Migratory and Antigen Presentation Functions of IFN-Producing Killer Dendritic Cells

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Abstract

The CD11cint B220+ NK1.1+ CD49+ subset of cells has recently been described as IFN-producing killer dendritic cells (IKDC), which share phenotypic and functional properties with both dendritic cells and natural killer cells. We have previously shown that IKDCs within murine bone marrow–derived DC preparations are essential for the antitumor activity of unpulsed DCs. Here we show that bone marrow–derived IKDCs (BM-IKDC) migrate in vivo into tumors and thence to tumor draining lymph nodes, where they highly express MHC class II and costimulatory molecules.

In vitro, freshly isolated BM-IKDCs, fluorescence-activated cell sorted to homogeneity, have no intrinsic antigen presentation function unless cocultured with tumor target cells. On killing of target cells, they can cross-present antigens to stimulate antigen-primed CD8 T cells and can also present antigens to antigen-primed CD4 cells. In vivo, in mice lacking class I–restricted antigen-presenting cell function, robust proliferation of antigen-specific T cells is achieved after adoptive transfer of BM-IKDCs at an injection site distant to the tumor site. Therefore, BM-IKDCs are capable of cytotoxic killing of tumor targets and also of potent antigen presentation after encountering antigen in the context of a viable target cell.

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Introduction

Adoptive transfer of dendritic cells (DC) has the potential to inhibit cancer growth through stimulation of both innate and adaptive immune responses (1–4). Presentation of tumor antigens by DCs leads to adaptive immunity responses after activation of tumor antigen–reactive T cells in lymph nodes. Within lymphoid tissues, DCs are attracted to sites of inflammation where they can take up antigen. Subsequent maturation of DCs results in the up-regulation of costimulatory molecules and MHC class II and migration to sentinel lymph nodes where they present antigens to T cells and stimulate innate responses.

Natural killer (NK) cells and DCs can exchange bidirectional activating signals in a positive feedback (cross talk). Further information on the cross talk of innate and adaptive responses has emerged after the recent description of IFN-γ–producing killer dendritic cells (IKDC), which showed both NK cell and antigen-presenting cell (APC) surface phenotypes and functions (5–7). Originally, IKDCs were described in mice as showing intermediate expression of CD11c coupled with high expression of B220, high expression of the NK cell markers NK1.1 and CD49b, and absence of Gr1 to distinguish them from conventional NK cells (NK1.1–/CD49b–/CD11c–/B220–) and plasmacytoid DCs (CD11c+K1.1+CD49b–Gr1–B220+) (refs. 5, 6). However, the distinction between IKDCs, DCs, and NK cells has been challenged in recent reports that have described the presence of CD11c and B220 in conventional NK cells and have failed to show the presentation of antigen by IKDCs, leading to the suggestion that IKDCs are an activated form of NK cells (8–10).

We have recently shown that IKDCs comprise 3% to 5% of cells from mouse bone marrow DC preparations. In adoptive transfer experiments, purified IKDCs from bone marrow (BM-IKDC) were effective at eliminating tumor cells implanted at distant s.c. sites, using a mechanism that is dependent on effector cells of the host immune system (11). We here show that highly purified IKDCs are capable of cross-presenting tumor antigens in vitro and that in vivo cross-presentation occurs during the process of tumor rejection by IKDCs.

Materials and Methods

Preparation of bone marrow–derived DCs, NK cells, and IKDCs. Recombinant murine granulocyte macrophage colony-stimulating factor (GM-CSF; 25 ng/mL) was added to fresh mouse bone marrow cells and then cultured for 2 or 3 d at 37°C in 5% CO2. On day 2 or day 3, bulk DCs were enriched for CD49b+ by magnetic separation using a CD49b-specific (DX5) monoclonal antibody (mAb; Miltenyi Biotech). CD49b+ cells were stained with mAb against CD11c, NK1.1, and B220 and subjected to fluorescence-activated cell sorting (FACS; MoFlo) for double-positive NK1.1 and B220+ activated cell sorting on CD11cint. Conventional DCs (cDC; CD11c–NK1.1–B220+) and NK cells (CD11c–B220–NK1.1+) were also FACS sorted. In some experiments, IKDCs, DCs, and NK cells were isolated from fresh bone marrow. For in vivo migration experiments, IKDCs were prepared from transgenic C57BL/6 expressing enhanced green fluorescent protein (eGFP). Purified NK cells and IKDCs were then used for functional experiments. Cell purity was 98% (IKDC) and 98% (cDC).

