Interleukin (IL)-15Rα–deficient Natural Killer Cells Survive in Normal but Not IL-15Rα–deficient Mice

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Abstract

Natural killer (NK) cells protect hosts against viral pathogens and transformed cells. IL-15 is thought to play a critical role in NK cell development, but its role in the regulation of peripheral NK cells is less well defined. We now find that adoptive transfer of normal NK cells into mice lacking the high affinity interleukin (IL)-15 receptor, IL-15Rα, surprisingly results in the abrupt loss of these cells. Moreover, IL-15Rα–deficient NK cells can differentiate successfully in radiation bone marrow chimera bearing normal cells. Finally, adoptively transferred IL-15Rα–deficient NK cells survive in normal but not IL-15Rα–deficient mice. These findings demonstrate that NK cell–independent IL-15Rα expression is critical for maintaining peripheral NK cells, while IL-15Rα expression on NK cells is not required for this function.

Key words: IL-15 • NK cell homeostasis • NK cell survival • NK cell development

Introduction

NK cells are innate immune cells that are cytotoxic and can secrete cytokines such as IFN-γ. Their primary function is to defend the host against cells bearing altered or foreign MHC molecules (1). Peripheral NK cells are thought to be mature cells that do not proliferate significantly unless stimuli such as viral pathogens are introduced. It is unclear, however, whether the lifespan of these cells are actively regulated in unperturbed animals and what factors might regulate NK cell survival.

IL-15 is a unique cytokine that plays essential roles in regulating the homeostasis of $CD8^+$ T cells, $TCR\gamma/\delta^+$ intraepithelial lymphocytes, NKT cells, and NK cells (2–7). This growth factor binds to a trimeric receptor comprised of IL-15Rα, IL-2Rβ, and γ_c (8–12). Binding of IL-15 to this receptor is mediated largely by the high affinity IL-15Rα chain, and intracellular signals are initiated by the cytoplasmic portions of the IL-2Rβ, and γ_c chains. While IL-15 is also able to stimulate primary cells through the IL-2Rβ, and γ_c chains of the IL-15R in vitro (unpublished data), the similar phenotypes of IL-15 and $IL15R\alpha^{-/-}$ mice suggests that most physiological IL-15 signals utilize IL-15Rα–bearing receptors (13, 14).

Prior studies have suggested important roles for IL-15 in supporting both NK precursor cell differentiation and peripheral NK cell function (15–18). During development, IL-15 is expressed by bone marrow (BM)* stromal cells and stimulates NK cell precursors to proliferate and differentiate into mature NK cells (19–23). The virtual absence of NK cells in IL-2Rβ–deficient ($IL\text{-}2R\beta^{-/-}$), IL-15–deficient ($IL\text{-}15^{-/-}$), and IL-15Rα–deficient ($IL\text{-}15R\alpha^{-/-}$) mice is consistent with the suggestion that IL-15 receptor signals are critical for NK cell development. In the periphery, IL-15 mRNA expression is induced during infectious stimuli and may regulate NK cell activation and/or proliferation (17, 18, 24). However, whether IL-15Rα is required for these NK cell responses is unknown. In addition, IL-15Rα's potential role in supporting the maintenance of peripheral NK cells in unstimulated mice has not been examined.

The ability of IL-15 to stimulate the survival and proliferation of T cells and NK cells in vitro has led to the suggestion that the physiological role of IL-15 is to bind directly to these cells and induce IL-15Rα–dependent signals. However, IL-15 and IL-15Rα mRNA are both broadly expressed by hematopoietic and nonhematopoietic cells, so IL-15Rα expression on accessory cells could indirectly regulate NK cell function in vivo. Furthermore, recent studies of poly inosine:cytosine (poly I:C) induced bystander activation of $CD8^+$ T cells in $IL\text{-}15R\alpha^{-/-}$ mice revealed the surprising result that IL-15Rα expression on cells other than $CD8^+$ T cells can regulate $CD8^+$ T cell

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*Abbreviations used in this paper: BM, bone marrow; CFSE, carboxyfluorescein diacetate succinimidyl ester.

proliferation (25). Thus, we have used IL-15Rα$^{-/-}$ mice to investigate whether and how IL-15Rα regulates the homeostasis of mature NK cells.

