

# IL-15R $\alpha$ chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation

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Natural killer (NK) cells are innate immune effectors that mediate rapid responses to viral antigens. Interleukin (IL)-15 and its high affinity IL-15 receptor, IL-15R $\alpha$ , support NK cell homeostasis in resting animals via a novel trans presentation mechanism. To better understand how IL-15 and IL-15R $\alpha$  support NK cell activation during immune responses, we have used sensitive assays for detecting native IL-15 and IL-15R $\alpha$  proteins and developed an assay for detecting complexes of these proteins. We find that IL-15 and IL-15R $\alpha$  are preassembled in complexes within the endoplasmic reticulum/Golgi of stimulated dendritic cells (DCs) before being released from cells. IL-15R $\alpha$  is required for IL-15 production by DCs, and IL-15 that emerges onto the cell surface of matured DCs does not bind to neighboring cells expressing IL-15R $\alpha$ . We also find that soluble IL-15–IL-15R $\alpha$  complexes are induced during inflammation, but membrane-bound IL-15–IL-15R $\alpha$  complexes, rather than soluble complexes, support NK cell activation in vitro and in vivo. Finally, we provide in vivo evidence that expression of IL-15R $\alpha$  specifically on DCs is critical for trans presenting IL-15 and activating NK cells. These studies define an unprecedented cytokine–receptor biosynthetic pathway in which IL-15R $\alpha$  serves as a chaperone for IL-15, after which membrane-bound IL-15R $\alpha$ –IL-15 complexes activate NK cells via direct cell–cell contact.

## CORRESPONDENCE

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Abbreviations used: BMDC, bone marrow–derived DC; DT, diphtheria toxin; HSC, hematopoietic stem cell; poly I:C, poly inosinic–polycytidylic acid; TLR, Toll-like receptor.

NK cells are innate immune effector cells that secrete cytokines and acquire cytolytic function within hours of viral infections. The activation of NK cells is regulated by the balance of inhibitory and activating signals and is dependent on DCs, cytokines, and co-stimulatory molecules (1–3). NK cell responses to microbes are triggered by Toll-like receptor (TLR) ligands, which stimulate secretion of type I IFNs that in turn support IL-15 and IL-15R $\alpha$  production (4–6).

IL-15 and its proprietary receptor IL-15R $\alpha$  are both essential for supporting NK cell homeostasis (7). An unusual aspect of IL-15 and IL-15R $\alpha$  binding is the extraordinarily high affinity with which these proteins interact ( $\sim 5 \times 10^{-11}$  M) (8, 9). Another notable observation is that the phenotypes of IL-15 (IL-15 $^{-/-}$ ) and IL-15R $\alpha$  (IL-15R $\alpha^{-/-}$ ) –deficient mice are indistinguishable, suggesting that physiologically relevant IL-15 signals require IL-15R $\alpha$

and that these molecules function in close cooperation (10, 11). On the other hand, some studies have reported secretion of soluble IL-15, and the ability of recombinant IL-15 to stimulate cells bearing IL-2/15R $\beta$  and  $\gamma c$  but lacking IL-15R $\alpha$  raises questions about whether and when IL-15 may perform IL-15R $\alpha$ –independent functions. Additional questions surrounding IL-15 and IL-15R $\alpha$  biology relate to the observations that IL-15 cDNAs are difficult to express heterologously unless leader sequences, ATG sites, and 3' mRNA sequences are altered (12). These findings suggest that complex intracellular processing regulates the bioavailability of IL-15.

Prior studies from our laboratory showed that IL-15R $\alpha$  functions in an unexpected non-cell-autonomous fashion in vivo (13–15). IL-15R $\alpha$  expression on responding cells does not enhance the responsiveness of IL-15-dependent cells in vivo (13–16). Soluble IL-15 can bind to IL-15R $\alpha$  on accessory cells that “trans present” IL-15 to responding cells in vitro (17).

The online version of this article contains supplemental material.

Trans presentation in vivo requires expression of IL-15 and IL-15R $\alpha$  by hematopoietic accessory cells and expression of IL-2/15R $\beta$  by IL-15-responsive NK cells and memory CD8 $^{+}$  T cells (18). Trans presentation appears to be the dominant mechanism by which IL-15 signals are delivered in vivo (13–16, 18).

Why IL-15 signals should be delivered via this somewhat complicated trans presentation mechanism is unclear. Some clues have been provided by recent studies revealing that coordinate expression of IL-15 and IL-15R $\alpha$  by the same accessory cells such as DCs is required for supporting both NK and CD8 $^{+}$  memory T cell homeostasis (18, 19). These results are not consistent with a mechanism in which IL-15 is secreted by DCs and subsequently bound to IL-15R $\alpha$  on the surface of other DCs to be trans presented to IL-15-responsive cells. Instead, they suggest a more complicated model of trans presentation.

The studies above focused on the functions of IL-15 and IL-15R $\alpha$  during noninflamed, homeostatic conditions. TLRs induce the production of both IL-15 and IL-15R $\alpha$  by myeloid cells (4–6, 20–22). Hence, to understand how endogenous IL-15 and IL-15R $\alpha$  proteins function during inflammatory responses, we have developed sensitive assays for detecting endogenous IL-15 and IL-15R $\alpha$  proteins. We have used these assays to further examine how these proteins are induced in DCs and how they support NK cell activation.

## RESULTS

### IL-15, IL-15R $\alpha$ , and complexes of IL-15–IL-15R $\alpha$ proteins are induced in DCs after TLR stimulation

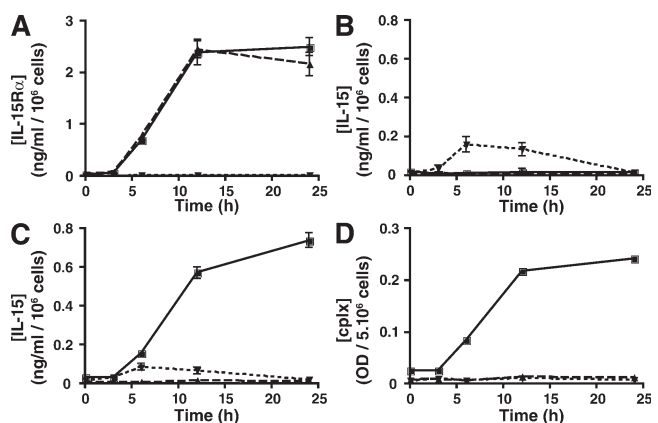
Analyses of the biology of IL-15 and IL-15R $\alpha$  have been challenging because the potent physiological effects of these proteins occur at relatively low levels of expression. To understand how IL-15 and IL-15R $\alpha$  function under physiological circumstances, we first optimized ELISAs for these proteins using recombinant IL-15 and IL-15R $\alpha$  proteins and found that we were able to detect picomolar levels of these proteins (unpublished data). Because IL-15 and IL-15R $\alpha$  bind to each other with high affinity and may function as a complex in physiological settings, we investigated whether the presence of either protein affects the detection of the other. Detection of IL-15R $\alpha$  protein was not affected by the presence of IL-15 (Fig. S1 A, available at <http://www.jem.org/cgi/content/full/jem.20071913/DC1>). In contrast, the detection of IL-15 protein was progressively diminished by the presence of increasing amounts of IL-15R $\alpha$  protein (Fig. S1 B). These results suggest that at least one of the anti-IL-15 antibodies used in our capture ELISA binds to an epitope that is masked when IL-15 binds to IL-15R $\alpha$ . Several commercially available antibodies to IL-15 shared this capacity for interference with IL-15R $\alpha$  binding (unpublished data).

mRNA transcripts for IL-15 and IL-15R $\alpha$  are induced by TLR ligands in DCs, but the translation of these proteins may not follow mRNA levels. We thus measured the levels of IL-15 and IL-15R $\alpha$  proteins before and after stimulation with LPS or poly inosinic–polycytidylic acid (poly I:C).

Negligible levels of IL-15R $\alpha$  were detected in cell lysates from nonstimulated bone marrow–derived DCs (BMDCs), and IL-15R $\alpha$  protein levels were similarly induced in lysates from WT or IL-15 $^{-/-}$  cell BMDCs (but not IL-15R $\alpha$  $^{-/-}$  cells) between 2 and 12 h (Fig. 1 A). Hence, IL-15R $\alpha$  is induced in DCs by TLR stimulation, and IL-15 does not influence the production of IL-15R $\alpha$ .

Somewhat surprisingly, IL-15 protein was only transiently detected at modest levels within IL-15R $\alpha$  $^{-/-}$  BMDCs and not at all in WT cells (Fig. 1 B). This occurred despite the fact that IL-15 mRNA levels, detected by real-time PCR, were markedly induced by poly I:C in both IL-15R $\alpha$  $^{-/-}$  and WT BMDCs (unpublished data). To understand why IL-15 protein was not detected in WT cells, we hypothesized that IL-15 in WT cells might be complexed with IL-15R $\alpha$  protein. As noted above, epitopes of IL-15 detected by several anti-IL-15 antibodies are blocked by high affinity interactions between IL-15 and IL-15R $\alpha$ . To investigate whether IL-15 is complexed with IL-15R $\alpha$  in poly I:C-stimulated BMDCs, we established conditions to dissociate these proteins without denaturing them before ELISA analyses. We boiled lysates from poly I:C-stimulated BMDCs in the presence of 0.01% SDS before analyzing IL-15 protein levels by ELISA. These experiments showed that IL-15 protein was indeed induced in WT cells at nanomolar levels in parallel with the induction of IL-15R $\alpha$  (Fig. 1 C). Similar results were obtained with LPS stimulation of DCs (unpublished data). Thus, IL-15 is induced by TLR stimulation and appears to be predominantly bound to another protein (e.g., IL-15R $\alpha$ ) inside DCs.

To further examine the possibility that endogenous IL-15 and IL-15R $\alpha$  proteins exist in complexes in normal BMDCs, we developed a “complex” ELISA in which an anti-IL-15 antibody that detects IL-15 in the presence of IL-15R $\alpha$  was



**Figure 1. Detection of IL-15 and IL-15R $\alpha$  proteins and IL-15–IL-15R $\alpha$  complex expression in lysates of TLR-stimulated DCs.** ELISA measurement of IL-15R $\alpha$  (A) and IL-15 (B) proteins and IL-15–IL-15R $\alpha$  complex (D) expression in lysates of poly I:C-stimulated BMDCs at the indicated time points. (C) ELISA determination of IL-15 protein levels after dissociation of IL-15–IL-15R $\alpha$  protein complexes with 0.01% SDS and boiling. Lines represent data from WT (■), IL-15 $^{-/-}$  (▲), and IL-15R $\alpha$  $^{-/-}$  (▼) BMDCs. All data are representative of at least three separate experiments.

used as a “capture” antibody and an anti-IL-15R $\alpha$  antibody was used as a “detection” reagent (Fig. S1 C). We used this complex ELISA to measure the levels of IL-15–IL-15R $\alpha$  complexes in cell lysates from BMDCs. IL-15–IL-15R $\alpha$  complexes were induced by poly I:C in WT BMDCs but not in *IL-15*<sup>−/−</sup> BMDCs or *IL-15R $\alpha$* <sup>−/−</sup> BMDCs (Fig. 1 D). The kinetics of IL-15–IL-15R $\alpha$  complex induction was very similar to the induction of IL-15 and IL-15R $\alpha$  proteins. Collectively, these studies suggest that IL-15 and IL-15R $\alpha$  proteins are induced in BMDCs by TLR ligands and exist largely in IL-15–IL-15R $\alpha$  complexes.