In vitro and in vivo T-cell proliferation assays. OT-1 CD8 T cells were activated in vitro for 5 d in the presence of 200 IU/mL interleukin-2 (IL-2) and SIINFEKL (MHC class I–restricted OVA peptide)–pulsed and irradiated splenocytes (primed OT-1) or were used without stimulation (naive OT-1). Sorted CD8 T cells from the spleens of 76-9 tumor–bearing mice treated with unpulsed DC vaccines were activated for 5 d with 76-9 tumor lysate–loaded and irradiated splenocytes in the presence of IL-2 (200 IU/mL). T-cell populations were of ≥98% purity and were free from DCs. Sorted NK cells, IKDCs, or DCs (2 × 105–5 × 105) were mixed with an equivalent number of Gr1 to distinguish them from conventional NK cells (NK1.1+).
of tumor target cells or peptide and with 0.5 $\times$ 10^7 to 2 $\times$ 10^7 carboxyfluorescein diacetate succinimydyl ester (CFSE)–labeled T-cell populations for 4 to 5 d. In any individual experiment, the numbers of APCs, tumor targets, and T cells were kept constant. In some experiments, OT-1 T cells were stimulated with concanavalin A (5 μg/mL; Sigma-Aldrich). For in vivo T-cell proliferation, B16-OVA tumor–bearing RAG^−/− j2-microglobulin^−/− j2-microglobulin^−/− mice were s.c. treated with IKDCs or NK cells in the contralateral flank at day 10 and day 14 after tumor inoculation. Forty-eight hours after the second treatment, 5 $\times$ 10^5 primed or naive CFSE-labeled OT-1 CD8 T cells were injected i.v. Seventy-two hours later, splenocytes, tumors, and draining lymph nodes (DLN) were harvested.

Antibodies, flow cytometry, and reagents. All the antibodies used in this study are from eBioscience, except for MHC class I blocking mAb, 34-5-8s (purchased from Abcam), and MCA298 rat anti-mouse MHC class I H-2hLipuq.v16 clone er-hr52 (from Serotec). The eBioscience antibodies were CD11c (clone N418), B220 (clone RA3-6B2), CD8 (clone GL1), CCR7 (clone 4B12), MHC class II (I-A/I-E; clone M5/114.15.2), Ly-6G (Gr1; clone RB6-8C5), CD4 (clone GK1.5), CD8 (clone 53-6.7), CD8 (clone YTS169.4), NK1.1 (clone PK136), NKG2D/CDA49 (clone C7), NKG2D/CDA49 (clone CX5), CD49b (clone HMa2), CD49b (DX5; clone DX5), and tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL; clone N2B2), conjugated to appropriate fluorochromes. Antibodies, flow cytometry, and reagents. All the antibodies used in this study are from eBioscience, except for MHC class I blocking mAb, 34-5-8s (purchased from Abcam), and MCA298 rat anti-mouse MHC class I H-2hLipuq.v16 clone er-hr52 (from Serotec). The eBioscience antibodies were CD11c (clone N418), B220 (clone RA3-6B2), CD8 (clone GL1), CCR7 (clone 4B12), MHC class II (I-A/I-E; clone M5/114.15.2), Ly-6G (Gr1; clone RB6-8C5), CD4 (clone GK1.5), CD8 (clone S3-6.7), CD8 (clone YTS169.4), NK1.1 (clone PK136), NKG2D/CDA49 (clone C7), NKG2D/CDA49 (clone CX5), CD49b (clone HMa2), CD49b (DX5; clone DX5), and tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL; clone N2B2), conjugated to appropriate fluorochromes.

