Isolation and characterization of plasmacytoid dendritic cells from Flt3 ligand and granulocyte-macrophage colony-stimulating factor–treated mice

Pia Björck

Interferon α/β plays an important role in the first-line defense against viral infections and can modulate cytokine responses by T-helper cells. Type 1 interferons (IFNs) are clinically important in infectious diseases and in the treatment of leukemia and lymphomas. Many different cell types have the capacity to produce IFN-α after encounter with virus and bacteria. The major, natural type 1 IFN–producing cell in humans was recently described as the plasmacytoid T cell, or pDC2, and it can differentiate into dendritic cells (DCs) on culture. This study describes the murine natural IFN-α–producing cell, or pDC2, that shares morphologic features with its human counterpart but has some distinct phenotypical characterisitcs. Murine plasmacytoid DCs can be differentially isolated based on their expression of CD11c, CD220 (CD45R), and Thy1.2 (CD90). They lack expression of myeloid (eg, CD11b) antigens and CD8α, a marker used to isolate lymphoid DCs. Like human pDC2, murine plasmacytoid DCs exhibit their maximal type 1 IFN–producing capacity at a precursor stage; pDCs isolated from bone marrow responded to viral stimulation with higher IFN-α production than cells of the same phenotype isolated from spleen. Mobilization of mice with Flt3 ligand (Flt3L) or Flt3L and granulocyte-macrophage colony-stimulating factor, hematopoietic factors that specifically enhance DC growth, resulted in strikingly increased numbers of pDC in bone marrow and spleen. The isolation of this novel murine DC subset may serve as a useful tool in the study of viral immunobiology and for the design of treatments for murine malignancies. (Blood. 2001;98:3520-3526)
exhibit a phenotype slightly different from that of its human counterpart in that it expressed CD11c, but they were similar in expressing CD45R and low levels of CD4 and IL-3R. Short-term culture of murine pDC in media containing IL-3 and anti-CD40 antibodies induced maturation, resulting in the evolution of long cellular protrusions and high expression of major histocompatibility complex class 2 and accessory molecules.

Materials and methods

Mice

Balb/c and C57BL/6 6- to 8-week-old female mice were purchased from Jackson Laboratory (Bar Harbor, ME). Animals were maintained according to institutional guidelines in the Central Animal Facility of the Biomedical Science Tower at the University at a Institutional Animal Care & Use Committee (IACUC)-approved protocol.

Reagents

Recombinant murine (rm) Flt3L and rmGM-CSF for in vivo use were the generous gifts of Pharmacia (St Louis, MO), rmGM-CSF and mIL-4 for in vitro culture were the kind gifts of Schering-Plough (Kenilworth, NJ). Herpes simplex virus (KOS strain) was a kind gift from Dr N. DeLuca (Department of Molecular Biology and Genetics, University of Pittsburgh). Stimulatory cytokine poly-guaiac (CGp) motifs specific for the induction of IFN-α (GGGGTGAAAGTTGAGGGGGG), IL-12 (CTCGGTTTTT- GTCGGTTTTGGTGGT), and a control Cpg motif (CTGGGCTTTCTG- GGTTTTGTTCTGGG) were the kind gifts of Dr A. Krieg (University of Iowa). rmIL-3 was purchased from R&D Systems (Minneapolis, MN). Staphylococcus aureus Cowan strain 1 (SAC, Pansorbin) was purchased from Calbiochem (La Jolla, CA), lipopolysaccharide (LPS) was obtained from Sigma, and stimulatory anti-CD40 (IgM) antibodies were obtained from Pharmingen (San Diego, CA). All antibodies for cell separation and fluorochrome-conjugated antibodies for FACs and analysis were purchased from Pharmingen. Brefeldin A was obtained from Sigma. MACS magnetic beads directly conjugated with antibodies to CD11c or CD11b or with anti-rat immunoglobulin were purchased from Miltenyi Biotec (Auburn, CA).

