Acid-Induced NK Cell Activation during Polyinosinic-Polyctidylic Acid-Induced NK Cell Activation

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Cutting Edge: A Dual Role for Type I IFNs during Polyinosinic-Polycytidylic Acid-Induced NK Cell Activation

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NK cells are cytotoxic lymphocytes that are most efficient at fulfilling their functions after a phase of priming provided by cytokines and/or accessory cells. Although type I IFNs are known to be important in this process, it remains unclear whether they act directly on NK cells or indirectly on accessory cells. We used adoptive transfer experiments and mixed bone marrow chimeras to dissect the requirement for type I IFN signaling in response to the dsRNA analog polyinosinic-polycytidylic acid. We demonstrate that optimal NK cell priming requires type I IFNs to signal on both NK cells and accessory cells. In the absence of IL-15, the residual NK cell activation was strictly dependent on cell-intrinsic IFNAR signaling in NK cells. Our results suggest that type I IFNs produced following viral infection simultaneously target accessory cells for IL-15 transpresentation and NK cells themselves and that these two pathways cooperate for NK cell priming. The Journal of Immunology, 2011, 187: 2084–2088.

Natural killer cells are lymphocytes of the innate immune system that rapidly act to eliminate virally infected or tumoral cells, a decision based on a balance between signals provided by activating and inhibitory receptors (1). NK cells have been classically considered as ready to go, capable of cytokine production and cytotoxic activity upon their first encounter with a target cell. Recently, it has been recognized that NK cells are, in fact, most potent to perform effector functions following an initial phase of activation or priming provided by cytokine signals (2) (IL-12, IL-18, IL-15, type I IFN) and/or direct contact with accessory cells such as dendritic cells (DCs) (2, 3). The requirement for cell–cell interaction during NK cell priming in vivo is suggested by the requirement for DCs to simultaneously express both IL-15 and transpresent IL-15 to NK cells (4, 5). Interestingly, two-photon imaging has revealed that NK cells become primed while maintaining a motile behavior (i.e., in the absence of a stop signal and of a long-term synapse) (6).

NK cell priming occurs during viral, bacterial, and parasitic infections and is also observed upon administration of TLR agonists (4, 7–9). In mice, injection of polyinosinic-polycytidylic acid (poly I:C) provides a useful model for studying NK cell priming. Poly I:C is an analog of viral dsRNA that can be recognized in endosomal compartments by TLR3 and in the cytosol by MDA-5 (10). Upon in vivo injection, poly I:C targets both hematopoietic and stromal components, primarily through interactions with TLR3 and MDA-5, respectively (11), and results in robust type I IFN production. Because poly I:C promotes both innate and adaptive immune responses, it represents an attractive adjuvant for vaccines.

Type I IFNs play a crucial role during NK cell priming, but the underlying mechanism, including the cell type targeted by IFNs, is controversial. NK cell activation following administration of TLR agonists has been shown to depend on type I IFN signaling in DCs but not in NK cells (4). Type I IFN signaling in DCs resulted in DC maturation and IL-15Rα upregulation (4). Moreover, IL-15 transpresentation was critical for NK cell priming (5, 12). Likewise, IFNAR signaling on DCs rather than on NK cells has been suggested to promote antitumor NK cell activity induced by poly I:C (13). In sharp contrast, using adoptive transfer experiments, NK cell activation induced by vaccinia virus (14) or an adenoviral vector (15) was shown to require solely NK cells to respond to type I IFN. In these studies, wild-type (WT; but not IFNAR−/−) NK cells transferred into IFNAR−/− recipients were efficiently primed upon viral infection. Moreover, addition of type I IFNs on NK cells in vitro is well known to upregulate their cytotoxic potential (16).

In the present report, we investigate the direct versus indirect contribution of type I IFN during NK cell priming in vivo. Using both adoptive transfers and mixed bone marrow chimeras, we demonstrate that optimal NK cell priming requires...
type I IFN to act simultaneously on NK cells and accessory cells. The dual role played by type I IFN in response to poly I:C enables NK cells to integrate signals from multiple cytokines including IFNs and IL-15 to become potent effectors.

