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Review

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IL-15 and IL-15Rα in CD4$^+$ T cell immunity

Tom Van Belle and Johan Grooten

Department for Molecular Biomedical Research, Flanders Institute for Biotechnology (VIB) and Laboratory for Molecular Biology, University of Ghent, Belgium

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Summary

The cytokine IL-15 performs numerous functions, such as promotion of growth and survival, on a plethora of cell types from both the lymphoid and non-lymphoid compartments. Therefore, mice genetically engineered to either lack or overexpress functional IL-15 display reduced immunological responses and leukemia, respectively. Surprisingly, IL-15 protein is hardly found in serum or body fluids. Due to the lack of a clear demonstration of its presence as protein, IL-15 was often referred to as a "ghost cytokine". Recently, however, membrane-bound IL-15 was detected in both a membrane-anchored form and an IL-15Rα-bound form on monocytes. Interestingly, the latter complex can be transpresented to cells expressing the intermediate-affinity IL-2/15Rβ-γ_C receptor and thereby support the survival and proliferation of T cells. Moreover, overlapping promoter elements indicate a model of co-regulation of IL-15 and IL-15Rα by which IL-15 activities are controlled in a cell-contact-dependent manner. In this review, recent reports on IL-15 are combined with previous observations and discussed in terms of their functional consequences for CD4$^+$ T cell responses.

Key words: **cytokines • T lymphocytes • IL-15 • transpresentation • apoptosis**

Abbreviations: **AA** – amino acid, **AICD** – activation-induced cell death, **BM** – bone marrow, **DC** – dendritic cell, **ds** – double-stranded, **ER** – endoplasmatic reticulum, **IFN** – interferon, **iIEL** – intestinal intraepithelial lymphocytes, **IRE** – interferon regulatory element, **IL** – interleukin, **LN** – lymph node, **LPS** – lipopolysaccharide, **LSP** – long signal peptide, **MHC** – major histocompatibility complex, **NK** – natural killer, **SP** – signal peptide, **SSP** – short signal peptide, **TCR** – T cell receptor, **Tg** – transgenic.

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Author's address: Tom Van Belle, Molecular Immunology Unit, Department for Molecular Biomedical Research, VIB, University Ghent, Technologiepark 927, 9052 Gent-Zwijnaarde, Belgium, e-mail: tom.vanbelle@dmbr.ugent.be

PHYSIOLOGICAL EXPRESSION OF IL-15

Discrepancy between transcript and secreted protein

Interleukin (IL)-15 mRNA is detected in several tissues (placenta, skeletal muscle, heart, lung, liver, brain, kidney, and bone marrow)[30] as well as in many cell types of both hematopoietic and non-hematopoietic origin[18, 28, 30, 33, 35, 42, 53, 54, 56, 69]. Furthermore, its cellular expression is regulated by various stimuli, such as type I interferons (IFNs), double stranded (ds)RNA, lipopolysaccharide (LPS), and pathogenic triggers[13, 18, 49, 52, 61, 82]. The expression patterns of IL-15 in various tissues and cell types, as well as its functions in (non)-lymphoid cell types, have been reviewed in detail elsewhere[24, 80].

In contrast to the widespread IL-15 mRNA expression, the detection of significant quantities of IL-15 protein in serum or supernatant of transcript-expressing cells has been extremely difficult[5]. From a physiological viewpoint, this suggests that IL-15 may exist in translationally inactive pools. By storing translationally quiescent IL-15 mRNA, cells might respond to intracellular infections or other stimuli by rapidly transforming IL-15 message into one that can be efficiently translated. Mechanistically, this discordance is explained by a complicated sequence of transcriptional and posttranscriptional regulation mechanisms that, in turn, have posttranslational trafficking consequences[41, 77, 80]. Multiple start codons in the 5' untranslated region, the unusually long and short signal peptides, and the negative regulator near the carboxyl terminus of the precursor IL-15 protein hamper the full translation to mature IL-15 protein[4, 5].

Signal peptides rule the way

These transcriptional and posttranscriptional regulatory mechanisms are differentially reflected in the various IL-15 isoforms generated by alternative splicing, both in man[2, 51, 66, 77] and in mouse[62, 67]. Although all transcripts ultimately result in a similar mature IL-15 protein, translational efficiency, intracellular trafficking, and secretion differ dramatically[41]. Basically, two transcript classes exist: the classical IL-15 transcript containing a 48-AA-long signal peptide (LSP) and the alternative transcript with a short SP (SSP) of 21 AA (or 26 AA in mouse)[1, 62, 67]. Transfection studies with constructs containing the IL-15 SSP, the IL-15 LSP, or a SP from an efficiently translated mRNA[5] showed that signal peptides guide IL-15 to alternative destinations through a very complex pattern of intracellular trafficking. The alternative SSP-IL-15 isoform does not enter the endoplasmatic reticulum (ER) and is not secreted. Rather, SSP-IL-15 appears diffusely in the cytoplasm and enters the nucleus[50, 51, 66]. The LSP-IL-15, on the other hand, appears to have a more complex pattern of intracellular trafficking into diverse cellular compartments, including the ER, cytoplasm, and nucleus. Initially, the LSP-IL-15 isoform was observed in the ER and appeared to be largely secreted[77]. More recently, however, experiments using combinations of the LSP and the mature proteins IL-2, IL-15, and green fluorescent protein revealed three different trafficking pathways for the classical transcript[41]. In one pathway, the LSP was unprocessed and IL-15 was not glycosylated, and it remained predominantly in the cytoplasm, where it was degraded. To a lesser extent, IL-15 was also found in the nucleus, where it appeared to have entered by diffusion rather than by an active process[41]. The second trafficking pathway involved ER entry, N-linked glycosylation, and alternative partial LSP processing. Noteworthy, this partial LSP cleavage is not equal to nor does it result in the SSP generated by alternative splicing (see above). This partially processed form might possibly reside in vesicles for subsequent intracellular release or, alternatively, for secretion with a final processing step occurring at its terminal stage[41]. The third pathway is the classical pathway of secretion that involves entry in the ER, followed by glycosylation, complete processing, and ultimately secretion[41].