Cytokine mobilization

Mice were mobilized with recombinant Flt3L ± GM-CSF or combinations thereof at a concentration of 20 μg/mouse per day in phosphate-buffered saline (PBS) by subcutaneous injections in the neck for 7 consecutive days under an IACUC-approved protocol.

Cell preparation

Bone marrow cells were obtained by flushing femurs and tibias with PBS using a 23-gauge needle. Red cells were hypotonically lysed, and the residual cells were washed and kept on ice until used. Spleens were isolated, and a single-cell suspension was made by passing the spleen through a nylon cell strainer (Falcon; Becton Dickinson, Franklin Lakes, NJ). After lysis of red cells by ammonium chloride solution, cells were incubated with antibodies to block FcR interactions (FcR-block, CD16/CD32, Pharmingen, at 1 μg/1 × 10^7 cells at 10 × 10^9/mL in PBS) for 15 minutes on ice. For isolation of CD11c^+ cells using directly conjugated MACS magnetic beads (Miltenyi Biotec), CD11c+ cells were added according to the manufacturer’s instructions. After incubation for 20 minutes at 4°C, cells were washed and passed over a MACS column. Positively selected cells were isolated and suspended in appropriate buffer. Purity was checked routinely by FACS and was found to be greater than 97% (not shown). For analysis by FACS, cells were stained with directly conjugated antibodies.

Fluorescence-activated cell sorting

Splenocytes were isolated as described above. After blocking of FcR-binding sites, T and B cells were depleted using antibodies to CD3 and surface immunoglobulin, respectively. After incubation, goat anti-rat-coated magnetic beads (MACS) were added, and, after further incubation, cells were passed over a MACS column. The negative fraction was collected, washed, and stained with antibodies to CD3-fluorescein isothio-cyanate (FITC), sIgM-FITC, CD11c-phcoerythrin (PE), and CD11b-APC. Cells were sorted on a FACS Star Plus (Becton Dickinson, Mountain View, CA). Gates were set to exclude debris and dead cells. T and B cells were further excluded by gating on the FITC-negative population. CD11c^+CD11b^+ and CD11c^+CD11b^- cells were collected. Cell purity usually exceeded 96% (data not shown). Alternatively, total DCs were isolated using directly conjugated CD11c antibody-coated magnetic beads. After they were washed, DCs were stained with antibodies to CD80-PE, B220-APC, and a cocktail of FITC-labeled antibodies (CD11b, sIg, CD3). Gates were set to exclude monocytes, T cells, and B cells, and DCs were sorted based on the expression of CD8α and B220. Purity exceeded 96% (data not shown).

Cell culture

All cell cultures were performed in complete RPMI-1640 (Gibco Life Technologies, Grand Island, NY) supplemented with 5% fetal calf serum (Gibco), 2 mM L-glutamine, penicillin, streptomycin, and 1 mM HEPES buffer (Gibco).

ELISA for detection of IFN-α and IL-12

ELISA was performed using standard procedures. In brief, for the detection of IFN-α, 96-well flat-bottomed plates (Costar, Corning, NY) were coated with 5 μg/mL sheep anti-mouse IFN-α/b (a kind gift from Schering-Plough Research Institute) overnight at 4°C. After blocking with 3% bovine serum albumin in PBS-Tween 20 for 1 hour at 37°C, plates were washed, and standard rmIFN-α2 (a kind gift from Schering-Plough) and culture supernatants were added (standard rmIFN-α2 had been titrated in parallel against a known rmIFN-α2 standard provided by the National Institute of Allergy and Infectious Diseases reference reagent repository). Plates were then incubated at 4°C overnight. After washing, a rat anti-mouse IFN-α2 antibody (4E-A1 [IgG1]; Seikagaku America, Falmouth, MA) was added, and plates were incubated at room temperature for 2 hours. After washing, peroxidase-conjugated goat anti-rat immunoglobulin antibody (Jackson Laboratory) was added, and plates were further incubated for 1 hour. After washing, plates were developed using tetramethylbenzidine (TMB) substrate (Kirkegaard & Perry, Gaithersburg, MD) and were stopped by the addition of 1 M sulfuric acid. For determination of IL-12 (p70), plates were coated with 5 μg/mL rat anti-mouse IL-12 (p70) (Pharmingen) in PBS overnight. After blocking with bovine serum albumin, supernatants and standard (rmIL-12, a kind gift from the Genetics Institute, Cambridge, MA) were added. After incubation overnight at 4°C, a biotinylated rat anti–mouse IL-12 (p40/p70) antibody was added, and plates were incubated for 2 hours at room temperature. After washing, extravidin-peroxidase (Sigma) was added and plates were further incubated for 1 hour. Finally, plates were developed using TMB substrate as above.

