Putative IKDCs are functionally and developmentally similar to natural killer cells, but not to dendritic cells

Irina Caminschi,1 Fatma Ahmet,1 Klaus Heger,1 Jason Brady,1 Stephen L. Nutt,1 David Vremec,1 Suzanne Pietersz,1 Mireille H. Lahoud,1 Louis Schofield,1 Diana S. Hansen,1 Meredith O’Keeffe,2 Mark J. Smyth,3 Sammy Bedoui,1 Gayle M. Davey,1 Jose A. Villadangos,1 William R. Heath,1 and Ken Shortman1

1The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, 3050, Australia  
2Bavarian Nordic GmbH, Munich D-82152, Germany  
3Cancer Immunology Program, Peter MacCallum Cancer Centre, East Melbourne, 3002, Victoria, Australia

Interferon-producing killer dendritic cells (IKDCs) have been described as possessing the lytic potential of NK cells and the antigen-presenting capacity of dendritic cells (DCs). In this study, we examine the lytic function and antigen-presenting capacity of mouse spleen IKDCs, including those found in DC preparations. IKDCs efficiently killed NK cell targets, without requiring additional activation stimuli. However, in our hands, when exposed to protein antigen or to MHC class II peptide, IKDCs induced little or no T cell proliferation relative to conventional DCs or plasmacytoid DCs, either before or after activation with CpG, or in several disease models. Certain developmental features indicated that IKDCs resembled NK cells more than DCs. IKDCs, like NK cells, did not express the transcription factor PU.1 and were absent from recombinase activating gene-2-null, common γ-chain-null (Rag2−/−Il2rg−/−) mice. When cultured with IL-15 and -18, IKDCs proliferated extensively, like NK cells. Under these conditions, a proportion of expanded IKDCs and NK cells expressed high levels of surface MHC class II. However, even such MHC class II+ IKDCs and NK cells induced poor T cell proliferative responses compared with DCs. Thus, IKDCs resemble NK cells functionally, and neither cell type could be induced to be effective antigen-presenting cells.
to express CD45R like pDCs and NK cells, and to express CD49b like NK cells, but to have low surface MHC class II expression. We have identified cells with this surface phenotype and isolated them from our DC preparations, as well as from the total spleen population. The IKDCs that we isolated were immediately capable of killing NK cell targets, but in response to CpG and a range of other stimuli, failed to up-regulate MHC class II and failed to efficiently activate naive T cells. In terms of developmental requirements and functional capacity, the IKDCs that we isolated resembled NK cells rather than DCs. Importantly, we show that mouse IKDCs and NK cells co-expressed CD11c and CD49b, and a proportion up-regulated MHC class II molecules upon exposure to IL-15 and -18 in cell culture, in respect aligning mouse and human NK cell biology; however, both cell types in our hands remained poor activators of naive T cells.

RESULTS

Surface phenotype analysis

IKDCs have been defined as cells that are similar to pDCs in that they express CD45R (B220) and intermediate levels of CD11c, but unlike pDCs, they also express CD49b (DX5) and NK1.1 (8, 9). Using our DC isolation procedure that enriches for splenic pDCs and cDCs (Fig. 1 A), we noted that a proportion of cells expressed CD49b+ (Fig. 1 B). These CD49b+ cells expressed variable levels of CD45R (and CD45RA; not depicted), ranging from undetectable to high (Fig. 1 C). Furthermore, the majority of CD49b+ cells expressed low to intermediate levels of CD11c, although a small proportion were CD11c high (Fig. 1 C). The subpopulation of the CD49b+ cells that were CD11c low/CD45R high therefore matched the description of IKDCs (Fig. 1 C). Additionally, there appeared to be another minor population of CD49b+ cells that expressed high levels of CD11c and MHC class II, like cDCs; we termed these CD49b+ cDCs. Further analysis showed that these IKDCs and NK cells, but not CD49b+ cDCs, pDCs, or cDCs, expressed NKG2D and NK1.1 (Fig. 1 D). The freshly isolated IKDCs expressed only low levels of MHC class II (Fig. 1 D), as previously reported (9). However, the level appeared a little lower than in the earlier study, which may reflect differences in reagents and staining strategies, or differences in the IKDC isolation procedures.

Figure 1.  Surface phenotype of IKDCs. Splenic DCs were isolated and enriched as described in the Materials and methods. The enriched DC preparation was stained with anti-CD11c-Cy7-PE, anti-CD45R (B220-FITC), and anti-CD49b (DX5-biotin), followed by the secondary reagent SA-Alexa Fluor 594, and then analyzed by flow cytometry. Dead cells were stained with PI. (A) Gating for cDCs (CD11c high CD45R low) and pDCs (CD11c intermediate CD45R+/-) without excluding CD49b+ cells. (B) Gating for CD49b+ within the DC-enriched preparation. (C) Expression of CD11c and CD45R on the CD49b+ cells, showing the gates used for IKDC and CD49b+ DC isolation. (D) The expression of NK1.1, NKG2D, and MHC class II on splenic DC subsets. For NK1.1 expression analysis, splenic DC preparations were stained with anti-NK1.1 (PK136-APC), anti-CD45R (B220-PE), and anti-CD11c (N418-Alexa Fluor 594), and anti-CD49b (DX5-biotin) followed by secondary reagent SA-Cy7-PE. For NKG2D expression analysis, splenic DC preparations were stained with anti-NKG2D (CX5-biotin), anti-CD49b (DX5-FITC), anti-CD11c (N418-Alexa Fluor 594), and anti-CD45R (B220-PE), followed by SA-Cy7-PE secondary reagent. For MHC class II expression analysis, splenic DC preparations were stained with anti-MHC class II (M5/114-FITC), anti-CD11c (N418-Alexa Fluor 594), and anti-CD49b (DX5-biotin) followed by secondary reagent SA-Cy7-PE. Spleen suspensions, which were depleted of red blood cells using 1.091 g/cm3 Nycodenz density centrifugation, were the source of NK cells. Splenocytes were stained with anti-NK1.1 (PK136-APC), anti-CD49b (DX5-Biotin); followed by secondary reagent SA-Cy7-PE, and anti-CD3 (KT3-Alexa-594), then NK cells sorted as NK1.1+/CD49b+/CD3-. The continuous line represents staining on gated cells and the dotted line represents background.
However, culturing our purified IKDCs with CpG and GM-CSF for 48 h resulted in <10% survival (Fig. 4 A), despite the presence of GM-CSF, which enhances the in vitro survival of cDC. This questioned the origin of the few remaining cells because the starting cultures of IKDCs were 95% pure, with 5% of cells likely to be DC contaminants. To circumvent problems associated with poor IKDC survival in culture, we isolated IKDCs and pDCs from transgenic mice expressing

**Figure 2.** IKDCs kill YAC-1 and CHO-K1 target cells. Splenic DCs were enriched and sorted based on their expression of CD11c, CD45R, and CD49b, as described in the Materials and methods. CD11c<sup>hi</sup>CD45R<sup>−</sup> (cDC), CD11c<sup>int</sup>CD45R<sup>−</sup>CD49b<sup>−</sup> (pDC), and CD11c<sup>int</sup>CD45R<sup>−</sup>CD49b<sup>+</sup> (IKDC) were purified and incubated at various ratios with 51Cr-labeled YAC-1 (A) and CHO-OVA (B) target cells in a 4-h 51Cr release assay, or in a 5-h 51Cr release assay at an E/T ratio of 2:1 (C). Lysis at each effector to target (E/T) cell ratio was determined as described in the Materials and methods. Each assay point was done in triplicate or duplicate, and the result is representative of a minimum of two experiments. Error bars indicate the SEM.