### IL-15–IL-15R $\alpha$ complexes are not detected unless IL-15 and IL-15R $\alpha$ are coordinately expressed by the same DCs

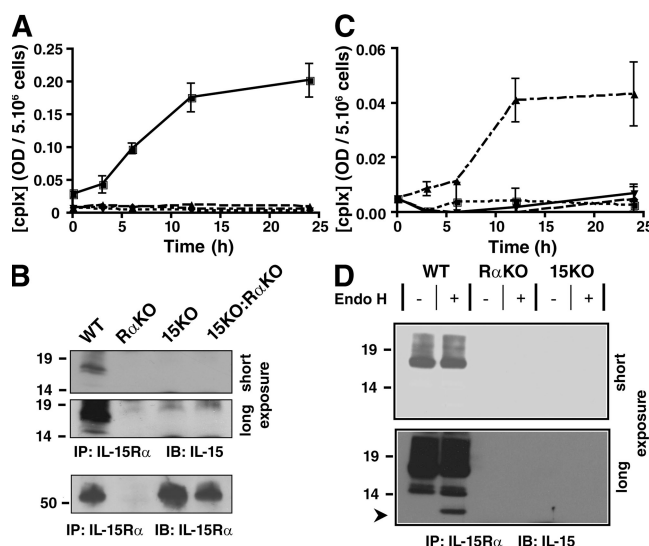
Exposure of DCs to TLR ligands is thought to stimulate IL-15 secretion from DCs, after which IL-15 may be subsequently bound to IL-15R $\alpha$  on the cell surface of DCs. The identification of IL-15–IL-15R $\alpha$  complexes in the lysates of stimulated DCs could thus reflect this sequence of events. Alternatively, our prior studies suggest that IL-15 and IL-15R $\alpha$  must be coordinately expressed on the same hematopoietic cells to support IL-15–dependent NK cells and memory CD8<sup>+</sup> T cells under homeostatic (non-inflamed) conditions (18). Thus, it is possible that IL-15R $\alpha$  and IL-15 must be coordinately expressed during TLR-induced inflammatory conditions as well. To test this hypothesis, we stimulated a 1:1 mixture of *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> BMDCs as well as uniform cultures of WT, *IL-15*<sup>−/−</sup>, and *IL-15R $\alpha$* <sup>−/−</sup> BMDCs with poly I:C and measured the production of IL-15–IL-15R $\alpha$  complexes in BMDC lysates by ELISA. As these cells were plated at concentrations at which *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> DCs were in direct contact, the production of freely soluble IL-15 protein from *IL-15R $\alpha$* <sup>−/−</sup> DCs should allow binding of IL-15 to IL-15R $\alpha$  on the surface of neighboring *IL-15*<sup>−/−</sup> DCs and potential recycling of these complexes. These experiments revealed that IL-15–IL-15R $\alpha$  complexes are detected only in cultures of poly I:C-stimulated WT BMDCs and not in 1:1 mixtures of *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> BMDCs (Fig. 2 A). IL-15–IL-15R $\alpha$  complexes were predictably absent from stimulated *IL-15*<sup>−/−</sup> cells and *IL-15R $\alpha$* <sup>−/−</sup> BMDCs (Fig. 2 A). Thus, IL-15 may not be secreted from *IL-15R $\alpha$* <sup>−/−</sup> cells. Moreover, IL-15 and IL-15R $\alpha$  proteins must be coordinately expressed within the same DCs to form IL-15–IL-15R $\alpha$  complexes—even after TLR stimulation.

To independently confirm that IL-15 and IL-15R $\alpha$  proteins must be coordinately expressed to form IL-15–IL-15R $\alpha$  complexes, we developed an immunoblotting assay to detect endogenous IL-15 bound to endogenous IL-15R $\alpha$  protein. We stimulated cultures of WT, *IL-15*<sup>−/−</sup>, *IL-15R $\alpha$* <sup>−/−</sup>, or a mixture of *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> DCs with LPS, lysed cells, immunoprecipitated endogenous IL-15R $\alpha$ , and immunoblotted for IL-15. As IL-15 is dissociated from IL-15R $\alpha$  by boiling in sample buffer (containing 1% SDS) before immunoblotting with anti-IL-15 antibody, this approach to detecting IL-15 is not compromised by potential masking of IL-15 epitopes by IL-15R $\alpha$ . Immunoprecipitation of IL-15R $\alpha$  from

WT DCs co-precipitated significant amounts of IL-15 protein, confirming that IL-15–IL-15R $\alpha$  complexes are induced in DCs by TLR stimulation (Fig. 2 B). In contrast, immunoprecipitation of IL-15R $\alpha$  yielded negligible amounts of co-precipitated IL-15 in resting WT DCs, or LPS-stimulated *IL-15*<sup>−/−</sup> DCs and *IL-15R $\alpha$* <sup>−/−</sup> DCs (Fig. 2 B and unpublished data). Importantly, IL-15 was not co-precipitated with IL-15R $\alpha$  from 1:1 mixtures of LPS-stimulated *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> DCs (Fig. 2 B). Therefore, IL-15 and IL-15R $\alpha$  must be coordinately expressed by the same cells to form IL-15–IL-15R $\alpha$  complexes—even under inflammatory conditions during which both proteins are induced.

### IL-15–IL-15R $\alpha$ complexes are preassembled in DCs in the absence of protein secretion

IL-15 protein is not efficiently produced in *IL-15R $\alpha$* <sup>−/−</sup> cells (Fig. 1 B). One mechanism by which IL-15R $\alpha$  could facilitate the production of IL-15 would be if IL-15R $\alpha$  directly bound newly synthesized IL-15 before being secreted (e.g.,



**Figure 2. Intracellular formation of IL-15–IL-15R $\alpha$  complexes within TLR-stimulated DCs before protein secretion.** (A) ELISA detection of IL-15–IL-15R $\alpha$  complexes in lysates from poly I:C-stimulated BMDCs of the indicated genotypes: WT (■), *IL-15*<sup>−/−</sup> (15KO; ▲), and *IL-15R $\alpha$* <sup>−/−</sup> (R $\alpha$ KO; ▼), and a 1:1 mixture of *IL-15*<sup>−/−</sup> with *IL-15R $\alpha$* <sup>−/−</sup> (15KO:R $\alpha$ KO; ◆) BMDCs. (B) Immunoblotting detection of IL-15R $\alpha$  co-precipitated IL-15 in LPS-stimulated BMDCs. Long and short exposures of IL-15-specific immunoblots are shown above. Note the absence of IL-15 protein in immunoprecipitates of mixed 15KO:R $\alpha$ KO BMDCs. Immunoblotting of immunoprecipitates for IL-15R $\alpha$  are shown below as a control (bottom). (C) ELISA detection of IL-15–IL-15R $\alpha$  complexes in the lysates of untreated WT DCs (◆), DCs treated with LPS plus brefeldin A (▲), DCs treated with LPS plus cycloheximide (■), and DCs treated with LPS plus brefeldin A plus cycloheximide (▼) for the indicated periods of time. (D) EndoH sensitivity of IL-15R $\alpha$  co-precipitated IL-15 from LPS-stimulated DCs. Note the appearance of an ~12.7-kD band corresponding to unglycosylated IL-15 in EndoH-treated WT DCs (arrow), but not in other control lanes. Short (top) and long (bottom) exposures of IL-15 expression are shown in the top panels. Data are representative of three separate experiments.

within the ER or Golgi) and stabilized IL-15 in a complex. To test this possibility, we pretreated BMDCs with brefeldin A (which blocks protein trafficking through the Golgi) before stimulation with LPS, and then tested lysates of these cells for the presence of IL-15–IL-15R $\alpha$  complexes. Significant levels of IL-15–IL-15R $\alpha$  complexes were induced in WT cells even in the presence of brefeldin A (Fig. 2 C). No IL-15R $\alpha$  protein was observed on the surface of these cells by flow cytometry, and no soluble IL-15R $\alpha$  (sIL-15R $\alpha$ ) was detected in supernatants, confirming that brefeldin A blocked the emergence of IL-15R $\alpha$  from the Golgi complex (Fig. S2, A and B, available at <http://www.jem.org/cgi/content/full/jem.20071913/DC1>). Thus, IL-15R $\alpha$  appears to bind IL-15 during biogenesis. Brefeldin A may also block the trafficking of proteins from recycling and/or late endosomes to the plasma membrane. To eliminate the possibility that brefeldin A exclusively unveiled recycling complexes in these experiments, we treated DCs with LPS plus cycloheximide, which should block the formation of nascent IL-15–IL-15R $\alpha$  complexes but should not block recycling complexes, and then asked whether IL-15–IL-15R $\alpha$  complexes were induced. These experiments revealed that cycloheximide prevented the formation of IL-15–IL-15R $\alpha$  complexes, suggesting that the complexes we detect in LPS plus brefeldin A-treated DCs are newly synthesized (Fig. 2 C).

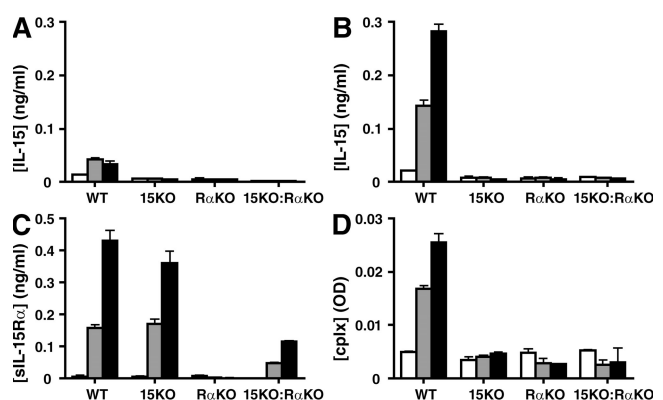
Like many transmembrane and secreted proteins, IL-15 undergoes N-linked glycosylation in the ER, a form that is sensitive to EndoH digestion (23). N-linked sugars are removed from IL-15 in the Golgi during further processing so that mature forms of IL-15 are resistant to EndoH. To determine whether IL-15–IL-15R $\alpha$  complexes may be assembled when IL-15 bears EndoH-sensitive N-linked sugars during biogenesis, we asked whether IL-15 that co-precipitated with IL-15R $\alpha$  from LPS-stimulated DCs is EndoH sensitive. LPS-stimulated DCs were lysed in NP-40 lysis buffer, immunoprecipitated with anti-IL-15R $\alpha$  antibodies, and treated with either EndoH or buffer alone before immunoblotting analysis for IL-15 expression. These experiments revealed that EndoH treatment of anti-IL-15R $\alpha$ –immunoprecipitated lysates produces a novel IL-15–specific band at ~12.7 kD, corresponding to un-glycosylated IL-15 (Fig. 2 D). Therefore, native IL-15–IL-15R $\alpha$  complexes contain EndoH-sensitive IL-15, indicating that these complexes form in the ER or early Golgi before the completion of Golgi processing.