Materials and Methods

Mice and Radiation BM Chimera. The generation and characterization of IL-15Rα$^{-/-}$ mice has been described (14). IL-15Rα$^{-/-}$ mice were backcrossed to C57BL/6J mice for at least eight generations. C57Bl/6J/SJL Ly5.2^{+} congenic mice were purchased from JAX Laboratories. To generate radiation BM chimera, individual mice were irradiated with 950 rads total body radiation, reconstituted with 4–6 × 10^6 BM cells (injected via retroorbital vein) within 1 h of irradiation, and allowed to recover for at least 8 wk before use in experiments.

Adoptive Transfers. For adoptive transfers of mature NK cells, splenocytes from donor mice were isolated as a single cell suspension, purified by negative depletion with anti-Ig and anti-CD4 magnetic beads (Dynal), and either labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE), or transferred directly into recipient mice via retroorbital vein. Cell purity was typically 12% NK cells. Each recipient mouse received 10–12 × 10^6 cells of which ~1 × 10^6 cells were NK cells. KO NK cells were enriched from KO BM→WT chimeric spleens and the purity of these cells was typically 2.5%. Recipient mice received 30 × 10^6 KO cells of which 8 × 10^5 were NK cells.

CFSE labeling was performed by incubating cells with 5 nM CFSE at room temperature in the dark. The reaction was quenched after 10 min with 100% FCS. Cells were washed, counted, and CFSE labeling was confirmed by flow cytometry.

5 μg of recombinant murine IL-15 (PeproTech) or PBS was administered per mouse, daily for four days, via intraperitoneal injection for NK cell proliferation studies.

Cellular Analyses. Single cell suspensions of lymphocytes from peripheral blood, spleens, lymph nodes, lungs, and livers were isolated as previously described and incubated with fluorescently conjugated or biotinylated antibodies specific for Ly5.1, Ly5.2, CD3, and NK1.1 (BD Biosciences/Becton Dickinson) before analyses on a FACSCalibur™ flow cytometer and CELLQuest™ software (14, 26). The percentage of donor NK cells was calculated as a percentage of total lymphoid cell population collected. Error bars depict one standard deviation from the mean. CFSE-labeled NK cells were analyzed from the peritoneum of recipient mice.

RT-PCR. mRNA was purified as described previously (14) from mouse spleens. Some mice received 150 ng of poly I:C via intraperitoneal injection, and were killed 1–4 d after treatment. RT-PCR for the secreted and non secreted IL-15 transcripts was performed as described previously (27).

Results

NK Cell–independent IL-15Rα Signals Are Critical for Maintaining NK Cells. To examine the potential roles of NK cell–independent IL-15Rα signals in supporting mature NK cell homeostasis, splenic NK cells from IL-15Rα$^{+/-}$ (hereafter designated wild-type, "WT") mice were transferred intravenously into either IL-15Rα$^{+/-}$ ("WT") or IL-15Rα$^{-/-}$ (knock-out, "KO") Ly5 congenic mice, and the percentages of transferred WT NK cells were studied by serial peripheral blood analyses. These mice were not irradiated before transfer, so radiation induced cytokines and lymphopenia were not induced. Transferred WT CD3^{-} NK1.1^{+} NK cells were found in the peripheral blood 3 h after transfer as ~0.15% of peripheral blood lymphocytes in either WT or KO mice (Fig. 1, A and B). Transferred NK cells persisted in the blood of WT mice for up to 30 d, falling gradually below the threshold of detection (0.01% of donor PBLs) beyond that time (Fig. 1 C). This finding is consistent with a peripheral NK cell half-life of ~7 d. By contrast, the percentage of transferred WT NK cells present in the peripheral blood of KO mice fell dramatically over the first 16 h after transfer, and were undetectable greater than 72 h after transfer (Fig. 1, B and C). The half-life of the transferred WT NK cells was thus shortened from 7 d in WT mice to ~10 h in KO mice. Thus, WT NK cells are actively maintained in the periphery of unperturbed mice, and IL-15Rα is critical for mediating these signals.