Migration assays. We resuspended CFSE-labeled CD11c^+ B220^− NK1.1^+ IKDCs or CD11c^+ B220^− NK1.1^+ NK cells in HBSS buffer plus 2% FCS at a density of 6 $\times$ 10^7/mL in the top chamber of 24-transwell chambers (5-μm pore size; Corning, Inc.). The bottom chamber was filled with 0.6 mL of the same chemotaxis buffer containing 1 $\times$ 10^7 cells/mL of the test cell lines. The plates were incubated for 4 h at 37°C in 5% CO2. Cells that migrated to the bottom chamber were harvested and FACS analyzed for CFSE. For in vivo migration assays, small s.c. tumors were generated after labeling 76-9 cells with lentivirus expressing dsRed.

Confocal microscopy. Samples of tumor and DLNs were immersed in optimum cutting temperature compound (Sakura) and frozen in liquid nitrogen. Cryostat sections of 7-μm thickness were cut on poly-l-lysine–coated slides (VWR), fixed for 20 min with 1% paraformaldehyde (BDH) in PBS, and rinsed in PBS. After thoroughly washing in PBS, the slides were mounted in 4,6-diamidino-2-phenylindole (DAPI)–containing Vectashield (Vector Laboratories) and examined using a laser scanning spectral confocal microscope (TCS SP2, Leica).

Results

IKDCs migrate to tumor and then to tumor DLNs. We have previously shown that distant injection of mouse BM-IKDCs could
inhibit the growth of the murine tumors B16F10, LL/2, and 76-9 in C57BL/6 mice (11). To investigate the role of the host NK cells and DCs in this effect, we first optimized a FACS strategy to allow isolation of IKDCs, NK cells, and cDCs by first enriching for NK cell marker CD49 and then staining with CD11c, B220, and NK1.1. We gated on the CD11cint population and then sorted by NK1.1+ B220" (IKDC), NK1.1+ B220" (NK), and NK1.1− B220" (cDC; Fig. 1A). We chose the CD11cint population of NK cells because these had previously been proposed to be activated NK cells, equivalent to IKDCs (8–10). When FACS-sorted BM-IKDCs or NK cells from wild-type mice were given to beige mice bearing 13-day tumors, BM-IKDCs were more potent in slowing growth compared with NK cells (Fig. 1B). Therefore, we confirm that BM-IKDCs are necessary and sufficient for strong tumoricidal effect in vivo, and host NK cells are not required for tumor rejection.

To address how and where BM-IKDCs could encounter tumor antigens to induce antigen-dependent cell death in vivo, we first studied their migratory properties in vitro in transwells. In the absence of tumor cells in the lower chamber, no CFSE-labeled BM-IKDCs migrated downward (data not shown). However, BM-IKDCs migrated toward murine cancer cell lines more efficiently than equivalent numbers of NK cells. This migration seemed to be specific for cancer cells because only very small numbers of NK cells or IKDCs migrated toward NIH3T3 cells (Fig. 2A).

We hypothesized that BM-IKDCs might be chemoattracted in vivo into lymph nodes and/or tumor. To test this, mice bearing dsRed-labeled 76-9 tumors were treated s.c. with BM-IKDCs from GFP transgenic mice in the contralateral flank. After 24 hours, BM-IKDCs were detectable by flow cytometry in tumors but not in other tissues (data not shown), whereas by 48 and 72 hours, they were detectable in much lower numbers at the tumor site but were now relatively abundant in the DLN (Fig. 2B), but not in other organs (data not shown). Fluorescence microscopy confirmed migration of IKDCs to DLNs by 72 hours after adoptive transfer but revealed no detectable dsRed tumor cells in the lymph node site (Fig. 2C). At the tumor site, BM-IKDCs could be visualized at 24 and 48 hours and were seen to be in close contact with tumor cells (Fig. 2D).

