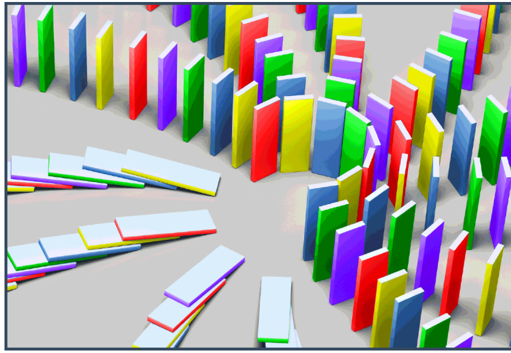
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J Immunol (2004) 173 (6): 3594–3598.
https://doi.org/10.4049/jimmunol.173.6.3594

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CUTTING EDGE

Cutting Edge: Murine Dendritic Cells Require IL-15Rα to Prime NK Cells[1]

Rima Koka, Patrick Burkett, Marcia Chien, Sophia Chai, David L. Boone, and Averil Ma[2]

***NK cells protect hosts against viral pathogens and transformed cells, and dendritic cells (DCs) play important roles in activating NK cells. We now find that murine IL-15Rα-deficient DCs fail to support NK cell cytolytic activity and elaboration of IFN-γ, despite the fact that these DCs express normal levels of costimulatory molecules and IL-12. By contrast, IL-15Rα expression on NK cells is entirely dispensable for their activation by DCs. In addition, blockade with anti-IL-15Rα and anti-IL-2Rβ but not anti-IL-2Rα-specific Abs prevents NK cell activation by wild-type DCs. Finally, presentation of IL-15 by purified IL-15Rα/Fc in* trans *synergizes with IL-12 to support NK cell priming. These findings suggest that murine DCs require IL-15Rα to present IL-15 in* trans *to NK cells during NK cell priming. The Journal of Immunology,* 2004, 173: 3594–3598.**

Natural killer cells defend the host against cells bearing altered or foreign MHC molecules. To become activated to perform these functions, NK cells initially receive priming signals and subsequently integrate target-specific signals from NK cell inhibitory and activating receptors (1). Recent studies suggest that dendritic cells (DCs)[3] may contribute to NK cell priming by enhancing both NK cell IFN-γ production and cytolytic activity (2–5). However, the mechanisms by which these interactions occur are incompletely defined.

IL-15 supports the homeostasis of CD8$^+$ T cells, TCR $\gamma\delta^+$ intraepithelial lymphocytes, NKT cells, and NK cells (6–9). IL-15 was originally thought to bind to a heterotrimeric IL-15R comprised of IL-15Rα, IL-2Rβ, and common γ-chain (γ_c), with the IL-15R α-chain conferring high affinity binding of IL-15, and the cytoplasmic domains of the low affinity IL-2Rβ and γ_c chains initiating cytoplasmic signals (10–13). The similar phenotypes of IL-15$^{-/-}$ and IL-15R$\alpha^{-/-}$ mice suggest that IL-15Rα is required for IL-15 signals (8, 9). Recent studies surprisingly indicate that noncell autonomous expression of IL-15Rα is essential for supporting bystander proliferation of memory phenotype CD8$^+$ T cells, homeostasis of CD8$^+$ memory T cells, and survival of NK cells in peripheral tissues (e.g., spleen, liver, lungs, and intestine), while the expression of IL-15Rα on these lymphocytes appears to play little or no role in supporting these cells (14–16). Although such indirect roles for IL-15Rα could involve multiple cell types, experiments with radiation chimera suggest that IL-15Rα expression on hemopoietic cells may be more important than nonhemopoietic cells in supporting both CD8$^+$ memory T cells and NK cells (15, 16). One potential mechanism for IL-15Rα function is suggested by the observation that IL-15Rα on activated human monocytes can present IL-15 in *trans* to IL-15-dependent T cell lines in vitro (17).

