decreased ability to produce Granzyme B after LPS or poly I:C stimulation; however, a similar effect was also observed with IL-15R α deletion in LysM+ cells [25]. This is significant as the role of macrophages in NK cell activation prior to this was unknown. Regarding IFN- γ production, our studies found that IL-15R α by DCs is sufficient to restore NK cell expression of IFN- γ after NK1.1 ligation [23]. Recently, it was revealed that different events in NK cell development (differentiation, Ly49 acquisition, functional maturation, and homeostasis) require different levels of IL-15 transpresentation [114]. This is interesting as, up until now, it wasn't clear whether the different events mediated by IL-15 were merely due to intrinsic differences in the responding cells as they progress through differentiation. The importance of the degree of IL-15 stimulation is also exemplified by the findings that chronic exposure to IL-15 stimulation generates NK cells with altered activation and functional capacity [115]. Altogether, these findings provide a mechanism for how various IL-15 transpresenting cells mediate discriminate events such as survival and activation. Therefore, dividing the labor of IL-15 transpresentation to different cell-types with varying levels of IL-15R α is likely important for normal NK cell development and functions.

6. IL-15 transpresentation in the intestinal epithelium

Intraepithelial lymphocytes (IELs) that reside within the intestinal epithelium of mice are comprised predominantly of CD8 T cells, which include a subset that is heavily dependent on IL-15. Surprisingly, these IL-15-dependent CD8 IELs are not the conventional memory CD8 T cells one might expect but rather are a unique group of CD8 T cells expressing CD8 $\alpha\alpha$ homodimers, instead of CD8 $\alpha\beta$ and consist of a mixture of TCR $\alpha\beta$ and TCR $\gamma\delta$ expressing cells. These T cells are also unique because of their unusual development and functions. In contrast to conventional T cells that undergo development entirely in the thymus, current evidence suggests that development of CD8 $\alpha\alpha$ IELs is partially thymus dependent with developmental cell intermediates leaving the thymus and completing development in the intestinal epithelium [7, 8]. As both CD8 $\alpha\alpha$ IEL subsets are dramatically deficient in the absence of IL-15 or IL-15R α , the question of the role of IL-15 in the developmental process has arisen.

Elucidating the role of IL-15 in the development of CD8 $\alpha\alpha$ IELs began by first identifying the cellular requirements for IL-15 and IL-15R α . Unlike other IL-15-dependent lymphocytes, IELs rely solely on non-hematopoietic cells expressing IL-15 and IL-15R α [20]. The lack of necessity for IL-15R α by hematopoietic cells is also clear evidence

that IELs themselves do not require IL-15R α indicating that Cis-presentation of IL-15 is not a critical mechanism used by CD8 $\alpha\alpha$ IELs for development. With their intimate association, intestinal epithelial cells are an obvious parenchymal cell type to provide IL-15 to CD8 $\alpha\alpha$ IELs; however, considering the thymic origin of IELs and their unclear developmental pathway, the potential for thymic epithelial cells needed to be addressed. Using thymic transplants, Liao's group demonstrated that an IL-15R α + thymus in *IL-15R α -/-* recipients did not restore CD8 $\alpha\alpha$ IEL development while an *IL-15R α -/-* thymus could support normal development of CD8 $\alpha\alpha$ IELs in IL-15R α + recipients [116]. To determine whether IL-15R α expression by enterocytes is sufficient for IEL development, our group used a similar Tg approach as described earlier to restrict IL-15R α expression to enterocytes via the Villin promoter in conjunction with an *IL-15R α -/-* background [117]. Within these mice, exclusive expression of IL-15R α by enterocytes restores all the deficiencies in the CD8 $\alpha\alpha$ TCR $\alpha\beta$ and CD8 $\alpha\alpha$ TCR $\gamma\delta$ subsets that exist in the global absence of IL-15R α [117]. Conversely, IEL subsets are not affected by CD11c-restricted expression of IL-15R α [22]. These studies demonstrated that enterocyte IL-15R α is sufficient for CD8 $\alpha\alpha$ IEL development. Studies showing that IL-15R α by enterocytes is required for IEL development was demonstrated using IL-15R α conditional deletion mice [25]. Deletion of IL-15R α in enterocytes (Villin-Cre X IL-15R $\alpha^{fl/-}$) leads to a ~50% decrease in CD8 $\alpha\alpha$ TCR $\alpha\beta$ IELs numbers and ~75% decrease in CD8 $\alpha\alpha$ TCR $\gamma\delta$ IELs, which is similar to the IEL deficiency present in the *IL-15/-* and *IL-15R α -/-* mice [7, 8, 25]. Altogether, these studies are strong evidence that IL-15R α expression by enterocytes regulates CD8 $\alpha\alpha$ IEL numbers via transpresentation.

