

Video Article

Assessing the Development of Murine Plasmacytoid Dendritic Cells in Peyer's Patches Using Adoptive Transfer of Hematopoietic Progenitors

Haiyan S. Li¹, Stephanie S. Watowich^{1,2}

¹Department of Immunology, The University of Texas MD Anderson Cancer Center

²The University of Texas Graduate School of Biomedical Sciences

Correspondence to: Stephanie S. Watowich at swatowich@mdanderson.org

URL: <https://www.jove.com/video/51189>

DOI: [doi:10.3791/51189](https://doi.org/10.3791/51189)

Keywords: Immunology, Issue 85, hematopoiesis, dendritic cells, Peyer's patch, cytokines, adoptive transfer

Date Published: 3/17/2014

Citation: Li, H.S., Watowich, S.S. Assessing the Development of Murine Plasmacytoid Dendritic Cells in Peyer's Patches Using Adoptive Transfer of Hematopoietic Progenitors. *J. Vis. Exp.* (85), e51189, doi:10.3791/51189 (2014).

Abstract

This protocol details a method to analyze the ability of purified hematopoietic progenitors to generate plasmacytoid dendritic cells (pDC) in intestinal Peyer's patch (PP). Common dendritic cell progenitors (CDPs, $\text{lin}^- \text{c-kit}^{\text{lo}} \text{CD115}^+ \text{Flt3}^+$) were purified from the bone marrow of C57BL/6 mice by FACS and transferred to recipient mice that lack a significant pDC population in PP; in this case, *Ifnar*^{-/-} mice were used as the transfer recipients. In some mice, overexpression of the dendritic cell growth factor Flt3 ligand (Flt3L) was enforced prior to adoptive transfer of CDPs, using hydrodynamic gene transfer (HGT) of Flt3L-encoding plasmid. Flt3L overexpression expands DC populations originating from transferred (or endogenous) hematopoietic progenitors. At 7-10 days after progenitor transfer, pDCs that arise from the adoptively transferred progenitors were distinguished from recipient cells on the basis of CD45 marker expression, with pDCs from transferred CDPs being CD45.1⁺ and recipients being CD45.2⁺. The ability of transferred CDPs to contribute to the pDC population in PP and to respond to Flt3L was evaluated by flow cytometry of PP single cell suspensions from recipient mice. This method may be used to test whether other progenitor populations are capable of generating PP pDCs. In addition, this approach could be used to examine the role of factors that are predicted to affect pDC development in PP, by transferring progenitor subsets with an appropriate knockdown, knockout or overexpression of the putative developmental factor and/or by manipulating circulating cytokines via HGT. This method may also allow analysis of how PP pDCs affect the frequency or function of other immune subsets in PPs. A unique feature of this method is the use of *Ifnar*^{-/-} mice, which show severely depleted PP pDCs relative to wild type animals, thus allowing reconstitution of PP pDCs in the absence of confounding effects from lethal irradiation.

Video Link

The video component of this article can be found at <https://www.jove.com/video/51189/>

Introduction

Here, we demonstrate a protocol to assess whether common dendritic cell progenitors (CDPs) are capable of giving rise to the plasmacytoid dendritic cell (pDC) population in Peyer's patch (PP). The overall goal of using this method was to evaluate the developmental regulation of pDCs in Peyer's patch (PP pDCs). The reason this is important is that PP pDCs differ from pDCs found in other tissues, including bone marrow, blood and spleen, and therefore it is unclear whether PP pDCs and other pDC populations are developmentally and/or functionally related. Specifically, pDCs are widely known for being the principal type I interferon (IFN) producers within the hematopoietic system, responding to Toll-like receptor 7 and 9 (TLR7/9) stimulation by rapid IFN secretion¹⁻³. However, PP pDCs are deficient in producing type I IFN in response to TLR agonist stimulation^{4,5}. Moreover, PP pDCs also differ from pDCs found in bone marrow and spleen in requiring signals from the type I interferon (IFN) receptor (IFNAR1) or the IFN signaling molecule STAT1 for their development and/or accumulation⁵. These data have suggested the possibility that distinct regulatory mechanisms control PP pDCs versus pDCs in other organs (e.g. bone marrow, spleen)⁵.