In addition to supporting NK cell survival and proliferation, IL-15 may support NK cell activation. NK cells express receptors for both IL-12 and IL-15, and IL-15 has been shown to synergize with IL-12 in inducing NK cell IFN-γ production (18–20). Furthermore, IL-12 and IL-15 cooperate to initiate NK cell-dependent IFN-γ-independent inflammatory responses against tumors in vivo (21, 22). Finally, IL-15 and IL-15Rα are widely expressed in multiple cell types and tissues, and their expression levels increase during infection. These findings suggest that IL-15 and IL-15Rα may support NK cell activation. Considered along with prior observations that IL-15Rα functions in a noncell autonomous fashion (14–17), and that DCs can activate NK cells (23), we hypothesized that IL-15Rα expression on DCs might support NK cell activation.

Materials and Methods

Mice

IL-15R$\alpha^{-/-}$ mice (8) were backcrossed to C57BL/6J and C57BL/6J/SJL (Ly5.2$^+$) congenic mice for ten generations. C57BL/6J/SJL (Ly5.2$^+$) congenic mice and RAG-1$^{-/-}$ C57BL/6J/SJL mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Radiation bone marrow chimera were generated as previously described (15).

Cell preparations

Bone marrow-derived DCs (BMDCs) were generated using 10 ng/ml IL-4 (PeproTech, Rocky Hill, NJ) and 20 ng/ml GM-CSF (PeproTech). Splenic NK cells were purified from RAG-1$^{-/-}$ mice using EasySep NK1.1 beads (StemCell Technologies, Vancouver, British Columbia, Canada), yielding cell purities >90%, or by flow cytometric sorting, yielding cell purities of >95%. NK cells (0.1×10^6) and BMDCs (0.2×10^6) were cocultured for 18 h in the presence or absence of LPS (1 μg/ml; Sigma-Aldrich, St. Louis, MO). Where indicated, BMDCs were incubated with LPS (1 μg/ml) for 18 h, washed, and

Department of Medicine, University of California, San Francisco, CA 94143

Received for publication May 4, 2004. Accepted for publication June 4, 2004.

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[1] This work was supported by National Institutes of Health Grants RO1AI45860, RO1AI59827, and T32DK60414 (to R.K.), and the Sandler Family Foundation.

[2] Address correspondence and reprint requests to Dr. Averil Ma, Department of Medicine, University of California, 513 Parnassus Avenue, S-1057, San Francisco, CA 94143-0451. E-mail address: ama1@itsa.ucsf.edu

[3] Abbreviations used in this paper: DC, dendritic cell; BMDC, bone marrow-derived DC; γ_c, common γ-chain.

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cultured with NK cells for an additional 18 h. Abs directed against IL-12, IL-2Rβ, IL-2Rα (BD Pharmingen, San Diego, CA), and IL-15Rα (R&D Systems, Minneapolis, MN) were used (5 μg/ml) where indicated. Cell-free IL-15Rα *trans* presentation experiments were performed by coating plates with either murine or human IL-15Rα-Fc (3 μg/ml), blocking with PBS plus FCS (10%), and then adding the indicated concentrations of murine IL-15. Wells were then washed, and NK cells were added in the presence or absence of rIL-12 (10 ng/ml). Cytokines were quantitated by ELISA (BD Pharmingen).

Cytotoxicity assays

A total of 5×10^{3} ^{51}Cr-labeled YAC-1 cells were incubated at the indicated E:T ratios with NK cells and BMDCs. Percentage of specific lysis was determined as described (8).

Immunofluorescence assays

Surface marker analyses were performed as previously described, except for surface IL-15Rα expression (R&D Systems), which was performed with 2% FCS and 0.01% NaN_3. Intracellular IFN-γ production was determined using a Cytofix/Cytoperm kit (BD Pharmingen).

Molecular analyses

IL-15Rα protein expression in total cell lysates was detected by Western blotting using an anti-murine IL-15Rα Ab as previously described (R&D Systems) (24).

