The unconventional expression of IL-15 and its role in NK cell homeostasis.

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Abstract

Natural killer (NK) cells are the founding members of the innate lymphoid cell (ILC) family and contribute to the rapid production of inflammatory mediators upon pathogen detection. The evolution of receptors for self MHC-I and stress-induced ligands also bestows upon NK cells an important effector role in the clearance of virus infected and transformed cells. NK cells are dependent on the pleiotropic cytokine IL-15 for their development, differentiation and optimal function. Here I review the regulation of IL-15 *in vivo*, its role in driving NK cell differentiation and discuss the function of NK cell diversification in regards to innate immunity.

Diverse IL-15 sources impact on NK cell homeostasis and differentiation

NK cell development in adults occurs in the bone marrow where lymphoid progenitors commit to making NK cells instead of B and T cells *via* extrinsic cues or stochastic gene expression[1, 2, 3]. Committed NK cell progenitors in the mouse have been identified by expression of the inhibitor of DNA-binding 2 (ID2) amongst $Sca1^{+}CD127^{+}CD117^{low}CD135^{-}$ cells[4]. This population also expresses markers of mature NK cells such as CD244 and CD27 but lacks expression of the lineage defining NK1.1 and NKp46 receptors. At this stage the β chain of the IL-2/15 receptor complex (IL-2Rβ, CD122) that is essential for IL-15 signal transduction and NK cell development and survival is not expressed[4, 5]. The ability to respond to IL-15 is a defining event in NK cell development and occurs at the NKP stage when pre-pro NK cells upregulate CD122. CD122 dimerizes with the common gamma chain (γ_c/IL-2Rγ/CD132) facilitating IL-15 binding and signal propagation via Jak1/Jak3 and STAT5α/β thus driving NK cell differentiation[6]. A third IL-15 receptor component, IL-15Rα, also exists and although IL-15-dependent cells can express heterotrimers (IL-15Rα/β/γ), intrinsic expression of IL-15Rα by these cells is not essential for their development[7 8 9]. This is in itself a curious result given IL-15Rα/β/γ binds IL-15 with 100-fold higher affinity than IL-15Rβ/γ[10 11, 12]

Given the critical role of IL-15 in NK cell homeostasis, it's worth outlining some key findings that have set the foundations of our understanding of this pathway. IL-15 was identified by its functional similarities to IL-2 in promoting T cell and NK cell proliferation, and signalling through an IL-2Rβ (IL-15Rβ) complex[13, 14]. IL-15 mRNA expression was detected in a broad range of tissues including adherent mononuclear blood cells, activated macrophages, epithelial and fibroblast cell lines, placenta and skeletal muscle [13] with a similar expression pattern being latter determined for IL-15Rα[11].

Germline deletion of IL-15 or IL-15Rα in mice resulted in a clear reduction in the frequency of IL-15-dependent immune cell including NK cells, NKT cells, γ/δ T cells and memory CD8 T cells[15, 16]. IL-15 dependent immune cell development and function was later shown to be rescued when IL-15$^{-/-}$ or IL-15Rα$^{-/-}$ haematopoietic progenitors were transplanted into wild-type mice or IL-15$^{-/-}$/IL-15Rα$^{-/-}$ mice or lymphocytes treated with IL-15 receptor agonists[8, 17, 18, 19, 20, 21 16, 22]. While for these findings multiple studies demonstrated that IL-15 dependent cells such as NK cells are not required to express IL-15Rα to respond to IL-15 *in vivo*, NK cell development from IL-15Rα$^{-/-}$ progenitors was significantly reduced compared to wild-type haematopoietic progenitors transplanted into either wild type, IL-15$^{-/-}$ or IL-15Rα$^{-/-}$ mice[8, 17, 18, 19, 20, 21]. These transplantation studies led to our current understanding of IL-15 trans-presentation (reviewed in [23, 24]) as they indicated that both haematopoietic and parenchymal cells could express IL-15 and IL-15Rα and that the same cells must express both these proteins to promote NK cell genesis. In this model, IL-15-expressing cells use IL-15Rα to chaperone IL-15 to the cell surface where it can bind IL-15 dependent, IL-15Rβ/γ expressing cells such as NK cells[25 17, 18, 19, 20, 21].