In a separate experiment, we analyzed the presence of GFP-positive BM-IKDCs and NK cells in disaggregated DLN by flow cytometry. Only IKDCs, but not NK cells, in DLN exhibited bright MHC class II, CD40, CD80, and CD86 expression (Supplementary Fig. S1A), consistent with an ability to acquire an APC-like phenotype in vivo. Finally, in another experiment, BM-IKDCs in DLN of tumor-bearing mice were shown to have dramatically higher MHC class II compared with non–tumor-bearing counterparts (Supplementary Fig. S1B), and by 7 days, the adoptively transferred GFP-positive IKDCs were seen in DLN, but not in the tumor site (Supplementary Fig. S2). These findings are consistent with a model in which BM-IKDCs cause growth inhibition in vivo of distantly implanted tumor cells through initial migration to tumor and subsequent migration to DLNs where they up-regulate MHC class II and costimulatory molecules, consistent with in vivo antigen presentation.

BM-IKDCs are capable of antigen presentation in vitro after killing tumor targets. We speculated that the up-regulation of MHC class II in the lymph nodes of tumor-bearing mice reflected
in vivo presentation of tumor antigens. We propose a model by which the BM-IKDCs kill tumor cells, take up tumor antigens at the tumor site, and then present tumor antigens in DLN. We tested this in an in vitro cross-presentation assay, in which responder cells were CFSE-labeled CD8 cells purified from spleens of OT-1 T-cell receptor transgenic mice, primed in vitro for 5 days with the OT-1-specific SIINFEKL ovalbumin peptide (OVA peptide). BM-IKDCs, NK cells, and cDCs were FACS sorted

Figure 3. IKDCs cross-present antigens in vitro from viable tumor target cells. A, responders (CFSE-labeled OT-1 CD8+ T cells), effectors (NK cells, DCs, or IKDCs), and targets (tumor cells or peptide) were cocultured for 4 d. A representative FACS plot of OT-1 cells after CD8 selection confirms the absence of CD11c contamination. B and C, equal numbers of FACS-purified APCs (sorted as in Fig. 1A) were added to peptide-primed OT-1 CD8 cells in the presence or absence of OVA peptide or B16-OVA (B) or in the presence of OVA-expressing B16 tumor cells (C). Gray histogram, CFSE-labeled OT-1 T cells before coculture; open histogram, CFSE-labeled OT-1 T cells after coculture. D, the proliferation of OT-1 CD8 T cells when cocultured with IKDCs, IKDCs + 2% DCs, or DCs alone (black line) or with NK cells (gray fill) is shown in response to B16-OVA tumor cells or OVA peptide.
to >98% purity, and there was no evidence of contaminating residual CD11c-positive cDCs within sorted OT-1 cells (Fig. 3A). In coculture assays, BM-IKDCs, unlike cDCs, were unable to cross-present OVA peptide to OT-1 T cells. However, BM-IKDCs (unlike NK cells or DCs alone) were able to cause robust proliferation of antigen-primed OT-1 T cells when cocultured with OVA-expressing tumor cells, although this effect was somewhat reduced compared with the NK/DC mix (Fig. 3B). BM-IKDCs were also capable of inducing the proliferation of naive OT-1 T cells when cocultured with B16-OVA tumor cells (Fig. 3C).

To exclude contamination of the BM-IKDC preparation with cDCs, we deliberately added cDCs to the BM-IKDCs. Addition of only 2% cDCs caused a significant proliferation of OT-1 cells in response to OVA peptide, in clear contrast with pure BM-IKDCs, indicating that there is no significant level of contamination of the BM-IKDC preparation with functional cDCs (Fig. 3D). Therefore, BM-IKDCs cause an antigen-specific proliferation of CD8 T cells in vitro but only in the presence of antigen-expressing target cells and not in the presence of peptide antigen alone.