The complex intracellular trafficking patterns of LSP-IL-15 with its impediments to translation as well as to secretion may be required because of the inflammatory potential of IL-15. In terms of a more positive role, Kurys et al.[41] propose that intracellular infection may relieve the burdens on translation and intracellular trafficking to yield mature IL-15. A similar scenario might be at the origin of the heightened IL-15 production and secretion that is observed in several autoimmune and chronic inflammatory pathological conditions, such as rheumatoid arthritis.

GETTING CONNECTED: IL-15 AND ITS RECEPTOR

Cytokines mostly show functional redundancy and pleiotropy, both explainable by shared usage of receptor subunits. Several cytokines share the γ_c receptor, including IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, and IL-21, all of which use additional receptor subunit(s) for specificity of signaling[17, 31, 43, 52]. The IL-2/15Rβ and γ_c chains are shared between IL-2 and IL-15 and are essential for the signaling of these cytokines[27, 29]. Neither IL-2Rα nor IL-2/15Rβ chains appear to be strictly required for IL-15 binding[14]. The $\beta\gamma_c$ complex constitutes, on its own, a functional receptor with intermediate affinity for cytokine signaling through both the Jak1/Jak3 and Stat3/Stat5

pathways[32]. Although IL-15 binds with intermediate affinity to the $\beta\gamma_c$ complex, its specific high-affinity receptor on T and natural killer (NK) cells consists of three chains, namely IL-15Rα, IL-2/15Rβ, and γ_c molecules. The IL-15Rα chain binds specifically to IL-15 and has structural similarity to IL-2Rα/CD25, its counterpart in the IL-2R system. The IL-2Rα and IL-15Rα molecules have a similar ligand-binding motif (i.e. Sushi domain) as well as a short intracellular tail (13 AA for human IL-2Rα, 41 AA for human IL-15Rα). It is widely accepted that the IL-2Rα chain does not participate in signal transduction by the IL--2R. Likewise, IL-15Rα may not be involved in signal transduction by IL-15 in T/NK cells in the context of the hetero-trimeric IL-15R[2]. In contrast to IL-2Rα, which displays a low affinity for IL-2 (K_d=10 nM) and is expressed mainly on activated T cells, IL-15Rα has a high affinity for IL-15 (K_d=10-50 pM) and its mRNA has a wide tissue distribution. Using tagged IL-15, IL-15Rα expression was shown to be expressed on (mitogen)-activated macrophages, NK cells, and $CD4^+$ and $CD8^+$ T cells, but not on resting cells[14]. Thus, while resting cells express IL-15Rα mRNA, receptor subunit expression on the membrane is only detectable after stimulation.

MEMBRANE-BOUND OR MEMBRANE-ANCHORED?

Except for pathological and chronic inflammatory conditions, almost no IL-15 could be detected in the serum or other body fluids (see above). While the extensive posttranscriptional control might be responsible for the discrepancy between transcript expression and biologically active IL-15 in serum, a membrane-bound form of the cytokine might equally explain this discrepancy. In $CD14^+$ monocytes, intracellularly stored IL-15 protein is mobilized to the plasma membrane upon LPS or GM-CSF stimulation[59]. This process is independent of *de novo* transcription and translation, and thus allows a rapid availability of biologically active IL-15. Interestingly, this membrane-bound IL-15 appeared more potent than the soluble form. Membrane-bound IL-15 may promote clustering of its cognate receptor, resulting in an enhanced signal compared with its soluble counterpart. In addition, IFN-γ treatment of human monocytes strongly and significantly upregulates cytoplasmic and membrane-bound IL-15[57]. In terms of mode of attachment to the plasma membrane, Musso et al.[57] proposed that IL-15 was present as an integral membrane protein, not in a receptor-bound manner. However, Dubois et al.[21] countered this hypothesis by stating that the monocyte-expressed IL-15 is not directly anchored in the plasma membrane, but is bound indirectly by association with IL-15Rα. Technical issues appear to account for this discrepancy. Using a more acidic buffer for elution than Musso et al.[57], Dubois et al.[21] showed that IL-15 was non-covalently attached to the monocyte surface. Conceivably, the very strong affinity of IL-15Rα for IL-15 required highly acidic conditions to disrupt the IL-15/IL-15Rα complex.

Recently it was reported that membrane-bound IL-15 is constitutively expressed on the cell surface of the PC-3 human prostate carcinoma cell line and on IFN-γ-activated monocytes[7]. Results obtained using techniques similar to those of Dubois et al.[21] suggested that IL-15 exists on the surface of activated monocytes in both the membrane-anchored and the IL-15Rα-bound forms, whereas PC-3 cells express only membrane-bound IL-15.

Taken together, these data support the notion that IL-15 is stored intracellularly in resting monocytes. Once activated, monocytes express IL-15 on the membrane both as a membrane-anchored and as part of a IL-15/IL-15Rα ligand/receptor complex. Membrane-bound IL-15 may promote receptor clustering, and might be more potent than soluble IL-15. Alternatively, because of the high affinity of IL-15Rα for IL-15 ($K_a=1\times10^{11}M^{-1}$), IL-15Rα may act as a molecular sink for excess IL-15, or it may associate with receptor components yet to be identified[29, 37].