The "trans-presentation" mechanism of IL-15 expression that has evolved in vertebrates is fascinating and is still relatively poorly understood. We still do not know what factors regulate IL-15 and IL-15Rα expression in haematopoietic and parenchymal cells during steady-state and inflammation. The recent generation of an IL-15 green fluorescent protein reporter mouse strain using a bacterial artificial chromosome (BAC) approach may be useful in this regard as it now allows IL-15-expressing cells to be readily visualized by flow cytometry or fluorescence microscopy. Initial studies using IL-15 reporter mice have revealed that CD8^{+} dendritic cells, basophils and early haematopoietic progenitors all have high IL-15 promoter activity and vesicular stomatitis virus infection up-regulates IL-15 expression in an type 1 interferon receptor dependent manner; however non-haematopoietic cells have not yet been examined in detail[26, 27]. This will be essential as non-haematopoietic IL-15 trans-presentation can contribute to NK cell development and is essential for the development of other IL-15-dependent immune cells types including

CD8αα intraepithelial lymphocytes (IELs) and NKT cells[8] [24] [22] [28] [29]. Indeed, deletion of IL-15Rα specifically in dendritic cells (CD11c-Cre) or macrophages (*via* LysM-Cre) only resulted in a 50% reduction in peripheral NK cells, namely in the KLRG1^{+} population whereas the number of bone marrow NK cells was not affected[30]. In line with this, restricting IL-15 trans-presentation to CD11c^{+} cells *in vivo via* ectopic IL-15Rα expression under control of the CD11c promoter in IL-15Rα$^{-/-}$ mice only partially rescued NK cell development, mainly in the immature NK cell compartment[29], whereas restricting IL-15 trans-presentation to intestinal epithelial cells *via* the Villin promoter in the same model fully rescued IL-15 dependent CD8αα IELs[28]. These data suggest that different lineages are dependent on different sources (haematopoietic *versus* parenchymal cells) of trans-presented IL-15. In the case of NK cells, it is likely that parenchymal, CD11c^{+} cells and additional haematopoietic cells are required for this process. Given the numerous sources of tissue-derived IL-15 and the lack of reagents to detect trans-presented IL-15, the IL-15 reporter strain should prove a valuable tool in addressing IL-15 regulation *in vivo* [20, 21].

This unconventional method of IL-15 presentation permits additional levels of regulation for NK cell development. IL-15 bioavailability would ultimately be the net balance between transcriptional regulation and co-ordinated expression of *Il15* and *Il15Rα,* their trafficking and membrane presentation/cleavage/secretion by the same cell. In this setting, it is unclear to what degree membrane bound *versus* cleaved/secreted IL-15/IL-15Rα complexes might contribute to the development of NK cells at steady-state[25]. IL-15/IL-15Rα complexes have been shown to be pre-assembled within the endoplasmic reticulum/Golgi of TLR stimulated DCs before being released from these cells [25]. IL-15Rα is reportedly cleaved by TACE/ADAM17 together with trans-presented IL-15 [31] and soluble IL-15/IL-15Rα complexes account for all the soluble IL-15 detectable in human serum [32]. Alternate N- and C-terminal splicing of human IL-15Rα determines whether IL-15 is secreted or remains bound to the cell membrane [33]. IL-15/IL-15Rα complexes are also detected in the sera of wild-type mice; however,

detection of these complexes depended on first treating the mice with TLR ligands to stimulate dendritic cell (DC) activation [25]. This type of stimulations is known to up-regulate IL-15Rα expression on DC in a type-I IFN dependent manner [34]. A number of experimental approaches have been developed to mimic physiological IL-15 trans-presentation. These include pre-ligation of IL-15 to IL-15Rα-Fc (IL-15/IL-15Rα–Fc) and IL-15 linked to the sushi domain of IL-15Rα (RLI)[35, 36]. Treating mice with either of these reagents results in an accumulation of NK cells, particularly mature NK cells[36, 37, 38] and can rescue IL-15-dependent processes in IL-15Rα$^{-/-}$ mice[22]. Furthermore, these studies highlight that IL-15/IL-15Rα complexes are clearly more potent than soluble IL-15 alone *in vivo*. However whether secretion of soluble IL-15/IL-15Rα complexes is the predominant mechanism for IL-15 trans-presentation is still unclear.