Results and Discussion

IL-15Rα expression is induced on the surface of murine BMDCs but is not required for BMDC differentiation or activation

To determine whether IL-15Rα expression by murine BMDCs may support NK cells, we first examined whether BMDCs express IL-15Rα protein. Western analyses of total cell lysates from BMDCs revealed that IL-15Rα protein expression is induced in BMDCs by LPS stimulation (Fig. 1*A*). No band corresponding to IL-15Rα is seen in IL-15Rα$^{-/-}$ DCs before or after LPS stimulation, confirming the specificity of this Ab (Fig. 1*A*). To determine whether IL-15Rα expression may be induced on the surface of DCs, we used the same Ab to analyze BMDCs by flow cytometry. These studies revealed that BMDCs express modest amounts of IL-15Rα on their cell surface at rest, and that IL-15Rα expression levels increase after LPS stimulation (Fig. 1*A*). To study the functional responses of IL-15Rα$^{-/-}$ DCs, we examined the activation markers of myeloid BMDCs from IL-15Rα$^{-/-}$ and IL-15Rα$^{+/-}$ mice. IL-15Rα$^{-/-}$ and IL-15Rα$^{+/-}$ BMDCs were obtained in similar numbers and expressed similar levels of CD11c, CD11b, and CD40 (data not shown). Thus, although IL-15 may play a role in the differentiation of Langerhans-type DCs, IL-15Rα is not required for BMDCs to differentiate into myeloid DCs (25).

We then examined the responses of IL-15Rα$^{-/-}$ and IL-15Rα$^{+/-}$ BMDCs to LPS. These experiments revealed that IL-15Rα$^{-/-}$ and IL-15Rα$^{+/-}$ BMDCs expressed comparable levels of the activation/maturation markers CD40 and CD86 both before and after LPS stimulation (Fig. 1*B*). In addition, the numbers of lymphoid (CD11c^{+}, CD11b^{-}) and myeloid (CD11c^{+}, CD11b^{+}) DCs obtained from spleens of IL-15Rα$^{-/-}$ and IL-15Rα$^{+/-}$ mice were also similar (data not shown), and both lymphoid and myeloid DCs from IL-15Rα$^{-/-}$ and IL-15Rα$^{+/-}$ mice up-regulated expression of CD40 similarly after in vivo treatment with poly(I:C) (Supplemental Fig. 1).[4] Thus, IL-15Rα$^{-/-}$ DCs up-regulate costimulatory molecules normally in vitro and in vivo. DCs elaborate IL-12 after stimulation with LPS, and IL-12 enhances NK cell activation. IL-12 production by IL-15Rα$^{-/-}$ and IL-15Rα$^{+/-}$ BMDCs was comparable during 18 h of LPS stimulation (data not shown). Hence, although IL-15$^{-/-}$ macrophages have been reported to exhibit defective IL-12 responses to bacterial LPS, IL-15Rα$^{-/-}$ BMDCs secrete normal levels of IL-12 in response to LPS (26). Macrophages and DCs also elaborate enhanced levels of IL-15 after LPS stimulation (26, 27). We thus measured IL-15 mRNA levels from IL-15Rα$^{-/-}$ and IL-15Rα$^{+/-}$ BMDCs after LPS stimulation by RNase protection assay. These studies revealed similar levels of IL-15 mRNA as well as other cytokines (e.g., IL-6 and IFN-γ) in IL-15Rα$^{-/-}$ and IL-15Rα$^{+/-}$ BMDCs (data not shown). Thus, IL-15Rα$^{-/-}$ BMDCs respond to LPS by expressing normal levels of costimulatory molecules and cytokines known to support NK cells.

IL-15Rα$^{-/-}$ and IL-15$^{-/-}$ BMDCs are unable to fully activate NK cells

Having established the functional responses of IL-15Rα$^{-/-}$ BMDCs, we next examined the ability of these cells to support NK cell activation. As noncell autonomous expression of IL-15Rα is critical for supporting NK cell survival in vivo (15), we first examined whether IL-15Rα expression on BMDCs was essential for NK cell survival in vitro. Splenic NK cells were purified from RAG-1$^{-/-}$ mice, cocultured with IL-15Rα$^{-/-}$ or IL-15Rα$^{+/-}$ BMDCs at a 2:1 DC:NK cell ratio or no DCs in the presence or absence of LPS, and then quantitated by annexin and propidium iodide staining and flow cytometry at the end of the 18-h culture period. NK cells cultured without DCs died rapidly, suggesting that DCs provide essential survival signals to NK cells in these cultures (data not shown). Similar numbers of viable NK cells were obtained from cocultures with IL-15Rα$^{+/-}$ and IL-15Rα$^{-/-}$ BMDCs, suggesting that such survival signals are not IL-15Rα dependent (data not shown).