Electron microscopy

Transmission electron microscopy was performed on sorted, CD11c^+ B220^- DCs. Cells were fixed in 2.5% glutaraldehyde in cacodylate buffer and postfixed in 1% OsO₄ solution. Cells were dehydrated in a series of alcohol solutions and embedded in epoxide. Sections were examined using a JEOL 1210 microscope (JEOL, Peabody, MA).

Staining for intracellular cytokines

Cells were cultured for 24 hours together with HSV (10 plaque-forming units [PFU]/cell), and Brefeldin A was added during the last 5 hours of culture. For preparation and staining of cytospins, cells were spun onto slides and fixed for 20 minutes with 2% formaldehyde. Thereafter, cells were permeabilized for 20 minutes with PBS containing 0.5% saponin, 5% fetal calf serum (FCS), and 2 mM HEPES. All subsequent steps were performed in buffer containing saponin. After washing and blocking with 10% goat serum and 5% FCS for 30 minutes, primary rat anti–mouse IFN-α

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Mobilization of mice with Flt3L results in elevated levels of IFN-α2 production from (A) BM and (B) spleen cell populations. BM (C) and spleen (D) cells were prepared from Flt3L–mobilized mice for 7 days with Flt3L were cultured for 24 hours in medium alone (white columns) or with HSV (black columns, 5 PFU/cell) or SAC (gray columns, 10 μg/mL). Supernatants were harvested and analyzed for their IFN-α (white columns) or with HSV (black columns) or SAC (gray columns, 10 μg/mL). Supernatants were collected and analyzed for IFN-α2 by ELISA. Data from 1 of at least 3 experiments yielding similar results are shown.

Bone marrow cells and splenocytes derived from mice mobilized with Flt3L+GM-CSF were isolated with magnetic beads directly conjugated with antibodies to CD11c or CD11b. Cells were cultured in medium alone or together with HSV or SAC, and supernatants were collected after 24 hours. The major IFN-α2–producing cells are present in the lymphoid, CD11c+CD11b+ (myeloid) and CD11c+CD11b– (lymphoid) DC populations. Bone marrow and spleen cells were analyzed by flow cytometry and were found to express high numbers of CD11c+CD11b+ (myeloid) and CD11c+CD11b– (lymphoid) DCs, respectively (data not shown). Because a functional characteristic of human plasmacytoid DCs (pDC2) is their high production of type 1 interferon after encounter with pathogens, isolated bone marrow and spleen cells were cultured overnight in the presence or absence of HSV or SAC. Cells maintained in medium alone were used as control. After 24 hours of culture, supernatants were harvested and analyzed for their IFN-α content by specific ELISA. As shown in Figure 1, bone marrow and, to a lesser degree, spleen cells from mice that had been mobilized with Flt3L showed greater production of IFN-α in response to HSV than nontreated mice. SAC induced low but reproducible levels of IFN-α. A significantly greater number of mobilized DCs could be detected when Flt3L was combined in vivo with GM-CSF, a factor known to support DC generation in vitro and also found to have an additive effect when coadministered with Flt3L (P.B. et al, unpublished observation, October 1999). Thus, the combination of Flt3L+GM-CSF was used throughout the rest of this study.