**Figure 3.** IKDCs cultured in the presence of OVA protein or peptide do not induce naive OVA-specific transgenic T cells to proliferate. Splenic pDCs (CD11c<sup>int</sup>CD45R<sup>−</sup>CD49b<sup>−</sup>), cDCs (CD11c<sup>hi</sup>CD45R<sup>−</sup>CD49b<sup>−</sup>), IKDCs (CD11c<sup>int</sup>CD45R<sup>−</sup>CD49b<sup>+</sup>), and NK cells (CD49b<sup>−</sup>NK1.1<sup>+</sup>CD3<sup>+</sup>) were isolated and cultured for 3 d with graded doses of class II–restricted OVA<sub>323-339</sub> peptide and 0.5 mg/ml whole OVA protein in the absence (A) or presence (B) of CpG and GM-CSF. APCs and CFSE-labeled OT-II cells were also incubated for 3 and 5 d with 0.5 mg/ml whole OVA protein in the absence (C) or presence (D) of CpG and GM-CSF. The proliferative response of OT-II cells was enumerated by flow cytometry, as described in the Materials and methods. Sample points were prepared in duplicates, and results are representative of several experiments.
Bcl-2 in their hematopoietic compartment (23). Cells from these mice are more resistant to factor deprivation–induced cell death, and 80% survival of IKDCs could be achieved after 48 h of culture (Fig. 4 A). Assessment of Bcl-2 transgenic IKDCs for up-regulation of MHC class II in response to CpG revealed no change in expression, although pDCs cultured under identical conditions clearly up-regulated MHC class II (Fig. 4 B). Similar experiments were performed using other stimulatory factors known to up-regulate MHC class II on DCs (LPS, IFN-γ, IL-4, and poly I:C), but all failed to enhance MHC class II levels on IKDC (not depicted).

Thus, the IKDCs we isolated did not up-regulate MHC class II and become APCs under conditions known to up-regulate MHC class II on DCs. However, it could not be excluded that under different conditions this might occur. Therefore, we attempted to activate IKDCs in three different experimental models. In the first, the IKDCs we isolated were incubated with target cells (CHO-OVA cells), which they efficiently kill (Fig. 2 B). We reasoned that IKDCs might first have to fulfill their cytolytic function, which might then provide the impetus for maturation into DCs. IKDCs were cocultured with graded numbers of CHO-OVA cells and CFSE-labeled OT-I or OT-II cells in the presence or absence of CpG. 3 d later, the number of transgenic cells that had proliferated were enumerated. IKDCs failed to induce naive OT-II cell proliferation (Fig. 5, A and B). We also tested cross-presentation of OVA in this assay, by incubation with OT-I T cells, and this demonstrated that IKDCs did not have this capacity (Fig. 5, C and D). However cDCs, which did not kill these target cells (Fig. 2 C), were able to initiate T cell responses, presumably by presenting antigen from the few CHO-OVA cells that died in culture (Fig. 5, C and D).

In the second model, sorted BALB/c IKDCs (purified using the same sorting gates as B6 IKDC; Fig. 1) were infected in vitro with influenza virus, and then cultured in the presence of CpG and CFSE–labeled influenza-specific transgenic CD4 (HNT) and CD8 (CL4) T cells for 3 d. Under these conditions, BALB/c IKDCs induced only minimal T cell proliferation compared with cDCs (Fig. 6, A and B). Thus, in vitro infection with virus did not enhance the ability of IKDCs to stimulate naive T cells to proliferate.

In the third model, we examined IKDCs in mice infected with malaria (*Plasmodium berghei*). We observed that such mice had increased numbers of IKDCs in the spleen (Fig. S3, available at http://www.jem.org/cgi/content/full/jem.20071351/DC1). Furthermore, infection with *P. berghei* results in systemic DC activation (24). We reasoned that the factors that governed the
We also followed the Chan et al. (9) procedure in using only anti-CD3 and -CD19 as depletion mAbs, but the IKDCs again failed to become effective APCs (Fig. S4 B). We also noted that some cells with the IKDC surface phenotype were denser than the 1.077 g/cm$^3$ used in this study and by Chan et al. (9) to enrich DCs, although all IKDCs appeared less dense than 1.082 g/cm$^3$. To ensure we recovered all spleen cells with the surface phenotype of IKDCs, we isolated IKDCs using a much denser cut, which recovered all spleen-viable cells (eliminating only erythrocytes and dead cells), as well as omitting anti-Thy1.1 mAb from the depletion mix. However, this “total” IKDC fraction likewise failed to acquire APC function on culture with CpG (Fig. S4 C). We concluded that the behavior of the IKDCs in our DC-enriched preparations reflected that of the total IKDC population.

Absence of IKDC in Rag2$^{-/-}$Il2rg$^{-/-}$ mice

The efficient lytic function of IKDC, but poor capacity to present antigen to naive T cells, suggested that IKDCs were a type of NK cell rather than a subtype of DCs. One important argument against this conclusion was the reported presence of IKDCs in recombinase-activating gene-2 null, common γ-chain null (Rag2$^{-/-}$Il2rg$^{-/-}$) mice, which are deficient in NK cells (8, 25). Therefore, we reexamined this issue. We found that a major complication in the analysis of Rag2$^{-/-}$Il2rg$^{-/-}$ mouse spleen is the high frequency of accumulation of IKDCs and systemic activation of DCs in the spleens of mice infected with malaria might also activate the antigen-presenting capacity of IKDCs. Thus, we isolated IKDCs from malaria-infected mice and assessed their ability to induce antigen-specific CD4$^+$ T cell proliferation by coculturing them with graded doses of MHC class II–restricted OVA peptide for 3 or 5 d. These IKDCs also failed to induce significant OT-II proliferation (Fig. 6, C and D). In comparison, and consistent with the results shown in Fig. 3, cDCs cultured with OVA peptide induced potent OT-II proliferation, whereas pDCs induced more moderate proliferation, regardless of whether they were isolated from malaria-infected mice or healthy control mice (Fig. 6, C and D).

