Trans-Presentation of IL-15 Dictates IFN-Producing Killer Dendritic Cells Effector Functions

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http://www.jimmunol.org/content/180/12/7887
Interferon-producing killer dendritic cells (IKDC) represent a rare but unique entity sharing hybrid features in-between dendritic and NK cells (1–7). They could be found in trace amounts in all lymphoid organs at the steady-state but accumulated during certain inflammatory processes, such as tumor regression under the influence of imatinib mesylate (IM) + IL-2 (2) or Listeria infection (1). In such circumstances, IKDC expressed high levels of MHC class II molecules and maintained CD11c expression while lacking CD19 and CD40 molecules, thereby diverging from bona fide B cells. Chan et al. (1) could demonstrate that IKDC represent a unique subset of innate effectors functionally distinguishable from conventional NK cells in their ability to promptly respond to IL-15-driven inflammatory processes.

Materials and Methods

Mice and cell lines

Female C57BL/6 wild-type (WT) mice were obtained from the Centre d’Elevage Janvier (Le Genest St. Isle, France) and used at 6–10 wk of age. IFNγ type 1R⁻/⁻, CCL2/MCP-1⁻/⁻, and CD45.1⁻ mice backcrossed on a C57BL/6 background were provided by Centre d’Elevage d’Orléans (Centre de distribution, type et archivage animal Orléans, France). IL-15Rα⁻/⁻, IL-15⁻/⁻, IL-2/IL-15⁻/⁻, IL-2⁻/⁻, and IL-15 Tg mice were backcrossed on a C57BL/6 six to eight times and maintained at the animal facility of S. Buffle-Paus (Research Center Borstel, Borstel, Germany). IL-2Rγ⁻/⁻ × Rag2⁻/⁻ were kindly provided by E. Vivier, Centre d’Immunologie de Marseille, France. Tg OTI and OTII mice were a kind gift by O. Lantz (Institut Curie, Paris, France). Animals were all maintained according to the Animal Experimental Ethics Committee Guidelines. B16F10 is a melanoma cell line syngeneic of C57BL/6 (provided by M. T. Lotze, Institut Gustave Roussy, and Laurence Zitvogel3*§).
University of Pittsburgh, PA) and was cultured in RPMI 1640 (Invitrogen) with 10% heat-inactivated FBS enriched with 5% l-glutamine, non-essential amino acids, sodium pyruvate, and antibiotics. MS-5-feeder cell lines (provided by W. Vainchenker, IGR, Villejuif, France) were cultured in IMDM (Sigma-Aldrich) containing 10% heat-inactivated FBS, 5% l-glutamine, sodium pyruvate, and antibiotics.
4500 mg/l of glucose, 5% L-glutamin, pyruvate, and enriched with antibiotic-coated 96-well plates in DMEM (Invitrogen) culture medium containing sorted CD3<sup>+</sup>/H11002 (1 cell per well) using the automated cell device unit. Although B220<sup>−</sup>20 ng/ml IL-15. Limiting dilution assays were also initiated in 96-well plates by coculture with 10<sup>5</sup> freshly sorted NK cells and MS-5 feeders and rIL-15 to allow fair comparisons with IKDC.

Cytokine profiling of IKDC and NK cells

Freshly cell sorted IKDC were cultured in the presence of murine stromal cells MS-5 (16). One or 2 days before coculture with IKDC, MS-5 cells were plated in a 96-well plate (7500 cells per well). Cultures of IKDC were initiated by seeding 10<sup>5</sup> freshly sorted IKDC in MS-5 pre-coated 96-well plates in DMEM (Invitrogen) culture medium containing 4500 mg/l of glucose, 5% Bovine Growth serum (Lot no. ANB 18298, HyClone), and 20 μM l-glutamine. A total of 10<sup>5</sup> freshly sorted NK cells and IKDC or IKDC15 (obtained at day 7 of ex vivo expansion) or NK and IKDC stimulated with rIL-15 (20 ng/ml; R&D Systems) for 24 h were further incubated with LPS at 100 ng/ml (InvivoGen) or CpG oligodeoxynucleotide (ODN) 1668 (MWG Biotech) at 5 μg/ml. These in vitro cultures were performed in 200 μl RPMI (Invitrogen) 10% FBS (Invitrogen) in 96 round-bottom well plates. After 24–36 h, cell supernatants were collected and commercial LUMINEX kits were used to determine cytokine and chemokine profiles (used according to the manufacturer’s conditions, Linco Research/BioSource International).