Materials and Methods
Mice and injections
C57BL/6 mice were purchased from Charles River Laboratories. IFNAR−/− mice backcrossed onto a C57BL/6 background (17), Ncr1GFP/+ (18), CD11c-YFP (19), IFN regulatory factor (IRF) 3/7−/− (20, 21), and IL-15−/− mice were bred in our animal facility. All mice experiments were performed according to institutional guidelines. Mice were anesthetized and injected i.v. with 100 μg poly I:C or polyadenylic-polyuridylic acid (Invivogen) or PBS.

Cell preparation and transfer
For adoptive transfer, NK cells were isolated from lymph nodes and spleens of WT or IFNAR−/− mice using depletion kits and an Automacs system (Miltenyi Biotec). Purified NK cells (>80%) were then labeled with 2.5 μM CFSE for 10 min at 37°C, and recipient mice (WT, IFNAR−/−), were adoptively transferred with 6 × 106 NK cells.

Mixed bone marrow chimeras
Bone marrow cells were collected from femurs and tibias of CD11c−/− × NCR1GFP/+ or IFNAR−/− mice. IFNAR−/− × control recipient mice were irradiated at 950 rad and injected 5 h later with a mixture of 2 × 106 BM cells composed of 90% IFN−/− or CD11c−/− cells and 10% CD11c-YFP × NCR1GFP/+ cells. After 5 to 6 wk, chimeras were injected i.v. with 100 μg poly I:C (Invivogen) or PBS.

Measurement of IFN-α levels
Blood was collected in heparin-coated capillary tubes at 6 and 24 h after PBS or poly I:C injection and centrifuged for 10 min. IFN-α was then quantified in the plasma using a Mouse IFN-α ELISA kit (PBL InterferonSource) according to the manufacturer’s instructions.

Flow cytometry
Spleens were harvested and incubated 15 min in RPMI 1640 containing 1 mg/ml collagenase D and 0.05 mg/ml DNAse. Spleocytes were then incubated with purified anti-CD16/32 (BD Biosciences) for 15 min at 4°C for Fc blocking. Cells were then stained with a mixture of the following mAbs: biotinylated anti-NKG2D followed by Pacific Blue-labeled streptavidin, Fc blocking. Cells were then stained with a mixture of the following mAbs: anti-CD16/32 (BD Biosciences), anti-CD69, anti-CD11c (BD Biosciences), anti-CD86, anti-I-Ab, and anti-CD81 (BioLegend). Intracellular stainings were performed using the BD Cytofix/Cytoperm kit (BD Biosciences) and anti-granzyme B (Invivogen). The dual role played by type I IFN in response to poly I:C was severely diminished (Fig. 1A, 1C, Supplementary Fig. 1A). In IFNAR−/− mice, however, upregulation of activation markers was most prominent on CD81+ cells, as measured 6 h after poly I:C injection (Fig. 1B). These changes were most prominent on CD81+CD11chi DCs and to a lesser extent on CD11b+CD11clow DCs and were

Results and Discussion
Type I IFN signaling during poly I:C-induced NK cell activation
NK cell priming following infection or in vivo injection of a TLR agonist is reflected by several phenotypical changes detected within 24 h, including upregulation of CD69, increase in intracellular granzyme B content, and results in enhanced cytolytic activity. To investigate the role of type I IFN recognition during poly I:C-induced NK cell activation, we took advantage of mice lacking the IFNAR1 (hereafter referred to as IFNAR−/− mice) and analyzed NK cell activation following i.v. poly I:C injection. In WT mice injected with poly I:C, NK cells upregulated CD69, NK1.1, NKG2D, and CD25 and displayed a strong increase in intracellular granzyme B content (Fig. 1A, 1C, Supplemental Fig. 1A). In IFNAR−/− mice, however, upregulation of activation markers was severely diminished (Fig. 1A, 1C, Supplemental Fig. 1A). To further assess the role of type I IFN in this response, we injected poly I:C in IFNRF3/7−/− mice (20). In the absence of these key transcription factors, poly I:C treatment did not result in type I IFN production, and there was no evidence of NK cell activation (Fig. 1A, 1B).