The rationale that led to the development of this method was based on recent advances in understanding dendritic cell (DC) biology. Most, if not all, DC subsets derive from hematopoietic progenitors that express the FMS-like tyrosine kinase 3 receptor (Flt3)⁶⁻¹⁰; however, DC development is not restricted to the classic myeloid and lymphoid pathways. For example, Flt3⁺ common myeloid progenitors (CMPs, $\text{lin}^- \text{IL-7R}^- \text{Sca-1}^- \text{c-kit}^+ \text{CD34}^+ \text{FcγR}^{\text{lo}}$) give rise to CDPs ($\text{lin}^- \text{c-kit}^{\text{lo}} \text{CD115}^+ \text{Flt3}^+$), which further differentiate into pDCs and conventional DCs (cDCs)^{9,10}. By contrast, Flt3⁺ common lymphoid progenitors (CLPs, $\text{lin}^- \text{IL-7R}^+ \text{Sca-1}^{\text{lo}} \text{c-kit}^{\text{lo}}$) develop primarily into pDCs¹¹. Therefore, prior studies indicate pDCs arise from at least 2 distinct hematopoietic progenitor populations under the regulation of Flt3L, although the typical analysis has been restricted to the bone marrow, spleen and/or blood pDC subsets. Thus, the progenitor population(s) that generates PP pDCs required investigation. Understanding the origins of PP pDCs will shed light on whether they share common developmental pathways with other pDC populations, or utilize distinct mechanisms during their generation in PP.

A unique advantage of the approach described herein is the use of mice that show a severe deficiency in PP pDCs as recipients for the adoptive transfer of hematopoietic progenitors. Mice with genetic deletion in the gene encoding IFNAR1 (*Ifnar*^{-/-} mice) or STAT1 (*Stat1*^{-/-}) revealed a striking depletion in PP pDCs⁵. Therefore, these strains provide an environment in which PP pDCs are reduced, allowing adoptive transfer studies to be performed in the absence of potent cell ablation regimes such as lethal irradiation. An additional strength of the method presented

here is the use of hydrodynamic gene transfer (HGT) to stimulate elevated circulating amounts of Flt3L. This provides a cost effective approach to induce Flt3L *in vivo*, versus injection of recombinant protein. Numerous studies, including those in our lab, have employed HGT to induce cytokine amounts in a variety of experimental conditions^{5,12,13}.

The division of labor and precise immune functions for DCs is of major interest in immunology. In particular, pDCs are important mediators of oral tolerance and systemic anti-viral responses, yet they also appear to contribute to the development and persistence of autoimmunity and cancer¹⁴⁻¹⁷. The protocol described herein will allow the developmental mechanisms regulating PP pDCs to be more fully explored. In addition, this approach may allow studies to assess PP pDC function, and may be extended to understanding the regulation and function of other immune populations within PPs.

Protocol

Institutional approval must be obtained in advance for all experimental manipulations described herein involving mice. These include the use of C57BL6 mice for isolation of bone marrow progenitor cells, *Ifnar*^{-/-} mice as recipients for adoptive transfer of hematopoietic progenitors and use of HGT for cytokine overexpression *in vivo*. Appropriate housing and animal care must also be provided by the investigator or institution. Furthermore, institutional approval may be required for the plasmids used in hydrodynamic gene transfer (*i.e.* recombinant DNA approval). The studies described here were approved by the Institutional Animal Care and Use Committee at UT MD Anderson Cancer Center.

1. Hydrodynamic Gene Transfer (HGT)

This step should be performed 2 days prior to the adoptive transfer of CDPs to induce circulating Flt3L for DC expansion *in vivo*⁵. Prepare at least 5 recipient mice/group.

1. To achieve efficient HGT, the blood vessels of the recipient *Ifnar*^{-/-} mice (CD45.2⁺) should be dilated by exposing mice to a heating lamp for 5-10 min.
2. Place the mouse in a restrainer device and disinfect its tail with 70% ethanol.
3. Inject 5 µg of plasmid encoding Flt3L in 2 ml of sterile PBS into the tail vein using a 27 G needle. For the control cohort, inject 5 µg of empty vector (pORF) via the tail vein as indicated.
4. Monitor mice for 15-30 min to ensure there are no deleterious effects of the tail vein injection. Return mice to housing facility for 2 days.