**Activation of OTII Tg T cells in vitro**

FACS sorted 10<sup>5</sup> CD4<sup>+</sup> resting OTII lymphocytes purified from naive OTII Tg mice were incubated at various effector:T cell ratios (1:1, 1:5, 1:20, and 1:100) with different effector cells (such as resting IKDC, B220<sup>−</sup> NK, bone marrow-derived DC (BMDC), IKDC15, or NK15 cells) after a 24-h coculture of effector cells with B16 tumor cells in the presence of 1 mg/ml OVA protein followed by extensive washing (three times in PBS 1× to remove resting traces of OVA protein). After a 24-h incubation period, T cells were stained with anti-CD3, anti-CD4, anti-CD8 and anti-CD69 Ab and analyzed by FACS.

Cytotoxicity assay

<sup>51</sup>Cr release assay was performed according to standard protocols using 2 × 10<sup>3</sup> Na<sup>2</sup>CrO<sub>4</sub>-labeled B16F10 tumor cells (T) incubated with various E:T ratios (1:1, 5:1, 10:1, 15:1, and 30:1) of effector (E) cells (NK vs IKDC stimulated or not with rIL-15 in trans-presentation) for 4, 8, or 12 h. Supernatants were harvested for the measurement of chromium release (E) using γ emission counting (Topcount NXT, Packard Instrument). Spontaneous <sup>51</sup>Cr release (S) was counted in target cells alone, maximal <sup>51</sup>Cr release (M) from target cells treated with 5% alkyltrimethyamine oxide and specific lysis was calculated according to the following:

\[ \text{Specific lysis} = \left( \frac{E - S}{M - S} \right) \times 100 \]

As an additional method, crystal violet assay was used. Effector and target cells were mixed at different ratios for 24 or 48 h. Live tumor cells were revealed using a crystal violet staining as previously reported (17). Cocultures of E:T were performed in the presence of neutralizing anti-TRAIL Ab (N2B2, provided by H. Yagita, Juntendo University School of Medicine, Tokyo, Japan), commercial anti-FasL mAb (eBioscience) at 10 μg/ml, or anti-FAS mAb (BD Biosciences) at 1 μg/ml, or anti-CD95L (eBioscience) at 10 μg/ml. Slides were incubated for 45 min at 37°C. Cells were fixed in 4%
Paraformaldehyde and permeabilized with 0.1% SDS. After 20 min of blocking in 10% FBS and washing, cells were stained with the appropriate anti-MHC class II (NIH-MHC; Southern Biotechnology Associates), anti-Perforin, and anti-Granzyme B mAbs (BD Pharmingen) in PBS containing 1% BSA for 1 h. Next, slides were extensively washed and incubated with the appropriate secondary Ab (Alexa Fluor 488 goat anti-rat IgG) for 1 h and, after an additional washing step, with DNA-labeling Topro3 (Invitrogen) for 10 min. Finally, 0.17-mm cover glasses were mounted on the slides. Stacks of confocal images were collected with a Zeiss LSM 510 laser scanning confocal microscope (Carl Zeiss) using a × 63 1.4 NA apochromat plan objective. Z-projection of slices and image analyses were performed using Zeiss LSM Image Examiner software.

Isolation of RNA and RT-PCR
RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. Low cell number samples were precipitated in the presence of 10 μg/sample GlycoBlue (Ambion). After RNA purification, samples were treated with DNase to remove contaminating genomic DNA (DNasel Amplification grade, 18068; Invitrogen) and Superscript II Reverse transcriptase (Invitrogen). Gene specific primers were purchased from NBS BIOTECH Scrl; sequences and detailed amplification protocols are available upon request. The iQ SYBR Green Supermix (Bio-Rad) was used to run relative quantitative real-time PCR of the samples according to the manufacturer’s instructions. Reactions were run in triplicate on an iCycler (Bio-Rad) and generated products analyzed with the iCycler iQ Optical System software (Version 3.0a; Bio-Rad). Gene expression was routinely normalized both based on β-Actin mRNA and 18S rRNA contents with overlapping results. The amounts of target mRNAs are expressed in arbitrary units calculated as the relative change compared with spleen samples. Data are displayed as 2−ΔΔCt values and are representative of at least three independent experiments.