Figure 4. Tumor cells do not contribute to in vitro cross-presentation. A, effector cells (NK, DC, NK/DC 50:50 mix, or BM-IKDC) were pretreated with anti–MHC class I antibody or isotype control before coculture with B16-wild-type or OVA-expressing tumor cells and CFSE-labeled OT-1 T cells. B, B16-OVA tumor cells and CFSE-labeled OT-1 T cells were cocultured with IFN-γ (0–50 ng/mL; gray) or concanavalin A (5 μg/mL; black). C, effector cells were pretreated with anti-NKG2D and anti-TRAIL antibodies before coculture with B16-OVA tumor cells and CFSE-labeled OT-1.
To show that antigen presentation was occurring via the class I pathway and to further compare the antigen presentation function of BM-IKDCs with those of NK cells and cDCs, we repeated the assay in the presence of MHC class I blocking antibodies. Only BM-IKDCs (or a mix of cDCs and NK cells) were capable of inducing robust proliferation of antigen-primed OT-1 cells when the APCs were cultured with viable B16-OVA tumor cells. The proliferation was completely inhibited by pretreatment of the APCs with MHC class I blocking antibodies. Furthermore, to exclude presentation by the tumor cells themselves, we cocultured B16-OVA and OT-1 CD8 T cells in the absence of IKDCs and showed no proliferation of OT-1 T cells (Fig. 4A). Moreover, coculture of B16-OVA with OT-1 T cells in the presence of different concentrations of IFN-γ and without any IKDCs did not induce any stimulation of T cells, excluding the possibility that IFN-γ released by IKDCs might be enough to induce T-cell proliferation (Fig. 4B), although adding IFN-γ to the coculture did cause up-regulation of MHC class I expression on the surface of B16-OVA tumor cells (data not shown).
We hypothesized that cDCs failed to stimulate OT-1 because the antigen was not released from the living B16-OVA cells and that both cell killing and uptake of antigen were required for robust proliferation response, a combined functional activity only present in the BM-IKDC population. We showed this further by mixing together cDCs and NK cells, which resulted in a slightly stronger proliferation response than with BM-IKDCs alone (Fig. 4A). It has been previously shown that both the NKG2D and TRAIL pathways are involved in IKDC killing, and thus, we combined blocking antibodies against both receptors and showed that this caused almost complete abrogation of the CD8 cell proliferation induced by BM-IKDCs (Fig. 4C).

This led us to speculate that BM-IKDCs can cross-present antigens but need to encounter intact target cells and/or kill those targets before they acquire antigen presentation function. To test this, we cocultured IKDCs, cDCs, or NK cells with antigen (76-9 lysate)–primed CD8+ T cells in the presence of viable 76-9 tumor targets and in the presence or absence of mAbs to block killing function. The lysate-primed CD8 cells underwent robust proliferation induced by coculture with both BM-IKDCs and tumor cells, but not with NK cells or cDCs cocultured with tumor cells, and a cocktail of blocking antibodies against NKG2D and TRAIL caused almost complete abrogation of CD8 cell proliferation (Fig. 5A). Purified BM-IKDCs could also induce proliferation of 76-9 F5 lysate–primed CD4 cells under the same conditions, and proliferation was inhibited by a MHC class II blocking antibody (Fig. 5B).

Therefore, unlike cDCs, BM-IKDCs purified to homogeneity cannot present peptide antigens to CD8 cells; however, they induce a robust CD8 proliferative response if cultured with tumor cells expressing the target antigens. cDCs and NK cells cannot individually induce a CD8 proliferative response when cultured with viable tumor cells; however, the combination of NK cells and cDCs recapitulates the proliferative response of IKDCs. Others have recently shown that activated splenic IKDCs coexpress markers of activation of both NK cells (IFN-γ) and DCs (CD86, MHC class II) within the same cell (14), and the same group previously identified an inverse relationship between NKG2D and MHC II levels of expression within the IKDC population (6). Together, these suggest a model in which IKDCs migrate to tumor, wherein their cytotoxic activity results in their activation and acquisition of professional APC function, leading to uptake of antigen from the lysed cells and migration to sentinel lymph nodes for initiation of immune responses.