T cell stimulatory capacity of total spleen IKDCs

It was possible our failure to activate IKDCs to become APCs was because we had isolated an IKDC population that was different from that of Chan et al. (9), despite the similarities in the isolation procedure. One difference was that, when depleting our DC preparations of lymphoid cells, we normally include several mAbs for maximum purity, including anti-CD90 (Thy1.1; T24/31.7), which Chan et al. (9) did not. Indeed, we found that ~50% of IKDCs expressed CD90, and that some of these were likely removed from our IKDC sample. However, when CD90 depletion was omitted, the resulting IKDCs in our DC preparations still failed to become effective APCs on activation with CpG (Fig. S2 B and Fig. S4 A, available at http://www.jem.org/cgi/content/full/jem.20071351/DC1). We also followed the Chan et al. (9) procedure in using only anti-CD3 and -CD19 as depletion mAbs, but the IKDCs again failed to become effective APCs (Fig. S4 B). We also noted that some cells with the IKDC surface phenotype were denser than the 1.077 g/cm$^3$ used in this study and by Chan et al. (9) to enrich DCs, although all IKDCs appeared less dense than 1.082 g/cm$^3$. To ensure we recovered all spleen cells with the surface phenotype of IKDCs, we isolated IKDCs using a much denser cut, which recovered all spleen-viable cells (eliminating only erythrocytes and dead cells), as well as omitting anti-Thy1.1 mAb from the depletion mix. However, this “total” IKDC fraction likewise failed to acquire APC function on culture with CpG (Fig. S4 C). We concluded that the behavior of the IKDCs in our DC-enriched preparations reflected that of the total IKDC population.

Figure 6. IKDCs exposed to two different pathogens do not acquire the ability to stimulate naive T cells into proliferation. Splenic pDCs (CD11c$^+$CD45R$^+$CD49b$^-$), cDCs (CD11c$^+$CD45R$^-$CD49b$^-$), IKDCs (CD11c$^-$CD45R$^-$CD49b$^-$), and NK cells (CD49b$^+$NK1.1$^+$CD3$^+$) were isolated and purified by flow cytometry. (A and B) These cells were infected in vitro with influenza (PR8 influenza virus), washed, and used at the indicated numbers to stimulate flow-cytometric transgenic CD4 (HNT; A) and CD8 (CL4) T cells (B). Proliferating T cells were identified and enumerated by flow cytometry. (C and D) Mice were infected with malaria (P. berghei) and killed 3 d later. Splenks from healthy or malaria-infected mice were used to isolate and purify pDCs (CD11c$^+$CD45R$^+$CD49b$^-$), cDCs (CD11c$^+$CD45R$^-$CD49b$^-$), IKDCs (CD11c$^-$CD45R$^-$CD49b$^-$), and NK cells (CD49b$^+$NK1.1$^+$CD3$^+$), and 10$^4$ APCs were cocultured for 3 (C) and 5 d (D) with graded doses of class II–restricted OVA peptide 323-339 peptide and 5 $\times$ 10$^4$ CFSE-labeled OT-II. Proliferating OT-II were enumerated as indicated in the Materials and methods. The presented data is representative of three independent experiments.
autofluorescent cells, which can generate false positives in the analysis of rare DC populations (10). Thus, particular care was taken to exclude autofluorescent cells and dead cells, and to avoid and gate out doublets and aggregates (Fig. 7 A). Using this approach, Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mice were shown to have pDCs and cDCs (Fig. 7 B), albeit at reduced total numbers compared with wild-type B6 mice (Fig. 7 E). This reduction likely reflects the absence of B cells (26) and the smaller spleen size. Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mouse spleen appeared to contain some CD49b\(^{+}\) cells (Fig. 7 C), although this population differed from the CD49b\(^{+}\) cells seen in normal B6 mice (Fig. 7 C). As expected (25), there were virtually no CD49b\(^{+}\)NK1.1\(^{+}\) NK cells in Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mice (Fig. 7 D). To enumerate IKDC, we used two separate definitions. First, we gated for CD49b\(^{+}\)CD11c\(^{int}\)CD45R\(^{+}\) cells and found very few cells that had this phenotype (Fig. 7 C). Because even these may have been residual autofluorescent cells or other noise, we then used the more stringent definition that specifies IKDC to be NK1.1\(^{+}\), as well as CD49b\(^{+}\)CD11c\(^{int}\)CD45R\(^{+}\) (Fig. 7 D), and found that these cells were at extremely low levels in Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mice (Fig. 7 E). Furthermore, the NK cell–sensitive targets RMA-S and RMA-S Rae-B, which are killed in a perforin- and NKG2D-dependent manner (27), were growth suppressed (RMA-S) and rejected (RMA-S Rae-B) in Rag\(^{-/-}\) mice, but not eliminated in Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mice, or in Rag\(^{-/-}\) mice depleted of NK1.1\(^{+}\) cells (Fig. S5, available at http://www.jem.org/cgi/content/full/jem.20071351/DC1). This argues that the residual CD49b\(^{+}\) cells present in Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mice are neither NK cells nor IKDCs, which have been shown to kill targets autologous and not autologous. Thus, particular care was taken to exclude autofluorescent cells and dead cells, and to avoid and gate out doublets and aggregates (Fig. 7 A). Using this approach, Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mice were shown to have pDCs and cDCs (Fig. 7 B), albeit at reduced total numbers compared with wild-type B6 mice (Fig. 7 E). This reduction likely reflects the absence of B cells (26) and the smaller spleen size. Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mouse spleen appeared to contain some CD49b\(^{+}\) cells (Fig. 7 C), although this population differed from the CD49b\(^{+}\) cells seen in normal B6 mice (Fig. 7 C). As expected (25), there were virtually no CD49b\(^{+}\)NK1.1\(^{+}\) NK cells in Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mice (Fig. 7 D). To enumerate IKDC, we used two separate definitions. First, we gated for CD49b\(^{+}\)CD11c\(^{int}\)CD45R\(^{+}\) cells and found very few cells that had this phenotype (Fig. 7 C). Because even these may have been residual autofluorescent cells or other noise, we then used the more stringent definition that specifies IKDC to be NK1.1\(^{+}\), as well as CD49b\(^{+}\)CD11c\(^{int}\)CD45R\(^{+}\) (Fig. 7 D), and found that these cells were at extremely low levels in Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mice (Fig. 7 E). Furthermore, the NK cell–sensitive targets RMA-S and RMA-S Rae-B, which are killed in a perforin- and NKG2D-dependent manner (27), were growth suppressed (RMA-S) and rejected (RMA-S Rae-B) in Rag\(^{-/-}\) mice, but not eliminated in Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mice, or in Rag\(^{-/-}\) mice depleted of NK1.1\(^{+}\) cells (Fig. S5, available at http://www.jem.org/cgi/content/full/jem.20071351/DC1). This argues that the residual CD49b\(^{+}\) cells present in Rag2\(^{-/-}\)Il2rg\(^{-/-}\) mice are neither NK cells nor IKDCs, which have been shown to kill targets autologous and not autologous.
via NKG2D (8, 9). Our overall conclusion was that IKDCs, like NK cells, are at a very low level or absent from Rag2−/− Il2rg−/− mice.