Statistical analyses
Aberrant values were excluded using Dixon’s test. Normality of distributions was assessed using the Shapiro-Wilk’s test. Normal distributions were compared by the Student’s t test; non-normal samplings were compared using the Mann-Whitney test. Statistical analyses of survival curves were performed using Log-rank (Mantel-Cox) test. Values of p inferior to 0.05 were considered significant. All tests were done using Prism 5 software (GraphPad).
Results

Phenotypic definition and isolation of IKDC

IKDC were previously described as CD11c<sup>+</sup>B220<sup>-</sup>NK1.1<sup>+</sup> cells mediating the antitumor effects of the combination therapy with IM and IL-2. IKDC represent ~2% of bone marrow CD11c<sup>+</sup> cells and 1–2% of spleen-derived CD11c<sup>+</sup> cells in resting C57BL/6 mice and increased by 4-fold during the combination therapy with IM + IL-2 (2). Phenotypically, IKDC are a specific cell population coexpressing CD11c, B220, NK1.1, and NKP46 (a recently described NK cell marker) (18) (Fig. 1A). To avoid possible contamination with plasmacytoid DC, conventional DC, B lymphocytes, or NK cells, we sorted IKDC in two steps. First, we performed a preselection of CD3<sup>-</sup>CD19<sup>-</sup>NK1.1<sup>+</sup> cells. Second, we gated on CD11c<sup>int</sup>B220<sup>-</sup>NK1.1<sup>+</sup> cells (defined as “IKDC”) and CD11c<sup>+</sup>B220<sup>-</sup>NK1.1<sup>+</sup> cells (defined as B220<sup>-</sup>NK cells henceforth).

Importantly, the expression of B220 molecules on bona fide B220<sup>-</sup>NK cells did not appear to represent a marker of activation (12, 13, 19) because a 24–48 h stimulation of NK cells with cytokines (such as IL-2, IL-12, IL-15, and IL-18) and/or maturing DC were not sufficient to convert B220<sup>-</sup>NK cells into B220<sup>+</sup>IKDC counterparts (Table I). Conversely, IKDC did not convert or differentiate into B220<sup>-</sup>NK cells neither in vitro (Table I) nor in vivo (Fig. 1B). Indeed, we performed an adoptive transfer of FACS sorted IKDC from CD45.2<sup>+</sup> donor mice into irradiated congenic CD45.1<sup>+</sup>C57BL/6 recipient mice. To rescue recipient hosts, coinjection of bone marrow derived from CD45.1<sup>x</sup>CD45.2<sup>x</sup> chimera was performed in parallel. IKDC were cell sorted either from spleen or bone marrow. A similar number of CD45.2<sup>+</sup>IKDC could be recovered in the spleen at day 12, whether originating from spleen or bone marrow (Fig. 1B). IKDC did not lose CD11c nor B220 cell surface markers and did not acquire Ly6C<sup>+</sup>Gr1, CD4, or CD8<sup>a</sup> molecules (Fig. 1B and our unpublished data). At later time points (3 wk), CD45.2<sup>+</sup>IKDC were almost undetectable (not shown). Therefore, these data support the notion that IKDC are terminally differentiated cells.

IKDC might be considered as a subpopulation of the so-called “NKDC” (containing all CD11c<sup>+</sup>NK1.1<sup>+</sup> cells) (20, 21). However, it is noteworthy that the B220<sup>-</sup> fraction of NKDC was not significantly different from bona fide NK cells for all the hallmark criteria that the manuscript will describe (our unpublished data).

Absolute requirements for IL-15 in IKDC homeostasis

IL-2Rγ-chain-dependent cytokines, such as IL-15, are critical to promote lymphoid homeostasis and, more specifically, to maintain survival and proliferation of NK cells. We have previously reported that CD11c<sup>+</sup>CD49b<sup>+</sup>B220<sup>-</sup> cells (described as such by Chan et al. in BALB/c littermates) (1) could be found in old (>3 mo) Rag<sup>−/−</sup>×IL-2R<sup>−/−</sup> mice. However, those cells did not express NK1.1 molecules (not shown). An almost complete deprivation in IKDC (as defined in Fig. 1A) was found in IL-15Rα<sup>−/−</sup> and IL-15<sup>−/−</sup> animals, supporting that IL-15 is a requirement for the differentiation of not only B220<sup>-</sup>NK cells.