#### TLR-stimulated DCs release soluble IL-15–sIL-15R $\alpha$ protein complexes

To follow the fate of IL-15–IL-15R $\alpha$  complexes after Golgi processing, we studied the expression of these proteins on the cell surface of stimulated DCs. Emergence of IL-15R $\alpha$  protein onto the surface of DCs occurs normally in the absence of IL-15, but emergence of IL-15 requires IL-15R $\alpha$  (Fig. S3, available at <http://www.jem.org/cgi/content/full/jem.20071913/DC1>). IL-15 may be secreted from cells, and a soluble form of IL-15R $\alpha$  (sIL-15R $\alpha$ ) can be released from cells (24, 25). Accordingly, we studied the levels of IL-15 and

sIL-15R $\alpha$  proteins in supernatants from poly I:C-stimulated BMDCs. These experiments revealed that minimal amounts of IL-15 were induced by poly I:C stimulation in the supernatants of WT BMDCs after 24 (Fig. 3 A, gray columns) and 48 (Fig. 3 A, black columns) h, but not in supernatants from IL-15 $^{-/-}$  and IL-15R $\alpha$  $^{-/-}$  cells. Given the interference of IL-15R $\alpha$  with ELISA-mediated detection of IL-15 in cell lysates (Fig. 1 B), we suspected that IL-15 might be present in IL-15–sIL-15R $\alpha$  protein complexes in these supernatants. We thus boiled these supernatants in the presence of 0.01% SDS before ELISA and found that IL-15 was indeed induced and progressively accumulated in the supernatants from WT but not IL-15 $^{-/-}$  cells (Fig. 3 B). Thus, IL-15 is secreted from WT DCs predominantly in the form of IL-15–sIL-15R $\alpha$  complexes. Importantly, no IL-15 was observed in the supernatants from IL-15R $\alpha$  $^{-/-}$  DCs, even after boiling of the supernatants (Fig. 3 B). Thus, IL-15R $\alpha$  is required for the emergence of IL-15 from cells.

Our assays of sIL-15R $\alpha$  levels in supernatants from poly I:C-stimulated DCs show that soluble IL-15R $\alpha$  accumulated in the supernatants from WT cells and was detected at similar levels in the supernatants from IL-15 $^{-/-}$  cells (Fig. 3 C). Hence, IL-15 is not required for the production or release of sIL-15R $\alpha$ . To directly examine whether coordinate expression of IL-15 and IL-15R $\alpha$  was required for producing soluble IL-15–sIL-15R $\alpha$  protein complexes in the supernatants of poly I:C-stimulated DCs, we assayed supernatants of 1:1 mixed cultures of IL-15 $^{-/-}$  and IL-15R $\alpha$  $^{-/-}$  DCs for the presence of these complexes. These experiments revealed that IL-15–sIL-15R $\alpha$  complexes were detected only in the supernatants of WT cells, but not when IL-15 and IL-15R $\alpha$  are produced by separate cells (Fig. 3 D). These results all mirror the findings we obtained in the cellular lysates of BMDCs. Thus, the



**Figure 3. Release of IL-15 and sIL-15R $\alpha$  proteins and IL-15–sIL-15R $\alpha$  complexes from TLR-stimulated BMDCs.** (A–D) ELISA of supernatants from stimulated BMDCs. WT, 15KO, R $\alpha$ KO, and mixed 15KO:R $\alpha$ KO BMDCs were stimulated with poly I:C for 0 (white bars), 24 (gray bars), and 48 h (black bars), after which supernatants were assayed for the production of (A) IL-15, (C) IL-15R $\alpha$ , and (D) IL-15–sIL-15R $\alpha$  complex expression. (B) Supernatants from TLR-stimulated DCs were supplemented with 0.01% SDS and boiled before ELISA for IL-15 expression. Data are representative of three independent experiments.



differences in IL-15, IL-15R $\alpha$ , and IL-15–IL-15R $\alpha$  protein levels are likely due to differences in protein production rather than differences in recycling or secretion from the cells.

### Coordinate expression of IL-15 and IL-15R $\alpha$ by DCs is required for supporting NK cell activation in vitro

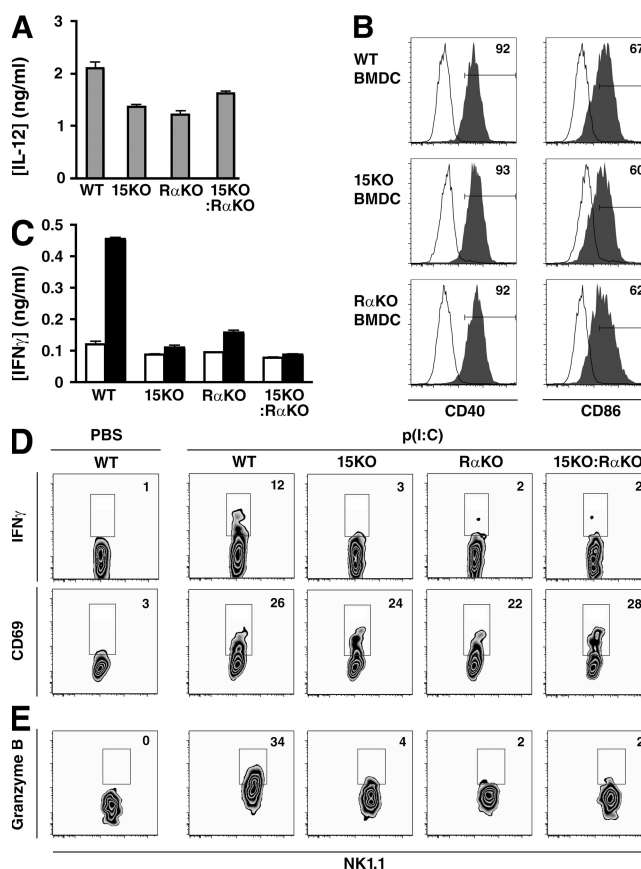
Our prior studies indicated that IL-15R $\alpha$  expression on DCs is required for NK cell activation (20). As IL-15 signaling in splenic DCs has been suggested to support their maturation, we first examined the activation of WT, *IL-15*<sup>−/−</sup>, and *IL-15R $\alpha$* <sup>−/−</sup> BMDCs (26). Stimulation of BMDCs with poly I:C led to modestly reduced levels of IL-12 production and normal levels of CD40 and CD86 up-regulation (Fig. 4, A and B). Thus, TLR-triggered activation of BMDCs proceeds largely normally in the absence of either IL-15 or IL-15R $\alpha$ .

To determine whether coordinated expression of IL-15 and IL-15R $\alpha$  by DCs is required for activating NK cells, we stimulated WT, *IL-15*<sup>−/−</sup>, *IL-15R $\alpha$* <sup>−/−</sup>, or a 1:1 mixture of *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> BMDCs with poly I:C and co-cultured these cells with NK cells. We then assayed NK cell activation in these cultures by testing the supernatants of these cultures for the production of IFN- $\gamma$  by ELISA. These studies revealed that IFN- $\gamma$  was secreted in significant amounts only in cultures containing WT BMDCs and not in cultures from *IL-15*<sup>−/−</sup>, *IL-15R $\alpha$* <sup>−/−</sup>, or a mixture of these DCs (Fig. 4 C). Flow cytometric analyses of these NK cells revealed that NK cells from all poly I:C-stimulated cultures up-regulated expression of the surface activation marker CD69 (Fig. 4 D). However, only NK cells co-cultured with WT DCs expressed significant levels of intracellular IFN- $\gamma$  and intracellular granzyme B, indicating that these cells were fully activated and armed with cytotoxic granules (Fig. 4, D and E). Thus, coordinate expression of IL-15 and IL-15R $\alpha$  in DCs is required for NK cell activation, and the failure of mixtures of *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> BMDCs to form IL-15–IL-15R $\alpha$  complexes leads to a failure of these cells to fully activate NK cells.

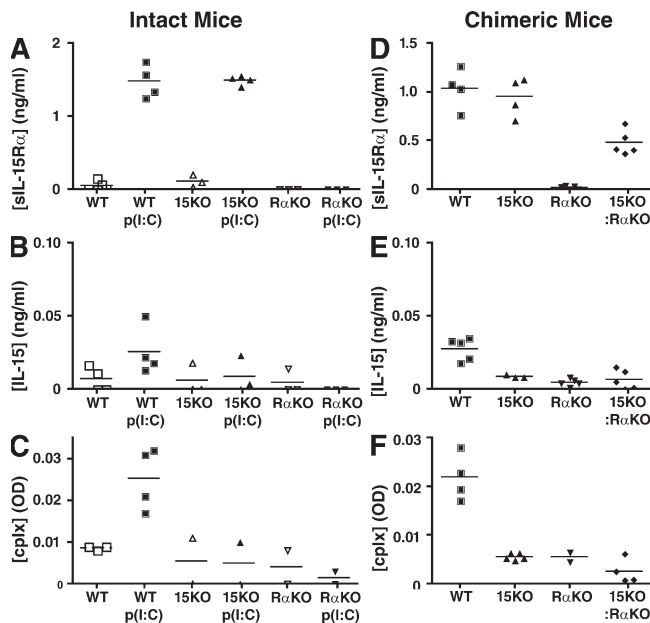
### sIL-15R $\alpha$ and IL-15–sIL-15R $\alpha$ complexes are induced in the sera of stimulated mice

Multiple cell types may be able to respond to TLR ligands and elaborate IL-15 and IL-15R $\alpha$ . To determine whether the mechanisms that we have elucidated in DCs apply to physiological responses in vivo, we studied the expression of IL-15, sIL-15R $\alpha$ , and IL-15–sIL-15R $\alpha$  complexes in the serum of resting and poly I:C-stimulated mice by ELISA. These experiments revealed that negligible levels of IL-15 and IL-15R $\alpha$  are present in the sera of resting mice. 24 h after stimulation with poly I:C, sIL-15R $\alpha$  was detected at similarly high levels in the serum of WT and *IL-15*<sup>−/−</sup> mice, but not in *IL-15R $\alpha$* <sup>−/−</sup> mice (Fig. 5 A). Similar results were obtained with LPS stimulation (unpublished data). Thus, sIL-15R $\alpha$  is physiologically released into the sera from TLR-stimulated mice. Minimal levels of soluble IL-15 were detected in the serum of poly I:C-treated WT mice, and no appreciable IL-15 was found in the sera from *IL-15*<sup>−/−</sup> mice

or *IL-15R $\alpha$* <sup>−/−</sup> mice (Fig. 5 B). It was not technically feasible to perform ELISA analyses on boiled sera samples and thereby quantitate IL-15 protein samples in complexes. However, ELISAs revealed that IL-15–sIL-15R $\alpha$  complexes are induced only in WT mice (Fig. 5 C). Thus, similar to our findings with cultured BMDCs, soluble complexes of IL-15 and sIL-15R $\alpha$  are induced by TLR stimulation in vivo.