**IKDCs migrate into tumors in vivo to cross-present antigen.** A possible criticism of demonstration of APC function in BM-IKDC preparations is that small numbers of bystander

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**Figure 6.** IKDCs migrate into tumors in vivo to cross-present antigen. APCs were injected s.c. at day 10 and day 13 after tumor challenge into the contralateral flank of tumor-bearing RAG−/− μ2-microglobulin−/− mice. On day 15, 5 × 10^6 CFSE-labeled naive or OVA peptide–primed OT-1 T cells were injected i.v. Spleen and tumors were harvested 48 h later. Percentage of expanding OT-1 cells is shown as a range from each group of five mice.
cDCs could be responsible for antigen presentation after NK cell activation. We next attempted to show BM-IKDC APC function in vivo and exclude the possibility of bystander APC function by FACs sorting the populations to >98% purity and by using mouse hosts lacking presentation function for class I–restricted antigens. We made use of RAG−/− β2-microglobulin−/− hosts lacking endogenous presentation via the class I pathway. FACs-sorted APCs (IKDCs or cDCs) from C57BL/6 wild-type mice were injected into the contralateral flank of B16-OVA or B16-wild-type tumor–bearing RAG−/− β2-microglobulin−/− mice. Forty-eight hours later, CFSE-labeled naive or antigen-primed OT-1 cells were injected via the tail vein. CFSE dilution was determined in tumor or spleen 48 hours after OT-1 transfer (lymph nodes were too small to analyze). IKDCs were able to induce proliferation of both primed and (to a lesser degree) naive OT-1 cells, but only in mice whose tumors expressed OVA. Strikingly, an equivalent number of cDCs or NK cells failed to induce any proliferation of antigen-primed OT-1 cells in B16-OVA tumor–bearing mice (Fig. 6). We cannot exclude the possibility that the injected naive T cells had encountered antigen in vivo before stimulation by IKDCs, and our data therefore do not unequivocally show stimulation of naive T cells by IKDCs.

Discussion

It is interesting to speculate that the migration to tumor cells in vitro and the apparent change in tropism from tumor to lymph node in vivo are related to an IKDC maturation process that involves up-regulation of MHC II and transition from a predominantly NK to an APC function. Little is known about the IKDC chemokine/receptor profile that might govern their migration capacity. Mignot and colleagues have recently shown that, after IL-15 stimulation, IKDCs express CCR2 and CCR5, which are potentially important in tumor and/or lymph node tropism (15). Such a maturation of IKDCs might have a counterpart in conventional NK cells. For example, Hayakawa and colleagues have shown that, both in mice and in humans, CD27high and CD27low subsets of NK cells have different properties, with the CD27high population displaying greater effector function and migration in response to chemokines (16). Furthermore, Zitvogel and colleagues, in a companion study (17), show that activation of IKDCs by tumor licensing is associated with a switch, specifically within the CD11b population, from NK-like to DC-like cells. It will be of interest to determine if the BM-IKDC population contains large numbers of the CD11b− CD27− IKDC subset.

The concept that IKDCs develop APC function only on activation and/or tumor cell killing may also explain why others have failed to show antigen presentation function within the IKDC population (8). With splenic IKDCs, activation with Cpg (6) or imatinib (5) preceded the demonstration of APC function. In our hands in vitro, BM-IKDCs have no APC function (e.g., to present peptide antigens) unless they are in the presence of tumor target cells, and they do not acquire APC function in the presence of tumor targets if the are cultured in the presence of NKG2D and TRAIL blocking antibodies. This might be a form of "licensing" in which the killing by BM-IKDCs is responsible for the development of APC function associated with up-regulation of MHC class II and costimulatory molecules.