To determine whether IL-15Rα expression is required for NK cell activation, we assayed supernatants from these cocultures for IFN-γ production (5). Consistent with prior studies, LPS-activated IL-15Rα$^{+/-}$ BMDCs stimulated NK cell production of IFN-γ (Fig. 2*A*). This level of IFN-γ secretion was not seen when either LPS, NK cells, or BMDCs were omitted from the coculture (Fig. 2*A*). By contrast, LPS-activated IL-15Rα$^{-/-}$ BMDCs were unable to fully stimulate NK cell

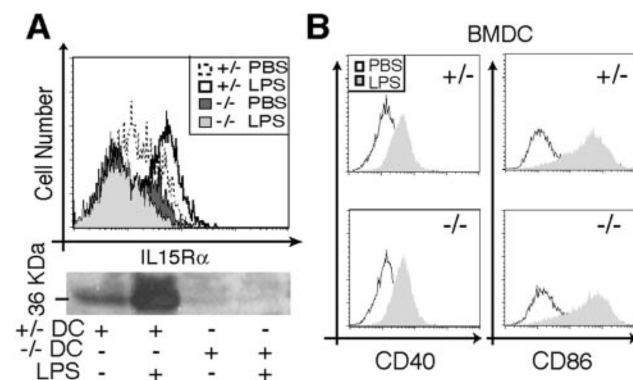

FIGURE 1. IL-15Rα$^{-/-}$-derived BMDCs express normal levels of costimulatory molecules. *A*, Flow cytometric (*upper panel*) and Western blot (*lower panel*) analyses of IL-15Rα expression on BMDCs. *B*, Flow cytometric analysis of CD40 and CD86 expression on LPS-stimulated IL-15Rα$^{+/-}$ and IL-15Rα$^{-/-}$ BMDCs. Data are representative of two separate experiments.

[4] The on-line version of this article contains supplemental material.

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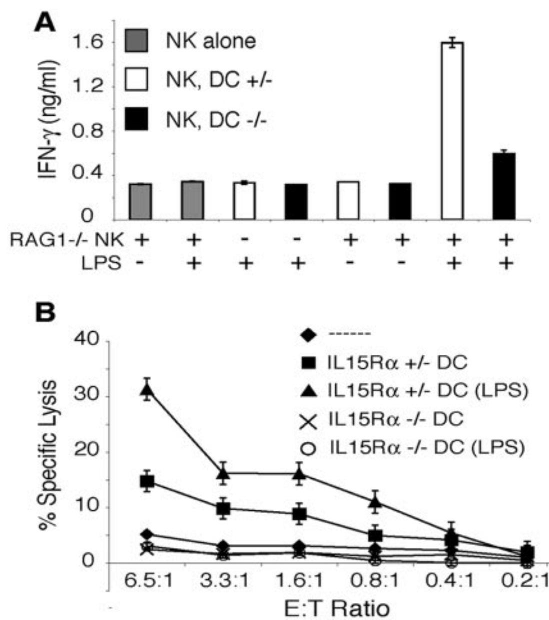

FIGURE 2. BMDCs expression of IL-15Rα is required for NK priming. *A*, IFN-γ secretion by NK cells cultured alone (▨) or with either IL-15Rα$^{+/-}$ (□) or IL-15Rα$^{-/-}$ (■) BMDCs was measured in the presence or absence of LPS and blocking Abs. *B*, Cytolytic activity of BMDC-activated NK cells. Data are representative of three separate experiments.

IFN-γ production (Fig. 2*A*). As the DC:NK cell ratio can affect the survival of DCs and the activation of NK cells (3), we repeated these experiments using DC:NK cell ratios of 1:1 and 0.5:1. These studies revealed that LPS-activated IL-15Rα$^{-/-}$ BMDCs were consistently less potent in activating NK cells than IL-15Rα$^{+/-}$ BMDCs at all ratios tested (data not shown). Finally, IL-15$^{-/-}$ BMDCs were also unable to induce NK cell production of IFN-γ, despite elaborating normal levels of IL-15Rα and IL-12, indicating that DC production of both IL-15Rα and IL-15 are required for NK cell activation (Supplemental Figs. 2 and 3).