**Lack of PU.1 expression by IKDCs**

To further determine whether the gene expression program of IKDCs is more closely related to DCs or to NK cells, we examined the expression of PU.1, which is a transcription factor involved in hematopoiesis. PU.1 is not expressed in mature NK cells, but is expressed at moderate and high levels in pDCs and cDCs, respectively (28, 29). Using the PU.1IRES reporter mice that express the GFP protein under the control of the endogenous PU.1 promoter, we assessed the levels of PU.1 expression in the IKDCs isolated from our DC preparations and compared these to NK cells and DCs. Like mature NK cells, these IKDCs failed to express PU.1, although, as expected, expression was detected in DCs (Fig. 8).

**Response of IKDCs to NK cell growth factors**

In our experiments, IKDCs failed to respond to DC growth or maturation factors such as CpG and GM-CSF. However, both cDCs and pDCs can efficiently be generated by culturing bone marrow with Flt-3 ligand (30). Thus, we analyzed Flt-3 ligand BM cultures for the presence of IKDCs (Fig. S6, available at http://www.jem.org/cgi/content/full/jem.20071351/DC1). Although both cDCs and pDCs were efficiently generated, IKDCs were not produced, further delineating the growth requirements of these cells from that of DCs.

Because IKDCs resembled NK cells by function and many surface markers, we tested their response to NK cell growth factors. When our IKDCs were cultured in the presence of IL-15 and −18, they proliferated extensively, like NK cells, whereas DCs did not (Fig. 9 A). Interestingly, a proportion of these expanded IKDCs then expressed surface MHC class II (Fig. 9 B). Importantly, a small proportion of expanded NK cells also expressed MHC class II (Fig. 9 B); this proportion was up to 25% under some culture conditions (Fig. S7, available at http://www.jem.org/cgi/content/full/jem.20071351/DC1).

**Discussion**

In our DC-enriched preparations, we found cells with the reported surface phenotype of IKDCs, namely CD11c+CD45R− CD49b+. These cells also expressed the NK cell markers NK1.1 and NKG2D. We found that these IKDCs had a potent ability to kill NK cell targets, even after preactivation. However, in our experiments, the IKDCs had little or no antigen presentation capacity, even after activation.

Despite the use of a range of DC activation stimuli (CpG, LPS, poly I:C, IL-4, and IFN-γ), and the use of bcl-2 transgenic IKDCs to overcome cell death in culture, in our hands, the IKDCs failed to up-regulate MHC class II under normal conditions of DC activation and failed to activate naive antigen-specific T cells, even after incubation with synthetic peptide antigen, which is in contrast to an earlier work (9). Although much higher levels of peptide were used in the earlier study, we titrated and tested levels of peptide beyond that which saturated DC responses, demonstrating that IKDCs were 100–1,000-fold less efficient than that induced by cDCs (Fig. 9 E), or by DCs labeled with equivalent levels of anti–MHC class II mAb. In addition, no response was obtained to whole OVA protein (Fig. 9 F). Consistent with the observation that MHC class II+ IKDCs and NK cells were poor activators of naïve T cells, these cultured cells did not express significant levels of the costimulatory molecules CD80 and CD86 (Fig. 9 D).
the IKDC surface phenotype, they showed the same lack of T cell stimulation capacity as the IKDC in our DC preparations. Another possible difference is the additional use of a positive bead selection of CD11c+ cells by Chan et al. (9). This may have excluded some types of IKDCs, but the selected IKDCs should have been included in our total spleen IKDC preparation, which included all spleen CD11c+ cells. It may be that the beads introduced some additional signals or generated cell doublets between IKDCs and pDCs. However, overlap of their IKDC fraction with true APCs is a more likely explanation for the IKDC surface phenotype, they showed the same lack of T cell stimulation capacity as the IKDC in our DC preparations. Another possible difference is the additional use of a positive bead selection of CD11c+ cells by Chan et al. (9). This may have excluded some types of IKDCs, but the selected IKDCs should have been included in our total spleen IKDC preparation, which included all spleen CD11c+ cells. It may be that the beads introduced some additional signals or generated cell doublets between IKDCs and pDCs. However, overlap of their IKDC fraction with true APCs is a more likely explanation for
the differences. One candidate contaminant is CD49b+DC. These cells express high levels of MHC class II, CD80, and CD86, and thus would be predicted to be potent APCs. It is notable that, in the B6 mice we mainly used, these CD49b+DCs were at a low level in the DC preparations, but their frequency varied in BALB/c DC preparations, on occasions reaching a 1:1 ratio with IKDC. Accordingly, they would be harder to exclude from the IKDC fraction. In addition, BALB/c mice lack the NK1.1 marker useful for distinguishing these two subpopulations. The developmental relationship between CD49b+DC and IKDC is not clear, but we obtained no transformation of IKDC to CD49b+DC on incubation with maturation factors such as CpG. However, it is possible that in culture, selective death of IKDCs could masquerade as transformation of one into the other, so giving the impression that IKDCs became effective APCs. A further possible contaminant which might lead to induced APC function is some residual pDC. We note that the IKDC fraction of Chan et al. (9) produced IFN-α on stimulation with CpG. Our recent study (10) suggests that pDCs, but not IKDCs, produce IFN-α under these conditions.

An alignment of IKDC with NK cells, rather than with DCs, was reinforced by our analysis of the expression of the hematopoietic transcription factor PU1. This factor is expressed at high levels in cDCs and at moderate levels in pDCs, but is not expressed on mature NK cells (28). Our IKDCs did not express PU.1, aligning their transcription factor expression pattern more closely with NK cells. Importantly, IKDC, like NK cells, are also dependent on the Id-2 transcription inhibitor, but unlike DCs, they do not express the transcription factor Spi-B (31). Therefore, both the cytokine signaling requirements and transcription factor expression profiles argue against a close association of IKDCs and DCs.