**Figure 4. Coordinate expression of IL-15 and IL-15R $\alpha$  is required for NK cell activation.** (A) ELISA measurement of IL-12 secretion by poly I:C-stimulated BMDCs of the indicated genotypes. (B) Flow cytometric analysis of DC activation after poly I:C stimulation. Histograms of poly I:C-stimulated DCs (gray-filled histograms) of the indicated genotypes indicating surface expression levels of the surface activation markers CD40 and CD86. (C) ELISA determination of IFN- $\gamma$  secretion by NK cells co-cultured with WT, 15KO, R $\alpha$ KO, or mixed 15KO:R $\alpha$ KO BMDCs. BMDCs were treated with PBS or poly I:C for 18 h, after which NK cells were co-cultured with activated DCs for an additional 6 h. Poly I:C-stimulated cultures are indicated by black bars, and PBS-stimulated control cultures are indicated by white bars. Note that significant poly I:C-induced NK cell activation occurs only in the presence of WT DCs. (D and E) Flow cytometric measurement of NK cell (NK1.1<sup>+</sup>) activation by poly I:C (p(I:C)) -stimulated DC–NK cell co-cultures. Elevated CD69 surface staining reflects initial (TLR-dependent, IL-15-independent) activation of NK cells. IFN- $\gamma$  and granzyme B expression by NK cells were performed by intracellular staining 6 and 12 h after stimulation, respectively. Numbers indicate the percentage of cells in the indicated gate. Note that NK cells express significant levels of both IFN- $\gamma$  and granzyme B only when activated by WT DCs. Plots are representative of three separate experiments.



**Figure 5. Soluble IL-15, IL-15R $\alpha$ , and IL-15-sIL-15R $\alpha$  complex detection in mouse sera.** ELISA determination of sIL-15R $\alpha$ , IL-15, and IL-15-sIL-15R $\alpha$  proteins in sera from intact (A–C, on left) or chimeric (D–F, on right) mice stimulated with 25  $\mu$ g/g poly I:C or PBS. Sera were collected 24 h after stimulation and IL-15 (B and E), sIL-15R $\alpha$  (A and D), and IL-15-sIL-15R $\alpha$  complex (C and F) protein levels were measured by ELISA. Each symbol reflects values obtained from individual mice.

We then asked whether a mixture of *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> hematopoietic cells could generate IL-15-sIL-15R $\alpha$  complexes in vivo after TLR stimulation. To address this question, we first interbred *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> mice to generate double-mutant mice. These double-mutant mice phenotypically resemble both *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> mice, reinforcing the idea that IL-15 and IL-15R $\alpha$  function together and do not perform significant functions independently from each other (unpublished data). We irradiated these double-mutant mice and reconstituted them with WT, *IL-15*<sup>−/−</sup>, *IL-15R $\alpha$* <sup>−/−</sup>, or a 1:1 mixture of *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> hematopoietic stem cells (HSCs). 6 wk after reconstitution, we stimulated these mice with poly I:C and assayed their sera for the presence of IL-15, IL-15R $\alpha$ , and IL-15-sIL-15R $\alpha$  complex proteins after 24 h by ELISA. These experiments revealed that modest levels of IL-15 were induced in chimeras reconstituted with WT cell HSCs, but not in the other chimera (Fig. 5 E). sIL-15R $\alpha$  was detected at significant levels in the sera of both WT and *IL-15*<sup>−/−</sup> HSC-reconstituted chimera, and at roughly half these levels when *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> HSCs were mixed at 1:1 (Fig. 5 D). Importantly, IL-15-sIL-15R $\alpha$  complexes were detected only in the sera of poly I:C-stimulated chimera reconstituted with WT cells and not in chimera reconstituted with a mixture of *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> HSCs (Fig. 5 F). Collectively, these results suggest that hematopoietic cells in intact mice regulate IL-15 and IL-15R $\alpha$  production in a similar fashion

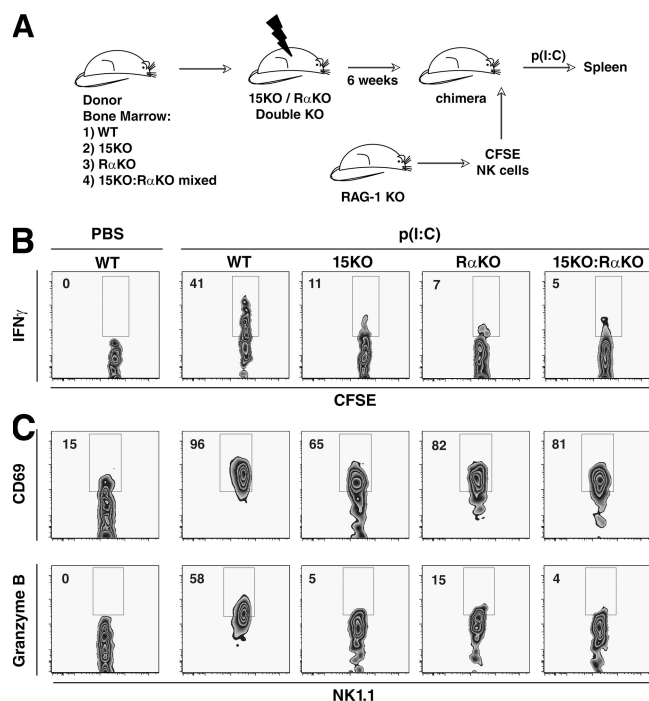
to cultured BMDCs. Therefore, the cell biology we have defined in BMDCs is an accurate reflection of physiological IL-15-sIL-15R $\alpha$  production in vivo.

#### Coordinate expression of IL-15 and IL-15R $\alpha$ by hematopoietic cells is required for supporting NK cell activation in vivo

To determine whether coordinated expression of IL-15 and IL-15R $\alpha$  is also required for activating NK cells in vivo, we tested the ability of the radiation chimera mice described above to activate NK cells. Consistent with our prior studies, only mice reconstituted with WT HSCs possessed appreciable numbers of NK cells (14, 18, 19). To have comparable NK cells to assay, we purified NK cells from *RAG-1*<sup>−/−</sup> mice, labeled them with CFSE, and adoptively transferred these labeled NK cells into the radiation chimera before stimulating the mice with poly I:C (see experimental design, Fig. 6 A). Production of IFN- $\gamma$  and granzyme B by the adoptively transferred NK cells was assayed by flow cytometry 6 and 12 h later, respectively. Importantly, although adoptively transferred (CFSE<sup>+</sup>) NK cells were stimulated to express CD69 in all poly I:C-stimulated mice, NK cells only expressed significant amounts of IFN- $\gamma$  and granzyme B in WT mice (Fig. 6, B and C). Thus, the failure of mixtures of *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> hematopoietic cells to form IL-15-sIL-15R $\alpha$  complexes correlates with a failure of these chimeric mice to activate NK cells in vivo.

#### Membrane-bound IL-15-IL-15R $\alpha$ complexes, rather than soluble IL-15-sIL-15R $\alpha$ complexes, support NK cell activation in vitro

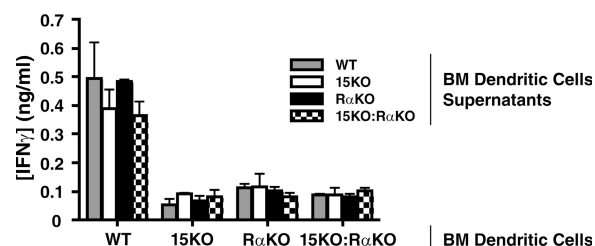
The findings above suggest that membrane-bound IL-15-IL-15R $\alpha$  complexes, soluble IL-15-sIL-15R $\alpha$  complexes, or both stimulate NK cells. To determine the relative contributions of these forms of IL-15-IL-15R $\alpha$ , we treated WT, *IL-15*<sup>−/−</sup>, *IL-15R $\alpha$* <sup>−/−</sup>, or a 1:1 mixture of *IL-15*<sup>−/−</sup> and *IL-15R $\alpha$* <sup>−/−</sup> BMDCs with poly I:C, after which supernatants of the four types of cultures were exchanged between cultures. NK cells were subsequently co-cultured with BMDCs and the exchanged supernatants, after which IFN- $\gamma$  production was measured by ELISA. These studies revealed that poly I:C-stimulated WT DCs activated NK cells regardless of the type of supernatant present, whereas no other DCs or mixtures of DCs were able to activate NK cells (Fig. 7). In contrast, soluble IL-15-sIL-15R $\alpha$  complexes found in WT supernatants did not augment NK cell activation any better than supernatants lacking these complexes (Fig. 7). Moreover, soluble IL-15-sIL-15R $\alpha$  complexes in supernatants of poly I:C-stimulated WT BMDCs were unable to support NK cell activation in the presence of *IL-15*<sup>−/−</sup> or *IL-15R $\alpha$* <sup>−/−</sup> DCs (Fig. 7). Similar results were obtained with LPS stimulation (unpublished data). Thus, membrane-bound IL-15-IL-15R $\alpha$  complexes are the critical mediators of IL-15-mediated NK cell activation. Meanwhile, soluble IL-15-sIL-15R $\alpha$  complexes are unable to either augment membrane-bound complexes or compensate for the lack of membrane-bound complexes.



**Figure 6. Full activation of NK cells requires coordinate expression of IL-15 and IL-15R $\alpha$  in vivo.** (A) Experimental design. Bone marrow HSCs of the indicated genotypes were used to reconstitute irradiated *IL-15*<sup>-/-</sup> *IL-15R $\alpha$* <sup>-/-</sup> double-mutant (15KO/R $\alpha$ KO DKO) mice. After 6 wk, NK cells from *RAG-1*<sup>-/-</sup> (RAG-1 KO) mice were CFSE labeled and transferred into the chimera. Mice were then activated with 25  $\mu$ g/g poly I:C and studied for NK cell activation. (B and C) Flow cytometric analyses of (B) surface CD69 expression and intracellular IFN- $\gamma$  after 6 h and (C) CD69 and intracellular granzyme B expression after 12 h. Note robust NK cell expression of intracellular IFN- $\gamma$  and granzyme B in WT HSC-reconstituted chimera, but not in chimera reconstituted with 15KO, R $\alpha$ KO, or mixed 15KO/R $\alpha$ KO HSCs. Results are representative of two independent experiments.