In addition to secreting IFN-γ, NK cells kill appropriate cellular targets based on signals from activating and inhibitory receptors. DCs have been shown to support NK cytolytic activity (2–4). To determine whether IL-15Rα$^{-/-}$ BMDCs can prime NK cells to become cytolytic effectors, BMDCs were pretreated with LPS or medium alone for 18 h and then cultured with fresh NK cells for a further 18 h. Subsequently, these NK cells were assayed for their ability to kill ^{51}Cr-labeled MHC-deficient YAC cells. These studies revealed that IL-15Rα$^{-/-}$ BMDC-primed NK cells are unable to lyse these targets, whether or not the BMDCs had been LPS activated (Fig. 2*B*). As NK cells can be cytolytic without IFN-γ, IL-15Rα's role in supporting cytotoxic function may be independent of its role in supporting NK cell IFN-γ secretion (21). Thus, IL-15Rα expression on BMDCs supports multiple aspects of NK cell activation. These studies provide the first evidence that IL-15Rα is critical for NK cell activation, independently of its previously described roles in NK cell survival and memory CD8^{+} T cell proliferation (6, 14–16, 28).

Ab-mediated blockade of IL-15R on normal BMDCs inhibits their ability to activate NK cells

The failure of IL-15Rα$^{-/-}$ BMDCs to support NK cell activation could be due to the absence of essential IL-15Rα signals during BMDC differentiation, or during BMDC activation of NK cells, or both. To further examine whether activated BMDCs use IL-15Rα to directly activate NK cells, we examined the ability of IL-15Rα$^{-/-}$ or IL-15Rα$^{+/-}$ BMDCs to activate normal NK cells in the presence of anti-IL-15Rα, anti-IL-2Rβ, or anti-IL-2Rα-specific Abs. Anti-IL-15Rα and anti-IL-2Rβ Abs inhibited the ability of LPS-matured IL-15Rα$^{+/-}$ BMDCs but not IL-15Rα$^{-/-}$ BMDCs to stimulate NK cell IFN-γ secretion, while anti-IL-2Rα-specific Abs and isotype control Abs had no effect (Fig. 3*A*, and data not shown). These findings indicate that inhibiting IL-15R but not IL-2R signals blocks BMDC-mediated NK cell activation. In addition, IL-15Rα is directly involved in the priming of NK cells by DCs, providing a molecular mechanism for this process (29, 30).

IL-12 is secreted by BMDCs and supports NK cell activation. Our prior data demonstrated that purified IL-15Rα$^{-/-}$ BMDCs express as much IL-12 as IL-15Rα$^{+/-}$ BMDCs in response to LPS (data not shown). However, it is possible that Ab-mediated blockade of IL-15R signals might inhibit NK cell signals to BMDCs, which in turn could elaborate less IL-12 and fail to support NK cells. We thus measured IL-12 levels in supernatants from Ab-treated BMDC-NK cell cocultures. These studies revealed similar levels of IL-12 from nontreated, anti-IL-15Rα-, anti-IL-2Rβ-, and anti-IL-2Rα-treated cocultures of IL-15Rα$^{+/-}$ BMDCs and NK cells (Supplemental Fig. 4). Finally, anti-IL-12 Ab treatment blocked IFN-γ secretion but not cytotoxicity of NK cells from IL-15Rα$^{+/-}$ BMDC-NK cell cocultures (Fig. 3, *A* and *B*). This result highlights the fact that DC-mediated NK cell cytotoxicity can occur independently of IL-12 production. Furthermore, these findings suggest that IL-15Rα signals from BMDCs support both NK cell IFN-γ production and cytotoxicity independently of IL-12 production.

The experiments above also demonstrate that the reduced ability of LPS-activated IL-15Rα$^{-/-}$ BMDCs to induce NK cell IFN-γ secretion was not further inhibited by anti-IL-15Rα