![Image](http://www.jimmunol.org/DownloadedFrom/)
FIGURE 4. IKDC15 lose their MHC class II–restricted Ag presenting capacities in vitro. A, Down-regulation of MHC class II mRNA in IKDC15. Quantitative RT-PCR was performed on freshly sorted IKDC and on IKDC15 (at day 7 of expansion). B and C, Activation of OTII Tg T cells in contact with IKDC but not IKDC15 in vitro. FACS sorted 10^6 CD4^+ resting OTII lymphocytes purified from naive OTII Tg mice were added at various effector/T cell ratios (as indicated) to different effector cells (either resting IKDC, B220^-NK, immature BMDC (C), or trans-IL-15-activated IKDC or NK cells (B)) or to a control without APC (B) after a 24-h coculture with B16 tumor cells in the presence of 1 mg/ml OVA protein. After a 20-h incubation period, cocultures were stained with anti-CD3, anti-CD4, anti-Vα, and anti-CD69 Ab and analyzed by FACS. A representative experiment is depicted of three yielding identical results. The mean ± SEM of the % of CD69^+ OTII cells is indicated on the graphs.

FIGURE 5. Trans-presentation of IL-15 licenses IKDC to respond to TLR3 and TLR4 ligands. A, IL-15 trans-presentation induced TLR3 and TLR4 expression in IKDC. Quantitative RT-PCR was performed on freshly sorted IKDC and on IKDC15 (at day 7 of expansion). B, Chemokine release by IKDC and IKDC15. After 24-h stimulation with medium, TLR3L (poly I:C), TLR4L (LPS), or TLR9L (CpG) multiplex analysis of chemokine release were performed on IKDC stimulated or not with trans-IL-15 presentation. IKDC0 meant freshly sorted cells without ex vivo stimulation. The experiments were performed at least twice with identical results.

Hence, IKDC critically depended upon IL-15/IL-15Rα for their homeostasis and CpG driven-proliferation in vivo.

Ex vivo expansion of IKDC required IL-15/IL-15Rα

There are trace numbers of IKDC in lymphoid organs of naive animals (~50,000/spleen). Based on the IL-15/IL-15Rα requirement for IKDC differentiation in vivo, we set up culture conditions allowing ex vivo IKDC proliferation and/or differentiation. Immunoblot analysis indicated that IKDC do not harbor IL-15Rα in contrast to B220^-NK cells or DC (Fig. 2A). However, cell surface expression of IL-15Rα was detectable only on MS-5 stromal cells and DC using FACS analyses (Fig. 2B). We used IL-15Rα expressing MS-5 to test the hypothesis of the role of trans-presentation of IL-15 by IL-15Rα in the biology of IKDC. “Trans-presentation” of IL-15 defines a phenomenon by which IL-15 is presented by IL-15Rα-expressing cells to bystander cells in their vicinity (22).

Accordingly, trans-presentation of rIL-15 by MS-5 to IKDC was successful and mandatory to promote ex vivo expansion (up to 10–30-fold) of spleen or bone marrow derived-IKDC within 7–12 days (Fig. 2, C and D). Indeed, IKDC proliferated only in the condition of IL-15 trans-presentation, neither on MS-5 alone, nor in rIL-15 alone (Fig. 2C). IKDC cultured in rIL-15 in the absence of MS-5 feeder cells expanded by 5-fold by day 7 but lost their proliferative potential afterward (Fig. 2C). IL-15 could not be substituted by rIL-2 and there was no additive effect with the combination of rIL-2 or rIL-18 + rIL-15 (Fig. 2C and unpublished data).

IKDC proliferation on the MS-5 stromal cells in the presence of rIL-15 was dependent on cell to cell contact, as shown by transwell
experiments (Fig. 2D), and could be abrogated by anti-IL-15Rα neutralizing Abs (Fig. 2D). Interestingly, this culture procedure did not allow the expansion of B220⁺ NK cells (Fig. 2D), although NK cell proliferation also depended on trans-presentation of IL-15 in vivo (Fig. 1E). The cloning efficiency of IKDC on MS-5 + rIL-15 as determined by limiting dilution analysis after single-cell sorting was ~20% (Fig. 2E). Clones derived from CD117⁺ or CD117⁻ bone marrow or spleen IKDC expanded exponentially in culture to colonies of 3 × 10³ cells by 7 days (not shown). Under these culture conditions, cloning efficiency of NK cells was not significant (Fig. 2E).