CO-REGULATION OF IL-15 AND IL-15Rα

If IL-15 were expressed on the surface of activated monocytes via binding to the high-affinity IL15Rα, a physiologically attractive combination would arise when IL-15 and IL-15Rα are co-expressed. Indeed, in contrast to IL-2 and IL-2Rα expression, IL-15 and IL-15Rα exhibit a largely overlapping and broad expression pattern[2, 30]. Besides, while both IL-15 and IL-15Rα mRNA have been detected in resting cells, the corresponding proteins appear to be available extracellularly only after activation of the cells[14, 49]. The promoter regions of both genes share critical transcriptional motifs: one NFκB site and two IFN regulatory elements (IRE)[3, 47]. Therefore, a common inflammatory stimulus may potentially induce both molecules in the same cell. This is corroborated by the simultaneous increase in expression of IL-15 and IL-15Rα-chain mRNA in splenic dendritc cells (DCs) from mice inoculated with dsRNA (poly (I:C)), LPS, or IFN-α/β and in purified murine splenic DCs treated with IFN-α/β *in vitro*[49]. Both transcripts are also coordinately upregulated in monocytes stimulated with LPS and IFN-γ[4, 47].

More recently, Waldmann's group generated a monoclonal anti-IL-15Rα antibody, thus allowing simul-

taneous monitoring of the membrane expression of IL-15 and IL-15Rα directly by flow cytometry[21]. The results demonstrated an upregulation of IL-15 and IL-15Rα protein in a concerted fashion on the membrane of IFN-γ- and LPS-stimulated monocytes, resulting in the formation of an IL-15/IL-15Rα complex on the monocyte surface[21].

TRANSPRESENTATION OF IL-15 VIA IL-15Rα

Passing IL-15 signals from one cell to another

The occurrence of membrane-bound IL-15 as an IL--15/IL-15Rα complex raises questions on the mode of action of IL-15 and how cells can benefit from this mode of action. In an elegant IL-15R subunit transfection study, Dubois et al.[21] showed that the stable IL-15/IL-15Rα complex leads to a continued presentation of IL-15 on the plasma membrane even after withdrawal of IL-15 from the medium. This retained IL-15 promoted a prolonged proliferative response of IL-15Rα-transfected CTLL-2 cells[21]. Moreover, transendosomal recycling of the IL-15/IL-15Rα complex, a process dependent on the cytoplasmic part of IL-15Rα, led to a continuous reappearance of biologically active IL-15 on the cell surface. Interestingly, in terms of intercellular communication, the IL-15Rα/IL-15 complex could be presented in trans to adjacent cells bearing only the intermediate-affinity IL-15Rβ/γ_c[21]. This resembles the transpresentation of IL-2 via IL-2/IL-2Rα complexes on donor cells to intermediate-affinity IL-2Rβ/γ_c receptor complexes on target cells[23].

Physiological impact of transpresentation

While these first studies mainly highlighted the possibility of transpresentation of IL-15, the presence of such a complex on activated monocytes, DCs, or even stromal cells implies a broad physiological impact. The concept of transpresentation may therefore explain why normal CD8$^+$ T cells transferred to an IL-15Rα$^{-/-}$ host lose their proliferative capacity to IL-15, whereas IL-15Rα$^{-/-}$ CD8$^+$ T cells do proliferate in a normal host environment[45]. At first, a positive feedback loop via IL-15Rα on other cells stimulating further IL-15 secretion was believed to be responsible. However, this seems unlikely, as IL-15 itself does not induce IL-15 mRNA (at least not in macrophages[82]) and no difference in total IL-15 mRNA levels[44] or the IL-15 isoform encoding secreted IL-15[11, 49] was detected between spleens from IL-15Rα$^{+/-}$ (wild-type) and IL-15Rα$^{-/-}$ mice. Therefore, it is more likely that CD8$^+$ T cells require transpresentation of membrane-bound IL-15 by monocyte/dendritic or stromal cells (environmental cells). Obviously, the environmental cells in the IL-15Rα$^{-/-}$ are incapable of transpresentation. In contrast, environmental cells from normal mice can efficiently (trans)-present IL-15 to CD8$^+$ T cells even if these T cells themselves do not express IL-15Rα. This concept of non-cell-autonomous transpresentation was shown to be valid for the generation and maintenance of memory CD8$^+$ T lymphocytes[11]. Similarly, the survival of NK cells was dependent on non-NK-cell IL-15Rα expression[38]. These accumulating data in fact buttress the possibility that transpresentation is the predominant mechanism by which IL-15Rα supports IL-15-dependent immune cells *in vivo*.

Interestingly, plate-bound IL-15Rα can also serve as a platform to present IL-15 to CD8$^+$ memory T cells *in vitro*, thereby indicating a direct action of transpresented IL-15 on the target cells[11]. This argues against a strict necessity for other signals from accessory cells (e.g. IL-7). Such an *in vitro* transpresentation system is also of interest for unraveling IL-15 signaling mechanisms, allowing distinction between soluble and transpresented IL-15 effects as well as between IL-15-dependent and -independent signals.

The source of transpresented IL-15/IL-15Rα complex changes with the responding cell type

Using bone marrow chimeras it was shown that the source of the cell transpresenting IL-15 via the IL-15Rα subunit changes with the target cell receiving the signal[75, 76] (Table 1). As such, memory CD8$^+$ T cell proliferation requires a bone marrow (BM)--derived (radiation-sensitive, hematopoietic) cell type to express IL-15Rα[11, 75]. Naive CD8$^+$ T develop-

Table 1. Source of IL-15 and IL-15Rα is determined by the lymphoid subsets receiving transpresented IL-15/IL-15Rα signals

Responding cell type	IL-15Rα	IL-15
Naive CD8$^+$ T cell development	BM-derived **or** parenchymal	?
Memory phenotype CD8$^+$ T cell	spleen: hematopoietic only lung: both BM-derived **and** parenchymal	BM-derived **or** parenchymal
TCRγδ IEL development (especially CD8αα Thy1$^-$ and Vγ5$^+$)	parenchymal	parenchymal
NK, NK-T maturation	hematopoietic more than parenchymal	hematopoietic more than parenchymal