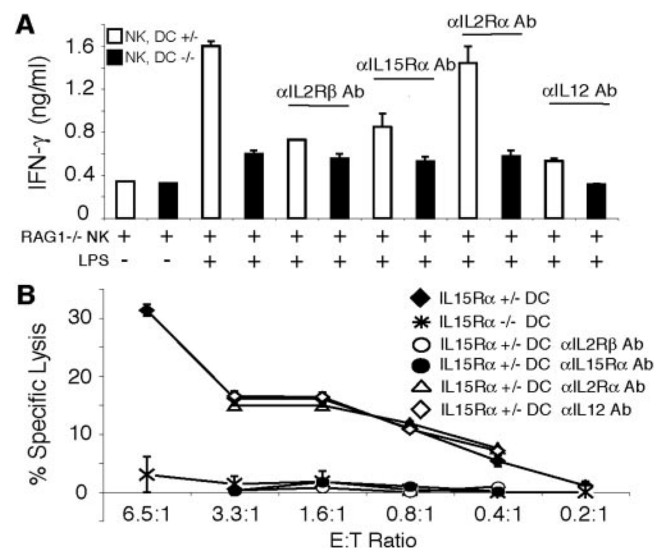

FIGURE 3. BMDC priming of NK cells is inhibited by blockade of IL-2Rβ and IL-15Rα. *A*, IFN-γ secretion by NK cells cultured with either IL-15Rα$^{+/-}$ (□) or IL-15Rα$^{-/-}$ BMDCs (■), in the presence or absence of LPS and indicated blocking Abs (5 μg/ml). *B*, Cytolytic activity of LPS-treated BMDC-activated NK cells in the presence or absence of blocking Abs (5 μg/ml). Data are representative of three separate experiments.

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Ab (Fig. 3*A*). This finding suggests that no significant IL-15R-mediated signaling occurs in BMDC-NK cell cocultures when BMDCs lack IL-15Rα. As IL-15R$\alpha^{-/-}$ BMDCs express normal amounts of IL-15 after LPS stimulation (data not shown), this result also suggests that BMDC-produced IL-15 does not activate NK cells when BMDCs lack IL-15Rα. Finally, both anti-IL-15Rα- and anti-IL-2Rβ-specific Abs dramatically inhibited the ability of IL-15R$\alpha^{+/-}$ BMDC-activated NK cells to kill YAC cells (Fig. 3*B*), again confirming an important role for IL-15Rα expression on BMDCs during NK cell activation.

Both BMDCs and NK cells express IL-15Rα, so the blockade of BMDC-NK interactions by anti-IL-15Rα Ab could indicate important roles for IL-15Rα on either or both of these cell types. To further examine whether anti-IL-15Rα Ab blocks BMDC-NK cell interactions by binding to IL-15Rα on the surface of BMDCs or NK cells, we generated IL-15R$\alpha^{-/-}$ NK cells in vivo by reconstituting lethally irradiated wild-type mice with congenic IL-15R$\alpha^{-/-}$ bone marrow (15). IL-15R$\alpha^{-/-}$ NK cells were purified from the chimera by flow cytometric sorting and cocultured with normal BMDCs in the presence or absence of anti-IL-15Rα-specific Ab. These studies revealed that IL-15R$\alpha^{+/-}$ BMDCs can stimulate IL-15R$\alpha^{-/-}$ NK cells to secrete IFN-γ, demonstrating that IL-15Rα expression on NK cells is not essential for BMDC-mediated NK cell activation (Fig. 4). In addition, anti-IL-15Rα Ab inhibited this ability of IL-15R$\alpha^{+/-}$ BMDCs to induce IL-15R$\alpha^{-/-}$ NK secretion of IFN-γ (Fig. 4). Thus, IL-15Rα expression on the surface of mature BMDCs, and not on NK cells, plays an essential role in NK cell activation. The most straightforward interpretation of these results is that IL-15Rα on the surface of BMDCs presents IL-15 in *trans* to IL-2Rβ and γ_c on the surface of NK cells, leading to NK cell activation.

Cell-free IL-15Rα-bound IL-15 supports NK cell activation

The findings above demonstrate that IL-15Rα expression by BMDCs is critical for acutely supporting NK cell activation. BMDCs express other proteins to support NK cell activation, so IL-15Rα-mediated signals may function in collaboration with these other signals. To directly examine the role of IL-15Rα-bound IL-15 in supporting NK cell activation in the absence of other BMDC signals, we coated tissue culture plates with rIL-15 and/or IL-15Rα/Fc and assayed the ability of these plates to induce NK cell production of IFN-γ in the presence or absence of IL-12. IL-12 or IL-15Rα/Fc-bound IL-15 stimulation modestly enhanced the proportion of IFN-γ-producing NK cells (Fig. 5). Neither soluble IL-15 nor plate-bound IL-15Rα/Fc alone increased the percentage of IFN-γ-producing cells (Fig. 5, and data not shown). By contrast, the combination of IL-15Rα/Fc-bound IL-15 and soluble IL-12 dramatically enhanced NK cell activation (Fig. 5).