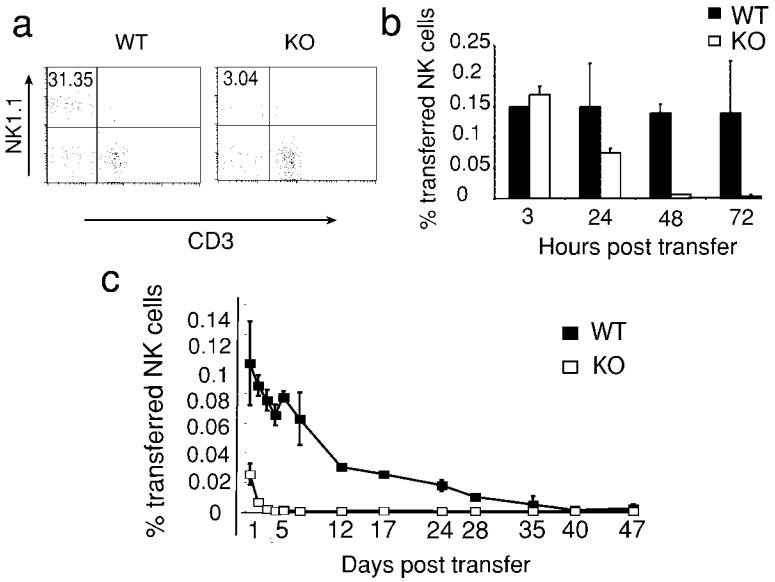

Figure 1. Adoptively transferred NK cells survive significantly longer in a WT host than in a KO host. (a) Representative FACS® plot of transferred WT NK cells in WT (left) and KO (right) hosts. Splenic WT NK cells were transferred intravenously into either WT or KO hosts, congenic for the Ly5 marker. 2 d after transfer, peripheral blood from these mice were analyzed for presence of NK cells using antibodies against Ly5.1, Ly5.2, NK1.1, and CD3. Events displayed are gated on donor cells among the lymphoid gate and presented as the percentage of NK cells among donor cells for this and all subsequent figures. (b) Peripheral blood (PBL) analysis of adoptively transferred WT NK cells in WT (■) or KO (□) mice at indicated times after transfer. Data presented is the percentage of transferred NK cells/total lymphoid cells collected for this and all subsequent NK cell transfer experiments. (c) Long term PBL analysis of adoptively transferred WT NK cells in WT (■) or KO (□) mice. Data are representative of three experiments, with three mice in each experiments.

IL-15Rα Supports NK Cell Survival, Rather than Proliferation or Homing. IL-15Rα may support T lymphocyte homing through the regulation of adhesion molecule expression (25, 28, 29). Hence, the dramatic loss of WT NK cells from the peripheral blood of KO mice might reflect differential homing of NK cells to peripheral tissues. We thus analyzed both secondary lymphoid and nonlymphoid tissues of recipient mice for the presence of transferred NK cells at two and fourteen days post transfer. The percentages of transferred WT NK cells found in spleens, lymph nodes, lungs or livers from recipient mice 48 h after transfer reflected the same trend observed in peripheral blood analyses (Fig. 2). No transferred WT NK cells were found in any peripheral tissues from KO mice 72 h after adoptive transfer. Thus, differential homing does not account for the loss of NK cells from the peripheral blood of KO mice. Instead, WT NK cells disappear entirely from KO mice.

IL-15 has been shown to support both NK cell survival and proliferation in vitro. Thus, the loss of WT NK cells after transfer into KO mice could reflect critical roles for IL-15 in supporting NK cell survival or proliferation. To distinguish between these possibilities, donor NK cells were labeled with CFSE before transfer. Analyses of tissues and peripheral blood from recipient WT mice revealed that transferred cells contained mostly nondiluted CFSE for 4 d, indicating that the transferred peripheral NK cells were nonproliferating cells (Fig. 3 A). This observation is consistent with prior reports that mature NK cells do not proliferate at a significant rate in unchallenged animals (30, 31) and demonstrates that our manipulation of splenic NK cells does not induce NK cell proliferation. Considered together with the absence of selective homing defects, the loss of WT NK cells after transfer into KO mice is likely due to the failure of KO mice to support survival of WT cells. Moreover, the critical role of IL-15Rα in supporting NK cell survival is correlated with IL-15Rα expression on cells other than NK cells.

Heterologous IL-15 Can Induce Peripheral NK Cell Proliferation in IL-15Rα$^{-/-}$ Mice. IL-15 may stimulate NK cell proliferation during physiological conditions such as viral infections (32, 33). As our current experiments indicate that IL-15Rα expression on cells other than NK cells regulate NK cell survival, we investigated whether IL-15Rα expression on other cells would also regulate proliferative responses of NK cells. Thus, we coinjected recombinant IL-15 and CFSE-labeled WT NK cells into either WT or KO mice. Analysis of peritoneal leukocytes from these mice 4 d after adoptive transfer revealed that transferred NK cells diluted CFSE comparably in both WT and KO recipients, indicating that WT peripheral NK cells can proliferate in response to IL-15 in KO mice (Fig. 3 C). The proportion (though not the total number) of transferred WT cells that proliferated was similar in WT and KO mice (Fig. 3, B and C). Thus, while IL-15Rα expression on non-NK cells is critical for maintaining survival of peripheral NK cells in unstimulated mice, it may not be required for supporting IL-15–mediated proliferation of these cells.