There are also conflicting data concerning the ability of IL-15Rα to promote or suppress IL-15 activity *in vivo*. Using an animal model for Psoriasis in which a chronic inflammatory skin disorder is mediated by effector T cells (primarily CD8^{+} and γ/δ T cells) and IL-15, the authors note a surprising result in that IL-15Rα$^{-/-}$ mice develop more severe skin inflammation than wild type and IL-15$^{-/-}$ mice when treated with Imiquimod[39]. IL-15Rα was highly expressed on keratinocytes and soluble IL-15Rα released *via* proteolytic cleavage was sufficient to antagonize DC-derived IL-15 and subsequent inflammation[39]. While this result is consistent with other studies showing recombinant soluble IL-15Rα can antagonize IL-15 responses *in vivo* [40, 41, 42] it is surprising that IL-15-dependent inflammation can be induced at all in IL-15Rα$^{-/-}$ mice given that IL-15 transpresentation would not be possible in these mice and IL-15 dependent immune cells are also reduced [15]. Aberrant IL-15 and IL-15Rα expression has been implicated in various autoimmune disorders including arthritis, celiac disease and inflammatory bowel disease [43, 44, 45, 46, 47]. In these cases, elevated IL-15 or IL-15Rα expression correlated with pathogenesis and disease severity. Elevated soluble IL-15Rα has also been reported in patients with T cell large granular lymphocyte (T-LGL) leukaemia [48]. In some of these patients, higher IL-15Rα expression on the

surface of CD8 T cells and monocytes was observed and correlated with enhanced proliferation to IL-15 *in vitro* compared to normal controls. Collectively, these data suggest that IL-15Rα may both positively and negatively regulate IL-15 bio-availability *in vivo* depending on the context.

Irrespective of the source or whether it is membrane bound or soluble, trans-presented IL-15 drives NK cell proliferation and differentiation *in vivo*. This process begins in the bone marrow where newly generated immature (Mac-1$^-$) NK cells proliferate rapidly and differentiate into mature (Mac-1$^+$) NK cells[49, 50, 51, 52]. The bone marrow NK cell population is enriched for immature NK cells whereas peripheral lymphoid organs contain very few immature NK cells and instead possess two functionally distinct mature populations in terms of both cytotoxicity and pro-inflammatory cytokine production[53]. Peripheral NK cell subsets were first identified and characterised in man by the groups of Lanier and Phillips in the 1980's[54, 55, 56, 57, 58, 59]. A similar fractionation of conventional murine NK cells was not described until the early to mid 2000's, when differential expression of a number of cell surface receptors including Mac-1, CD27 and KLRG1 amongst peripheral NK cell subsets were found to correlate with clearly distinct NK cell responses[49, 50, 51, 52].

Curiously, NK cell subsets possess differential responsiveness to IL-15. Mac-1$^-$ NK cells proliferate to a greater extent than Mac-1$^+$ NK cells during steady state and following intravenous transfer into alymphoid *Rag2*$^{-/-}\gamma_c^{-/-}$ mice[60 49, 52, 61]. This is also true in response to soluble IL-15 *in vitro*[49]. Interestingly, NK cells do not proliferate when transferred into *Rag2*$^{-/-}$ mice despite the fact they lack T cell populations, which require IL-15 for survival (eg. NKT and CD122$^+$ CD8 T cells) and would potentially compete with NK cells for this factor [60]. This suggests that the presence of endogenous NK cells is a limiting factor in donor NK cell proliferation. Donor NK cells do survive when transferred into wild-type or *Rag2*$^{-/-}$ mice unless these mice lack IL-15 or IL-15Rα[62 20 63], suggesting the level of bioavailable IL-15 is sufficient to up-regulate anti-apoptotic Mcl-1 and mediate cell survival but

insufficient to drive donor NK cell proliferation. It is currently not resolved how the differential responsiveness to IL-15 arises during NK cell differentiation. Higher expression of IL-2Rβ has been reported on immature human NK cells compared to mature NK cells[64] and we observe a similar expression pattern in mouse (unpublished observation) however the levels of IL-15 signalling proteins or negative regulators of this pathway such as the SOCS family of proteins have not been investigated to date.