So what is the role of IL-15 transpresentation by enterocytes in IEL development? As with other IL-15-responsive lymphocytes, IL-15 mediates survival of CD8 $\alpha\alpha$ IELs by increasing Bcl-2 [118]. In the absence of IL-15 signals, decreased Bcl-2 is evident in IELs at multiple stages and correlates with decreased cell numbers [104, 117]. When Bcl-2 is transgenically expressed in the absence of IL-15, IEL numbers are partially restored [104]. When IL-15R α is expressed solely by enterocytes, Bcl-2 expression is likewise restored among CD8 $\alpha\alpha$ IELs [117]. IL-15 also has the potential to regulate IEL numbers by promoting proliferation, which it has been shown to do *in vitro* [119]. Unexplainably, BrdU incorporation in CD8 $\alpha\alpha$ IELs was increased in *IL-15R α -/-* mice compared to wildtype mice and Villin-IL-15R α Tg mice [117] suggesting IL-15 mainly regulates IEL numbers *in vivo* by maintaining survival. Despite the ability of IL-15 to regulate Bcl-2, expression of transgenic Bcl-2 in *IL-15/-* mice only partially restored CD8 $\alpha\alpha$ TCR $\gamma\delta$ IEL numbers [104] suggesting other IL-15-mediated responses help regulate CD8 $\alpha\alpha$ TCR $\gamma\delta$ IEL numbers; whether the same is true for CD8 $\alpha\alpha$ TCR $\alpha\beta$ IELs is currently not known. Our studies also provided evidence that IL-15 influences IEL differentiation. Unlike conventional T cells, a large portion of both CD8 $\alpha\alpha$ IEL subsets have low Thy1 expression. Although the significance of low Thy1 expression is unknown, we found that IL-15R α expression by enterocytes promotes Thy1 downregulation in CD8 $\alpha\alpha$ IELs [117]. In addition, transfer of putative IEL precursors (CD4- CD8-Thy1+) isolated from the thymus lead to development of Thy1-CD8 $\alpha\alpha$ IELs, which was dependent on IL-15R α expression by enterocytes [117]. Overall, it appears that IL-15 transpresentation mediates differentiation and survival of CD8 $\alpha\alpha$ IELs.

The function of CD8 $\alpha\alpha$ IELs is not entirely clear but, in general, these IELs are thought to protect the integrity of the epithelial layer. CD8 $\alpha\alpha$ TCR $\gamma\delta$ IELs protect the epithelium by promoting healing through their production of Keratinocyte Growth Factor and by eliminating transformed cells [120–122]. In contrast, CD8 $\alpha\beta$ TCR $\alpha\beta$ are immunosuppressive in nature via production of IL-10 and TGF- β [123, 124] and exhibit regulatory activities similar to Tregs [123, 125]. While CD8 $\alpha\alpha$ TCR $\gamma\delta$ IELs express immunosuppressive cytokines, they are also capable of expressing proinflammatory factors, like IFN- γ and TNF- α . These unique functions of CD8 $\alpha\alpha$ IELs are presumably obtained

during development, which is another potential way IL-15 influences IEL development. Indeed, evidence that IL-15 influences acquisition of functions is seen in studies where CD8 $\alpha\alpha$ IEL numbers are restored by Tg Bcl-2 in the *IL-15*^{-/-} mice; however, IFN- γ and cytotoxic activity of $\gamma\delta$ IELs are not recovered [104]. Moreover, Tbet and Eomesodermin expression is also decreased in IELs in the absence of IL-15 but unaffected by Tg Bcl-2 [104]. Eomesodermin and Tbet regulate IFN- γ , Granzyme B, and Perforin in CD8 T cells and NK cells [100, 126–128] and hence appear to act in an analogous manner in IELs. Considering IL-15 enhances Tbet expression in CD8 T cells and NK cells, these studies suggest that the ability of IL-15 to modulate Tbet and Eomesodermin may also facilitate the acquisition of cytolytic functions in IELs. Interestingly, the signals that promote the ability to produce KGF, IL-10, and TGF- β in IELs are still unknown. Overall, multiple functions of IL-15 in CD8 $\alpha\alpha$ IELs development include regulating survival, differentiation, cytolytic activity, and possibly proliferation.