Several lymphoid cell subsets are dependent on IL-15 signals for development and/or survival. The cellular origin of transpresented IL-15/IL--15Rα complexes changes with the responding cell subset[11, 38, 76, 77].

ment and NK and NK-T cell generation and maintenance depend on IL-15Rα that is present on BM or parenchymal (radiation-resistant) cells[38, 76]. Development of intestinal intraepithelial lymphocyte (iIEL) subsets is completely dependent on parenchymal transpresentation[76]. The generation and maintenance of memory CD4$^+$ T cells is not strictly dependent on IL-15 and, consequently, IL-15 transpresentation[78]. So employing CD4$^+$ T cell adoptive transfer to IL-15Rα$^{-/-}$, as done for strictly IL-15-dependent cell types, cannot reveal whether the IL-15 transpresentation mechanism is valid during CD4$^+$ T cell survival and/or activation. IL-15 signals can, however, be advantageous in certain stages of CD4$^+$ T cell life[19, 79]. At that time, transpresentation might be of significance. Taken together, *in vivo* survival and/or homeostasis of IL-15-dependent cells are assured by periodic exposure of these cells to both hematopoietic and/or non-hematopoietic cells presenting the IL-15/ /IL-15Rα complex in trans.

Why transpresent IL-15?

The observation that IL-15 can be transpresented to opposing cells challenges the traditional view of how cytokines mediate their effects. As mentioned earlier, IL-15 is widely expressed at the transcript level, but multiple posttranscriptional constraints effectively hamper its extracellular emergence. Given the pro-inflammatory nature of IL-15 and its role in autoimmune diseases as rheumatoid arthritis, membrane-associated IL-15, either membrane-anchored or IL-15Rα-bound, might represent just another regulation of IL-15 activity. Upon infection, IL-15 protein can be trafficked to endosomes where IL-15/IL--15Rα complexes are preformed before exposure on the plasma membrane. Alternatively, secreted IL-15 is trapped on the cell surface by IL-15Rα. Because the complex is present in a cellular context and not just as a soluble factor, intercellular communications via adhesion molecules and/or T cell receptor (TCR):MHC interactions allow control on the target specificity of the IL-15 effect. However, an imbalance in the equilibrium between IL-15 and IL-15Rα might result in pathological conditions. When secretion of IL-15 is excessive, an insufficient IL-15Rα supply entails that IL-15 no longer can be trapped by IL-15Rα on the membrane or in preformed (endosomal) complexes. IL-15, now present in a soluble form in the serum or tissue, can act without control on cellular specificity otherwise imposed by transpresentation. Consequently, other cell types might respond to the secreted pro-inflammatory IL-15, leading to positive feedback loops that result in aggravation of the inflamed condition and (auto)immune disease.

LESSONS FROM IL-15 KNOCK-OUT AND TRANSGENIC MICE

In vivo deficiency

A first clue to the necessity of IL-15 in NK cell development originated from IRF$^{-/-}$ mice, which lack inducible IL-15 expression[64, 65]. Further, significant lymphopenia is observed in mice with targeted genetic alterations in IL-15Rα, created using a targeting construct designed to delete exons 2 and 3 of the IL-15Rα gene; these exons encode ligand-binding and proline/threonine-rich extracellular domains[44]. These IL-15Rα$^{-/-}$ C57BL/6 mice were deficient in NK cells, due to both developmental and functional defects, and deficient in NK-T cell differentiation (Table 2). This was confirmed more recently by Wu et al.[81] using a targeting construct designed to delete exon 1 of the IL-15Rα gene. These mice exhibited a great reduction in thymic and peripheral NK-T (CD3$^+$DX5$^+$) and NK cells (CD3$^-$DX5$^+$), as did IL-15$^{-/-}$ mice generated using a gene-targeting vector to delete IL-15 exons 3–5[15, 36].

Total intraepithelial lymphocytes (IELs), and especially CD8αα TCRγδ IELs, were reduced in numbers in IL-15Rα$^{-/-}$ mice with targeted exons 2 and 3[44]. Using different parameters, the predominant decrease of specific IEL populations that arise or mature independently of the thymus was largely confirmed in IL-15$^{-/-}$ mice[36].

In the adaptive immune compartment, B cell numbers in thymus, fetal liver, bone marrow, or spleen were not affected in both types of IL-15Rα$^{-/-}$ mice[44, 81] and in IL-15$^{-/-}$ mice[36], indicating that IL-15 is not required for the development and maintenance of B cells.

Results from the thymic characterization of IL-15Rα$^{-/-}$ and IL-15$^{-/-}$ mice are different. In exon 2–3-deleted IL-15Rα$^{-/-}$ mice[44], thymic hypocellularity was present in all single-positive CD8$^+$, single-positive CD4$^+$, and double-positive CD4$^+$CD8$^+$ T cell fractions, but the reduction in single-positive CD8$^+$ was clearly the most pronounced. In contrast, exon 1-deleted IL-15Rα$^{-/-}$ mice showed normal cell numbers and composition of the thymocyte compartment[81], as did IL-15$^{-/-}$ mice[36].