Elevated levels of type I IFN in WT mice were detected in the blood of WT and to a lesser extent in IFNAR−/− mice, as measured 6 h after poly I:C injection (Fig. 1B). Thus, type I IFN production and signaling are critical to trigger several aspects of NK cell priming in response to poly I:C.

Given the putative role of DCs in driving NK cell priming, we assessed the phenotypical changes following poly I:C injection in distinct DC subsets: myeloid DCs (CD11b+CD11clow), lymphoid DCs (CD8α+CD11clow), and plasmacytoid DCs (B220+CD11c+). Poly I:C administration resulted in MHC class II, CD86, and IL-15Rα upregulation within the total CD11c+ population (Fig. 1D). These changes were most prominent on CD8α+CD11clow DCs and to a lesser extent on CD11b+CD11clow cells and were
dependent on type I IFN, as they were not observed in IFNAR−/− mice (Fig. 1B) and IRF3/7−/− mice (Supplemental Fig. 1B). Little to no induction of these markers was seen on plasmacytoid DCs. These results confirm and extend previous studies (4, 13, 22) showing the major role for type I IFN, as they were not observed in either WT or IFNAR−/− mice. After 18 h, mice were treated with 100 μg poly I:C. A. Splenic NK cells were assessed 24 h later for expression of CD69 and granzyme B. Histograms were gated on CFSE+CD3+ NK1.1+ cells. B. Graphs show the percentage of CD69+ and granzyme B high cells and the mean fluorescence intensity (MFI) for granzyme B fluorescence on gated CFSE+CD3+ NK1.1+ cells. Results are representative of two independent experiments.

FIGURE 2. Type I IFN targets both NK cells and accessory cells for optimal priming of adoptively transferred NK cells. NK cells purified from WT or IFNAR−/− mice were labeled with CFSE and then adoptively transferred into either WT or IFNAR−/− mice. After 18 h, mice were treated with 100 μg poly I:C. A. Splenic NK cells were assessed 24 h later for expression of CD69 and granzyme B. Histograms were gated on CFSE+CD3+ NK1.1+ cells. B. Graphs show the percentage of CD69+ and granzyme B high cells and the mean fluorescence intensity (MFI) for granzyme B fluorescence on gated CFSE+CD3+ NK1.1+ cells. Results are representative of two independent experiments.

FIGURE 3. IFNAR signaling on NK cells can partly compensate for the lack of IL-15 during priming by poly I:C. NK cells were purified from the spleens of WT or IFNAR−/− mice, labeled with CFSE, and transferred in IL-15−/− recipients. Mice were injected i.v. 18 h later with 100 μg poly I:C. A. Splenic CFSE+CD3+ NK1.1+ cells were analyzed 24 h later by flow cytometry. A, Histograms showing CD69 expression and intracellular granzyme B content were gated on CFSE+CD3+ NK1.1+ (black lines). Filled histograms correspond to PBS-injected mice. B, Percentage of CD69+ and granzyme B high cells and mean fluorescence intensity (MFI) for granzyme B fluorescence are graphed for WT and IFNAR−/− NK cells.

Type I IFN targets multiple cell types for optimal priming of adoptively transferred NK cells

To determine on which cells type I IFNs were acting, we adoptively transferred WT or IFNAR−/− NK cells in WT or IFNAR−/− recipients (Fig. 2). Optimal NK cell priming in response to poly I:C in vivo was observed when WT NK cells were adoptively transferred in WT recipients. Interestingly, NK cell priming also occurred, albeit at a lower efficiency, when WT NK cells were transferred in IFNAR−/− mice or when IFNAR−/− NK cells were transferred in WT recipients (Fig. 2). NK cell priming was, however, abolished when IFNAR−/− NK cells were transferred in an IFNAR−/− recipient. These experiments suggested that both type I IFN signaling on NK cells and accessory cells could contribute to NK cell priming but that optimal NK cell activation required the simultaneous action on both cell types.