Bone marrow and spleen cells from day 7 Flt3L+GM-CSF–mobilized mice were then sorted by FACs based on their expression of the CD11c+CD11b+ (myeloid) and CD11c+CD11b– (lymphoid) phenotypes using fluorochrome-labeled antibodies. Gates were set to exclude dead cells and debris, and T and B cells were further excluded by staining with antibodies to slg-FITC and CD3-FITC. The purity of sorted cells exceeded 97% (Figure 2A). Sorted cells were cultured overnight in the presence or absence of HSV or SAC. Supernatants were collected and analyzed for their IFN-α2 content.

Measurement of T-cell stimulation

For alloreaction, serial dilutions of irradiated DC (2000 cGy) that had been preactivated for 24 hours with stimulatory CD40 antibodies (IgM subclass; Pharmingen), and 2 × 105 total allogeneic CD4+ T cells were cultured for 6 days in round-bottomed, 96-well plates at a final volume of 200 μL. Cells were maintained in medium alone were used as control. After 24 hours of culture, supernatants were collected and analyzed for IFN-α2. Data from 1 of 3 experiments with similar results are shown.

Results

Isolation of murine plasmacytoid dendritic cells from spleen and bone marrow

Mice were mobilized for 7 days with Flt3L, a hematopoietic growth factor known to specifically expand DCs in vivo9,11 and were killed 1 day after the last cytokine injection. Bone marrow cells were obtained by flushing resected femurs with PBS. Spleens were harvested and dispersed into single-cell suspensions. Total
by specific ELISA. CD11c+CD11b-, but not CD11c+CD11b+, DCs were found to produce IFN-α after stimulation with virus (Figure 2B). SAC induced lower but significant IFN-α production yet strongly promoted the production of IL-12 from both CD11c+CD11b- (lymphoid) and CD11c+CD11b+ (myeloid) DC subsets.

### Isolation of the major type 1 IFN–producing dendritic cell subset

The CD11c+CD11b- DC subset contains a population of CD11c+CD8α+ DCs that has been previously shown to be the major IL-12–producing DC subset; however, CD11c+CD8α+ DCs do not produce significant levels of IFN-α after HSV infection. Because this suggested that there was heterogeneity within the CD11c+CD11b- subset responsible for the production of IFN-α versus IL-12, DCs were sorted in a manner biased by the phenotype recently reported for human pDC2. Human pDC2 expresses CD45R, CD4, and the receptor for IL-3, but FACS staining of murine CD11c+CD11b- DCs using antibodies recognizing IL-3R or CD4 showed only weak expression of these antigens (Table 1). However, antibodies to B220 (mouse CD45R) could be readily used to subdivide the DC population further given that flow cytometry of total CD11c+ DCs revealed a distinct subpopulation of CD11c+B220+ cells in day 7 Flt3L+GM-CSF–mobilized mice (Figure 3A). This was also observed to a lesser extent in mice treated with Flt3L alone (data not shown). This CD11c+B220+ population could be found in bone marrow (BM), peripheral blood, spleen, and lymph nodes of mobilized mice. Total CD11c+ cells were isolated by directly conjugated anti-CD11c magnetic beads and were sorted by flow cytometry using fluorochrome-labeled antibodies to CD11b, Fc, CD8α–PE, and B220–APC. Anti–CD3- and slg–FITC–labeled antibodies were used to further exclude contaminating T and B cells. CD11b+ DCs (myeloid) were excluded, and gates were set on CD8α+– and B220+–expressing cells, respectively (Figure 3B). FACs analysis of sorted DCs showed that though both populations expressed the CD11c antigen, the CD8α+ population was typically CD11cbright and the B220+ population was CD11cdim (Table 1). B220+CD11c+ cells expressed Thy1.2 and low, but detectable, levels of IL-3R and CD4. Furthermore, they expressed MHC class 1 (H2-K^d^) and class 2 (I-A^d^) and accessory and adhesion molecules CD86, CD11a, and CD54, but no myeloid antigens or CD19. The expression of Gr-1 varied and was low or undetectable on freshly isolated cells, but it was slightly up-regulated after overnight culture. The CD8α+CD11c+ DC population expressed high levels of the MHC class II and CD86 molecules but lacked expression of the CD4, IL-3R, and Thy1.2 antigens. Table 1 provides a summary of the phenotype of the distinct murine DC subpopulations either directly after FACs sorting or after overnight culture in complete medium.