A compelling argument delineating IKDCs from NK cells had been the observation that IKDCs, like DCs, appeared to be present in Rag2−/−Il2rg−/− mice (8), whereas NK cells are absent (25). However, our analysis now suggests that IKDC cells were markedly reduced, if present at all, in such mice. One possible explanation for the discrepancy is the disproportionate number of autofluorescent cells in DC preparations from these mice, which may give the false-positive signals in the IKDC gates. Multiple markers are required to clearly delineate an IKDC population.

Responses to growth factors can clarify the lineage relationships between different cell types. In our hands IKDCs did not respond to the DC growth factor GM-CSF. Administration of Flt-3 ligand in vivo enhances DC numbers, and did not respond to the DC growth factor GM-CSF. Administration of Flt-3 ligand in vivo enhances DC numbers, and did not respond to the DC growth factor GM-CSF. However, it is possible that in culture, selective death of IKDCs could masquerade as transformation of one into the other, so giving the impression that IKDCs became effective APCs. A further possible contaminant which might lead to induced APC function is some residual pDC. We note that the IKDC fraction of Chan et al. (9) produced IFN-α on stimulation with CpG. Our recent study (10) suggests that pDCs, but not IKDCs, produce IFN-α under these conditions.

An alignment of IKDC with NK cells, rather than with DCs, was reinforced by our analysis of the expression of the hematopoietic transcription factor PU1. This factor is expressed at high levels in cDCs and at moderate levels in pDCs, but is not expressed on mature NK cells (28). Our IKDCs did not express PU.1, aligning their transcription factor expression pattern more closely with NK cells. Importantly, IKDC, like NK cells, are also dependent on the Id-2 transcription inhibitor, but unlike DCs, they do not express the transcription factor Spi-B (31). Therefore, both the cytokine signaling requirements and transcription factor expression profiles argue against a close association of IKDCs and DCs.

A compelling argument delineating IKDCs from NK cells had been the observation that IKDCs, like DCs, appeared to be present in Rag2−/−Il2rg−/− mice (8), whereas NK cells are absent (25). However, our analysis now suggests that IKDC cells were markedly reduced, if present at all, in such mice. One possible explanation for the discrepancy is the disproportionate number of autofluorescent cells in DC preparations from these mice, which may give the false-positive signals in the IKDC gates. Multiple markers are required to clearly delineate an IKDC population.

Responses to growth factors can clarify the lineage relationships between different cell types. In our hands IKDCs did not respond to the DC growth factor GM-CSF. Administration of Flt-3 ligand in vivo enhances DC numbers, and has been reported to increase a population of “NKDCs,” a subset of which expresses CD45R, and so might be classed as an IKDC. However, our kinetic study of cultures of bone marrow with Flt-3 ligand, which efficiently generates both cDCs and pDCs, indicated that IKDCs were not produced. Secondary events of Flt-3 ligand administration may cause the elevation of IKDC numbers in vivo. Our GM-CSF and Flt-3 ligand culture results suggest IKDC are not responsive to DC growth factors. In contrast, IKDCs, like NK cells, but unlike DCs, proliferated vigorously in culture to IL-15 and -18.

Culture of IKDCs in IL-15 and -18 not only induced cell proliferation, but produced some cells expressing high levels of MHC class II. Importantly, a proportion of cultured NK cells showed a similarly high surface expression of MHC class II. To our knowledge, this is the first study of growth factors directly inducing MHC class II expression in mouse NK cells and provides some alignment with human NK cells, which express MHC class II upon activation (19–21). In addition, mouse NK cells and NKDCs can be induced to express MHC class II in vivo (32). These findings are in contrast to our inability to induce MHC class II by activation of IKDC with CpG and other DC stimuli in vitro. However, despite the induction of MHC class II, our culture-expanded IKDCs did not significantly up-regulate the costimulator molecules CD80 and CD86, and they still failed to induce naive T cell proliferation. They could not be classed as effective APCs.

In conclusion, in our studies, IKDCs resemble NK cells in their lytic function, and do not acquire the antigen-presenting functions of DCs even after activation. As we previously reported (10), they produce IFNγ like NK cells, but do not produce IFNα like pDCs. Their transcription factor profile and response to cytokines resembles that of NK cells, not that of DCs. Their developmental relationship to NK cells is not entirely clear. They have the appearance of an activated form of NK cell, but one study (31) points to differences in early stages of development. In either case, IKDCs should be considered as a subtype of NK cells, rather than a subtype of DCs.

MATERIALS AND METHODS

Mice. C57BL/6j or B6 (B6), BALB/c, Rag2−/− Il2rg−/− (backcrossed onto B6), and various transgenic mice (vav-bcl-2, PU.1gfp, OT-I, OT-I bm-1, OT-II, DO11.10, CL4, and HNT) were bred under specific pathogen-free conditions at the Walter and Eliza Hall Institute. Female mice were used at 6–12 wk of age and handled according to the guidelines of the National Health and Medical Research Council of Australia. Experimental procedures were approved by the Animal Ethics Committee, Melbourne Health Research Directorate.

Cell lines and media. YAC-1 and CHO-K1 cell lines were maintained in DMEM or RPMI-1640 containing 10% FCS, and then harvested during exponential growth phase for use in assays. Freshly isolated cells were cultured in DC culture medium (modified RPMI-1640 medium containing 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10−3 M 2-mercaptoethanol). Where indicated, 50 ng/ml IL-18 (R&D Systems) and 50 ng/ml IL-15 (R&D Systems) were added to DC culture medium.

Antibodies. The following fluorescent-conjugated mAbs were used: anti-CD11c (N418)-APC, -Cy5, or -FITC; anti-CD45RA (14.8) or anti-CD45R (CD45R; RA-6B2)-PE or -APC; anti-CD49b (DX5)-biotin or -FITC; anti-NK1.1 (PK136)-biotin or APC; anti-NK2GD (CX5-biotin); anti-MHC class II (M5/114)-FITC, PE, or Alexa Fluor 594. For biotin conjugates, streptavidin (SA)-PE, SA-Alexa Fluor 594, or SA-Cy7-Pe was used in the second-stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen). To assure comparative MHC class II staining, our M5/114 conjugates contained sufficient unconjugated mAb to saturate the stage reaction (BD PharMingen).
blocking with rat Ig anti-Fc receptor mAb (2.4G2) was used to reduce nonspecific staining.