In the peripheral T cell compartment, both IL-15Rα$^{-/-}$ [44, 81] and IL-15$^{-/-}$ [36] mice showed mainly a significant reduction in CD8$^+$ T cells that was most drastic in the memory CD8$^+$ T cell subset. The effect on CD4$^+$ T cells, however, might have been underestimated and requires a closer look. In exon 2–3-delet-

Table 2. Comparison of different IL-15Rα$^{-/-}$ and IL-15$^{-/-}$ mice

	IL-15Rα$^{-/-}$ (exon 2–3) Lodolce et al.[44]	IL-15Rα$^{-/-}$ (exon 1) Wu et al.[82]	IL-15$^{-/-}$ Kennedy et al.[36]
Innate			
NK cells	deficient	great reduction	reduced
NK-T cells	deficient	great reduction	reduced
IELs			
total	reduced to 50%	NT	reduced to 50%
TCRαβ:TCRγδ (WT 1:1)	in total IEL: 7:1	NT	in CD3$^+$ IEL: 2:1
Thy-1$^-$ IEL (WT 50%)	NT	NT	5% of CD3$^+$ IEL
TCRαβ CD8αα	NT	NT	reduced
TCRγδ CD8αα	reduced 5–10-fold	NT	NT
Adaptive thymus	hypocellularity	normal numbers	normal thymic development
SP CD8$^+$	hypocellularity (most pronounced)	normal composition	normal composition
SP CD4$^+$	hypocellularity	normal composition	normal composition
DP CD4$^+$CD8$^+$	hypocellularity	normal composition	normal composition
Lymph node		30% fewer LN cells	30% fewer LN cells
B cells	not affected	not affected	not affected
CD3$^+$	reduced to 50%		
CD4$^+$	reduced to 75%	10–15% decrease	not affected
CD8$^+$	reduced to 25%	reduced to 40%	reduced to 40%
memory CD8$^+$ T cells	dramatically reduced	CD44^{hi}CD8$^+$: reduced to <30%	
Spleen		similar white cell numbers	similar cell numbers
B cells	not affected	not affected	not affected
CD4$^+$	reduced to 55%	30% increased	not affected
CD8$^+$	reduced to 30%	reduced to 50%	reduced to 50%
memory CD8$^+$ T cells	dramatically reduced		reduced to 20%

NT – not tested, WT – wild-type, LN – lymph node, DP – double-positive, SP – signal peptide, IEL – intraepithelial lymphocyte.

ed IL-15Rα$^{-/-}$ mice, peripheral lymph node (LN) cellularity was reduced to approximately 50% of that of littermate controls, but both IL-15Rα$^{-/-}$ and wild-type had a similar CD3$^+$ T cell percentage. The CD4 and CD8 portions were 37 and 24% in IL-15Rα$^{-/-}$ mice compared with 55.4 and 11.2% in wild-type controls, respectively. This indicates that the absolute LN CD4$^+$ T cell numbers were 75% of the littermate control. Although this is less dramatic than the reduction of CD8$^+$ T cells to 25% of wild-type mice, it does not preclude a role for IL-15 in CD4$^+$ T cell maintenance. Likewise, total splenic cell numbers were reduced by 20%, but the CD3$^+$ portions dropped from 30% for wild-type to 20% for IL-15Rα$^{-/-}$ mice. Within this T cell compartment, the respective CD4 and CD8 proportions changed from 18.7 and 9.1% in the wild-type to 13 and 3.9% in the IL-15Rα$^{-/-}$ mice. Recalculating the absolute splenic cell numbers reveals that CD4$^+$ T cell numbers dropped to about 55% and CD8$^+$ T cell numbers decreased to 30% of wild-type controls. So while it was concluded by the authors that CD8$^+$ T cell development and memory CD8$^+$ T cells are selectively compromised, the calculations above, showing reduced absolute CD4$^+$ T cell numbers in thymus, spleen, and LN, indicate a role for IL-15 in the development, differentiation, and maintenance of CD4$^+$ T lymphocytes as well. The exon 1-deleted IL-15Rα$^{-/-}$ mice showed a 50% decrease in splenic CD8$^+$ T cells, but a contrasting 20–30% increase in CD4$^+$ T cells[81]. Finally, 30% fewer total LN cell numbers and a small increase (7%) in CD4$^+$ T lymphocyte percentage was reported, suggesting a 15% decrease in absolute LN CD4$^+$ T cell numbers, corroborating the calculations shown above. CD4$^+$ T cell generation and peripheral survival was not affected in IL-15$^{-/-}$ mice[36].

Thus the exon 1-deleted IL-15Rα$^{-/-}$ show a less severe phenotypic abnormality than the exon 2–3-targeted IL-15Rα$^{-/-}$ mice, and bear greater resemblance to the IL-15$^{-/-}$ engineered by Kennedy et al.[36]. The different immunological constitutions generated by targeting IL-15 or IL-15Rα, by deleting exon 1 or exon 2–3, raise a number of questions on the mechanism(s) behind these phenotypic differences. Answering these questions will require a systematic comparison of IL-15$^{-/-}$ and IL-15Rα$^{-/-}$ mice with exactly matched genetic backgrounds and housed under comparable conditions.

In conclusion, although these gene knock-out experiments clearly identify a number of lymphoid cells that are strictly dependent on IL-15/IL-15Rα for differentiation and/or survival, the data obtained from

IL-15Rα$^{-/-}$ mice do not exclude a function for IL-15 in CD4$^+$ T cell development and/or maintenance, while IL-15$^{-/-}$ indicate that IL-15 is not uniquely required in these processes. Conceptually it is important to emphasize that the function of a molecule such as IL-15 is not simply the inverse of the phenotype seen when that molecule is absent, since necessity and sufficiency are not always coupled in physiological processes.