Following intravenous transfer into alymphoid *Rag2*$^{-/-}\gamma_C^{-/-}$ mice, immature NK cells rapidly differentiate into mature NK cells[49, 60] and it was using through using this *in vivo* transfer assay that we identifyied KLRG1 as a marker of mature, fully differentiated NK cells [49]. 13 days after transfer into *Rag2*$^{-/-}\gamma_C^{-/-}$ mice, 80% of donor splenic NK cells were KLRG1$^+$ whereas about only 30% were KLRG1$^+$ prior to transfer[49]. Subsequent investigations revealed that KLRG1 expression was acquired late in NK cell maturation and was restricted in NK cells that no longer expressed the TNF-receptor superfamily member CD27 [50] [49.] Based on the finding of Kim *et al* [52] that immature NK cells do not express Mac-1[52], it was then possible to identify three NK cells subsets: Immature (Imm; Mac-1$^-$CD27$^+$KLRG1$^-$), Mature 1 (M1; Mac-1$^+$CD27$^+$KLRG1$^-$) and Mature 2 (M2; Mac-1$^+$CD27$^-$KLRG1$^+$). Adoptive transfer of FACS purified Imm NK cells into *Rag2*$^{-/-}\gamma_C^{-/-}$ mice gave rise to all three NK cell subsets, whereby transfer purified M1, NK cells gave rise to M1 and M2 NK cells. Transfer of FACS sorted M2 NK cells only gave rise to M2 NK cells. Thus a strong precursor-product relationship exists between these subsets [49].

The current nomenclature defining the different murine NK cell subsets is as follows:

Imm NK cells are actively proliferating *in vivo*[52], undergo extensive homeostatic expansion following transfer into *Rag2*$^{-/-}\gamma_C^{-/-}$ mice and are highly sensitive to IL-15 concentrations *in vitro* [49]. These

cells are preferentially found in the bone marrow and represent the majority of NK cells in 1 week old mice, following NK cell depletion using diphtheria toxin (Ncr1-DTR mice) and following bone marrow transplant into sub-lethally irradiated recipient mice[49, 50, 65, 66]. These Imm NK cells also possess poor effector functions compared to mature NK cells (Mac-1$^+$)[52].

M1 NK cells (Mac-1$^+$CD27$^+$KLRG1$^-$) appear shortly after Imm NK cells in NK cell repopulation experiments mentioned above[49, 50, 65, 66] and are found in all organs with a preference for bone marrow and lymph nodes[50, 66]. They display reduced proliferation in response to IL-15 *in vitro* and *in vivo* in steady state and lymphopenic settings compared to Imm. NK cells. They are functionally the most proficient of all NK cell subsets[49, 50].

M2 NK cells (Mac-1$^+$CD27$^-$KLRG1$^+$) represent the final stage of NK cell differentiation. M2 NK cells are lacking in newborn mice and are observed last during NK cell repopulation *in vivo* following depletion or bone marrow transplant[49, 50, 66]. M2 NK cells posses impaired effector functions (both cytotoxicity and cytokine production) compared to M1 NK cells[49, 50, 67] and appear almost terminally differentiated or exhausted with negligible proliferation in response to IL-15 *in vitro* and in both steady-state and lymphopenic setting *in vivo* [49, 68].

Thus what has emerged is the complex manner in which IL-15 is regulated and the contrasting requirement by different IL-15-dependent cell types for distinct physiological sources of IL-15 during development. NK cells can use both parenchymal and haematopoietic trans-presented IL-15 for development. What remains to be understood is the regulation of IL-15 expression in the different cell types and its importance in installing optimal NK cell effector responses *in vivo*. The change in responsiveness to IL-15 during NK cell differentiation and its relationship to the effector responses of mature KLRG1$^+$ and CD27$^+$ NK cells is yet to be appreciated. Future studies into these parameters are likely to reveal novel roles for NK cells in innate immunity.

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1. Seaman WE, Blackman MA, Gindhart TD, Roubinian JR, Loeb JM, Talal N. beta-Estradiol reduces natural killer cells in mice. *Journal of immunology* 1978, **121**(6)**:** 2193-2198.

2. Kumar V, Ben-Ezra J, Bennett M, Sonnenfeld G. Natural killer cells in mice treated with 89strontium: normal target-binding cell numbers but inability to kill even after interferon administration. *Journal of immunology* 1979, **123**(4)**:** 1832-1838.