IL-15Rα$^{-/-}$ Mice Express Normal Levels of IL-15 mRNA. As the WT NK cells transferred into KO mice above are IL-15Rα competent, and as IL-15 has been suggested to play a direct role in supporting NK cell survival, one possible explanation for the failure of WT NK cells to survive in these mice is that KO mice elaborate less IL-15 than WT mice. IL-15 secretion is regulated both transcriptionally and posttranscriptionally (34, 35). In the absence of a reliable antibody for measuring endogenous murine IL-15 levels, we examined mRNA levels of the preferentially secreted isoform of IL-15 (27). These studies revealed that IL-15 mRNA is constitutively expressed and induced by poly I:C in splenocytes from both WT and KO mice (Fig. 4). Thus, reduced IL-15 mRNA expression does not appear to explain the inability of KO mice to support peripheral NK cell survival.

Hematopoietic IL-15Rα Competent Cells Can Support Mature NK Cells in Chimeric Mice. The experiments above suggest that IL-15Rα expression on cells other than the NK cells is critical for supporting the survival of mature NK cells. IL-15Rα is expressed on multiple cell types and

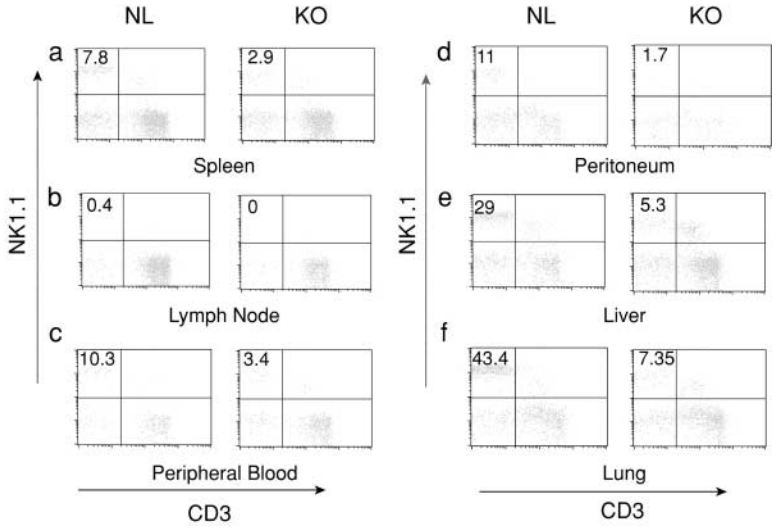

Figure 2. The disappearance of adoptively transferred NK cells from peripheral blood is not due to differential homing to specific tissues. Quantitation of adoptively transferred WT NK cells from various tissues, (a) spleen, (b) LN, (c) PBL, (d) peritoneum, (e) liver, (f) lung of WT (left) or KO (right) mice 48 h after adoptive transfer. Events displayed in histogram represent percentage of transferred CD3$^-$ NK1.1$^+$ cells within the lymphoid gate. Data are representative of two experiments with two mice each.

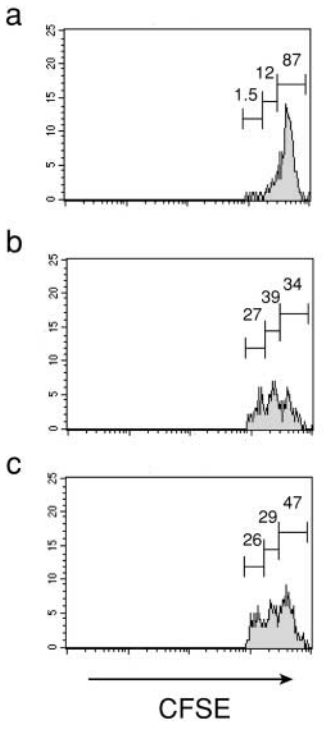

Figure 3. Adoptively transferred splenic NK cells do not proliferate in the absence of specific stimuli. (a) Proliferation of WT NK cells in response to PBS 4 d after transfer into WT host. Events displayed in histogram represent transferred $CD3^-$ $NK1.1^+$ cells obtained from the peritoneum of recipient mice. (b) Proliferation of WT NK cells in response to IL-15 4 d after transfer into WT host. (c) Proliferation of WT NK cells in response to IL-15 (5 μg/day) 4 d after transfer into KO host.