Freshly isolated and sorted spleen CD11c+CD8α+ or CD11c+CD8α+ DCs were cultured overnight with or without HSV, SAC, anti-CD40 antibodies, and CpG oligodeoxynucleotides (ODN) motifs to analyze the impact of these stimuli on cytokine production. Culture supernatants were then harvested and examined for their content of secreted IFN-α and IL-12 (p70). Figure 3C shows that splenic CD11c+CD8α+ DCs produced IFN-α after viral stimulation. Interestingly, SAC induced both IL-12 and IFN-α from this DC subset. However, CD11c+CD8α+ lymphoid DCs did not produce IFN-α after HSV stimulation (Figure 3D), even at high viral multiplicity of infection (ie, 50 PFU/cell), but they could produce low levels after stimulation with SAC. SAC were also the only stimuli that induced the production of IL-12 from lymphoid, myeloid, and CD11c+CD8α+ DCs as measured by intracellular staining and flow cytometry. The major IL-12–producing DC subset was the CD11c+CD8α+ lymphoid DC that also responded to stimulatory anti-CD40 antibodies or LPS activation (Figure 4).

The key markers used to isolate these 3 different mouse DC subsets are detailed in Figure 5. To obtain myeloid DCs, PE–conjugated antibodies to CD11b were used and CD8α–FITC–expressing cells were excluded.

### Table 1. Phenotype of splenic plasmacytoid, lymphoid, and myeloid murine DCs

<table>
<thead>
<tr>
<th>Cell surface marker</th>
<th>Plasmacytoid DC</th>
<th>Lymphoid DC</th>
<th>Myeloid DC</th>
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<tbody>
<tr>
<td></td>
<td>CD11c+CD11b+</td>
<td>CD11c+CD8α+</td>
<td>CD11c+CD8α+</td>
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<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
<td>Day 0</td>
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<tr>
<td>CD2</td>
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<td>CD4</td>
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<td>CD5</td>
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<td>CD8α</td>
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<tr>
<td>CD11a</td>
<td>+</td>
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<td>CD11b</td>
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<tr>
<td>CD11c</td>
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<tr>
<td>CD19</td>
<td>–</td>
<td>–</td>
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<tr>
<td>CD40</td>
<td>+</td>
<td>+</td>
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<tr>
<td>CD54</td>
<td>+</td>
<td>+</td>
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<tr>
<td>CD86L</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>CD95 (Fas)</td>
<td>±</td>
<td>±</td>
<td>±</td>
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<tr>
<td>B220 (CD45R)</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Thy1.2</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>Gr-1</td>
<td>±</td>
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<tr>
<td>H-2K^d^ (MHC class I)</td>
<td>+</td>
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<tr>
<td>I-A^b^ (MHC class II)</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>IL-3R</td>
<td>+</td>
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Expression ranges from ± (low) to +++ (strong). Lymphoid DCs are characterized by a CD11c+CD11b+CD8α+ phenotype, and myeloid DCs exhibit a CD11c+CD11b−CD8α+ phenotype. Cells were analyzed directly after FACs sorting or after overnight culture in complete medium. ND indicates not determined.
Sorted cells were typically more than 96% pure. (C) Sorted splenic CD11c and sIg were used to exclude myeloid DCs, T cells and B cells in FACS sorting. CD11c-conjugated magnetic beads. FITC-conjugated antibodies to CD11b, CD3, 1 population is distinct from CD11c produce IFN-α on stimulation with HSV, but SAC induced low levels of IFN-α. (D) CD11c-CD11b– lymphoid DCs did not proliferate significantly in culture, regardless of the content by specific ELISA. Major HSV-responsive, IFN-α producers of type 1 interferon after viral stimulation but can also lymphoid or CD11b myeloid DC markers. They are the major lymphoid DC markers. They are the major

Mouse pDC develops into dendritic cells after culture and acquires the capacity to stimulate T cells

Visual inspection of cytospins of the CD11c 1 population is distinct from CD11c 1 population is distinct from CD11c