Ex vivo expanded IKDC (referred to as “IKDC15” henceforth) acquired a large blastic cytoplasm and contained numerous granules and vacuoles (Fig. 3A). At days 7–10 after expansion, IKDC15 still failed to harbor membrane expression of IL-15Rα.
IKDC REQUIRE IL-15 FOR PROLIFERATION AND ACTIVATION

FIGURE 7. IKDC15 have immunizing potential and resist to TGF-β-induced immunosuppression. A total of 10⁶ IKDC15 or NK15 cells (maintained 7 days in MS-5 + IL-15) were incubated with B16OVA at a 10:1 E:T ratio for 16 h before inoculation into the footpad of C57BL/6 mice. Rechallenge was performed 10 days later with a lethal tumorigenic dose of B16OVA (3 × 10⁵ cells) s.c. in the flank of mice (A and B). Controls included untreated mice (PBS) or immunization with 10⁵ B16OVA tumor cells incubated 15 h with 5 μM doxorubicin (23) (apoptotic B16OVA). The same experiments were performed adding TGF-β to IKDC15 or apoptotic tumor cells for 6 h before washing and injection into the footpads (C). Graphs show survival curves of animals (n = 8–12 per group) from at least four independent experiments. Statistical analyses were performed using Mantel Cox test. The inset of C depicts the TGF-β reduced killing of IKDC15 in vitro. IKDC15 incubated for 24 h with B16OVA in medium alone or with TGF-β (2 ng/ml) were subjected to a 24-h crystal violet assay against B16OVA. Results of a representative experiment of three are depicted as means ± SEM.

IL-15 trans-presentation is a prerequisite for IKDC responsiveness to TLR3 and TLR4 ligands

Resting IKDC did not express basal levels of mRNA encoding any of the 11 mouse TLR and could not respond to TLR stimuli (Fig. 5A and our unpublished data). However, trans-presentation of IL-15 significantly up-regulated the transcription levels of TLR3 and TLR4 in IKDC (Fig. 5A). Thus, IKDC15 acquired the capacity to respond to TLR4 ligands (LPS) by producing high levels of CCL2 (MCP-1) and CXCL1 (KC-GROα) (Fig. 5B). It is important to note that this responsiveness of IKDC required trans-presentation of IL-15 (not shown). Importantly, IKDC15 acquired the capacity to produce CCL2 even after 2–3 days culturing in IL-15/MS-5 (not shown). In addition, IKDC15 responded to TLR3 ligands (poly(I:C)) for the production of CCL5 (RANTES, Fig. 5B). Moreover, trans-presentation of IL-15 lead to responsiveness of IKDC to IL-2 and IFNα for the secretion of high amounts of CCL2 and CCL5 (23).

IL-15 trans-presentation promoted IKDC effector functions

The basal transcription level of the killing machinery (perforin/granzyme B/FasL), which was detectable in resting NK cells (not shown), was absent in resting IKDC (Fig. 6A). However, upon IL-15 trans-presentation, the transcription of perforin, granzyme B, CD95L, and TRAIL was dramatically induced in IKDC (Fig. 6, A and B). Interestingly, the transcription levels of perforin increased by 1000-fold in ex vivo expanded IKDC15 compared with freshly sorted IKDC. At the protein level, similar conclusions could be drawn in that IKDC15 contained high amounts of granules of perforin and granzyme B compared with resting IKDC as observed in confocal microscopy (Fig. 6B) or flow cytometry (not shown). Accordingly, the lytic activity of IKDC against B16F10 was markedly enhanced by trans-presentation of IL-15 (Fig. 6C).
The lytic activity of IKDC15 was mainly dependent on TRAIL molecules (Fig. 6E). The side by side comparisons between IKDC and NK cells both stimulated for short (36 h) or long (7 days) periods of time with MS-5/IL-15 revealed qualitative but not quantitative differences. Although NK cell-mediated killing was dependent on granule exocytosis, IKDC lytic functions mostly rely on TRAIL molecules (Fig. 6, D and E).