the types of cells that support NK cell survival are unknown. To determine whether IL-15Rα competent hematopoietic cells can complement the environmental defect of KO mice, we reconstituted lethally irradiated $Ly5.2^+$ KO mice with either WT (WT BM→KO chimera) or KO (KO BM→KO chimera) $Ly5.1^+$ BM precursors. Analyses of radiation chimera 8 wk after reconstitution failed to reveal any residual host NK cells in these chimera (unpublished data). WT NK cells purified from normal $Ly5.2^+$ mice (i.e., NK cells congenic to the Ly5 genotype of the reconstituting BM precursors) were then transferred into these chimera, after which serial peripheral blood analyses and terminal tissue analyses were performed to quantitate the transferred WT NK cells. These studies revealed that adoptively transferred WT NK cells survived in WT BM→KO chimera for ~30 d. By contrast, transferred WT NK cells disappeared from KO BM→KO chimera within 48 h after adoptive transfer (Fig. 5). Tissue analyses of these mice confirmed that the percentages of NK cells in spleens, livers, and lungs all reflected the percentages of NK cells in the peripheral blood of these animals (unpublished data). As these WT BM→KO chimera contain WT BM-derived hematopoietic cells in their peripheral organs, this finding demonstrates that WT hematopoietic cells can support survival of peripheral NK cells in KO mice.

IL-15Rα Competent Cells Support KO NK Cells in Chimeric Mice. If IL-15Rα–dependent cells play an important role in supporting NK cell survival, then it is possible that peripheral NK cells are absent from KO mice partly due to the inability of these mice to support these cells in the periphery (in addition to potential defects in NK cell development). To address this point, we used congenic KO BM to reconstitute either lethally irradiated WT (KO BM→WT chimera) or KO (KO BM→KO chimera) mice. Analyses of spleens, livers, and lungs from these chimeric mice 8 wk after reconstitution revealed that far greater percentages of BM derived KO NK cells were obtained from KO BM→WT than from KO BM→KO chimera (Fig. 6). This finding indicates that KO NK cells can differentiate successfully in KO BM→WT chimera. Thus, WT cells can support the differentiation and/or survival of KO NK cells.

IL-15Rα Expression on NK Cells Is Not Required for Survival of Peripheral NK Cells. While IL-15Rα competent cells are critical for indirectly supporting peripheral NK cells, the signal(s) that directly support NK cell survival in vivo are unknown. As earlier studies suggested that IL-15 can support NK cell survival and/or proliferation by binding directly to NK cells (17, 18, 24), we investigated whether IL-15Rα expression on NK cells might also be important for regulating NK cell survival in vivo. For these experiments, we took advantage of the fact that KO NK cells are present in the periphery of KO BM→WT chimeric mice (Fig. 6), and asked whether these KO NK cells would persist in greater numbers after adoptive transfer into WT versus KO mice. KO NK cells were harvested

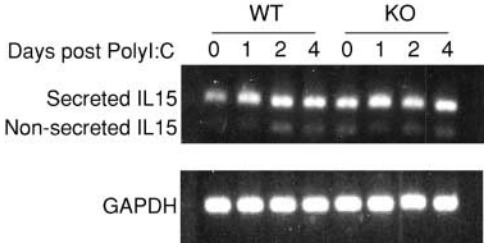

Figure 4. WT and KO mice express similar amounts of IL-15 mRNA. Reverse transcriptase-PCR analysis of secreted and nonsecreted forms of IL-15 mRNA was performed on splenic RNA from mice treated with poly I:C for the indicated number of days.

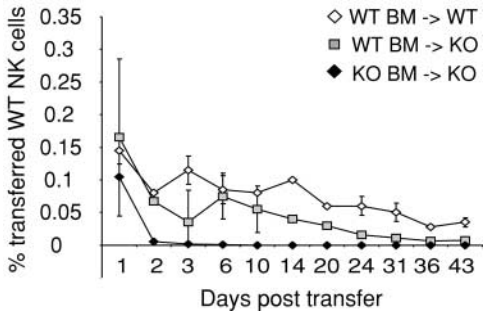

Figure 5. Long term survival of transferred WT NK cells in WT BM→KO radiation BM chimera ◇ WT BM→WT, ■ WT BM→KO, ◆ KO BM→KO. Radiation BM chimeric mice were prepared as described in Materials and Methods, allowed to recover for at least 8 wk, and were then used as recipients for WT splenic NK cells. Adoptively transferred cells were congenic for the radiation sensitive compartment of the chimeric mice. Data are representative of two experiments, with two mice each.