Figure 3. Major murine IFN-α-producing DC subset expresses a CD11c 1, B220 2 phenotype and lacks CD8α expression. (A) Staining of total splenocytes derived from Flt3L + GM-CSF–treated mice show that, in addition to myeloid CD11c 1, CD11b– DC, a subset of CD11c 1 DC coexpresses the B220– antigen. This population is distinct from CD11c 1, CD8α– lymphoid DCs. (B) The CD11c 1, CD11b– DC subset was further subdivided using antibodies to CD8α–PE and B220–APC. Total CD11c 1 cells were isolated from the spleens of Flt3L + GM-CSF–treated mice using CD11c–conjugated magnetic beads. FITC–conjugated antibodies to CD11b, CD3, and sIg were used to exclude myeloid DCs, T cells and B cells in FACS sorting. Sorted cells were typically more than 96% pure. (C) Sorted splenic CD11c 1, B220 2 DCs were cultured for 24 hours alone or with HSV, SAC, CD40 antibodies, or 2 μg/mL CpG (ODN). Supernatants were harvested and examined for their IFN-α and IL-12 content by specific ELISA. Major HSV-responsive, IFN-α–producing cells were found in the CD11c 1, B220 2 subset. These cells exhibited the capacity to produce IFN-α and IL-12 in response to SAC. (D) CD11c 1, CD8α– lymphoid DCs did not produce IFN-α on stimulation with HSV, but SAC induced low levels of IFN-α and high levels of IL-12. At least 5 experiments performed gave comparable results.

T-cell costimulation

A defining characteristic for most DC subsets is T-cell stimulatory capacity. CD11c 1, B220 2 DCs were examined for their capacity to stimulate T-cell proliferation. Isolated DCs were cultured overnight with stimulatory anti-CD40 antibodies (25 μg/mL). After washing, DCs were irradiated (2000 cGy), and serial dilutions of these APCs were cultured with total allogeneic CD4+ T cells (2 × 10^5)/well). T-cell cocultures with CD11c 1, CD11b– and CD11c 1, CD8α– DCs were generated in parallel. As shown in Figure 6F, CD40-activated pDC induced T-cell proliferation to levels similar to those observed for CD11c 1, CD8α– lymphoid DCs. Human cultured pDC has also been shown to induce a more potent T-cell stimulation than freshly isolated.4 As expected, myeloid DCs induced comparably strong T-cell stimulation.

In summary, the mouse plasmacytoid DCs exhibited a CD11c 1, B220 2 phenotype but did not express the CD8α lymphoid or CD11b myeloid DC markers. They are the major producers of type 1 interferon after viral stimulation but can also produce both IFN-α and IL-12 when activated by bacterial proteins. Table 2 outlines the characteristics of murine and human DC subsets. Murine pDC are not the strong stimulators of allogeneic T cells their human counterparts are and may possibly serve a regulatory function similar to that of murine lymphoid DCs.

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Discussion

The current study describes the identification of the murine natural type 1 IFN–producing cell or plasmacytoid DC, with the capacity to produce IFN-α after viral challenge. Murine pDC can also produce IL-12 in response to bacterial antigen such as SAC. The kinetics of the pDC response to SAC is different from that of lymphoid CD11c<sup>+</sup>CD8α<sup>+</sup> DC, the major IL-12 producing DC subset, in that it is immediate and transient (not shown). Activating anti-CD40 antibodies were unable to induce IL-12 production in murine pDC, whereas lymphoid CD11c<sup>+</sup>CD8α<sup>+</sup> DCs responded readily to this stimuli and to stimulation using LPS. Moreover, as shown for human natural IFN–producing cells, murine pDC produced low but significant levels of IFN-α in response to SAC. This may have in vivo relevance because both IFN-α and IL-12 have been reported to promote CD8<sup>+</sup> cytotoxic T-lymphocyte and natural killer activity, thereby providing a link between innate and adaptive immunity. Experiments are under way to analyze what signals regulate IFN-α versus IL-12 production in murine pDC.