#### Membrane-bound IL-15–IL-15R $\alpha$ complexes, rather than soluble IL-15–sIL-15R $\alpha$ complexes, support NK cell activation ex vivo

To determine whether membrane-bound or soluble IL-15–sIL-15R $\alpha$  complexes support NK cell activation ex vivo, we tested whether serum containing these complexes could support NK cell activation. We generated radiation chimera in which *IL-15*<sup>-/-</sup> *IL-15R $\alpha$* <sup>-/-</sup> double-mutant mice were reconstituted with WT, *IL-15*<sup>-/-</sup>, *IL-15R $\alpha$* <sup>-/-</sup>, or a 1:1 mixture of *IL-15*<sup>-/-</sup> and *IL-15R $\alpha$* <sup>-/-</sup> HSCs. 24 h after stimulation with poly I:C, IL-15–sIL-15R $\alpha$  complexes were present only in the sera of WT HSC-reconstituted chimera, consistent with our findings above (Fig. 5). We incubated this undiluted serum with purified NK cells in vitro for 6 h and assayed the NK cells for expression of CD69 and intracellular IFN- $\gamma$ . These studies showed that serum from poly I:C-stimulated mice caused NK cells to express CD69 but did not induce IFN- $\gamma$  expression (Fig. S4, available at <http://www.jem.org/cgi/content/full/jem.20071913/DC1>). Reinforcing our findings above with supernatants from activated BMDCs (Fig. 7), these studies indicate that membrane-bound



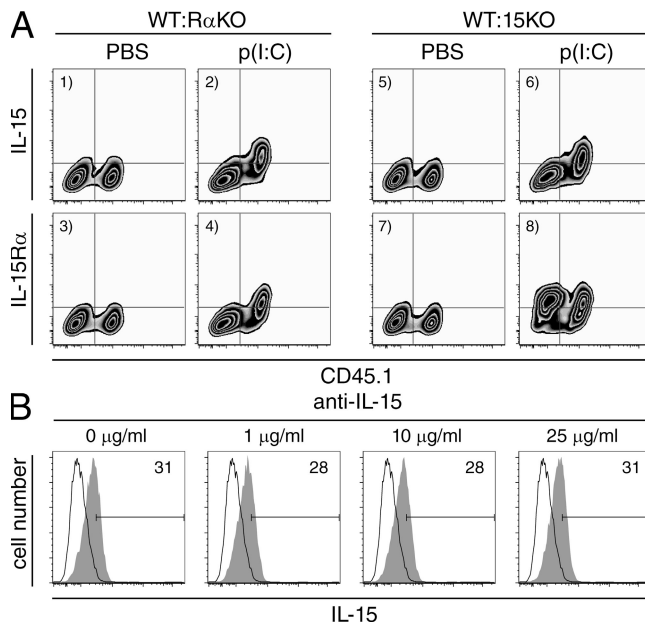
**Figure 7. Soluble IL-15 complexed with sIL-15R $\alpha$  fails to activate NK cells.** Activation of IFN- $\gamma$  secretion by NK cells co-cultured with various combinations of poly I:C-stimulated BMDCs and supernatants from these BMDCs. BMDCs from various genotypes (indicated along the x axis of graph) were activated with poly I:C and then supplemented with supernatants exchanged from similarly activated BMDCs. Genotypes of DCs from which supernatants were derived are indicated by individual columns (gray, WT; white, 15KO; black, R $\alpha$ KO; checked, mixture of 15KO and R $\alpha$ KO). ELISA quantitation of NK cell IFN- $\gamma$  secretion is indicated on the y axis. Note that WT DCs activate NK cells regardless of the type of supernatant added. Note also that WT DC-derived supernatants containing IL-15–sIL-15R $\alpha$  complexes fail to support or augment NK cell activation.

IL-15–IL-15R $\alpha$  complexes and not soluble IL-15–sIL-15R $\alpha$  complexes provide the critical signal for stimulating IFN- $\gamma$  production by NK cells in vitro and in vivo.

#### IL-15 that emerges from normal DCs does not bind to IL-15R $\alpha$ on neighboring DCs

Free IL-15 has been reported in certain pathological conditions, and soluble recombinant IL-15 can be added to cells and bind to IL-15R $\alpha$ . Hence, an additional question is whether IL-15–IL-15R $\alpha$  complexes that emerge onto cell surfaces dissociate at an appreciable rate to release free soluble IL-15 that can bind to IL-15R $\alpha$  on other cells. To address this question, we stimulated 1:1 mixtures of congenic CD45.1<sup>+</sup> WT DCs and either CD45.2<sup>+</sup> (CD45.1<sup>-</sup>) *IL-15*<sup>-/-</sup> (15KO) DCs or CD45.2<sup>+</sup> (CD45.1<sup>-</sup>) *IL-15R $\alpha$* <sup>-/-</sup> (R $\alpha$ KO) DCs with poly I:C. Cell surface expression levels of IL-15 and IL-15R $\alpha$  were examined after 24 h. In mixtures of WT and *IL-15R $\alpha$* <sup>-/-</sup> DCs, both IL-15 and IL-15R $\alpha$  proteins are readily induced by poly I:C on the surface of WT (CD45.1<sup>+</sup>) cells, whereas neither protein is seen on the surface of *IL-15R $\alpha$* <sup>-/-</sup> (CD45.1<sup>-</sup>) cells (Fig. 8 A, panels 1–4, and Fig. S5, which is available at <http://www.jem.org/cgi/content/full/jem.20071913/DC1>). In mixtures of WT (CD45.1<sup>+</sup>) and *IL-15*<sup>-/-</sup> (CD45.1<sup>-</sup>) DCs, IL-15R $\alpha$  protein is induced to identical levels on the surfaces of both types of cells by poly I:C (Fig. 8 A, panels 7 and 8, and Fig. S5). Remarkably, IL-15 protein was induced only on the surface of WT (CD45.1<sup>+</sup>) cells and was not found on *IL-15*<sup>-/-</sup> (CD45.1<sup>-</sup>) cells, despite the fact that *IL-15*<sup>-/-</sup> cells express identical levels of surface IL-15R $\alpha$  (Fig. 8 A, panels 5 and 6, and Fig. S5). Thus, IL-15 that emerges from WT cells is not released in appreciable amounts to bind to contiguous, neighboring cells.

To further examine whether IL-15–IL-15R $\alpha$  complexes on the surface of DCs might release free soluble IL-15 protein, even if transiently, we tested the ability of an anti-IL-15



**Figure 8. IL-15 that emerges from normal DCs does not bind to IL-15R $\alpha$  on neighboring DCs.** (A) Flow cytometric analyses of surface IL-15 and IL-15R $\alpha$  expression in mixed DC cultures. 1:1 mixtures of congenic WT (CD45.1<sup>+</sup>) and IL-15R $\alpha$ <sup>-/-</sup> BMDCs (WT:R $\alpha$ KO; panels 1–4) or 1:1 mixtures of congenic WT (CD45.1<sup>+</sup>) and IL-15<sup>-/-</sup> BMDCs (WT:15KO; panels 5–8) were stimulated in vitro with PBS or poly I:C for 24 h. Surface expression of IL-15 and IL-15R $\alpha$  (and CD45.1) proteins was then analyzed by flow cytometry. Note that poly I:C-stimulated IL-15<sup>-/-</sup> BMDCs express normal levels of IL-15R $\alpha$  (panel 8), but fail to capture or bind IL-15 even though virtually all of their neighboring WT DCs possess elevated levels of IL-15 in the same cultures (panel 6). (B) Flow cytometric analysis of surface IL-15 expression on normal BMDCs stimulated with poly I:C in the presence of the indicated doses of anti-IL-15 antibody. Note that WT BMDCs induce similar amounts of surface IL-15 protein (gray, filled histograms) regardless of the amount of inhibitor anti-IL-15 antibody in the cultures. Numbers indicate the percentage of cells whose fluorescence intensity lies beyond the unstimulated control cells. All data are representative of at least two independent experiments.

monoclonal antibody that binds only free IL-15 to compete with the formation of membrane IL-15–IL-15R $\alpha$  complexes on the surface of poly I:C-stimulated DCs. We incubated progressively higher doses of anti-IL-15 antibody with WT DCs at the same time that they were stimulated with poly I:C and measured the amount of membrane-bound IL-15 after 24 h. These experiments revealed that the number of DCs expressing surface IL-15 and the intensity of surface staining were unchanged by doses of anti-IL-15 antibody of up to 25  $\mu$ g/ml (Fig. 8 B). Hence, negligible amounts of membrane-bound IL-15 are released by membrane IL-15R $\alpha$ . Therefore, physiologically induced IL-15–IL-15R $\alpha$  complexes on DC surfaces do not dissociate at appreciable rates.

#### IL-15R $\alpha$ expression on DCs is essential for NK cell activation in vivo

Our recent work showed that IL-15R $\alpha$  expression by DCs is essential for DC-mediated NK cell activation in vitro, and re-

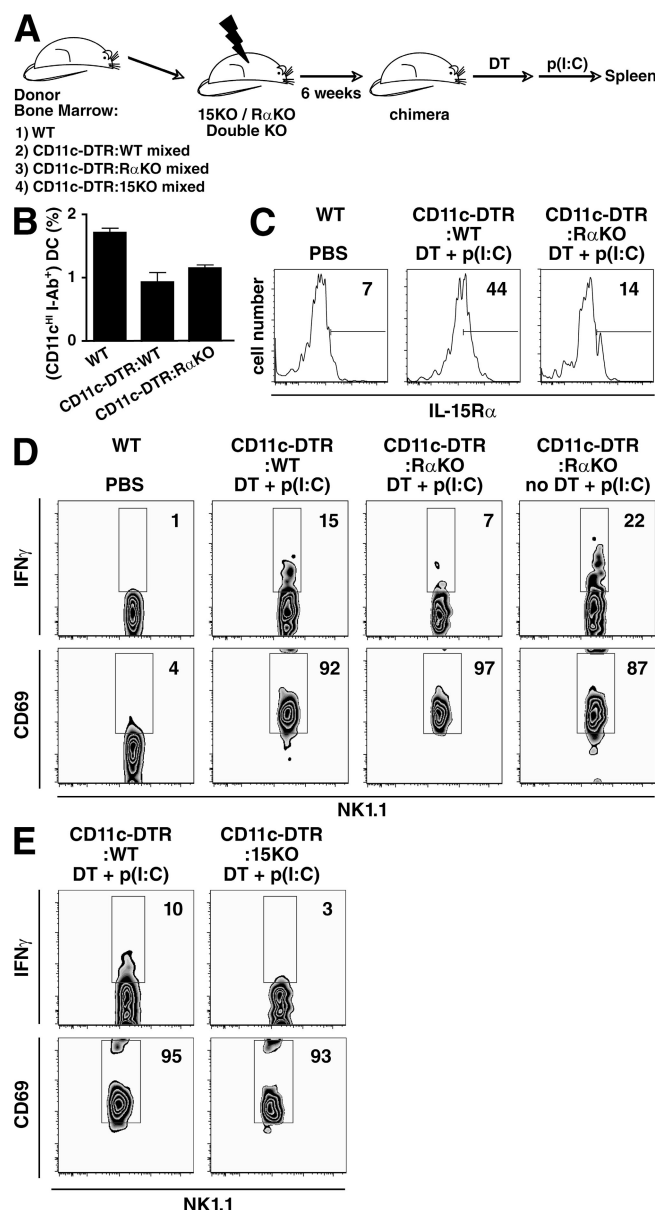
cent studies have suggested that both IL-15 and DCs are critical for NK cell activation in vivo (20, 22). However, these studies leave open the possibility that IL-15 and DCs might independently support NK cell activation in vivo. For example, IL-15 might be produced by macrophages to stimulate DCs to activate NK cells in vivo. To more directly investigate whether IL-15R $\alpha$  expression by DCs mediates NK cell activation in vivo, we reconstituted irradiated IL-15<sup>-/-</sup> IL-15R $\alpha$ <sup>-/-</sup> double-mutant mice with a 1:1 mixture of HSCs from CD11c–diphtheria toxin (DT) receptor (CD11c–DTR) transgenic mice and HSCs from either WT or IL-15R $\alpha$ <sup>-/-</sup> mice. After hematopoietic reconstitution, DT was injected into the chimera to transiently eliminate DCs. 24 h later, mice were injected with poly I:C. Finally, 6 h later, the status of CD11cHi DCs and the activation of NK cells were studied by flow cytometry (see experimental scheme, Fig. 9 A). These experiments revealed that DT eliminated approximately half of the CD11cHi DCs in chimera reconstituted with a 1:1 mixture of CD11c–DTR and other HSCs (Fig. 9 B). The remaining CD11cHi DCs expressed IL-15R $\alpha$  after poly I:C stimulation in chimera reconstituted with WT HSCs and did not express IL-15R $\alpha$  in chimera reconstituted with IL-15R $\alpha$ <sup>-/-</sup> HSCs (Fig. 9 C). Thus, this experiment created chimeric mice in which IL-15R $\alpha$ <sup>-/-</sup> DCs could be compared directly to IL-15R $\alpha$ <sup>+</sup> DCs in their ability to activate NK cells.