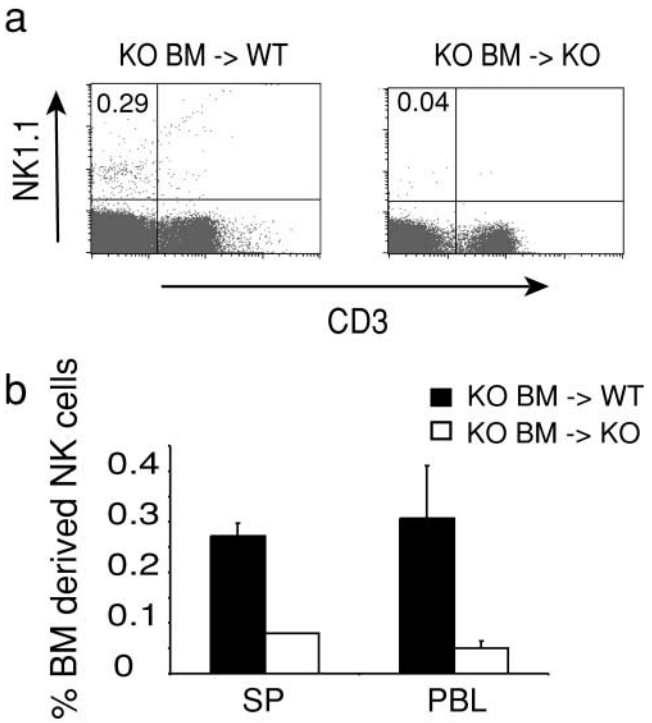

Figure 6. KO NK cells can survive in the peripheral tissues of KO BM→WT chimera mice. Radiation BM chimeric mice were prepared as described in Materials and Methods and allowed to recover for at least 8 wk before analysis. (a) Representative FACS® plot of BM-derived NK cells in spleens of KO BM→WT (top panel) versus KO BM→KO chimera (bottom panel). (b) Percentage of BM derived NK cells obtained from spleens and peripheral blood of KO BM→WT (■) and KO BM→KO (□) chimera. Data are representative of five mice.

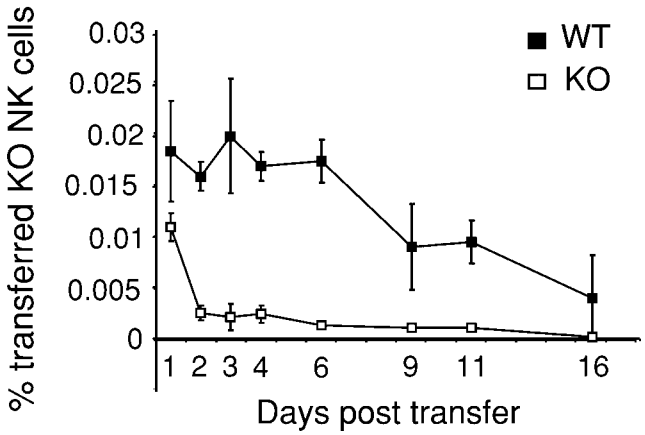

Figure 7. IL-15Rα on NK cells is not required for their peripheral maintenance. Long term serial peripheral blood analysis of adoptively transferred KO NK cells after transfer into either WT (■) or KO (□) hosts. Data are representative of two experiments, with two mice each.

from spleens of reconstituted KO BM→WT chimera and transferred into either KO or WT congenic mice. Serial peripheral blood analyses of these mice revealed that KO NK cells survive for up to 30 d in WT mice, but disappear rapidly in KO mice (Fig. 7). Thus, IL-15Rα expression on peripheral NK cells does not mediate the survival signal required by these cells. This surprising finding indicates that peripheral NK cell survival is unlikely to be mediated by the interaction of soluble IL-15 with IL-15Rα on the surface of NK cells.

Discussion

In this study, we have investigated the role of IL-15Rα, the high affinity receptor chain for IL-15, in regulating peripheral NK cells. Our experiments indicate that the survival of peripheral NK cells is actively maintained by extrinsic signals and that IL-15Rα expression on cells other than NK cells mediates this function. Surprisingly, IL-15Rα expression on NK cells does not mediate this survival function.