When combined with the in vivo migration data, this strongly suggests that BM-IKDCs breach innate and adaptive immunity by first migrating to tumor and killing tumor cells, then becoming activated, taking up tumor antigen, and migrating to DLNs where they become effective APCs to induce adaptive responses. Our previous data showed that IKDCs were present in high amounts in bone marrow DC preparations and that there was an absolute requirement of IKDCs within therapeutic DC preparations to induce a tumor response to unpulsed DCs. We also showed that the tumoricidal activity of BM-IKDCs is lost in hosts lacking functional T cells and NK cells, implying that induction of secondary immune responses is vital for BM-IKDC tumoricidal effects (11).

IKDCs, as first described in 2006, added to the literature of known mouse and human cells with an overlap of NK cell and DC markers and functions. The distinction between IKDCs, DCs, and NK cells has been challenged in recent reports that have described the presence of CD11c and B220 on conventional NK cells and have shown lack of presentation of antigen by IKDCs, leading to the suggestion that IKDCs represent an activated form of NK cells (8–10). Moreover, it has been claimed that none of the early IKDC studies convincingly showed both NK and antigen presentation functions in the same cell type. Together, all of these studies have focused on IKDCs derived predominantly from splenocytes, and further work is required to determine whether there are significant functional and phenotypic differences between splenic and BM-IKDCs.

Regarding ontogeny, there is evidence that IKDCs share a similar developmental pathway as NK cells, for example, IL-15 and common γ-chain dependence (8–10), similarity of the transcriptional signature of IKDCs and NK cells (18), and the expression of NK-specific transcription factors in IKDCs (9), coupled with the absence of expression of the hematopoietic transcription factor PU.1, known to be expressed in DCs (8). However, others have recently shown that the transcriptional expression of antigen presentation genes is significantly higher in freshly isolated IKDCs than in NK cells (14). Moreover, IKDCs have a unique developmental pathway from lymphoid precursors (19). IKDCs seem to be part of a spectrum of NK-type cells, which increase expression of DC markers such as CD11c, MHC class II, and B220 on activation. Human NK cells can induce both CD4 and CD8 cell proliferation in response to antigen-specific stimulation through a process that depends on costimulatory receptors and is associated with bright expression of human MHC class II (20–23). CD56bright human NK cells are found in T-cell–enriched areas of lymphoid organs (24), and it will be interesting to investigate whether these cells have potent antigen presentation properties.

Similarly, a growing body of evidence has identified cytotoxic activity within DC populations. Immature DCs generated in vitro from blood monocytes (MoDC) were reported to induce apoptosis in hematopoietic and nonhematopoietic tumor cells (25, 26) without affecting normal cells (27, 28). A large number of studies further showed that activation/maturation of MoDCs with type I IFN (29–31), IFN-γ (31), lipopolysaccharide, double-stranded RNA, or various viruses (32–34) enhanced or induced cytotoxicity. This activity was mostly mediated by TRAIL, which could be induced by type I IFN produced by DCs. In addition, MoDCs generated in vitro by GM-CSF and IFN-α, instead of IL-4, exhibited expression of TRAIL and granzyme B and killed K562 cells (35). DCs generated from CD34 cells also acquired killing potential on IFN-β stimulation (29). Cooperation between TRAIL, Fas ligand, TNF-α, and lymphotoxin α1β2 in the killing mechanisms of MoDCs has also been shown (36). Additional and to date unknown mechanisms of MoDC-mediated killing have also been reported (28, 37).
In summary, BM-IKDCs, like certain NK cell and DC populations, show evidence of a degree of combined antigen presentation and NK cell function within the same cell. However, within the experimental systems, we have investigated significant levels of both killing and antigen presentation function, which could only be observed in either the IKDC purified population or the combined populations of DCs and NK cells. This identifies IKDCs as a unique population capable of both functional killing and functional stimulation of adaptive immune responses via classic antigen presentation.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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