Much of this discussion focused on CD8 $\alpha\alpha$ IELs; however, conventional CD8 $\alpha\beta$ T cells also reside in the intestinal epithelium. As CD8 $\alpha\beta$ IELs display a memory phenotype (CD44^{hi}), one would suspect that these IELs would also be influenced by IL-15 transpresented in the intestinal epithelium. Oddly, CD122 (IL-2/15R β) expression in these CD8 T cells becomes down-regulated after entering the IEL compartment decreasing their IL-15 responsiveness [129]. Therefore, these memory CD8 T cells are likely ignorant of IL-15 signals available in the intestinal epithelium. In contrast to CD8 $\alpha\beta$ T cells in the epithelium, CD8 $\alpha\beta$ T cells in the lamina propria of the intestines do not have down regulated CD122 and likely have similar requirements for IL-15 transpresented by DCs and macrophages present within the lamina propria microenvironment; however, this needs to be determined.

Whereas CD8 $\alpha\alpha$ and CD8 $\alpha\beta$ IELs represent distinct populations in mice, analogous IEL populations have not been identified in the human intestinal epithelium. Nonetheless human IELs are responsive to IL-15 and thus, there is a possibility that transpresentation regulates IL-15 responses in human IELs. Interestingly, dysregulation of IL-15 is associated with human intestinal pathologies such as Celiac disease and inflammatory bowel diseases [41, 130–132]. Hence, discerning whether dysregulation of IL-15 involves transpresentation or one of the other alternative mechanisms could provide insight into the aberrant immune responses occurring in these different conditions.

7. Conclusions

This review focused heavily on how IL-15 transpresentation regulates lymphocytes during the steady state as this is an area where the role of transpresentation is undisputed. Regrettably, some issues are not covered herein. Whereas we limited our discussion to IL-15 responses in lymphocytes, there are also reports that IL-15 can affect other immune cells such as DCs, macrophages, neutrophils and mast cells [133–135]. These myeloid cells are feasible targets of IL-15 as they express IL-2R β / γ C. For instance, IL-15 can mediate survival of DCs and alters DC differentiation [133, 134]. In addition, IL-15 stimulates mast cell proliferation and induces chemokine expression in neutrophils [136–139]. Unfortunately, studies have not clearly shown a crucial role for IL-15 by these cells *in vivo*. In addition, how these myeloid cells access IL-15 has not been demonstrated. At least for DCs and macrophages, these cells could receive IL-15 stimulation via cis-presentation or after cleavage of IL-15R α /IL-15 complexes because they express both IL-15 and IL-15R α . Outside the immune system, IL-15 has also been implicated as a regulator of adipocytes and an anabolic factor for muscle cells [140, 141]. Indeed, absence of IL-15R α leads to altered metabolic activity in muscles of mice [141]. In addition, *in vivo* IL-15 expression levels

inversely correlate with body weight [140]. Future studies will need to determine how IL-15 stimulates these various cell types.

We also limited our discussion to those events that occur during homeostasis. This is because our understanding of IL-15 transpresentation during immune activation and inflammation is still in its infancy. When transpresentation was first proposed, it provided a logical mechanism for maintaining IL-15 expression at a low level and restricting IL-15 responses to specific lymphocytes. Limiting IL-15 responses is important to prevent excessive lymphocyte activation[142]. As such, it is possible that transpresentation is not active during states of immune activation, which contributes to enhanced IL-15 expression that is sometimes observed. Are these situations when cis-presentation occurs or soluble IL-15R α /IL-15 complexes are produced? Alternatively, transpresentation may still be a major mechanism inducing IL-15 responses during immune activation but at an enhanced level. More studies will be needed to answer these questions.

Lastly, there has been minimal mention of whether transpresentation is a mechanism stimulating IL-15 responses in humans. It will likely be difficult to firmly establish whether IL-15 transpresentation is active in human or its dominance in mediating IL-15 responses. Some groups have argued that human cells require cis-presentation rather than transpresentation [35]. While this is an interesting idea, more studies are needed to elucidate this. As human DCs still express IL-15R α and IL-15, one cannot rule out transpresentation as a mechanism that stimulates human lymphocytes.