3. Seaman WE, Gindhart TD, Greenspan JS, Blackman MA, Talal N. Natural killer cells, bone, and the bone marrow: studies in estrogen-treated mice and in congenitally osteopetrotic (mi/mi) mice. *Journal of immunology* 1979, **122**(6)**:** 2541-2547.

4. Carotta S, Pang SH, Nutt SL, Belz GT. Identification of the earliest NK-cell precursor in the mouse BM. *Blood* 2011, **117**(20)**:** 5449-5452.

5. Suzuki H, Duncan GS, Takimoto H, Mak TW. Abnormal development of intestinal intraepithelial lymphocytes and peripheral natural killer cells in mice lacking the IL-2 receptor beta chain. *The Journal of experimental medicine* 1997, **185**(3)**:** 499-505.

6. Di Santo JP. Natural killer cell developmental pathways: a question of balance. *Annu Rev Immunol* 2006, **24:** 257-286.

7. Koka R, Burkett P, Chien M, Chai S, Boone DL, Ma A. Cutting edge: murine dendritic cells require IL-15R alpha to prime NK cells. *Journal of immunology* 2004, **173**(6)**:** 3594-3598.

8. Schluns KS, Nowak EC, Cabrera-Hernandez A, Puddington L, Lefrancois L, Aguila HL. Distinct cell types control lymphoid subset development by means of IL-15 and IL-15 receptor alpha expression. *Proceedings of the National Academy of Sciences of the United States of America* 2004, **101**(15)**:** 5616-5621.

9. Lodolce JP, Burkett PR, Boone DL, Chien M, Ma A. T cell-independent interleukin 15Ralpha signals are required for bystander proliferation. *The Journal of experimental medicine* 2001, **194**(8)**:** 1187-1194.

10. Waldmann TA, Tagaya Y. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annual review of immunology* 1999, **17:** 19-49.

11. Giri JG, Kumaki S, Ahdieh M, Friend DJ, Loomis A, Shanebeck K*, et al.* Identification and cloning of a novel IL-15 binding protein that is structurally related to the alpha chain of the IL-2 receptor. *The EMBO journal* 1995, **14**(15)**:** 3654-3663.

12. Giri JG, Ahdieh M, Eisenman J, Shanebeck K, Grabstein K, Kumaki S*, et al.* Utilization of the beta and gamma chains of the IL-2 receptor by the novel cytokine IL-15. *The EMBO journal* 1994, **13**(12)**:** 2822-2830.

13. Grabstein KH, Eisenman J, Shanebeck K, Rauch C, Srinivasan S, Fung V*, et al.* Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. *Science* 1994, **264**(5161)**:** 965-968.

14. Carson WE, Lindemann MJ, Baiocchi R, Linett M, Tan JC, Chou CC*, et al.* The functional characterization of interleukin-10 receptor expression on human natural killer cells. *Blood* 1995, **85**(12)**:** 3577-3585.

15. Lodolce JP, Boone DL, Chai S, Swain RE, Dassopoulos T, Trettin S*, et al.* IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity* 1998, **9**(5)**:** 669-676.

16. Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M*, et al.* Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *The Journal of experimental medicine* 2000, **191**(5)**:** 771-780.

17. Dubois S, Mariner J, Waldmann TA, Tagaya Y. IL-15Ralpha recycles and presents IL-15 In trans to neighboring cells. *Immunity* 2002, **17**(5)**:** 537-547.

18. Cooper MA, Bush JE, Fehniger TA, VanDeusen JB, Waite RE, Liu Y*, et al.* In vivo evidence for a dependence on interleukin 15 for survival of natural killer cells. *Blood* 2002, **100**(10)**:** 3633-3638.

19. Koka R, Burkett PR, Chien M, Chai S, Chan F, Lodolce JP*, et al.* Interleukin (IL)-15R[alpha]-deficient natural killer cells survive in normal but not IL-15R[alpha]-deficient mice. *The Journal of experimental medicine* 2003, **197**(8)**:** 977-984.

20. Sandau MM, Schluns KS, Lefrancois L, Jameson SC. Cutting edge: transpresentation of IL-15 by bone marrow-derived cells necessitates expression of IL-15 and IL-15R alpha by the same cells. *Journal of immunology* 2004, **173**(11)**:** 6537-6541.