Turning to NK cell activation in these chimera, we found that NK cells up-regulated CD69 in all poly I:C-stimulated mice, so both WT and IL-15R $\alpha$ <sup>-/-</sup> DCs support this initial step of NK cell activation (Fig. 9 D). In contrast, WT DCs stimulated much greater numbers of NK cells to produce IFN- $\gamma$  when compared with IL-15R $\alpha$ <sup>-/-</sup> DCs (Fig. 9 D, compare second to third columns). Mice bearing a mixture of CD11c–DTR and IL-15R $\alpha$ <sup>-/-</sup> DCs also supported NK cell production of IFN- $\gamma$  in the absence of DT (Fig. 9 D, compare third to fourth columns). Parallel experiments performed with chimeric mice reconstituted with mixtures of CD11c–DTR and either WT or IL-15<sup>-/-</sup> HSCs revealed similar results, i.e., IL-15<sup>-/-</sup> DCs are also unable to activate NK cells in vivo (Fig. 9 E). Therefore, DCs must express both IL-15 and IL-15R $\alpha$  to activate NK cells under physiological conditions.

#### DISCUSSION

Our current studies reveal a novel cell biological mechanism by which IL-15 is coexpressed with IL-15R $\alpha$  in DCs after TLR stimulation and binds IL-15 within the ER–Golgi complex. Plasma membrane-bound IL-15–IL-15R $\alpha$  complexes are highly stable complexes that do not release appreciable amounts of free IL-15 to neighboring IL-15R $\alpha$ -bearing cells. These membrane-bound complexes trans present IL-15 to responsive cells during cell–cell contact, mediating the critical stimulatory signal to NK cells. Soluble IL-15–sIL-15R $\alpha$  complexes are released from the cell surface, but these complexes fail to provide agonistic signals. We have coupled these cell biological studies with in vivo studies that have confirmed the physiological validity of our experiments with cultured DCs. Finally, we have demonstrated that IL-15R $\alpha$





**Figure 9. DC-specific expression of IL-15Rα is required for NK cell activation in vivo.** (A) Experimental scheme. The indicated mixtures of HSCs were used to reconstitute double-mutant 15KO/RαKO mice. After 6 wk, chimeric mice were treated with DT and then activated with poly I:C. (B) Flow cytometric quantitation of splenic DCs (CD11c<sup>HI</sup> I-Ab<sup>+</sup>) after DT treatment. The percentage of splenic DCs from the chimera reconstituted with the indicated HSCs is shown. (C) Selective loss of CD11c-DTR DCs in mixed radiation chimera. Surface expression of IL-15Rα after DT treatment and poly I:C was analyzed by flow cytometry. Note that elimination of CD11c-DTR DCs by DT treatment leaves elevated surface expression of IL-15Rα on the remaining WT DCs in CD11c-DTR:WT mixed chimera (middle), whereas DT treatment of CD11c-DTR:RαKO mixed chimera leaves residual IL-15Rα<sup>-/-</sup> DCs (right). Splenic DCs from PBS-treated mice is shown as a control (left). (D) NK cells require IL-15Rα expression on DCs for full activation in vivo. Splenic NK cells from the mice above were analyzed for CD69 and IFN-γ expression by flow cytometry. Note that chimeras containing WT DCs (second column) or CD11c-DTR DCs (no DT treatment, fourth column) but not chimera containing RαKO DCs (third

expression specifically on DCs plays an essential role in supporting NK cell activation in mice. As membrane-bound IL-15Rα–IL-15 complexes on DCs appear to play a dominant role in supporting NK cell activation in vivo, these studies highlight the notion that cytokine signals may be delivered by specific cell types in cell contact-dependent fashion.

### IL-15Rα, a chaperone for IL-15

We have found that EndoH-sensitive forms of IL-15 are bound to IL-15Rα in LPS-stimulated DCs, and that IL-15–IL-15Rα complexes can be found in brefeldin A-treated but not cycloheximide-treated cells. Considered together with our discovery that DCs must express IL-15Rα to secrete IL-15, it is likely that IL-15Rα binds to IL-15 and stabilizes this protein in the ER and/or Golgi apparatus. Such complexes probably prevent free IL-15 from undergoing ER-associated protein degradation, a well-established process by which “misfolded” proteins in the ER are proteolyzed in ubiquitin- and proteasome-dependent processes (27). Recycling and trans presentation of these membrane-bound complexes may then stimulate responding cells (14). Hence, our results suggest that IL-15Rα functions both as an intracellular chaperone for IL-15 as well as an extracellular scaffold that presents IL-15 to responsive cells.

We have shown that IL-15–IL-15Rα complexes that emerge onto the surface of DCs fail to release soluble IL-15 to neighboring IL-15Rα-expressing DCs. These surprising observations suggest that IL-15–IL-15Rα complexes on DC surfaces are highly stable, a finding consistent with the high affinity of IL-15Rα for IL-15 ( $\sim 5 \times 10^{-11}$  M) (8). Thus, although heterologous IL-15 can be added to IL-15Rα-expressing cells, form cell surface complexes, and stimulate IL-15-dependent responses, the physiological production and trans presentation of IL-15 by DCs appears to predominantly involve IL-15–IL-15Rα complexes that form intracellularly during biosynthesis and remain intact for prolonged periods (e.g., 24 h) (17).

### Preassembly of IL-15–IL-15Rα protein complexes in DCs explains a requirement for coordinate expression of IL-15 and IL-15Rα to support NK cell activation

Our finding that IL-15–IL-15Rα complexes are preassembled within DCs provides an explanation for why coordinate expression of these molecules is required for NK cell activation

column) support NK cell production of IFN-γ (top panels). Note that mice containing significant residual DCs after DT treatment induce NK cell expression of CD69 after poly I:C (columns 2–4). WT HSC-reconstituted chimera treated with PBS (first column) are included as a control. Results are representative of two independent experiments, with eight mice used for each experiment. (E) NK cells require IL-15 expression on DCs for full activation in vivo. Chimera reconstituted with a 1:1 mixture of CD11c-DTR HSCs with either WT HSCs (left) or IL-15<sup>-/-</sup> HSCs (right) were treated as in A and D above, and flow cytometric analyses of NK cell expression of CD69 and IFN-γ are shown.

in vitro and in vivo. Coordinate expression of IL-15 and IL-15R $\alpha$  by DCs is required for the formation of IL-15–IL-15R $\alpha$  complexes and the activation of NK cells in vitro and in vivo after TLR stimulation. This result parallels prior findings that coordinate expression of these molecules is necessary to perform IL-15–dependent homeostatic functions in resting mice (18, 19). Hence, the cell biology of IL-15 and IL-15R $\alpha$  interactions during inflammatory conditions resembles the homeostatic condition.

In addition to identifying intracellular complexes of IL-15 and IL-15R $\alpha$ , we have identified soluble extracellular IL-15–sIL-15R $\alpha$  complexes that are released from DCs after TLR stimulation. A prior study described the presence of soluble IL-15R $\alpha$  in the serum of resting mice, and proposed that IL-15R $\alpha$  could be proteolytically cleaved from the cell surface (24). Our current experiments extend and contrast with the prior results in two respects. First, we find that the production and release of soluble sIL-15R $\alpha$  is induced by TLR stimulation, rather than being constitutively present at high levels (e.g., 40 ng/ml) in resting C57BL/6J strain mice (24). Second, we find that the majority of soluble IL-15R $\alpha$  is complexed with IL-15. Therefore, although free IL-15 is not released from DCs, soluble IL-15–sIL-15R $\alpha$  complexes, which may represent IL-15 bound to a proteolytic product of IL-15R $\alpha$ , are released into solution.

Our results with both cultured DC supernatants and mouse sera indicate that membrane-bound IL-15–IL-15R $\alpha$  complexes on DCs and not soluble IL-15–sIL-15R $\alpha$  complexes support NK cell production of IFN- $\gamma$  and granzyme B. Although soluble IL-15–sIL-15R $\alpha$  complexes might perform other functions, this important finding suggests that trans presentation of IL-15 requires cell–cell contact between IL-15–presenting and IL-15–responsive cells. The cell contact–dependent nature of IL-15 trans presentation also implies that IL-15 signals may be delivered in the context of other signals between the two cells, akin to antigen presentation between DCs and T cells. Finally, our findings imply that IL-15 signals are delivered via direct cell–cell contact rather than via soluble proteins. Hence, cytokine-mediated survival, growth, and activation signals may be delivered by specific cell types and in specific locations.

Although the physiological functions of soluble IL-15–sIL-15R $\alpha$  complexes are unclear, it is important to note that these complexes are neither agonistic nor antagonistic in our DC–NK cell co-culture assays (Fig. 7 A). In this regard, non-agonistic soluble IL-15–sIL-15R $\alpha$  complexes contrast with agonistic soluble IL-6–IL-6R complexes (28). This observation provides important clues about their function. If soluble complexes bound to IL-2/15R $\beta$ / $\gamma$ c receptors as well as membrane-bound complexes, one might expect them to display either agonist or antagonist activities (via competitive inhibition). Thus, soluble IL-15–sIL-15R $\alpha$  complexes may not bind well to cell surface IL-2/15R $\beta$  receptors on cell surfaces under physiological circumstances. This failure of IL-15–sIL-15R $\alpha$  complexes to bind IL-2/15R $\beta$  receptors could be due to differences between the structures of the soluble IL-15–

sIL-15R $\alpha$  complex and the membrane-bound IL-15–IL-15R $\alpha$  complex. Alternatively, membrane-bound IL-15–IL-15R $\alpha$  complexes may possess a competitive advantage over soluble IL-15–sIL-15R $\alpha$  complexes due to the increased local concentration or valency of membrane-bound complexes during cell–cell contact.