FIGURE 4. Type I IFN signaling on DCs promotes their maturation and IL-15Ra upregulation in a cell-autonomous manner. IFNAR−/− mice were irradiated and reconstituted with a mixture of bone marrow cells from Ncr1GFP/+ × CD11c-YFP (10%) and IFNAR−/− (90%) mice. Five weeks later, chimeras were injected i.v. with poly I:C or PBS. The following day, YFP+ (IFNAR−/−) and YFP− (IFNAR−/+ ) DCs were analyzed for I-A^b, CD86, and IL-15Ra expression. Representative FACS plots (A) and mean fluorescence intensity (MFI) for these three markers (B) are shown. Results are representative of two independent experiments.

FIGURE 5. IFNAR signaling on NK cells is required for optimal priming of endogenous NK cells. IFNAR−/− mice were irradiated and reconstituted with a mixture of BM cells from Ncr1GFP/+ × CD11c-YFP (10%) and IFNAR−/− (90%) mice. Five weeks later, chimeras were injected i.v. with poly I:C or PBS. The following day, splenic NK cells were assessed for expression of CD69 and granzyme B. A. FACS profile showing CD69 or granzyme B expression as a function of GFP on gated CD3+ NK1.1+ cells. B, The percentage of CD69+ and granzyme B high cells and the mean fluorescence intensity (MFI) for granzyme B fluorescence are graphed for GFP− (IFNAR−/−) and GFP+ (IFNAR−/+ ) NK cells. Results are representative of two independent experiments.
NK cell priming in response to poly I:C occurs through two partly independent pathways.

A previous study has established that type I IFN acts on DCs to promote IL-15 transpresentation to NK cells (4). To determine if IL-15 transpresentation was strictly required for NK cell priming, we analyzed the response of WT or IFNAR−/− NK cells transferred in an IL-15−/− recipient injected with poly I:C (Fig. 3). Although NK cell activation in IL-15−/− recipients was clearly reduced compared with that seen in WT mice (compare Fig. 1 and Fig. 3), we could still detect upregulation of CD69 and granzyme B on WT but not on IFNAR−/− NK cells. Thus, NK cells can receive activation signals in the absence of IL-15 through IFNAR signaling. These results suggest that NK cell priming occurs via two complementary pathways associated with the NK cell recognition of IL-15 and type I IFN, respectively.

Dual role for type I IFN for priming of endogenous NK cells in response to poly I:C

It remained possible that the requirement for activating adoptively transferred NK cells were different from those for the activation of endogenous NK cells. It is in fact our experience that adoptively transferred NK cells show higher expression of activation markers than endogenous NK cells from the recipients upon poly I:C injection (Supplemental Fig. 2). To circumvent this potential caveat and permit direct comparison between WT and IFNAR−/− NK cells, we generated mixed bone marrow chimeras. IFNAR−/− mice were irradiated and reconstituted with a mixture of IFNAR−/− and CD11c-YFP × NCR1GFP/+ bone marrow cells. In these mixed chimeras, IFNAR-deficient cells including DCs and NK cells were unlabeled, whereas IFNAR-sufficient DCs and NK cells expressed YFP and GFP, respectively. This strategy allowed us to assess the cell-intrinsic role of IFNAR signaling in both DCs and NK cells in response to poly I:C. First, we analyzed DCs from the mixed chimeras after poly I:C injection. As shown in Fig. 4, we observed upregulation of CD86, MHC class II, and IL-15Rα on WT (YFP+) but not IFNAR−/− (YFP−) DCs. These results extend previous findings (22) by showing that DC activation, IL-15Rα upregulation, and possibly IL-15 transpresentation rely on IFNAR signaling in a cell-autonomous manner. In the same animals, we analyzed NK cell activation by monitoring CD69 and granzyme B expression. Although WT (GFP+) NK cells were primed efficiently as indicated by upregulation of CD69 and intracellular granzyme B, IFNAR−/− NK cells were not significantly activated in response to poly I:C (Fig. 5). This result further emphasizes the crucial role for direct IFNAR signaling on NK cells for efficient activation. Interestingly, the defect in the priming of IFNAR−/− NK cells was even more pronounced in the mixed bone marrow chimera experiments than with the adoptive transfer. These differences may reflect the tendency of transferred NK cells to be more easily activated (Supplemental Fig. 2) or to contain a more mature population of NK cells. Alternatively, differences could be explained by the absence of IFNAR signaling on stromal cells in the chimeras (as the recipients of the BM marrow chimeras were IFNAR−/−) and lower levels of type I IFNs. At any rate, the fact that WT NK cells were efficiently activated in the mixed BM chimeras further indicates that IFNAR signaling on stromal cells is not essential for NK cell priming. Together, these experiments suggest that only DCs capable of IFNAR signaling can upregulate IL-15Rα (thus permitting transpresentation of IL-15) and, importantly, that NK cells need to receive active signals via IFNAR for efficient priming by poly I:C.