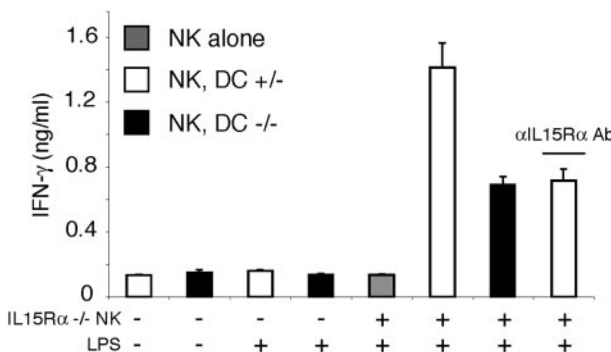

FIGURE 4. IL-15Rα expression on BMDCs is required for optimal NK cell IFN-γ production. IFN-γ secretion by IL-15R$\alpha^{-/-}$ NK cells cocultured with either IL-15R$\alpha^{+/-}$ or IL-15R$\alpha^{-/-}$ BMDCs, in the presence or absence of LPS. Blocking Ab against IL-15Rα (5 μg/ml) was included where indicated. Data are representative of three separate experiments.

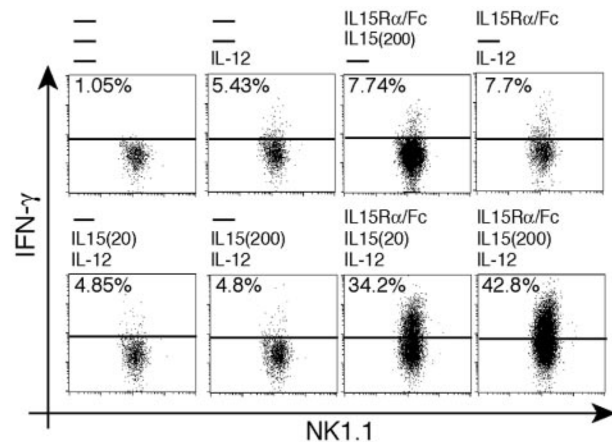

FIGURE 5. Soluble IL-12 and IL-15Rα/Fc-bound IL-15 synergistically induce NK cell production of IFN-γ in vitro. Cytoplasmic staining of IFN-γ production by NK cells 24 h after activation by plate-bound IL-15Rα/Fc and rIL-15 (20 ng/ml or 200 ng/ml), with or without soluble IL-12 (10 ng/ml). Data are representative of three separate experiments.

In summary, these experiments provide the first evidence that IL-15Rα expression by murine DCs is critical for NK cell activation. As both IL-15 and IL-15Rα expression are rapidly induced in BMDCs in response to microbial pathogens, BMDC IL-15Rα-mediated presentation of IL-15 to NK cells may provide an early priming step that synergizes with IL-12 to activate NK cells in vivo. Although an earlier study failed to detect soluble IL-15 in such cultures, and thus deduced that IL-15 might not be involved in this process (23), a recent study suggested that IL-15Rα-bound IL-15 might be presented in *trans* to responding cells (e.g., NK cells) (17). In this scenario, bioavailable IL-15 might not be readily measured in supernatants. In addition, at least one component of DC-mediated NK cell activation appears to be cell-contact dependent (5, 23). In this context, the potential ability of IL-15Rα on DCs to "*trans* present" IL-15 to NK cells is an attractive molecular explanation for this cell-contact-dependent activation of NK cells by DCs. Our observations that IL-15Rα expression is critical on the surface of BMDCs and dispensable on the surface of NK cells together suggest that BMDC-bound IL-15Rα binds IL-15 and presents it in *trans* to IL-2Rβ and γ_c receptors on NK cells to activate the latter. Hence, these studies provide a novel molecular basis for BMDC-mediated priming of NK cells.

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