IL-15Rα and Peripheral NK Cell Survival. The acute loss of NK cells after adoptive transfer into KO mice indicates that NK cells must receive tonic extrinsic signals to survive for even short periods in the periphery. This finding is consistent with a recent study demonstrating a requirement for IL-15 cytokine in supporting peripheral NK cells (36). However, the prior studies did not distinguish between the requirement for IL-15Rα expression on NK cells versus other cells. Our experiments show that this critical function relies upon IL-15Rα (in addition to IL-15 and IL-2Rβ) and is apparent in unperturbed, lymphoid replete animals. It is not dependent upon the induction of lymphopenic states or radiation-induced cytokines, and is therefore directly relevant to the physiologic state of resting animals. This survival function may be related to the ability of IL-15 to support Bcl-2 expression in NK cells (19, 36). Hence, in addition to supporting the differentiation of NK cell precursors and the activation and/or proliferation of peripheral NK cells, IL-15Rα plays a critical role in supporting the survival of peripheral NK cells in unstimulated animals.

IL-15Rα$^{-/-}$ (KO) NK Cells: Implications for Development and Peripheral Maintenance. NK cells are markedly depleted (<2% of normal) in the periphery of IL-15Rα$^{-/-}$ (KO) mice (14). Hence, our finding that substantial numbers (~25% of normal) of KO NK cells can differentiate and persist in KO BM→WT chimeric mice implies that IL-15Rα expression on NK cell precursors is less critical for NK cell differentiation than was suggested by the virtual absence of peripheral NK cells in KO mice. While it is possible that radiation resistant IL-15Rα competent BM cells may also augment the differentiation of KO NK cells in KO BM→WT chimera, it is more likely that KO NK cells differentiate comparably in KO BM→KO and KO BM→WT chimera. When KO NK cells emerge from the BMs of these chimera, these KO NK cells may survive in the periphery of KO BM→WT chimera because of the presence of IL-15Rα competent cells, but die in KO BM→KO chimera. This data also hinted that peripheral KO NK cells resemble WT NK cells in requiring IL-15Rα–dependent cells other than NK cells for survival.

Thus, in addition to the differential survival of adoptively transferred peripheral NK cells in WT and KO mice, the presence of KO NK cells in the periphery of KO BM→WT chimera provides further evidence that IL-15Rα plays a physiological and critical role in supporting peripheral NK cell survival.

The presence of significant numbers of KO NK cells in KO BM→WT chimeric mice also provides novel opportunities to study the functions of IL-15Rα on the surface of peripheral NK cells. While our findings indicate that IL-15Rα expression on NK cells is not required for maintaining survival of resting peripheral NK cells, it remains possible that IL-15Rα expression on NK cells may be required for NK cell effector functions such as cytolysis or cytokine secretion. In this context, our finding that IL-15 can stimulate WT NK cell proliferation in KO mice suggests that IL-15Rα expression on NK cells could mediate NK cell activation, cytolytic activity, cytokine elaboration, and/or proliferation responses during immune challenges in vivo. Hence, future studies of NK cell responses to viruses and other immune stimuli in KO BM→WT chimeric mice will address how IL-15Rα expression on NK cells may regulate these functions.

NK Cell–independent IL-15Rα Expression and Peripheral NK Cell Survival. The finding that IL-15Rα expression on cells other than NK cells support peripheral NK cell survival prompts reevaluation of the mechanism(s) by which IL-15Rα may support NK cells. The loss of NK cells after transfer into KO mice could theoretically reflect loss of IL-15Rα–dependent cells and/or loss of tonic IL-15Rα signals. Depletion of peripheral NK cells by repeated injections of anti-IL-2Rβ specific Fab' fragments suggested that tonic IL-15Rα signals in mature cells may be the primary mechanism maintaining NK cells (36).

IL-15Rα mRNA is expressed in most tissues and is thought to be expressed in multiple cell types (4). Thus, the IL-15Rα dependent cell types that support NK cells may include multiple hematopoietic or nonhematopoietic cells. The fact that WT BM→KO chimera possess mature NK cells demonstrates that BM derived hematopoietic cells support these cells in the periphery. The finding that WT BM→KO chimera support fewer peripheral NK cells than WT BM→WT chimera suggests that nonhematopoietic stromal cells might also serve this function. However, we cannot definitively confirm a role for nonhematopoietic cells since rare hematopoietic cells can survive the lethal irradiation protocols we have used. In addition, the presence of largely normal numbers of peripheral NK cells in both RAG-$1^{-/-}$ and RAG-$2^{-/-}$ mice suggest that adaptive T and B lymphocytes do not play a major role in supporting basal NK cell survival. Thus, one may deduce that the IL-15Rα–dependent cells that support NK cell survival include nonlymphoid hematopoietic cells.