In vivo abundance

An alternative approach to elucidating the potential roles of IL-15 includes genetic engineering of IL-15 transgenic (Tg) mice[25, 48, 63] (Table 3). In summary, four types of IL-15 Tg mice were created. While some compared the *in vivo* usage of the long or short signal peptide[63], others transgenetically introduced constructs to obtain efficient translation and secretion of IL-15[25, 48]. Fehniger et al. created an IL-15 Tg mouse strain using a construct with systematic elimination of the three primary posttranscriptional checkpoints[25, 26]. In these IL-15 Tg mice, the percentages and absolute numbers of NK cells are consistently and significantly increased. Moreover, these mice exhibit peripheral blood lymphocytosis with significant expansion of both NK cells and memory phenotype CD8$^+$ TCRαβ T cells early in life. This increase in lymphocyte numbers leads to the development of fatal lymphocytic leukemia with age. The lymphocyte expansions are predominantly clonal, but in the remainder of IL-15 Tg mice that lack clonal lymphocytic expansion and that are otherwise characterized by an identical clinical course, other processes, such as autoimmune reactions, may contribute to the observed polyclonal lymphocyte expansions and multi-organ infiltration. While CD8$^+$ T cells were significantly increased and showed a mem-

Table 3. Design and phenotypes of mice transgenetically engineered to overexpress IL-15

	Fehniger et al.[25, 26]	Marks-Konczalik et al.[48]	Nishimura et al.[64]	
			normal IL-15 Tg	alternative IL-15 Tg
Design				
efficiency enhancement	elimination of the three primary transcriptional checkpoints (AUGs in 5'UTR, inefficient SP, negative regulator in C-terminus)	comparison LSP, IL-2 SP, preprolactin SP, fused to human IL-15 cDNA (mature peptide)	normal exon 5 (**LSP**)	alternative exon 5 (**SSP**)
promotor	MHC class I:	human EF-1α:	MHC class I:	MHC class I:
IL-15 levels	186.7 ± 41.8 pg/ml*	650–800 pg/ml*	constitutive significant amount of IL-15	large amount of IL-15, not secreted
Innate				
NK cells	10-fold increase ($DX5^+CD3^-$ in peripheral blood)	BM, spleen and liver: 10×, 2× and 2× ↑ respectively ($NK1^+CD3^-$); thymus: no increase	spleen: unaltered ($NK1.1^+CD3^-$)	reduced to 70%
NK-T cells (in lymphocytes)		BM, spleen, liver, and thymus: 7×, 2×, 3×, and 3× ↑, respectively	spleen: unaltered ($NK1.1^+CD3^+$)	spleen: 2-fold ↓
γδ DETC	NT	10-fold increase	NT	NT
Adaptive				
$CD8^+$ T cells	peripheral blood: 10-fold increase; mostly memory ($CD44^{high}$ $CD62L^{low}$ $CD69^-Ly6C^{high}$)	BM, spleen, liver, and thymus: NT, 2× ↑, 2× ↑, and unaltered, respectively; mostly memory type	lymph node: 1.5× ↑ (in $CD3^+$); memory $CD8^+$ 2.5× ↑	unaltered
$CD4^+$ T cells	peripheral blood: absolute numbers unaltered	BM, spleen, liver, and thymus: unaltered	lymph node: slight reduction (in $CD3^+$); memory $CD4^+$: slight increase	unaltered
Other features	progressive alopecia and multi-organ lymphocytic infiltration	resistance to AICD by $CD4^+$ T cells	resistant to *Salmonella* infection (due to IFN-γ production)	susceptible to *Salmonella* infection (impaired IFN-γ production)

AICD – activation-induced cell death, BM – bone marrow, LSP – long signal peptide, SSP – short signal peptide, UTR – untranslated region, EF – elongation factor, NK – natural killer, DETC – dendritic epidermal T cell, NT – not tested. *Determined by human IL-15 ELISA.

ory phenotype ($CD44^{high}CD62L^{low}$ $CD69^{-}Ly6C^{high}$), there was no difference in absolute $CD4^+$ T cell counts between IL-15 Tg and wild-type mice.

These results confirmed the work of the laboratory of Waldmann and Tagaya[80], who have been instrumental in IL-15 research throughout the years. IL-15 Tg mice expressing human IL-15 cDNA with the prepro-lactin SP driven by the human EF-1α promoter displayed increased NK cells, $CD44^{high}CD8^+$ memory T cells, and γδ T cells[48]. Remarkably, overexpression of IL-15 did not lead to a rise in NK cells or $CD8^+$ T cells in the thymus. However, in BM, spleen, and liver, NK cells ($NK1.1^+CD3^-$) were increased, as were $CD8^+$ T cells in the secondary lymphoid organs. These $CD8^+$ T cells showed a memory phenotype. Although $CD4^+$ T cell numbers were unaffected in IL-15 Tg mouse, the overproduction of IL-15 did affect their function. In an activation-induced cell death (AICD) assay, $CD4^+$ T cells from IL-15 Tg mice were less susceptible to apoptosis than those from wild-type mice. Moreover, this resistance of IL-15 Tg $CD4^+$ T cells to AICD was abrogated using an anti-IL-15 antibody, thus suggesting that this phenotype was reversible. In support, our laboratory demonstrated that IL-15 treatment induced resistance to AICD in $CD4^+$ T cells[19, 20].