21. Burkett PR, Koka R, Chien M, Chai S, Boone DL, Ma A. Coordinate expression and trans presentation of interleukin (IL)-15Ralpha and IL-15 supports natural killer cell and memory CD8+ T cell homeostasis. *The Journal of experimental medicine* 2004, **200**(7)**:** 825-834.

22. Castillo EF, Acero LF, Stonier SW, Zhou D, Schluns KS. Thymic and peripheral microenvironments differentially mediate development and maturation of iNKT cells by IL-15 transpresentation. *Blood* 2010, **116**(14)**:** 2494-2503.

23. Schluns KS, Stoklasek T, Lefrancois L. The roles of interleukin-15 receptor alpha: trans-presentation, receptor component, or both? *The international journal of biochemistry & cell biology* 2005, **37**(8)**:** 1567-1571.

24. Castillo EF, Schluns KS. Regulating the immune system via IL-15 transpresentation. *Cytokine* 2012, **59**(3)**:** 479-490.

25. Mortier E, Woo T, Advincula R, Gozalo S, Ma A. IL-15Ralpha chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation. *The Journal of experimental medicine* 2008, **205**(5)**:** 1213-1225.

26. Colpitts SL, Stoklasek TA, Plumlee CR, Obar JJ, Guo C, Lefrancois L. Cutting edge: the role of IFN-alpha receptor and MyD88 signaling in induction of IL-15 expression in vivo. *Journal of immunology* 2012, **188**(6)**:** 2483-2487.

27. Colpitts SL, Stonier SW, Stoklasek TA, Root SH, Aguila HL, Schluns KS*, et al.* Transcriptional regulation of IL-15 expression during hematopoiesis. *Journal of immunology* 2013, **191**(6)**:** 3017-3024.

28. Ma LJ, Acero LF, Zal T, Schluns KS. Trans-presentation of IL-15 by intestinal epithelial cells drives development of CD8alphaalpha IELs. *Journal of immunology* 2009, **183**(2)**:** 1044-1054.

29. Castillo EF, Stonier SW, Frasca L, Schluns KS. Dendritic cells support the in vivo development and maintenance of NK cells via IL-15 trans-presentation. *Journal of immunology* 2009, **183**(8)**:** 4948-4956.

30. Mortier E, Advincula R, Kim L, Chmura S, Barrera J, Reizis B*, et al.* Macrophage- and dendritic-cell-derived interleukin-15 receptor alpha supports homeostasis of distinct CD8+ T cell subsets. *Immunity* 2009, **31**(5)**:** 811-822.

31. Budagian V, Bulanova E, Orinska Z, Ludwig A, Rose-John S, Saftig P*, et al.* Natural soluble interleukin-15Ralpha is generated by cleavage that involves the tumor necrosis factor-alpha-converting enzyme (TACE/ADAM17). *The Journal of biological chemistry* 2004, **279**(39)**:** 40368-40375.

32. Bergamaschi C, Bear J, Rosati M, Beach RK, Alicea C, Sowder R*, et al.* Circulating IL-15 exists as heterodimeric complex with soluble IL-15Ralpha in human and mouse serum. *Blood* 2012, **120**(1)**:** e1-8.

33. Muller JR, Waldmann TA, Kruhlak MJ, Dubois S. Paracrine and transpresentation functions of IL-15 are mediated by diverse splice versions of IL-15Ralpha in human monocytes and dendritic cells. *The Journal of biological chemistry* 2012, **287**(48)**:** 40328-40338.

34. Lucas M, Schachterle W, Oberle K, Aichele P, Diefenbach A. Dendritic cells prime natural killer cells by trans-presenting interleukin 15. *Immunity* 2007, **26**(4)**:** 503-517.

35. Mortier E, Quemener A, Vusio P, Lorenzen I, Boublik Y, Grotzinger J*, et al.* Soluble interleukin-15 receptor alpha (IL-15R alpha)-sushi as a selective and potent agonist of IL-15 action through IL-15R beta/gamma. Hyperagonist IL-15 x IL-15R alpha fusion proteins. *The Journal of biological chemistry* 2006, **281**(3)**:** 1612-1619.