2. Isolation of Hematopoietic Progenitors from Mouse Bone Marrow

This step should be performed 2 days after HGT. Use congenic CD45.1⁺ mice as the source of bone marrow progenitors for adoptive transfer into recipient *Ifnar*^{-/-} animals (CD45.2⁺). In this protocol, congenic strains are required to distinguish donor and recipient-derived DCs, as well as to avoid immune-mediated depletion due to MHC mismatch. Approximately 10-20 mice will be required to provide sufficient numbers of hematopoietic progenitor cells (10⁵ cells/recipient mouse) for the transfer experiments.

1. Euthanize 10 congenic CD45.1⁺ mice by CO₂ asphyxiation and cervical dislocation.
2. Place each carcass on a dissection tray and sterilize the abdomen and legs with 70% ethanol.
3. Make an incision at the mid-abdomen and cut through the skin from the abdomen to each leg, cutting skin down the length of the leg.
4. Gently remove the skin from each leg, and cut and remove the legs from the carcass at the hip joint using sharp scissors.
5. Carefully remove the muscles from the femur and tibia of each leg using a sharp blade.
6. Remove both ends of the femur and tibia with a sharp scalpel and place bones in a culture dish containing complete RPMI (RPMI with 10% heat-inactivated fetal bovine serum, 1% penicillin/streptomycin, 1 mM sodium pyruvate and 50 µM β-mercaptoethanol).
7. Prepare a syringe containing 1 ml of complete RPMI, connected to a 27 G needle.
8. Insert the 27 G needle into one end of the femur or tibia. Flush the bone marrow from the femur or tibia by gently injecting the complete RPMI into the bone. Ensure that bone marrow cells are being removed from the bone; this can be accomplished by visually detecting expelled media that appears cloudy.
9. Repeat flushing each femur and tibia 3x to thoroughly remove bone marrow cells.
10. Prepare a single cell suspension by gently pipetting cells up and down 3-5x in the culture dish.
11. Remove debris by passing the bone marrow cells through a 40 µm cell strainer, placing the exuded cells into a new culture dish. Disrupt any cell clumps that appear on the strainer by gentle pressure with the end of a sterile syringe plunger.
12. Lyse the red blood cells (RBC) present in the total bone marrow cell suspension with commercial RBC lysis buffer per the manufacturer's instructions. Use 2 ml RBC lysis buffer/4-6 x 10⁷ cells (generally cells from one mouse) and an incubation time of 5 min at RT.
13. Wash the bone marrow cells by pelleting cells by centrifugation for 4 min at 500 x g, gently aspirating the culture medium, resuspending in 10 ml FACS buffer (1x PBS + 2 mM EDTA + 1% FBS), pelleting cells by centrifugation and gently aspirating wash buffer.
14. Repeat the wash, as described in step 2.13, for a total of 2 washes.

3. Fluorescence-activated Cell Sorting (FACS) to Isolate CDPs

This step requires access to a MidiMACS cell separator and MACS LD columns for an initial negative selection procedure, as well as a FACS machine with at least 3 lasers to purify the multicolor progenitor subset after staining with fluorescently conjugated antibodies.

1. After the second wash in step 2.14, count the bone marrow cells. Pellet the cells by centrifugation as described in step 2.13 and resuspend in FACS buffer to achieve a final concentration of 4 x 10⁷ cells per 30 µl of FACS buffer.
2. To prepare cells for FACS, lineage-negative cells are first enriched by a negative selection technique that removes lineage-positive cells from the bone marrow mixture using magnetic bead column chromatography. For the negative selection procedure, add 1 µg of each of the

following commercial rat anti-mouse antibodies (Abs) that recognize hematopoietic lineage markers: CD3, CD19, CD11c, CD11b, and Ter-119 Abs. The Abs should be added in a volume of 2 μ l/Ab to the 30 μ l cell suspension in FACS buffer.