Another study addressed the role of alternatively spliced isoforms of IL-15[63]. Thus, IL-15 Tg mice were engineered to express murine IL-15 with LSP (usage of normal exon 5) or SSP (usage of alternative exon 5) under the control of MHC class I promoter. The normal IL-15 isoform with the LSP was already shown to be secreted in cell culture supernatants[77]. In accord, LSP IL-15 Tg mice constitutively produced significant amounts of IL-15. On the other hand, in SSP IL-15 Tg mice a large amount of intracellular IL-15 protein was detected, but little was secreted. As a consequence, these SSP IL-15 Tg mice closely resembled the wild-type mice. The LSP IL-15 Tg mice, however, possessed about a quarter more thymic cells at early age, with no preference for a particular thymocyte subset. Although cell numbers were unaltered, lymph nodes of LSP IL-15 Tg mice showed a preferential expansion of $CD8^+CD44^+$ cells, T cells with a memory phenotype ($CD25^-CD69^-Ly6C^+CD62L^+CD45RB^+$), a phenomenon not observed in SSP IL-15 Tg mice. Moreover, splenic NK ($NK1.1^+CD3^-$) and NK-T ($NK1.1^+$ $CD3^+$) cell numbers were doubled. Taken together, the LSP IL-15 Tg but not the SSP IL-15 Tg mice resembled the phenotype of the IL-15 Tg mice generated by Fehniger et al.[25] and Nishimura et al.[63]. The preferential expansion of the NK, NK-T, and $CD8^+$ T cell wing of cellular immunity in normal IL-15 Tg was reflected in an enhanced resistance to *Salmonella* infection. SSP IL-15 Tg mice were susceptible to *Salmonella* infection due to impairment of IFN-γ production. Interestingly, $CD4^+$ T cells were the main source of the protective IFN-γ production in LSP IL--15 Tg mice. Thus, besides protection against AICD, IL-15 arms $CD4^+$ T cells to help in clearing bacterial infection.

From these IL-15 overexpressing mice it can be concluded that IL-15 primarily sustains and enhances NK, NK-T, and memory $CD8^+$ T cell numbers, but fails to influence $CD4^+$ T cell numbers under steady--state conditions. Indeed, naive and memory $CD4^+$ T cells fail to express IL-2Rβ, thus excluding a role for IL-15 in their homeostatic proliferation[78]. However, this does not necessarily exclude IL-15 from any $CD4^+$ T cell function. As IL-15 Tg $CD4^+$ T cells exhibit increased resistance to AICD[48] and resistance to *Salmonella* of LSP IL-15 Tg mice is driven by IFN-γ derived from $CD4^+$ T cells, a role for IL-15 may be revealed under $CD4^+$ T cell stimulatory conditions. Additionally, faster activation kinetics of $CD8^+$ T cell responses may further enshroud IL-15 mediated modulation of $CD4^+$ T cell activation. Finally, systemic presence of IL-15 (transgenic or by administration) could be in contrast with the targeted IL-15 transpresentation as proposed by Dubois et al.[21].

IL-15'S INFLUENCES ON $CD4^+$ T CELL RESPONSES

Really a growth factor?

Originally, IL-15 was identified by 2 independent groups based upon its ability to stimulate proliferation of the IL-2-dependent CTLL-2 T cell line in the presence of neutralizing anti-IL-2 antibodies[6, 12, 30]. These initial studies suggested that IL-15 was just another T cell growth factor, exerting stimulatory activity on antigen (Ag)-dependent T cell clones[30], activated double-negative and double-positive thymocytes, $CD4^+$ and $CD8^+$ mature T cells[82], and epidermal γδ T cells[22]. Importantly, the outcome of addition of IL-15 to T cells depends on the T cell subset, the amount of IL-15, and the activation status of the T cells. IL-15 is considered mitogenic *per se* for $CD8^+$ T cells and indispensable for basal homeostasis-driven proliferation of memory phenotype $CD8^+$ T cells[25, 40]. However, the story for $CD4^+$ T cells is more complex, often concealed by differences in T cell activation status and IL-15 doses applied. First, as demonstrated by our group, the activation status of $CD4^+$ T cells critically determines the nature of IL-15 activity. In the absence of a simultaneous TCR stimulus, IL-15 has the ability to keep Ag-experienced $CD4^+$ T cells in a quiescent state, characterized by

$G_{0/1}$ arrest and decreased levels of CD25, CD69, and CD71[19]. Mechanistically, IL-15 leads to phosphorylation of Akt but not STAT-5, thus ensuring survival, but not proliferation (our unpublished results). This was confirmed in a human $CD4^+$ T cell clone, where IL-15 did not induce proliferation, but assured long-term survival of the cells[46]. Phenotypes of mice genetically engineered to overexpress IL-15 did not reveal any obvious effect of IL-15 on $CD4^+$ T cell numbers[25, 48, 63]. Thus, these "steady-state" phenotypes confirm the *in vitro* observations that IL-15 *per se* does not lead to proliferation of $CD4^+$ T cells. Furthermore, in the absence of TCR triggering, IL--15 initiated proliferation only when given at high doses[60]. Although supporting proliferation, these high IL-15 concentrations did not allow production of (effector) cytokines by type 1 and type 2 $CD4^+$ T cells (and $CD8^+$ T cells)[60]. Finally, in the presence of TCR triggering, IL-15 enhanced $CD4^+$ T cell proliferation. As such, IL-15 increased the proliferative response of human peripheral blood lymphocytes stimulated with PHA, ConA, or immobilized anti--CD3 monoclonal antibodies[9, 16, 30, 39]. Also, Ag/APC--stimulated murine primary $CD4^+$ T cells showed an enhanced proliferative response in the presence of IL-15, even at low doses[19]. In these conditions, IL-15 increased effector cytokine production[55] of human effector memory $CD4^+$ T cells (and $CD8^+$ T cells). It was suggested that a similar IFN-γ upregulation plays a role in the resistance of IL-15 transgenic mice to *Salmonella* infection[63]. These effects of IL-15 on activated $CD4^+$ T cells may explain the recent observation in rhesus macaques that IL-15 enhanced the generation of tetanus toxoid-specific effector $CD4^+$ T cells[79], indicating the potential use of this IL-15 effect for $CD4^+$ T cell-directed vaccinations.

In summary, in the case of $CD4^+$ T cells, low amounts of IL-15 *per se* do not lead to proliferation, but act as a viability preserving factor. At high concentrations, IL-15 is able to support proliferation, but not effector cytokine production. In combination with a TCR--trigger, IL-15 markedly enhances both proliferation and effector cytokine production. An analogous outcome of IL-15 effects is also observed in murine iIEL, where IL-15 stimulation promotes cell proliferation only when combined with TCR signals, but not when applied alone.