36. Rubinstein MP, Kovar M, Purton JF, Cho JH, Boyman O, Surh CD*, et al.* Converting IL-15 to a superagonist by binding to soluble IL-15R{alpha}. *Proceedings of the National Academy of Sciences of the United States of America* 2006, **103**(24)**:** 9166-9171.

37. Huntington ND, Legrand N, Alves NL, Jaron B, Weijer K, Plet A*, et al.* IL-15 trans-presentation promotes human NK cell development and differentiation in vivo. *The Journal of experimental medicine* 2009, **206**(1)**:** 25-34.

38. Elpek KG, Rubinstein MP, Bellemare-Pelletier A, Goldrath AW, Turley SJ. Mature natural killer cells with phenotypic and functional alterations accumulate upon sustained stimulation with IL-15/IL-15Ralpha complexes. *Proceedings of the National Academy of Sciences of the United States of America* 2010, **107**(50)**:** 21647-21652.

39. Bouchaud G, Gehrke S, Krieg C, Kolios A, Hafner J, Navarini AA*, et al.* Epidermal IL-15Ralpha acts as an endogenous antagonist of psoriasiform inflammation in mouse and man. *The Journal of experimental medicine* 2013, **210**(10)**:** 2105-2117.

40. Nguyen KB, Salazar-Mather TP, Dalod MY, Van Deusen JB, Wei XQ, Liew FY*, et al.* Coordinated and distinct roles for IFN-alpha beta, IL-12, and IL-15 regulation of NK cell responses to viral infection. *Journal of immunology* 2002, **169**(8)**:** 4279-4287.

41. Khan IA, Moretto M, Wei XQ, Williams M, Schwartzman JD, Liew FY. Treatment with soluble interleukin-15Ralpha exacerbates intracellular parasitic infection by blocking the development of memory CD8+ T cell response. *The Journal of experimental medicine* 2002, **195**(11)**:** 1463-1470.

42. Wei XQ, Leung BP, Arthur HM, McInnes IB, Liew FY. Reduced incidence and severity of collagen-induced arthritis in mice lacking IL-18. *Journal of immunology* 2001, **166**(1)**:** 517-521.

43. DePaolo RW, Abadie V, Tang F, Fehlner-Peach H, Hall JA, Wang W*, et al.* Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. *Nature* 2011, **471**(7337)**:** 220-224.

44. Mention JJ, Ben Ahmed M, Begue B, Barbe U, Verkarre V, Asnafi V*, et al.* Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. *Gastroenterology* 2003, **125**(3)**:** 730-745.

45. Liu Z, Geboes K, Colpaert S, D'Haens GR, Rutgeerts P, Ceuppens JL. IL-15 is highly expressed in inflammatory bowel disease and regulates local T cell-dependent cytokine production. *Journal of immunology* 2000, **164**(7)**:** 3608-3615.

46. McInnes IB, Leung BP, Sturrock RD, Field M, Liew FY. Interleukin-15 mediates T cell-dependent regulation of tumor necrosis factor-alpha production in rheumatoid arthritis. *Nature medicine* 1997, **3**(2)**:** 189-195.

47. Bernardo D, Garrote JA, Allegretti Y, Leon A, Gomez E, Bermejo-Martin JF*, et al.* Higher constitutive IL15R alpha expression and lower IL-15 response threshold in coeliac disease patients. *Clinical and experimental immunology* 2008, **154**(1)**:** 64-73.

48. Chen J, Petrus M, Bamford R, Shih JH, Morris JC, Janik JE*, et al.* Increased serum soluble IL-15Ralpha levels in T-cell large granular lymphocyte leukemia. *Blood* 2012, **119**(1)**:** 137-143.

49. Huntington ND, Tabarias H, Fairfax K, Brady J, Hayakawa Y, Degli-Esposti MA*, et al.* NK cell maturation and peripheral homeostasis is associated with KLRG1 up-regulation. *Journal of immunology* 2007, **178**(8)**:** 4764-4770.

50. Hayakawa Y, Smyth MJ. CD27 dissects mature NK cells into two subsets with distinct responsiveness and migratory capacity. *Journal of immunology* 2006, **176**(3)**:** 1517-1524.

51. Hayakawa Y, Huntington ND, Nutt SL, Smyth MJ. Functional subsets of mouse natural killer cells. *Immunological reviews* 2006, **214:** 47-55.