Isolation of DC, IKDC, and NK cells. The isolation of DC subpopulations has been previously described (10, 33, 34). In brief, tissues were chopped, digested with collagenase and DNase at room temperature, and treated with EDTA. Low-density cells were enriched by density centrifugation (1.077 g/cm³ Nycodenz, mouse osmolarity). In some experiments, which were designed to select IKDCs from all viable nucleated spleen cells, spleen cells were centrifuged instead in 1.091 g/cm³ Nycodenz medium, eliminating only the pellet of dead cells and erythrocytes. Non-DC lineage cells were coated with mAbs (KT3-1.1, anti-CD3; T24/31.7, anti-Thy-1; TEER199, anti-erythrocytes; ID3, anti-CD19; and IA8, anti-Ly6G or RB6-8C5, anti-Ly6C/G), and then removed using anti-rat Ig magnetic beads (BioMag beads; QIAGEN). Coating with RB6-8C5 mAb (anti-Gr-1) did not result in the depletion of pDCs (34). In some experiments, anti-Thy-1 mAb was omitted from the depletion mix, and in others only anti-CD3 and -CD19 were used, without changing the IKDC functional results. Dead cells were stained with propidium iodide (PI) and gated out. Freshly isolated splenic DCs were fluorescent cell sorted based on the expression of CD11c, CD45RA (14.8), or CD45R (B220) and CD49b, into CD11c<sup>hi</sup> CD45RA<sup>*</sup> (pDC), CD11c<sup>int</sup>CD45RA<sup>*</sup> (pDC), and CD11c<sup>int</sup>CD45RA<sup>*</sup>CD49b<sup>*</sup> (IKDC) subsets. Splenic NK cells were isolated by chopping and enzymatic digestion, as mentioned earlier in this section, and removing red and dead cells by density centrifugation (1.091 g/cm³ Nycodenz, mouse osmolarity). Other irrelevant cells were then coated with mAbs against MHC class II (M5/114, CD24 (M1/69), CD45 (Gr1.1K), and CD8 (53-6.7) and removed using anti-rat Ig magnetic beads. Remaining cells were stained with mAb against CD3ε-PE (BD PharmMingen), CD49b-FITC, and NK1.1-APC, and NK cells (CD49b<sup>*</sup>NK1.1<sup>-</sup>CD3<sup>-</sup>) were isolated to >99% purity by sorting. Depending on the fluorochromes used, sorting was performed on a DiVa instrument (Becton Dickinson), FACStar Plus (Becton Dickinson), or FACSAna (Becton Dickinson), but most commonly on a MoFlo instrument (DakoCytonation); the data generated with the sorted populations was consistent between instruments. Analysis was performed on LSR1 (Becton Dickinson), FACScan (Becton Dickinson), or FACStar Plus instruments.

Purification of transgenic T cells. CL4 and HNT, OT-I, and OT-II transgenic T cells were isolated from lymph node cell suspensions by staining irrelevant cells with mAbs (anti-erythrocytes, TER-119; anti-Gr1, RB6-6C5; anti-Mac-1, M1/70; anti-class II MHC, M5/114; anti-F4/80, and anti-CD8, 53-6.7; or anti-CD4, Gr1.1K), and then removing these using anti-rat Ig-coupled magnetic beads at a 1:10 cell/bead ratio; purity was verified to be ~95%.

CFSE-labeled T cell proliferation assays. Purified T cells were washed once in 0.1% BSA PBS and resuspended at 10<sup>5</sup> cells/ml. 5 mM CFSE was added (1 μl/10<sup>5</sup> cells), and incubated at 37°C for 10 min. RPMI-1640 medium containing 2.5% FCS was added, and cells were washed twice. T cells (5 × 10<sup>5</sup> cells/well) were incubated with APCs (10<sup>4</sup> cells, or as otherwise stated) in U-bottomed 96-well plates in 200 μl DC culture medium. Where indicated, 10 ng/ml GM-CSF and 0.5 μM CpG 1668 were added. Graded doses (2–2,000 ng/ml) of MHC class II–specific OVA peptide (323–339) or whole OVA (0.5 mg/ml) were added to the cells. To enumerate T cells after culture, 2.5 × 10<sup>4</sup> calibration beads (BD Bioscience) were added per well, and T cells were visualized by staining with appropriate markers (OT-1 or OT-II cells were stained with anti-TCR-VαVε2 mAb [B20.1-PE; BD PharmMingen] and HNT or CL4 transgenic T cells were stained with anti-CD4 [GR1.5-APC] or anti-CD8 [53-6.7-APC], respectively). Dead cells were excluded using propidium iodide. Analysis was performed on a FACScan or FACS caliber (Becton Dickinson). Proliferating T cells were identified by loss of CFSE fluorescence and enumerated relative to the beads, thus allowing a count of total proliferating T cells per well.

IKDC and DC activation/maturation. Isolated DCs and IKDCs (up to 10<sup>6</sup> cells/ml) were cultured in U-bottomed 96-well plates for 18–44 h in DC culture medium with 10 ng/ml GM-CSF and 0.5 μM CpG-1668 (Geneworks). The level of MHC class II expression by the various DC populations was assessed before and 1–2 d after activation.

<sup>3</sup>H-Chromium release lysis assay. Purified effector cells were washed in DC culture medium to remove residual EDTA, and then plated in serial dilution in U-bottomed 96-well plates in medium with without GM-CSF and CpG. Target cells (YAC-1 or CHO-K1-OVA) were harvested in exponential growth phase, washed, and resuspended in DC culture medium at 10<sup>7</sup>/ml. 1 million target cells were incubated at 37°C for 1.5 h with 100 μl of sodium chromate (106Cr, 100 μCi). Labeled targets were washed 4 times and plated at 10<sup>4</sup> cells/well onto prediluted effector cells. Cells were centrifuged for 1 min at 350 g to facilitate cell–cell contact, and incubated for 4 h at 37°C. The plates were centrifuged (for 5 min at 400 g), and 100 μl of supernatant was removed and counted for 1 min for γ emission. Spontaneous (S) chromium release was determined by incubating target cells in the absence of effectors, and maximum (M) cell lysis was obtained by exposing target cells to 10% Triton X-100. Specific lysis was calculated according to the following formula: percentage of lysis = 100 × (E – S)/(M – S), where E is the experimental chromium release. All effector/target cell ratios were conducted in duplicate or triplicate. The spontaneous chromium release was routinely <10%.

Influenza infection of APCs. For in vitro infections, purified IKDCs, CD4<sup>+</sup>DCs, pDCs, cDCs, and NK cells were incubated with 5 PFU/cell of PR8 influenza virus for 60 min at 37°C. Cells were washed three times, and then resuspended in culture media at 1.5 × 10<sup>6</sup> cells/ml. Graded doses of APC were incubated with 50,000 purified CFSE-labeled HNT or CL4 T cells, and after 60 h of coculture, proliferating T cells were enumerated.

Malaria infection of mice. Mice were injected intraperitonally with 10<sup>5</sup> P. berghei ANKA-infected erythrocytes. Parasitemia was assessed from Giemsa-stained smears of tail blood prepared after 2–3 d. 3 d after infection, spleens were extracted, and DCs, IKDCs, and NK cells were isolated and purified by flow cytometry.