Viability-preserving factor

Although IL-15 *per se* is not stimulatory for $CD4^+$ T cells, IL-15 does transfer survival signals to these cells. Thus, IL-15 can prevent spontaneous cell death of activated T cell lines[71], human $CD4^+$ T cells[73], primary $CD4^+$ T cells[19], and a $CD4^+CD7^-$ memory subset[68]. IL-15 is also very potent in rescuing synovial T cells from apoptosis *in vitro*[74]. In these cases, IL-15 does not protect from passive apoptosis by inducing proliferation, but acts as a genuine survival factor. More important, the anti-apoptotic effect of IL-15 can protect against active apoptosis-inducing stimuli. It was suggested that IL-15 can block TNFRp55-mediated apoptosis in fibroblasts by blocking protein-protein interactions of the TNFRp55 with adaptor proteins, by inhibiting the recruitment of cytosolic adaptor proteins to the stimulated TNFRp55, and/or by dissociating already bound adaptor proteins from the TNFRp55 complex[8]. In this fibroblast test system, IL--15Rα was shown to scavenge TRAF-2, but not FADD, TRADD, or RIP[8]. However, it is not known whether this protective mechanism is valid in $CD4^+$ T cells.

FasL/Fas-mediated inhibition of $CD4^+$ and $CD8^+$ antiviral T cells by viral Ag-positive "veto" cells was restored by the addition of IL-15 to the coculture, leading to restoration of the anti-retroviral response[70]. While IL-15 protects human activated T and B cells *in vitro* from apoptosis induced by anti--Fas, anti-CD3, dexamethasone, and/or anti-IgM in a manner dependent on RNA-synthesis[10], its anti-apoptotic activity *in vivo* is less clear. *In vivo*, IL-15 rescues hepatocytes from a lethal Fas-mediated -apoptosis[10]. In contrast, some reports show no protective effect of IL-15 on Fas-induced apoptosis of $CD4^+$ T cells[58], whereas others report that IL-15 potently inhibits CD95/Fas-induced apoptosis of effector-memory $CD4^+$ and $CD8^+$ T cells from HIV--infected individuals[55]. This discrepancy might be explained by the focus on effector memory $CD4^+$ T cells in the latter study, thereby enlarging a protective effect that may be concealed in the former study.

The CD95 (APO-1/Fas) system also plays a critical role in AICD of T cells, in which excessive activation through the TCR results in apoptosis. Fas/FasL interaction is believed to be the actual trigger of TCR--induced death in mature T lymphocytes[34]. In our laboratory it was shown that IL-15 protects $CD4^+$ T cells from AICD induced by a tolerizing immobilized anti--CD3 stimulus. However, the rescued $CD4^+$ T cells were converted to an anergic phenotype[20], showing hypo-responsiveness to TCR stimuli, but capable of proliferation in response to high-dose IL-2. Clearly, Fas/FasL-induced apoptosis is extremely important for the maintenance of tolerance in the secondary lymphoid organs, and sensitization for this Fas death pathway by IL-2 is accordingly a key regulatory event in avoidance of autoimmunity. Inhibition of this tolerizing death pathway by IL-15 might result in the establishment of a less absolute form of tolerance, namely anergy instead of depletion.

Taken together, the growth-promoting activity of IL-15 during TCR stimulation of $CD4^+$ T cells, combined with the protection against AICD, allows a larger amount of Ag-specific memory $CD4^+$ T cells to remain present after an immune response. These cells can be kept alive by IL-15, which protects them from spontaneous apoptosis. Thus, IL-15 co-administration can be beneficial in vaccination strategies, as was already shown in a pilot experiment on rhesus macaques[79]. A drawback to this combined growth-promoting and anti-apoptotic activity is obviously the possibility of allowing autoimmune responses to emerge. For instance, rheumatoid arthritis is an autoimmune disease that combines augmented levels of IL-15 with a strong $CD4^+$ T cell response. In a model of collagen-induced arthritis, blocking IL-15 using soluble IL-15Rα profoundly suppressed the T_{H1} cell-mediated anti-collagen response[72], indicating an important role for IL-15 in autoimmune $CD4^+$ T lymphocyte responses.

CONCLUSIONS

IL-15, a cytokine discovered as a T cell growth factor, shows distinct functions that recommend its inclusion in immune therapies. While IL-15 is abundantly present in the body as a transcript, its presence in serum or culture supernatant could hardly be demonstrated. However, recent data from several groups showing that IL-15 protein is present extracellularly as a membrane-anchored form[57, 59] and/or a transpresented form via IL-15Rα[21] may explain these detection problems. In addition, this cell-constrained expression of IL-15 might stand for a built-in control on the broad activities of the cytokine, ranging from broadly pro-inflammatory to growth stimulatory and anti-apoptotic for T cells. In the case of IL-15Rα-mediated transpresentation, an overproduction of IL-15 in excess to IL-15Rα may allow roaming by the cytokine, targeting every cell type it encounters. That would allow uncontrolled inflammatory responses to be initiated, in turn leading to T cell responses that get out of control due to increased growth-factor activity and decreased susceptibility to AICD. Subsequently, effector cytokines produced by these activated T cells can stimulate other cell types, further aggravating inflammation and leading to pathology. Thus, diseases such as rheumatoid arthritis might result from a vicious cycle initiated by an excessive IL-15 stimulus.

From a positive viewpoint, the use of IL-15 in vaccination therapy might enhance $CD4^+$ T cell responses that are otherwise compromised due to age or infection, such as HIV. Indeed, IL-15 promotes growth of T cells – both $CD4^+$ and $CD8^+$ – and protects them from AICD that would normally reduce T cell numbers and shut down the immune response.

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