All murine DC subsets described to date express the CD11c antigen.

Table 2. Summary of the characteristics of mouse and human DC subsets

<table>
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<th>Mouse</th>
<th>Human</th>
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<td>Myeloid CD11c&lt;sup&gt;+&lt;/sup&gt;CD11b&lt;sup&gt;+&lt;/sup&gt; (CD8α&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>Myeloid DC1 major IL-12–producing DCs, CD11c&lt;sup&gt;+&lt;/sup&gt;IL3R&lt;sup&gt;+&lt;/sup&gt;, induce strong MLR. Primarily T-cell stimulatory function.</td>
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<tr>
<td>Lymphoid CD11c&lt;sup&gt;+&lt;/sup&gt;CD11b&lt;sup&gt;+&lt;/sup&gt; (CD8α&lt;sup&gt;+&lt;/sup&gt;) can express Fas ligand, produce high amounts of IL-12, and induce weak MLR. Primarily T-cell regulatory function.</td>
<td>Plasmacytoid DC2 major type I IFN–producing cells, CD11c&lt;sup&gt;+&lt;/sup&gt;IL3R&lt;sup&gt;+&lt;/sup&gt;CD45R&lt;sup&gt;+&lt;/sup&gt;CD4&lt;sup&gt;+&lt;/sup&gt;, induce strong MLR and mature into DCs with Th2-stimulating capacity on culture with CD40 antibodies and IL3. Primarily T-cell regulatory function?</td>
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<tr>
<td>Plasmacytoid CD11c&lt;sup&gt;+&lt;/sup&gt;CD11b&lt;sup&gt;+&lt;/sup&gt;CD8α&lt;sup&gt;+&lt;/sup&gt;B220&lt;sup&gt;+&lt;/sup&gt;Thy1.2&lt;sup&gt;+&lt;/sup&gt;, but low expression of IL-3R</td>
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whereas those for human DCs do not. Murine pDC also express the CD45R (B220) antigen but at lower levels of CD4 and IL-3R than its human counterpart. In addition, murine pDC expressed Thy1.2, a marker expressed on T cells, but did not express the CD8α antigen under any conditions evaluated in this study. Recently, CD8α has been found on activated, myeloid DCs, suggesting it may not be a hallmark of lymphoid DC. Instead, expression of CD11c in the absence of CD11b might serve as a more reliable index for distinguishing lymphoid DCs from myeloid DCs.

The existence of the natural IFN–producing cell has been known for some time. Although its hematopoietic origin is still a matter of debate, a recent study identified certain transcription factors that regulate T- and B-cell development and pDC2 development, but not DC1, suggesting a lymphoid origin for pDC2. Murine pDC express markers associated with lymphoid lineage cells but lack expression of myeloid markers (ie, CD11b) and express low levels of Gr-1 when isolated from BM but not from spleen. However, a low expression of Gr-1 could be found on splenic pDC after overnight culture. A BM-derived cell with the phenotype CD45−Gr-1−IL-7Rα− also that low expression of B220 and Thy-1 was isolated previously and found to give rise to both B- and T-lineage cells and to myeloid cells on adoptive transfer. Whether these are the same cells as the plasmacytoid DC described here merits further study. Murine pDC also do not express CD19, a marker that can be found coexpressed with CD11c on a small subset of cells in BM; this may define an additional subset of DCs.

Flt3L with or without GM-CSF was found to induce and enhance the frequency of the CD11c+B220+DC subset that was correlated with the mobilization of the IFN-α–producing cell population. In nontreated mice these cells were found principally in the BM, whereas in Flt3L-treated mice, much of the IFN-α production was relocated to the spleen. This finding may prove to be of considerable importance in clinical trials of Flt3L, in which the migration of pDC to peripheral sites is desired.

Type I interferons produced by pDC would be anticipated to provide important insights in their pathogenesis. Most recent data suggest the involvement of pDC in allergic asthma and in the autoimmune condition systemic lupus erythematosus. Targeting pDC in murine models for these diseases may provide important insights in their pathogenesis.

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References

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