52. Kim S, Iizuka K, Kang HS, Dokun A, French AR, Greco S*, et al.* In vivo developmental stages in murine natural killer cell maturation. *Nature immunology* 2002, **3**(6)**:** 523-528.

53. Huntington ND, Vosshenrich CA, Di Santo JP. Developmental pathways that generate natural-killer-cell diversity in mice and humans. *Nature reviews Immunology* 2007, **7**(9)**:** 703-714.

54. Nagler A, Lanier LL, Cwirla S, Phillips JH. Comparative studies of human FcRIII-positive and negative natural killer cells. *Journal of immunology* 1989, **143**(10)**:** 3183-3191.

55. Lanier LL, Le AM, Civin CI, Loken MR, Phillips JH. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *Journal of immunology* 1986, **136**(12)**:** 4480-4486.

56. Lanier LL, Le AM, Phillips JH, Warner NL, Babcock GF. Subpopulations of human natural killer cells defined by expression of the Leu-7 (HNK-1) and Leu-11 (NK-15) antigens. *Journal of immunology* 1983, **131**(4)**:** 1789-1796.

57. Lanier LL, Phillips JH. Ontogeny of natural killer cells. *Nature* 1986, **319**(6051)**:** 269-270.

58. Lanier LL, Phillips JH, Warner NL, Babcock GF. A human natural killer cell-associated antigen defined by monoclonal antibody anti-Leu (NKP-15): functional and two-color flow cytometry analysis. *Journal of leukocyte biology* 1984, **35**(1)**:** 11-17.

59. Phillips JH, Lanier LL. A model for the differentiation of human natural killer cells. Studies on the in vitro activation of Leu-11+ granular lymphocytes with a natural killer-sensitive tumor cell, K562. *The Journal of experimental medicine* 1985, **161**(6)**:** 1464-1482.

60. Prlic M, Blazar BR, Farrar MA, Jameson SC. In vivo survival and homeostatic proliferation of natural killer cells. *The Journal of experimental medicine* 2003, **197**(8)**:** 967-976.

61. Jamieson AM, Isnard P, Dorfman JR, Coles MC, Raulet DH. Turnover and proliferation of NK cells in steady state and lymphopenic conditions. *Journal of immunology* 2004, **172**(2)**:** 864-870.

62. Ranson T, Vosshenrich CA, Corcuff E, Richard O, Muller W, Di Santo JP. IL-15 is an essential mediator of peripheral NK-cell homeostasis. *Blood* 2003, **101**(12)**:** 4887-4893.

63. Huntington ND, Puthalakath H, Gunn P, Naik E, Michalak EM, Smyth MJ*, et al.* Interleukin 15-mediated survival of natural killer cells is determined by interactions among Bim, Noxa and Mcl-1. *Nature immunology* 2007, **8**(8)**:** 856-863.

64. Nishikawa K, Saito S, Morii T, Kato Y, Narita N, Ichijo M*, et al.* Differential expression of the interleukin 2 receptor beta (p75) chain on human peripheral blood natural killer subsets. *International immunology* 1990, **2**(6)**:** 481-486.

65. Walzer T, Blery M, Chaix J, Fuseri N, Chasson L, Robbins SH*, et al.* Identification, activation, and selective in vivo ablation of mouse NK cells via NKp46. *Proceedings of the National Academy of Sciences of the United States of America* 2007, **104**(9)**:** 3384-3389.

66. Chiossone L, Chaix J, Fuseri N, Roth C, Vivier E, Walzer T. Maturation of mouse NK cells is a 4-stage developmental program. *Blood* 2009, **113**(22)**:** 5488-5496.

67. Robbins SH, Nguyen KB, Takahashi N, Mikayama T, Biron CA, Brossay L. Cutting edge: inhibitory functions of the killer cell lectin-like receptor G1 molecule during the activation of mouse NK cells. *Journal of immunology* 2002, **168**(6)**:** 2585-2589.

68. Sciume G, De Angelis G, Benigni G, Ponzetta A, Morrone S, Santoni A*, et al.* CX3CR1 expression defines 2 KLRG1+ mouse NK-cell subsets with distinct functional properties and positioning in the bone marrow. *Blood* 2011, **117**(17)**:** 4467-4475.