Therefore, trans-presentation of IL-15 endowed IKDC with TRAIL-dependent killing capacities, a biological attribute not shared by conventional NK cells.

Ex vivo expanded IKDC mediated a TGF-β resistant-protective immunity against tumor cells

We previously reported that IKDC invade tumor beds and were necessary and sufficient to prevent tumor outgrowth after adoptive cell transfer in Rag-/- x IL-2Rγ-/- deficient hosts (2). However, the immunizing potential of IKDC in nonimmunocompromised animals remained to be assessed. In as much as NK and IKDC diverge in their mechanisms of killing tumor cells, we addressed the differential immunizing potential of both innate effectors. We used B16OVA as target cells incubated with IKDC15 or B220- NK cells (equally activated in MS-5/IL-15) for 16 h before s.c. inoculation as immunization protocols. The ex vivo killing of B16OVA was comparable to that of B16F10 (Figs. 6 and 7C, inset). When mice were rechallenged 10 days later with a lethal dose of B16OVA, only those vaccinated with IKDC15, but not with 24 h or 7 day IL-15/IL-15Rα stimulated B220- NK cells or 1 x 10^5 dying tumor cells (24–26), exhibited delayed tumor outgrowth associated with a significantly prolonged survival compared with untreated animals (Fig. 7, A and B). It is noteworthy that inoculation of an increased number of at least 3 x 10^6 doxorubicin-treated B16OVA tumor cells could confer a significant protection after rechallenge (24). Because IKDC invade tumor beds and could theoretically be subjected to TGF-β-induced immunosuppression, we analyzed the effects of recombinant human (rh) TGF-β on their killing potential and their immunogenicity in vivo. TGF-β could substantially reduce the killing potential of IKDC against B16OVA in vitro (Inset, Fig. 7C), but did not abrogate their protective activity against tumor challenge in vivo (Fig. 7C). The prophylactic effects of IKDC15 treated with TGF-β were not observed in Nude counterparts, suggesting that IKDC15/ TGF-β mediated T cell-based antimtumor immunity (not shown).

In this study, we demonstrate that ex vivo expanded IKDC15 not only gained lytic capacity in vitro (Fig. 6), but also protective antimtumor function in vivo (Fig. 7A) that even resists to immunosuppressive TGF-β. These data support the hypothesis that IKDC15 could link innate and cognate immunity and, therefore, would be capable of inducing an antitumor immune response resistant to tumor-induced tolerance.

Discussion

This manuscript describes for the first time the pivotal role of IL-15 trans-presentation in the biology of IKDC, a novel subset of innate effectors sharing markers of both NK cells and conventional DC (1, 2). We initially reported that IKDC were B220-CD11c- NK1.1+ cells expressing MHC class II molecules during treatment with IM + IL-2 and invading tumor beds to kill in a TRAIL-dependent fashion, whereas Chan et al. (1) described that lymph node IKDC in BALB/c mice were endowed with MHC class II-restricted Ag presenting function in vitro. Therefore, IKDC may be considered as a MHC class II expressing NK cell subset or alternatively as a DC endowed with TRAIL-dependent killing capacities. This view has been recently challenged by several authors supporting the notion that IKDC, rather, represent an activated state of conventional NK cells (12, 13, 27). This manuscript aimed at clarifying the functional differences between IKDC and NK cells.

First, IKDC exhibited marked proliferative potential in vitro and in vivo following IL-15/IL-15Rα-driven stimulation (Figs. 1 and 2). Indeed, we showed that CpG ODN, rIL-15 (Fig. 1), and even IM + IL-2 (23) all drove IKDC proliferation in vivo in an IL-15Rα-dependent manner. Interestingly, despite their cloning expansion capacity, IKDC appeared to represent fully differentiated cells because they did not convert into bona fide B220-CD11c- NK cells after adoptive transfer into congenic animals (Fig. 1B). In sharp contrast, B220- NK cells failed to proliferate in vitro during stimulation with IL-15/IL-15Rα and their CpG driven-proliferation in vivo was IL-15Rα-independent. Although harboring intracytosolic IL-15Rα (Fig. 2A), why did B220- NK cells fail to respond to IL-15 for ex vivo proliferation? Several hypotheses can be drawn to account for the IL-15-driven proliferation of IKDC and not NK cells. There are several isoforms of IL-15Rα. The full-length sIL-15Rα ectodomain resulting from the proteolytic degradation of IL-15Rα is inhibitory when binding to IL-15. In contrast, some isoforms, such as the sushi sIL-15Rα resulting from an alternative splicing of the mRNA of IL-15Rα, are agonists (28). Moreover, there is a reciprocal activation of IL-15Rα with a tyrosine kinase receptor Axl leading to the phosphorylation of both receptors upon binding of IL-15 or Gas6 (the ligand for Axl) and survival effects of the transduced cell type (29). Hence, it is plausible that NK cells might secrete the antagonist form of IL-15Rα and would not be able to benefit from IL-15 and/or that IKDC do secrete a sushi-like isoform of IL-15Rα. Likewise, it is unlikely that Axl plays a dominant role because Axl was not found in Western blot analyses, neither in IKDC nor NK cells (not shown).