The failure of endogenous soluble IL-15–sIL-15R $\alpha$  complexes to activate NK cells can also be contrasted with recent studies indicating that certain cytokine-specific antibodies can form complexes with their cognate cytokines, stabilize cytokines, and amplify the immunological effects of these cytokines (29–31). There are multiple differences between antibody–cytokine complexes and receptor–cytokine complexes. For example, different anti-IL-15 antibodies or forms of IL-15R $\alpha$  may interfere with IL-15 binding to IL-2/15R $\beta$  and  $\gamma$ c receptors. In addition, antibodies, but not soluble cytokine receptors, can also bind to Fc receptors on multiple cells. Recent structural studies of IL-15 and IL-15R $\alpha$  proteins have revealed important insights into how these proteins interact and should facilitate future investigations into how such receptor–cytokine complexes differ from antibody–cytokine complexes (32). Combining these biochemical insights with the divergent biological properties of these complexes should facilitate future attempts to engineer these proteins for therapeutic benefit (33, 34).

#### DCs require IL-15R $\alpha$ to trans present IL-15 to NK cells during NK cell activation

We have used mixed radiation chimera to selectively unveil the role of IL-15R $\alpha$  expression by DCs during NK cell activation in vivo. Our findings extend our prior work that IL-15R $\alpha$  on BMDCs is critical for activating NK cells in vitro (20). Our current findings are also consistent with a recent study using both IL-15 $^{-/-}$  mice and CD11c–DTR mice to show that both IL-15 and DCs are required for NK cell activation in vivo (22). By using mixed radiation chimera generated from both IL-15R $\alpha$  $^{-/-}$  and CD11c–DTR HSCs, we have extended the prior work to show that IL-15R $\alpha$  expression specifically on DCs mediates NK cell activation in vivo. Further elucidation of these interactions should be facilitated by the generation of mice bearing lineage-specific deletions of IL-15 or IL-15R $\alpha$ . Considered together with our data showing that DCs trans present IL-15 via cell–cell contact, and with recent work showing the localization of NK cells to lymphoid structures, a model of NK cell activation emerges in which NK cells are recruited to DCs and receive critical IL-15–IL-15R $\alpha$ –dependent activation signals during DC–NK cell contact (35, 36). In this regard, DC activation of NK cells broadly resembles DC activation of naive T cells, during which an immunological synapse constitutes a sophisticated cell contact–dependent activation mechanism. Indeed, direct evidence for a DC–NK cell synapse directing IL-15 signaling to NK cells has recently been reported (37).

Recent structural studies have revived the notion that IL-2R $\alpha$  may trans present IL-2 (38–40). Although IL-2 signals clearly perform distinct biological functions from IL-15

signals in vivo, and although IL-2R $\alpha$  has not shown the capacity to bind and trans present IL-2 in vivo, directed cytokine presentation may nevertheless be a feature of IL-15, IL-2, and perhaps other cytokines (7, 10, 38, 41, 42). This mode of cell contact–dependent delivery of cytokine signals would greatly restrict the cells that receive cytokine signals. Finally, given the rapidity with which memory CD8 $^{+}$  T cells are reactivated in vivo, the requirement for DCs to reactivate memory CD8 $^{+}$  T cells, the selective expression of IL-2/15R $\beta$  receptors by these cells, and the IL-15 dependence of these cells, it is possible that memory CD8 $^{+}$  T cell reactivation involves a similar IL-15–dependent activation event mediated by DCs (43).

In summary, we have shown that IL-15 and IL-15R $\alpha$  are preformed as complexes in DCs in response to TLR stimulation. IL-15–IL-15R $\alpha$  complexes progress to the DC surface where they deliver critical activating signals to NK cells during cell–cell contact. IL-15–IL-15R $\alpha$  complexes that are cleaved from the surface of DCs may be byproducts of a termination mechanism for this potent stimulus. This novel series of cell biological events refines our understanding of IL-15R $\alpha$ –mediated trans presentation, and highlights how the immune system delivers cytokine signals in a highly regulated and cell type– and geographically restricted fashion.

## MATERIALS AND METHODS

### Mice, radiation chimera, adoptive transfers, and in vivo DC ablation.

*IL-15R $\alpha^{-/-}$*  (R $\alpha$ KO) mice were described previously (10). All strains were backbred to a C57B1/6J background for at least 12 generations. *IL-15 $^{-/-}$*  (15KO) and *RAG-1 $^{-/-}$*  mice on a C57BL/6J background were purchased from Taconic Laboratories. CD11c–DTR transgenic mice were purchased from The Jackson Laboratory (44). Double-mutant *IL-15 $^{-/-}$  IL-15R $\alpha^{-/-}$*  (15KO/R $\alpha$ KO) mice were generated by interbreeding in our facility. Radiation bone marrow chimeras were produced as described previously, except that mixed 15KO:R $\alpha$ KO, CD11c–DTR:WT, and CD11c–DTR:R $\alpha$ KO radiation chimera were generated by using mixtures (1:1) of bone marrow cells from congenic donors of the indicated genotypes of mice (18). All mice were used between the ages of 6 and 12 wk of age. All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco.

For in vivo stimulation experiments, mice were injected intraperitoneally with 10 g/kg LPS (Sigma-Aldrich) or 25 g/kg poly I:C (GE Healthcare). For adoptive transfer experiments, NK cells were purified from the spleens of *RAG-1 $^{-/-}$*  mice by adherence and magnetic bead depletion of myeloid cells, and in some experiments, labeled with CFSE before intravenous injection into recipient mice as described previously (18). In vivo depletion of CD11c $^{+}$  DTR $^{+}$  DCs was performed using a single injection of 2–4  $\mu$ g/kg body weight of DT (EMD) (44).

**In vitro DC preparations and assays.** BMDCs were generated in culture as described previously (18). The purity of BMDC cultures was checked by analyzing an aliquot of each culture for surface expression of CD11c and CD11b by flow cytometry (BD Biosciences). Splenic DCs were enriched by digesting spleens with 2.5 mg/ml collagenase B (Boehringer Mannheim) and 0.3 mg/ml DNase I (Sigma-Aldrich), followed by isolation on percoll gradients as described previously (18). TLR stimulation of DCs was performed with either 1  $\mu$ g/ml LPS or 25  $\mu$ g/ml poly I:C. To inhibit protein secretion, BMDCs were treated with Golgi Plug (brefeldin A; BD Biosciences) during the LPS stimulation per the manufacturer's instructions. To inhibit protein synthesis, BMDCs were treated with 10  $\mu$ g/ml cycloheximide during LPS stimulation.

For DC-mediated activation of NK cells in vitro,  $2 \times 10^5$ /ml BMDCs were cultured for 24 h in the presence of 1  $\mu$ g/ml LPS or 25  $\mu$ g/ml poly I:C, followed by incubation with  $10^5$ /ml NK cells as described previously (20).

**Detection of endogenous IL-15 and IL-15R $\alpha$  proteins.** For ELISA-based quantitation of proteins, IL-15R $\alpha$  was quantified using the Duo-set kit (R&D Systems) according to the manufacturer's instructions. IL-15 was quantified by using anti-IL-15 AIO3 antibody (eBioscience) as a capture reagent and biotinylated BAF447 antibody (R&D Systems) as the detection reagent. For IL-15–IL-15R $\alpha$  complex detection, anti-IL-15 AF447 antibody (R&D Systems) was used as the capture antibody and anti-IL-15R $\alpha$  (from the Duo-set kit) as the detection antibody. For ELISA-based analyses of intracellular IL-15 and IL-15R $\alpha$  proteins,  $5\text{--}10 \times 10^6$  BMDCs per condition were lysed in NP-40 lysis buffer (50 mM Hepes, 120 mM NaCl, 1 mM EDTA, 0.1% NP-40, and protease inhibitor cocktail [Roche]). IFN- $\gamma$  and IL-12 were quantified using a commercial assay kit according to the manufacturer's instructions (BD Biosciences).

For immunoblotting detection of intracellular IL-15 and IL-15R $\alpha$ ,  $5\text{--}10 \times 10^6$  BMDCs were stimulated for 12 or 24 h with 0.1  $\mu$ g/ml LPS washed in PBS and lysed in NP-40 or RIPA lysis buffer (0.5% SDS, 0.1% deoxycholic acid and 1% NP-40 in PBS, 150 mM NaCl, 50 mM Tris, pH 7.5, 1 mM NaVO $_4$ , 1 mM NaF, 1 mM PMSF, and protease inhibitors [Roche]), cleared by centrifugation (14,000 g for 20 min at 4°C), and immunoprecipitated with a mixture of anti-IL-15R $\alpha$  AF 551 (R&D Systems) and anti-IL-15R $\alpha$  N19 (Santa Cruz Biotechnology, Inc.) with a mixture of protein A– and protein G–coupled Sepharose beads. Immunoprecipitates were then washed in lysis buffer and subjected to SDS-PAGE. Immunoblotting of transferred proteins was performed with either rat anti-IL-15 antibody (Amgen) and goat anti-rat IgG horseradish peroxidase (Jackson ImmunoResearch Laboratories) or biotin-conjugated anti-IL-15R $\alpha$  antibody BAF 551 (R&D Systems) and streptavidin–horseradish peroxidase (BD Biosciences). EndoH sensitivity assays were performed by treating immunoprecipitates with EndoH per the manufacturer's instructions (New England Biolabs, Inc.).

Surface IL-15 expression was detected using biotinylated anti-IL-15 antibody (PeproTech). IL-15R $\alpha$  expression on cell surfaces was detected by using biotinylated anti-IL-15R $\alpha$  antibody BAF551 (R&D Systems) as described previously (15). IFN- $\gamma$  and granzyme B production by NK cells was determined by intracellular cytokine staining as described previously (Caltag and eBioscience) (12). Cell surface expression of NK1.1, CD69, CD40, CD86, CD11b, CD11c, and I-A $^b$  was performed using commercial antibodies (BD Biosciences). Cells were analyzed with an LSR II cytometer (Becton Dickinson) and FloJo software.

**Online supplemental material.** Fig. S1 shows the detection of IL-15 and IL-15R $\alpha$  proteins and the IL-15–IL-15R $\alpha$  complex. In Fig. S2, brefeldin A treatment blocks the emergence of IL-15R $\alpha$  onto the surface of cells or into the supernatants of DCs. Fig. S3 shows the expression of IL-15 and sIL-15R $\alpha$  proteins on the cell surface of TLR-stimulated BMDCs. Fig. S4 shows that soluble IL-15 complexed with sIL-15R $\alpha$  fails to activate NK cells ex vivo. In Fig. S5, IL-15 that emerges from normal DCs does not bind to IL-15R $\alpha$  on neighboring DCs. The online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20071913/DC1>.