All in all, transpresentation is an ideal mechanism for delivering IL-15 to a precise set of lymphocytes for their continual development and survival. While recent studies have begun to elucidate the cell types transpresenting IL-15, this analysis is not yet complete. More importantly, we are not yet clear on what parameters dictate specific cell transpresentation of IL-15 or the type of responses induced in the responding lymphocytes. In addition, it is not known if there are times when the level of transpresented IL-15 increases and induces a more potent signal. Considering there are alternative mechanisms that can mediate IL-15 signals, the sole function of transpresentation may simply be to provide low levels of IL-15 for lymphocyte homeostasis.

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Highlights

- Transpresentation is the mechanism mediating IL-15 responses during homeostasis.
- Other mechanisms can induce IL-15 responses but their relevance in vivo is unclear.
- Memory CD8 T cells depend on IL-15 transpresented by DCs and macrophages.
- Development of CD8 $\alpha\alpha$ IELs requires enterocytes transpresent IL-15.
- Hematopoietic and non-hematopoietic cells transpresent IL-15 to NK and INKT cells

CD8 T cell Differentiation

Figure 1. IL-15 transpresentation during CD8 T cell differentiation

Cartoon depicts various cell types transpresenting IL-15 through the differentiation of CD8 T cells. Cell surface IL-15R α (dark blue) is shown presenting IL-15 (light blue diamond) in trans to IL-15R β / γ C receptor complex (red/orange subunits) on the T cells. Naïve CD8 T cells require IL-15 for survival and receive this signal from unidentified hematopoietic and radiation-resistant stromal cells. T cell activation induces the differentiation of effector CD8 T cells, which does not require IL-15 transpresentation. As effector cells transition into memory CD8 T cells, macrophages transpresenting IL-15 facilitate survival and further differentiation into memory CD8 T cells. Once memory CD8 T cells are generated, DCs transpresenting IL-15 preferentially support CD62L+ memory CD8 T cells while macrophages preferentially support CD62L- memory CD8 T cells. Transpresentation of IL-15 mediates homeostasis of memory CD8 T cells in part by promoting homeostatic proliferation and survival.

iNKT cell Development

Figure 2. IL-15 transpresentation during iNKT cell development

Cartoon shows proposed scheme of iNKT cell development in the thymus and liver including the cells that transpresent IL-15 at the specific stages. iNKT cells transition from CD44^{low} NK1.1⁻ T cells (stage I) to CD44^{high} NK1.1⁻ T cells (stage II) and then finally into CD44^{high} NK1.1⁺ T cells (stage III). In the thymus, IL-15 transpresentation is crucial for either the differentiation and/or survival of stage III (CD44^{high} NK1.1⁺) iNKT cells and is provided by medullary thymic epithelial cells. Some stage II iNKT cells (CD44^{high} NK1.1⁻) leave the thymus and migrate to the liver where their survival, proliferation, and differentiation is supported by IL-15 transpresented by Kupffer cells, non-hematopoietic Stellate cells, DCs. DCs transpresenting IL-15 can also promote functional maturation of iNKT cells, making them capable of producing IFN-γ as well as induce homeostatic proliferation.

NK cell Development

Figure 3. IL-15 transpresentation during NK cell development

NK cell development begins as common lymphoid progenitors differentiate into NK precursors (NKp) (CD122⁺NK1.1⁻), which are the earliest cells to express IL-15Rβ (CD122). NKp then differentiate into immature NK cells (immNK; CD122⁺NK1.1⁺), which require IL-15 transpresentation that is efficiently provided by radiation-resistant stromal cells. Further differentiation into M1 (CD122⁺NK1.1⁺CD11b^{low}, CD49b⁺) and M2 (CD122⁺NK1.1⁺CD11b^{high}, CD27⁺) NK cells requires IL-15 transpresentation mediated primarily by unidentified hematopoietic cells. When M1 and M2 NK cells migrate into the peripheral tissues, such as the spleen and liver, these mature NK cells utilize IL-15 transpresented by both macrophages and DCs for survival, proliferation, and further maturation. Maturation can involve differentiation from the M1 to M2 stage as well as from the M2 stage to an even more mature state, identified by low CD27 expression.