General conclusions

NK cell activation involves the coordinated action of several cytokines including type I IFNs, IL-12, and IL-15 (23, 24). Through a careful assessment of the role of type I IFNs during NK cell priming, we have demonstrated a dual role for IFNAR signaling. Indeed, our data support the idea that optimal NK cell activation requires type I IFNs to act on two cell types: directly on NK cells and indirectly on DCs (or other accessory cells) to promote IL-15 transpresentation. These findings may help reconcile some of the conflicting data observed in the literature with respect to IFNAR signaling on NK cells. Based on the experimental approaches used, it may not be fully required, as seen upon NK cell adoptive transfer, or play a more decisive role as observed in the bone marrow chimera experiments. This work together with previous studies (4, 11, 22) highlights that TLR agonists, such poly I:C, trigger innate immune responses through a complex process that targets both the stromal and hematopoietic compartments to produce cytokines that act in concert. In addition, cell-surface molecules, such as the recently described IRF3-dependent NK activating molecule, may participate in DC-mediated NK cell activation (25). Thus, optimal NK cell priming in vivo requires the integration of a variety of signals by NK cells that are controlled in large part by the pleiotropic effects of type I IFNs.

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Disclosures

The authors have no financial conflicts of interest.

References


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Supplemental figure 1.

A) WT and IFNAR−/− mice were injected i.v. with 100 µg poly I:C. Percentage of CD69+, granzyme Bhigh NK cells together with the MFI for granzyme B expression are shown for WT and IFNAR−/− mice treated or not with poly I:C. B) WT, IFNAR−/− or IRF3/7−/− mice were injected i.v. with 100 µg poly I:C (black line) or PBS (filled gray). At 24hr, dendritic cells (gated on CD11c+ cells) were analyzed by flow cytometry as described in figure 1. C-D) WT or IFNAR−/− mice were injected i.v. with 100 µg poly I:C (black line), poly A:U (dashed line) or PBS (filled gray). At 24hr after poly I:C, poly A:U or PBS injection, NK cell (C) and DC (D) phenotypes were assessed by flow cytometry.
Supplemental figure 2: Activation of endogenous and adoptively transferred NK cells in response to poly I:C.

NK cells purified from WT mice were labeled with CFSE and adoptively transferred into WT mice. After 18hr, mice were treated with 100µg poly I:C. A) Splenic NK cells were assessed 24hr later for expression of CD69, granzyme B and IFN-γ. Plot were gated on CD3−NK1.1+. B) Graphs show the percentage of CD69+, granzyme B^{high} and IFN-γ^{+} cells on gated CFSE^{−}CD3^{−}NK1.1^{+} or CFSE^{+}CD3^{−}NK1.1^{+}cells.