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## REFERENCES

- Lanier, L.L. 2006. Natural killer cells: roundup. *Immunol. Rev.* 214:5–8.
- Yokoyama, W.M., and S. Kim. 2006. Licensing of natural killer cells by self-major histocompatibility complex class I. *Immunol. Rev.* 214:143–154.
- Degli-Esposti, M.A., and M.J. Smyth. 2005. Close encounters of different kinds: dendritic cells and NK cells take centre stage. *Nat. Rev. Immunol.* 5:112–124.
- Zhang, X., S. Sun, I. Hwang, D.F. Tough, and J. Sprent. 1998. Potent and selective stimulation of memory-phenotype CD8<sup>+</sup> T cells in vivo by IL-15. *Immunity*. 8:591–599.
- Nguyen, K.B., T.P. Salazar-Mather, M.Y. Dalod, J.B. Van Deusen, X.Q. Wei, F.Y. Liew, M.A. Caligiuri, J.E. Durbin, and C.A. Biron. 2002. Coordinated and distinct roles for IFN- $\alpha$  beta, IL-12, and IL-15 regulation of NK cell responses to viral infection. *J. Immunol.* 169:4279–4287.
- Mattei, F., G. Schiavoni, F. Belardelli, and D.F. Tough. 2001. IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation. *J. Immunol.* 167:1179–1187.
- Ma, A., R. Koka, and P. Burkett. 2006. Diverse functions of IL-2, IL-15, and IL-7 in lymphoid homeostasis. *Annu. Rev. Immunol.* 24:657–679.
- Giri, J.G., S. Kumaki, M. Ahdieh, D.J. Friend, A. Loomis, K. Shanebeck, R. DuBose, D. Cosman, L.S. Park, and D.M. Anderson. 1995. Identification and cloning of a novel IL-15 binding protein that is structurally related to the  $\alpha$  chain of the IL-2 receptor. *EMBO J.* 14:3654–3663.
- Mortier, E., A. Quemener, P. Vusio, I. Lorenzen, Y. Boublik, J. Grotzinger, A. Plet, and Y. Jacques. 2006. 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  $\times$  IL-15R  $\alpha$  fusion proteins. *J. Biol. Chem.* 281:1612–1619.
- Lodolce, J.P., D.L. Boone, S. Chai, R.E. Swain, T. Dassopoulos, S. Trettin, and A. Ma. 1998. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity*. 9:669–676.
- Kennedy, M.K., M. Glaccum, S.N. Brown, E.A. Butz, J.L. Viney, M. Embers, N. Matsuki, K. Charrier, L. Sedger, C.R. Willis, et al. 2000. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med.* 191:771–780.
- Bamford, R.N., A.P. DeFilippis, N. Azimi, G. Kurys, and T.A. Waldmann. 1998. The 5' untranslated region, signal peptide, and the coding sequence of the carboxyl terminus of IL-15 participate in its multifaceted translational control. *J. Immunol.* 160:4418–4426.
- Lodolce, J.P., P.R. Burkett, D.L. Boone, M. Chien, and A. Ma. 2001. T cell-independent interleukin-15R $\alpha$  signals are required for bystander proliferation. *J. Exp. Med.* 194:1187–1194.
- Koka, R., P.R. Burkett, M. Chien, S. Chai, F. Chan, J.P. Lodolce, D.L. Boone, and A. Ma. 2003. Interleukin (IL)-15R $\alpha$ -deficient natural killer cells survive in normal but not IL-15R $\alpha$ -deficient mice. *J. Exp. Med.* 197:977–984.
- Burkett, P.R., R. Koka, M. Chien, S. Chai, F. Chan, A. Ma, and D.L. Boone. 2003. IL-15R  $\alpha$  expression on CD8<sup>+</sup> T cells is dispensable for T cell memory. *Proc. Natl. Acad. Sci. USA.* 100:4724–4729.
- Schluns, K.S., K.D. Klonowski, and L. Lefrancois. 2004. Transregulation of memory CD8 T-cell proliferation by IL-15R $\alpha$ <sup>+</sup> bone marrow-derived cells. *Blood*. 103:988–994.
- Dubois, S., J. Mariner, T.A. Waldmann, and Y. Tagaya. 2002. IL-15R $\alpha$  recycles and presents IL-15 in trans to neighboring cells. *Immunity*. 17:537–547.
- Burkett, P.R., R. Koka, M. Chien, S. Chai, D.L. Boone, and A. Ma. 2004. Coordinate expression and trans presentation of interleukin (IL)-15R $\alpha$  and IL-15 supports natural killer cell and memory CD8<sup>+</sup> T cell homeostasis. *J. Exp. Med.* 200:825–834.
- Sandau, M.M., K.S. Schluns, L. Lefrancois, and S.C. Jameson. 2004. Cutting edge: transpresentation of IL-15 by bone marrow-derived cells necessitates expression of IL-15 and IL-15R  $\alpha$  by the same cells. *J. Immunol.* 173:6537–6541.
- Koka, R., P. Burkett, M. Chien, S. Chai, D.L. Boone, and A. Ma. 2004. Cutting edge: murine dendritic cells require IL-15R  $\alpha$  to prime NK cells. *J. Immunol.* 173:3594–3598.
- Ferlazzo, G., M. Pack, D. Thomas, C. Paludan, D. Schmid, T. Strowig, G. Bougras, W.A. Muller, L. Moretta, and C. Munz. 2004. Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs. *Proc. Natl. Acad. Sci. USA.* 101:16606–16611.
- Lucas, M., W. Schachterle, K. Oberle, P. Aichele, and A. Diefenbach. 2007. Dendritic cells prime natural killer cells by trans-presenting interleukin 15. *Immunity*. 26:503–517.
- Kurys, G., Y. Tagaya, R. Bamford, J.A. Hanover, and T.A. Waldmann. 2000. The long signal peptide isoform and its alternative processing direct the intracellular trafficking of interleukin-15. *J. Biol. Chem.* 275:30653–30659.
- Budagian, V., E. Bulanov, Z. Orinska, A. Ludwig, S. Rose-John, P. Saftig, E.C. Borden, and S. Bulfone-Paus. 2004. Natural soluble interleukin-15R $\alpha$  is generated by cleavage that involves the tumor necrosis factor- $\alpha$ -converting enzyme (TACE/ADAM17). *J. Biol. Chem.* 279:40368–40375.
- Mortier, E., J. Bernard, A. Plet, and Y. Jacques. 2004. Natural, proteolytic release of a soluble form of human IL-15 receptor  $\alpha$ -chain that behaves as a specific, high affinity IL-15 antagonist. *J. Immunol.* 173:1681–1688.
- Ohteki, T., K. Suzue, C. Maki, T. Ota, and S. Koyasu. 2001. Critical role of IL-15-IL-15R for antigen-presenting cell functions in the innate immune response. *Nat. Immunol.* 2:1138–1143.
- Meusser, B., C. Hirsch, E. Jarosch, and T. Sommer. 2005. ERAD: the long road to destruction. *Nat. Cell Biol.* 7:766–772.
- Scheller, J., N. Ohnesorge, and S. Rose-John. 2006. Interleukin-6 trans-signalling in chronic inflammation and cancer. *Scand. J. Immunol.* 63:321–329.
- Boyman, O., M. Kovar, M.P. Rubinstein, C.D. Surh, and J. Sprent. 2006. Selective stimulation of T cell subsets with antibody-cytokine immune complexes. *Science*. 311:1924–1927.
- Rubinstein, M.P., M. Kovar, J.F. Purton, J.H. Cho, O. Boyman, C.D. Surh, and J. Sprent. 2006. Converting IL-15 to a superagonist by binding to soluble IL-15R $\alpha$ . *Proc. Natl. Acad. Sci. USA.* 103:9166–9171.
- Stoklasek, T.A., K.S. Schluns, and L. Lefrancois. 2006. Combined IL-15/IL-15R $\alpha$  immunotherapy maximizes IL-15 activity *in vivo*. *J. Immunol.* 177:6072–6080.
- Chirifu, M., C. Hayashi, T. Nakamura, S. Toma, T. Shuto, H. Kai, Y. Yamagata, S.J. Davis, and S. Ikemizu. 2007. Crystal structure of the IL-15-IL-15R $\alpha$  complex, a cytokine-receptor unit presented in trans. *Nat. Immunol.* 8:1001–1007.
- Waldmann, T.A. 2006. The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. *Nat. Rev. Immunol.* 6:595–601.
- Sato, N., H.J. Patel, T.A. Waldmann, and Y. Tagaya. 2007. The IL-15/IL-15R $\alpha$  on cell surfaces enables sustained IL-15 activity and contributes to the long survival of CD8 memory T cells. *Proc. Natl. Acad. Sci. USA.* 104:588–593.
- Fernandez, N.C., A. Lozier, C. Flament, P. Ricciardi-Castagnoli, D. Bellet, M. Suter, M. Perricaudet, T. Tursz, E. Maraskovsky, and L. Zitvogel. 1999. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses *in vivo*. *Nat. Med.* 5:405–411.
- Bajénoff, M., B. Breart, A.Y. Huang, H. Qi, J. Cazareth, V.M. Braud, R.N. Germain, and N. Glaichenhaus. 2006. Natural killer cell behavior in lymph nodes revealed by static and real-time imaging. *J. Exp. Med.* 203:619–631.
- Brilot, F., T. Strowig, S.M. Roberts, F. Arrey, and C. Münz. 2007. NK cell survival mediated through the regulatory synapse with human DCs requires IL-15R $\alpha$ . *J. Clin. Invest.* 117:3316–3329.
- Eicher, D.M., and T.A. Waldmann. 1998. IL-2R  $\alpha$  on one cell can present IL-2 to IL-2R  $\beta$ / $\gamma$ (c) on another cell to augment IL-2 signaling. *J. Immunol.* 161:5430–5437.



39. Rickert, M., X. Wang, M.J. Boulanger, N. Goriatcheva, and K.C. Garcia. 2005. The structure of interleukin-2 complexed with its alpha receptor. *Science*. 308:1477–1480.
40. Stauber, D.J., E.W. Debler, P.A. Horton, K.A. Smith, and I.A. Wilson. 2006. Crystal structure of the IL-2 signaling complex: paradigm for a heterotrimeric cytokine receptor. *Proc. Natl. Acad. Sci. USA*. 103:2788–2793.
41. Willerford, D.M., J. Chen, J.A. Ferry, L. Davidson, A. Ma, and F.W. Alt. 1995. Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity*. 3:521–530.
42. Ku, C.C., M. Murakami, A. Sakamoto, J. Kappler, and P. Marrack. 2000. Control of homeostasis of CD8+ memory T cells by opposing cytokines. *Science*. 288:675–678.
43. Zammit, D.J., L.S. Cauley, Q.M. Pham, and L. Lefrancois. 2005. Dendritic cells maximize the memory CD8+ T cell response to infection. *Immunity*. 22:561–570.
44. Jung, S., D. Unutmaz, P. Wong, G. Sano, K. De los Santos, T. Sparwasser, S. Wu, S. Vuthoori, K. Ko, F. Zavala, et al. 2002. *In vivo* depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. *Immunity*. 17:211–220.