3. Incubate the cell suspension at 4 °C for 30 min. Following the incubation, wash the cells twice with 10 ml of FACS buffer as described in step 2.13.
4. Resuspend cells to achieve a final concentration of 4×10^7 cells/40 μ l of FACS buffer. Add 20 μ l of goat anti-rat IgG magnetic microbeads per each 40 μ l of cell suspension. Mix gently and incubate at 4 °C for 30 min. Wash cells with 10 ml of FACS buffer as described in step 2.13.
5. Resuspend up to 10^8 bone marrow cells in 500 μ l of FACS buffer.
6. Load a MACS LD column onto a MidiMACS cell separator per the manufacturer's instructions and prerinse the column with 2 ml FACS buffer.
7. Apply the bone marrow cell suspension to the column, loading up to 5×10^8 cells in 2.5 ml of FACS buffer. Ensure a collection tube (15 ml conical tube) is placed under the column. Wash the column 3x with 2 ml of FACS buffer/wash, and collect the cells that pass through the column, which will be enriched for lineage-negative cells. Count the cells in the material eluted (wash-through) from the column.
8. To perform positive selection of hematopoietic progenitor subsets by FACS, stain cells with the following fluorescently conjugated Abs: IL-7R (Pacific Blue), Flt3 (PE), Sca-1 (PE.Cy7), CD115 (APC), c-kit (APC.Cy7) Abs, plus a mixture of lineage marker Abs directed against CD3, CD19, CD11c, CD11b, F4/80 and Ter119 (all labeled with PerCP Cy5.5). Use 0.5-1 μ l of each Ab in a total volume of 100 μ l. Incubate cell suspension at 4 °C for 20-30 min.
9. Wash cells as indicated in step 2.13 and resuspend in FACS buffer at a concentration of $2-3 \times 10^7$ cells/ml. Filter cells through a 35 μ m cell strainer cap tube to remove cell clumps. This last step is crucial to avoid clogging the FACS machine.
10. Place the cell suspension in a FACS tube on the FACS stage and sort CDPs ($\text{lin}^- \text{c-kit}^+ \text{CD115}^+ \text{Flt3}^+$) on the basis of the indicated marker profile. Collect purified cells in a 15 ml tube containing 5 ml of complete RPMI.
11. Record the absolute number of sorted cells at the end of the FACS run. Pellet the cells by centrifugation as indicated in step 2.13 and resuspend in sterile PBS for adoptive transfer experiments.

4. Adoptive transfer of CDPs

This step is typically done in the animal facility where the recipient mice are housed. Depending on the location of the FACS machine, it may involve transport of the purified progenitor cell populations into the animal facility prior to adoptive transfer. Progenitor cell suspensions should be kept sterile and transported on ice.

1. Prepare cell suspensions for injection by diluting 10^5 purified CDPs in a total volume of 100 μ l sterile PBS. Place cell suspension in a syringe attached to a 27 G needle.
2. Expose the recipient *lfnar*^{-/-} mice (CD45.2⁺) to a heating lamp for 5-10 min to achieve efficient injection via the tail vein.
3. Place the mouse in a restrainer device and disinfect its tail with 70% ethanol.
4. Inject 10^5 FACS-purified CDPs in 100 μ l PBS into the tail vein using a 27 G needle.
5. Monitor mice for 15-30 min to ensure there are no deleterious effects of the tail vein injection. Return mice to housing facility.

5. Isolation of PP and Measurement of pDC Amounts

1. At 7-10 days following adoptive transfer, euthanize the recipient mice. Gently open the mouse by dissection and expose the intestine.
2. Remove the entire intestine and place it on PBS-soaked paper towels. Collect all visible PPs along the wall of the small intestine using fine forceps and scissors. C57BL6 and *lfnar*^{-/-} mice typically have 5-10 PP/mouse, which are different from isolated lymphoid follicles found along the small intestine¹⁸. Note that the PPs are often structurally distinct even within a single mouse, so carefully ensure all PPs are identified and dissected.
3. Place PPs in a Petri dish containing PBS, and use forceps to remove feces. Repeat this procedure twice to clean PPs.
4. Digest PPs with 1 mg/ml collagenase IV in 10 ml 1x Hank's balanced salt solution (HBSS) in a 50 ml flask with vigorous stirring for 1 hr at 37 °C.
5. Place the digested PP in the top compartment of a 40 μ m cell strainer and force cells through strainer with a sterile syringe plunger. Collect the PP cell suspension in a 15 ml conical tube.
6. Pellet strained PP cells by centrifugation at 500 x g for 5 min and resuspend in 6 ml of 37% Percoll solution (37% Percoll in RPMI medium). Gently place 6 ml of 70% Percoll solution (70% Percoll in RPMI medium) underneath the cell suspension, to form a Percoll step gradient.
7. Centrifuge cells for 20 min at 800 x g with the centrifuge break off. Mononuclear cells will migrate to the 37/70% interface. After centrifugation, the mononuclear cell population should be collected by careful pipetting at the interface region. Pellet collected cells by centrifugation and wash twice in 50 ml of complete RPMI/wash. A large volume is used to ensure complete Percoll removal.
8. Stain the collected PP cells with the following antibodies to detect murine pDCs that derive from the adoptively transferred progenitors (CD45.1⁺) or recipient mice (CD45.2⁺): CD45.1 (APC.Cy7), CD45.2 (PE.Cy7), CD11c (Pacific Blue), CD11b (PerCP Cy5.5), B220 (APC), Siglec-H (PE) and PDCA-1 (FITC) Abs. Perform flow cytometry analysis of PP cells as indicated in steps 3.9 and 3.10. Identify pDCs by their CD11c⁺ CD11b⁻ B220⁺ Siglec-H⁺ PDCA-1⁺ phenotype. Absolute pDC numbers can be determined by the following equation: % pDCs x total PP cell number = PP pDCs.