Generation of OVA-FLAG expressing CHO cells. Flag-tagged, membrane-bound OVA (OVA-FLAG) protein was expressed on the surface of CHO cells as a C-terminal (extracellular) FLAG-tagged protein. To generate the FLAG–tagged proteins, OVA cDNA was amplified using Advantage high fidelity 2 polymerase (CLONTECH Laboratories, Inc.) and the primers 5′-TAGTAGATGCGCGCCATGATCAAGCTAGATCAG-3′ and 5′-TAGTAGACCGGCTAGGAAGTATCATCAGCATTC-3′, and 5′-TAGTAGACCGGCTAGGAAGTATCATCAGCATTC-3′, using the previously described membrane-bound OVA (first 118 amino acids of transferrin receptor linked to amino acids 138–385 of OVA) (35) as a template. The amplified cDNA was restriction digested with Ascl and Mlu-I and cloned into the Ascl site of a pEF-BoS vector modified to contain the FLAG epitope (donated by T. Willson, Walter and Eliza Hall Institute, Melbourne, Australia). CHO cells were cotransfected with the pEF-BoS-OVA-FLAG and a pGK-neo plasmid containing the neomycin phosphotransferase gene by electroporation (Gene Pulser; Bio-Rad Laboratories) and transfectants selected with 1 mg/ml G418 (Geneticin; Life Technologies). OVA-FLAG–positive cells were stained with biotinylated anti-FLAG mAb 9H10 (36), followed by SA-PE, and then isolated by flow cytometric sorting.

Online supplemental material. Fig. S1 shows that BALB/c IKDCs do not activate naïve DC4 T cells. Fig. S2 shows that IKDCs preactivated with CpG did not acquire APC function. Fig. S3 shows that IKDCs accumulate in the spleens of mice infected with malaria. Fig. S4 shows that total spleen IKDCs do not activate naïve T cells. Fig. S5 shows that parental and Rae-1β–expressing RMA-S tumor cells are rejected by NK cells in Rag2<sup>−/−</sup> mice, but not by cells in Rag2<sup>−/−</sup>Iz2g<sup>−/−</sup> mice. Fig. S6 shows that Flt3 ligand bone marrow cultures produce pDCs and cDCs, but not IKDCs. Fig. S7
shows that a proportion of purified NK cells cultured in IL-15 and -18 up-regulate MHC class II expression. There is also a Supplemental materials and methods section. The online version of this article is available at http://www.jem.org/cgi/content/full/jem.20071351/DC1.

M.J. Smyth thanks Ms. Shannon Griffinths for technical support. We would like to thank Dr. Li Wu for her helpful discussions.

This work was supported by the National Health and Medical Research Council, Australia. I. Caminschi was supported by the Melbourne University Career Interruption Fellowship. S. Bedoui was supported by the Deutsche Forschungsgemeinschaft BE 3285/1-1 and 1-2. K. Heger was supported by the Dr. Interruption Fellowship. S. Bedoui was supported by the Deutsche Australia. I. Caminschi was supported by the Melbourne University Career Submitted: 2 July 2007

1. Steinman, R.M. 1991. The dendritic cell system and its role in immunity. Annu. Rev. Immunol. 9:271–296.

2. Reis e Sousa, C. 2006. Dendritic cells in a mature age. Nat. Rev. Immunol. 6:476–483.

3. Villadangos, J.A., and W.R. Heath. 2008. Life cycle, migration and antigen presenting functions of spleen and lymph node dendritic cells: limitations of the Langerhans cell paradigm. Semin. Immunol. 17:262–272.

4. Shortman, K., and S.H. Naik. 2007. Steady-state and inflammatory dendritic-cell development. Nat. Rev. Immunol. 7:19–30.

5. O’Keeffe, M., H. Hochrein, D. Vremec, B. Scott, P. Hertzog, L. Tatarczuch, and K. Shortman. 2003. Dendritic cell precursor population of mouse blood: identification of the murine homologues of human blood plasmacytoid pre-DC2 and CD11c+ DC1 precursors. Blood. 101:1453–1459.

6. Probst, H.C., and M. van den Broek. 2005. Priming of CTLs by lymphocytic choriomeningitis virus depends on dendritic cells. J. Immunol. 174:3920–3924.

7. Jung, S., D. Unutmaz, P. Wong, G. Sano, K. De los Santos, T. Sparwasser, S. Wu, S. Vuthoori, K. Ko, F. Zavala, et al. 2002. In vivo depletion of CD11c(+) dendritic cell argorates priming of CD8(+) T cells by exogenous cell-associated antigens. Immunity. 17:211–220.

8. Taieb, J., N. Chaput, C. Menard, L. Apetoh, E. Ullrich, M. Bonmort, T. Yokota, M. Ceppon, and J.P. Di Santo. 1999. Dissecting NK cell development using a novel alymphoid mouse model: investigating the role of the c-abl proto-oncogene in murine NK cell differentiation. J. Immunol. 162:2761–2765.

9. Wilson, N.S., G.M. Behrens, R.J. Lundie, C.M. Smith, J. Waithman, L. Young, S.P. Forehan, A. Mount, R.J. Stptoe, K.D. Shortman, et al. 2006. Systemic activation of dendritic cells by Toll-like receptor ligands or malaria infection impairs cross-presentation and antiviral immunity. Nat. Immunol. 7:165–172.

10. Colucci, F., C. Soudais, E. Rosmaraki, L. Vanes, L.V. Tybulewicz, and J.P. Di Santo. 1999. Dissecting NK cell development using a novel alymphoid mouse model: investigating the role of the c-abl proto-oncogene in murine NK cell differentiation. J. Immunol. 162:2761–2765.

11. Kabashima, K., T.A. Banks, K.M. Ansel, T.T. Lu, C.F. Ware, and J.G. Cyster. 2005. Intrinsinc lymphoxygen-beta receptor requirement for homeostasis of lymphoid tissue dendritic cells. Immunity. 22:430–450.

12. Hayakawa, Y., J.M. Kelly, J.A. Westwood, P.K. Darcy, A. Diefenbach, D. Rautela, and M.J. Smyth. 2002. Cutting edge: tumor rejection mediated by NKG2D receptor-ligand interaction is dependent upon perforin. J. Immunol. 169:5377–5381.

13. Nutt, S.L., D. Metcalf, A. D’Amico, M. Polli, and L. Wu. 2005. Dynamic regulation of PU.1 expression in multipotent hematopoietic progenitors. J. Exp. Med. 201:221–231.

14. Dakac, A., L. Wu, and S.L. Nutt. 2007. Is PU.1 a dosage-sensitive regulator of haematopoietic lineage commitment and leukemogenesis? Trends Immunol. 28:108–114.