The IL-15Rα–dependent factor that supports NK cells is unclear. Because prior data suggested that heterologous IL-15 could directly support these cells, we investigated the possibility that KO mice might express less IL-15 than normal mice. Multiple posttranscriptional and posttranslational mechanisms regulate IL-15 protein secretion (33, 34, 37), indicating that the measurement of endogenous IL-15 protein levels would be the preferable means of addressing this question. However, in the absence of reliable antibody reagents for detecting endogenous murine IL-15 protein, we are left with measurements of the preferentially secreted isoforms of IL-15 mRNA. As we detected similar levels of mRNA specific for these IL-15 isoforms, we have no evidence that KO mice secrete less IL-15 than normal mice. Indeed, if IL-15 was secreted at roughly normal levels in IL-15Rα$^{-/-}$ mice, then one would intuitively expect that the amount of soluble IL-15 would be present at far greater levels in these mice since no high affinity receptors would be present to bind IL-15. Moreover, our observation that adoptively transferred KO NK cells, presumably unable to bind soluble IL-15, survive in WT but not KO mice is not consistent with the hypothesis that reduced levels of soluble IL-15 cause less IL-15Rα–mediated stimulation of NK cells in KO mice (see below). Other cytokines that share the γ_c receptor, such as IL-2, IL-4, and IL-7, can support NK cells, and we have observed similar levels of mRNA specific for these cytokines in KO mice. This observation, combined with the largely normal numbers of NK cells in mice deficient for these cytokines or their receptors suggest that these other cytokines do not support peripheral NK cell survival. It is also possible that IL-15Rα expression on cells other than NK cells could mediate the stimulation of other unidentified soluble or membrane bound factors that directly stimulate NK cell survival.

IL-15Rα Expression on NK Cells and Peripheral NK Cell Survival. Our finding that IL-15Rα expression on cells other than NK cells regulates the survival of peripheral NK cells did not address whether IL-15Rα expression on NK cells may also contribute to this function. Indeed, prior evidence that IL-15Rα alone binds IL-15 with high affinity and that soluble IL-15 can stimulate mature NK cell survival and proliferation led to the widespread presumption that IL-15Rα on the surface of NK cells should mediate NK cell responses to IL-15. However, our observation that adoptively transferred KO NK cells survive well in WT but not KO mice indicates that IL-15Rα expression on NK cells does not regulate this process. Thus, the stereotypical interaction of soluble IL-15 with IL-15Rα/IL-2Rβ/γ_c trimeric receptors on NK cells is unlikely to be the signal that supports NK cell survival in unchallenged animals.

One intriguing possible mechanism of IL-15Rα's action is raised by a recent study indicating that IL-15Rα can present IL-15 in trans to other cells bearing IL-15Rβ and γ_c receptor chains (38). In this context, membrane bound IL-15 has been documented on multiple cell types (39, 40). Thus, if IL-15Rα on the surface of non-NK cells bound IL-15 and presented this cytokine to IL-2Rβ and γ_c receptor chains on NK cells, then IL-15Rα expression on non-NK cells could be required for NK cell survival. In this scenario, IL-15Rα expression on NK cells would also be dispensable. Other possible explanations for IL-15Rα's indirect role in regulating NK cell survival could include other IL-15Rα induced genes in accessory cells.

Recent studies indicate that $CD8^+$ memory phenotype T cells, antigen-specific memory $CD8^+$ T cells, and CD1-restricted NKT cells all require IL-15 or IL-15Rα for survival and/or proliferation in peripheral tissues (6, 41, 42, 43, 44). Their common dependence upon IL-15 or IL-15Rα has led to the suggestion that IL-15 bioavailability may comprise a biological niche shared by these diverse cell types. If substantiated by further studies, this concept could provide a novel mechanism by which innate and adaptive immune responses can be coordinated and regulated. Nevertheless, when combined with prior observations that $CD8^+$ T cell proliferation is indirectly regulated by IL-15Rα signals (25), our current data definitively demonstrate that a critical role for IL-15Rα expression exists on cells other than the responding lymphocytes, and that IL-15Rα expression on NK cells as well as $CD8^+$ T cells does not mediate the survival and proliferation signals ascribed to IL-15. Thus, a simple pool of soluble IL-15 that is shared by various immune cell types is unlikely to mediate this niche. These surprising findings not only force a reevaluation of how IL-15 and IL-15Rα regulate the homeostasis of both innate and adaptive immune cells, but also provide novel insights into the mechanisms of immune homeostasis.

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