Second, following trans-presentation of IL-15, IKDC acquired high lytic capacities (against B16F10 (Fig. 6C) and B16OVA (Fig. 7)) that were fully abrogated in the presence of anti-TRAIL neutralizing Ab (N2B2, Fig. 6). In contrast, B220- NK cells exhibited high basal transcription levels of perforin (in contrast to IKDC, not shown) and killed target cells using secretory granules and not TRAIL molecules (Fig. 6, D and E). TRAIL-dependent cytotoxicity was shown to play a dominant role in the prevention and treatment of neoplasia (30, 31). IKDC15 became capable of sensitizing and killing tumor cells mainly through TRAIL molecules while also up-regulating their levels of perforin and granzyme B (Fig. 6). Although previous observations tend to demonstrate that IL-15 can up-regulate TRAIL and boost TRAIL-dependent cytotoxicity of murine NK cells in vitro, it remains to be determined whether the IKDC component of the mouse NK cell pool was in fact mediating these TRAIL-dependent effects (32).

Third, following trans-presentation of IL-15, B16OVA-lysing IKDC mediated T cell-dependent protective effects in vivo, even in the presence of TGF-β. Such prophylactic immunization properties were not found with IL-15/IL-15Rα-stimulated NK cells (displaying equivalent quantitative killing capacities as IKDC). One of the main issues remains whether IKDC could not only play a scavenger role by mediating tissue destruction but also a role in T cell priming. Because we have shown that IL-15 trans-presentation skews IKDC toward cytotoxic effector cells rather than APC, we suggest that footpad inoculation of IKDC15 encountering B16OVA may indirectly promote recruitment and activation of conventional DC that will prime naive T lymphocytes. Given that IKDC15 differ from NK15 in their TRAIL-dependent killing of targets, we anticipate that programmed cell death triggered by the extrinsic (membrane bound, TRAIL-mediated) as opposed to the intrinsic (mitochondrial perforin/granzyme-mediated) cell death
pathways could matter in the outcome of the prophylactic potential of both effectors. Indeed, our group has reported that apoptosis mediated by anthracyclines, oxaliplatinum, or X Rays was immunogenic, whereas other cytotoxic agents failed to promote an immunogenic cell demise. This was due to the ability of some cytotoxic compounds to induce ecto-calreticulin (CRT) at the plasma membrane of dying cells (26) and to release HMGB1 alarmins to interact with TLR4 harbored on DC (33). Although ecto-calreticulin was required for phagocytosis by DC of dying tumor cells, HMGB1 was involved in the processing of apoptotic material by DC. Therefore, whether the immunogenicity of IKDC-mediated cell death is TRAIL-, HMGB1-, and/or CRT-dependent needs to be addressed.

Fourth, as recently demonstrated trans-presentation of IL-15 allowed CCR2 expression on IKDC but not on B220−NK cells, likely contributing to their CCL2-dependent intratumoral trafficking (23).

It is interesting to note that B220 and CD11c molecules were not acquired by conventional NK cells after 24–48 h of stimulation with a variety of cytokines or DC (Table I), presumably because such NK cells do not enter cell cycle in vitro. Moreover, IL-15 or IL-12 down-regulated MHC class II transcription levels on IKDC, supporting the notion that B220, CD11c, and MHC class II unlikely correspond to activation markers because they were differentially modulated by these activating cytokines.

IL-15 is a pivotal cytokine for the development and function of innate immune cells such as NK, NKT, and TCRα/β (47). Therefore, one of the major challenges will be addressed.

References