15. Trintie, B., C. Vosiene, H. Yagita, and R. Josien. 2000. A subset of cytolytic dendritic cells in rat. J. Immunol. 165:4202–4208.

16. Schmitz, M., S. Zhao, Y. Deuse, K. Schakel, R. Wehner, H. Wolner, K. Holig, F. Wienforth, A. Kissing, M. Bornhauser, et al. 2005. Tumoricidal potential of natural blood dendritic cells: direct tumor cell killing and activation of NK cell-mediated cytotoxicity. J. Immunol. 174:4127–4134.

17. Fanger, N.A., C.R. Maliszewski, K. Schooley, and T.S. Griffith. 1999. Human dendritic cells mediate cellular apoptosis via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). J. Exp. Med. 190:1155–1164.

18. Stary, G., C. Bangert, M. Tauber, R. Strohal, T. Kopf, and G. Stingl. 2007. Tumoricidal activity of TLR7/8-activated inflammatory dendritic cells. J. Exp. Med. 204:1441–1451.

19. Hanna, J., T. Gonen-Gross, J. Fitchett, T. Rowe, M. Daniels, T.I. Arnon, R. Gazzit, A. Joseph, K.W. Schjetne, A. Steinle, et al. 2004. Novel APC-like properties of human NK cells directly regulate T cell activation. J. Clin. Invest. 114:1612–1623.

20. Zingoni, A., T. Sornase, B.G. Cockx, Y. Tanaka, A. Santoni, and L.L. Lanier. 2004. Cross-talk between activated human NK cells and CD4+ T cells via OX40-OX40 ligand interactions. J. Immunol. 173:3716–3724.

21. Roncarolo, M.G., M. Bigler, J.B. Haazen, H. Ysel, R. Bacchetta, J.E. de Vries, and H. Spits. 1991. Natural killer cell clones can efficiently process and present protein antigens. J. Immunol. 147:781–787.

22. Spits, H., and L.L. Lanier. 2007. Natural killer or dendritic: what’s in a name? Immunity. 26:11–16.

23. Ogóry, S., D. Metcalfe, C.G. Print, M.L. Bath, A.W. Harris, and J.M. Adams. 1999. Constitutive Bcl-2 expression throughout the hematopoietic compartment affects multiple lineages and enhances progenitor cell survival. Proc. Natl. Acad. Sci. USA. 96:14943–14948.

24. Wilson, N.S., G.M. Behrens, R.J. Lundie, C.M. Smith, J. Waithman, L. Young, S.P. Forehan, A. Mount, R.J. Stptoe, K.D. Shortman, et al. 2006. Systemic activation of dendritic cells by Toll-like receptor ligands or malaria infection impairs cross-presentation and antiviral immunity. Nat. Immunol. 7:165–172.

25. Nutt, S.L., D. Metcalf, A. D’Amico, M. Polli, and L. Wu. 2005. Dynamic regulation of PU.1 expression in multipotent hematopoietic progenitors. J. Exp. Med. 201:221–231.

26. Dakec, A., L. Wu, and S.L. Nutt. 2007. Is PU.1 a dosage-sensitive regulator of haematopoietic lineage commitment and leukemogenesis? Trends Immunol. 28:108–114.

27. Naik, S.H., A.I. Proietto, N.S. Wilson, A. Dakac, P. Schrorrner, M. Fuchsberger, M.H. Lahoud, M. O’Keeffe, Q.X. Shao, W.F. Chen, et al. 2005. Cutting edge: generation of splenic CD8+ and CD8+ dendritic cell equivalents in Fms-like tyrosine kinase 3 ligand bone marrow cultures. J. Immunol. 174:6592–6597.

28. Wehner, R.S., R. Pelayo, K.P. Garrett, X. Chen, S.S. Perry, H.X. Sun, B.L. Kee, and P.W. Kincade. 2007. Interferon-producing killer dendritic cells (IKDCs) arise via a unique differentiation pathway from primitive c-kit+CD122+ lymphoid progenitors. Blood. 109:4825–4831.

29. Chaudhry, U.T., P.T. Kingham, G. Platas, S.C. Katz, J.R. Raab, and R.P. DeMatteo. 2006. Combined stimulation with interleukin-18 and CpG induces murine natural killer dendritic cells to produce IFN-gamma and inhibit tumor growth. Cancer Res. 66:10497–10504.
33. Vremec, D., J. Pooley, H. Hochrein, L. Wu, and K. Shortman. 2000. CD4 and CD8 expression by dendritic cell subtypes in mouse thymus and spleen. J. Immunol. 164:2978–2986.

34. O’Keeffe, M., H. Hochrein, D. Vremec, I. Caminschi, J.L. Miller, E.M. Anders, L. Wu, M.H. Lahoud, S. Henri, B. Scott, et al. 2002. Mouse plasmacytoid cells: long-lived cells, heterogeneous in surface phenotype and function, that differentiate into CD8<sup>+</sup> dendritic cells only after microbial stimulus. J. Exp. Med. 196:1307–1319.

35. Boyle, J.S., C. Koniaras, and A.M. Lew. 1997. Influence of cellular location of expressed antigen on the efficacy of DNA vaccination: cytotoxic T lymphocyte and antibody responses are suboptimal when antigen is cytoplasmic after intramuscular DNA immunization. Int. Immunol. 9:1897–1906.

36. Caminschi, I., A.J. Corbett, C. Zahra, M. Lahoud, K.M. Lucas, M. Sofi, D. Vremec, T. Gramberg, S. Pohlmann, J. Curtis, et al. 2006. Functional comparison of mouse CIRE/mouse DC-SIGN and human DC-SIGN. Int. Immunol. 18:741–753.
Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Caminschi, I; Ahmet, F; Heger, K; Brady, J; Nutt, SL; Vremec, D; Pietersz, S; Lahoud, MH; Eld, LS; Hansen, DS; O'Keeffe, M; Smyth, MJ; Bedoui, S; Davey, GM; Villadangos, JA; Heath, WR; Shortman, K

Title:
Putative IKDCs are functionally and developmentally similar to natural killer cells, but not to dendritic cells

Date:
2007-10-29

Citation:
Caminschi, I; Ahmet, F; Heger, K; Brady, J; Nutt, SL; Vremec, D; Pietersz, S; Lahoud, MH; Eld, LS; Hansen, DS; O'Keeffe, M; Smyth, MJ; Bedoui, S; Davey, GM; Villadangos, JA; Heath, WR; Shortman, K, Putative IKDCs are functionally and developmentally similar to natural killer cells, but not to dendritic cells, JOURNAL OF EXPERIMENTAL MEDICINE, 2007, 204 (11), pp. 2579 - 2590

Persistent Link:
http://hdl.handle.net/11343/212913

File Description:
Published version