Representative Results

Our results show the gating strategy for the isolation of CDPs from mouse bone marrow cells (**Figure 1**), as detailed in Protocols 2 and 3. CDPs comprise approximately 0.1% of total bone marrow cells, and roughly $4-6 \times 10^4$ CDPs can be isolated from one mouse. Upon adoptive transfer, CDPs differentiate into pDCs and cDCs¹⁰.

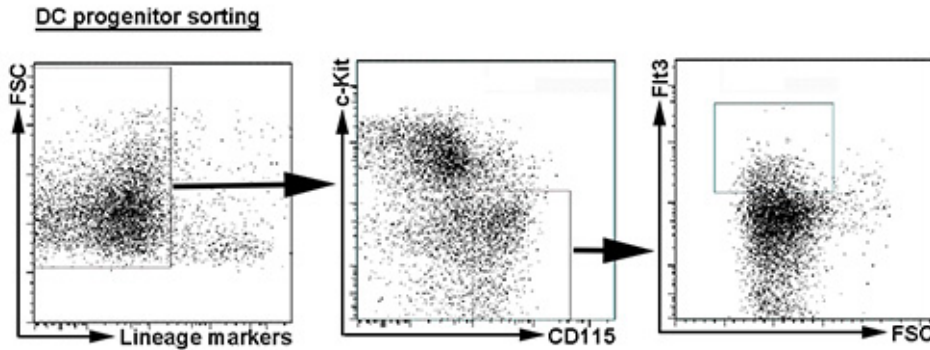


Figure 1. Gating strategy for FACS purification of CDPs. Bone marrow cells were collected from C57BL6 mice. Lineage-positive cells were depleted with Abs against lineage markers (CD3, CD19, CD11b, CD11c, Ter119) using MACS microbead-mediated selection. The enriched lineage-negative bone marrow population was stained with fluorescently-labeled Abs for CDP and lineage markers, and purified by FACS as shown. [Please click here to view a larger version of this figure.](#)

To identify pDCs in PPs, we use an initial forward and side scatter gating strategy, followed by gating for CD11c⁺ Siglec-H⁺ cells (**Figures 2A** and **2B**). *Ifnar*^{-/-} mice have a significant reduction in pDCs in PPs relative to wild type mice (**Figure 2B**)⁵. By contrast, CD11c⁺ Siglec-H⁺ cDCs are found at similar amounts in both genotypes (**Figure 2B**). Hence, *Ifnar*^{-/-} mice provide an opportunity to examine PP pDC reconstitution without effects of lethal irradiation⁵. For example, the adoptive transfer of wild type CDPs into *Ifnar*^{-/-} mice, as described in step 4, stimulates an increase in PP pDCs (**Figure 2B**). Moreover, pretreatment with Flt3L HGT (step 1) further enhanced pDC expansion in PPs (**Figure 2B**), implying transferred CDPs and possibly endogenous Flt3⁺ progenitors respond to Flt3L by inducing PP pDCs. Both conditions also stimulated CD11c⁺ Siglec-H⁺ cDCs amounts, consistent with the developmental origin of cDCs⁶. pDCs in PPs express traditional pDC markers including PDCA-1, Siglec-H and B220, and lack CD11b (**Figures 2B-D**)^{4,5}. In addition, analysis of PPs from *Ifnar*^{-/-} mice that received both Flt3L HGT and transferred CDPs showed that the majority (~70%) of pDCs were derived from donor (CD45.1⁺) mice (**Figure 2E**). Collectively, these data demonstrate that adoptive transfer of CDPs induces the PP pDC population in response to Flt3L-mediated signals *in vivo*.

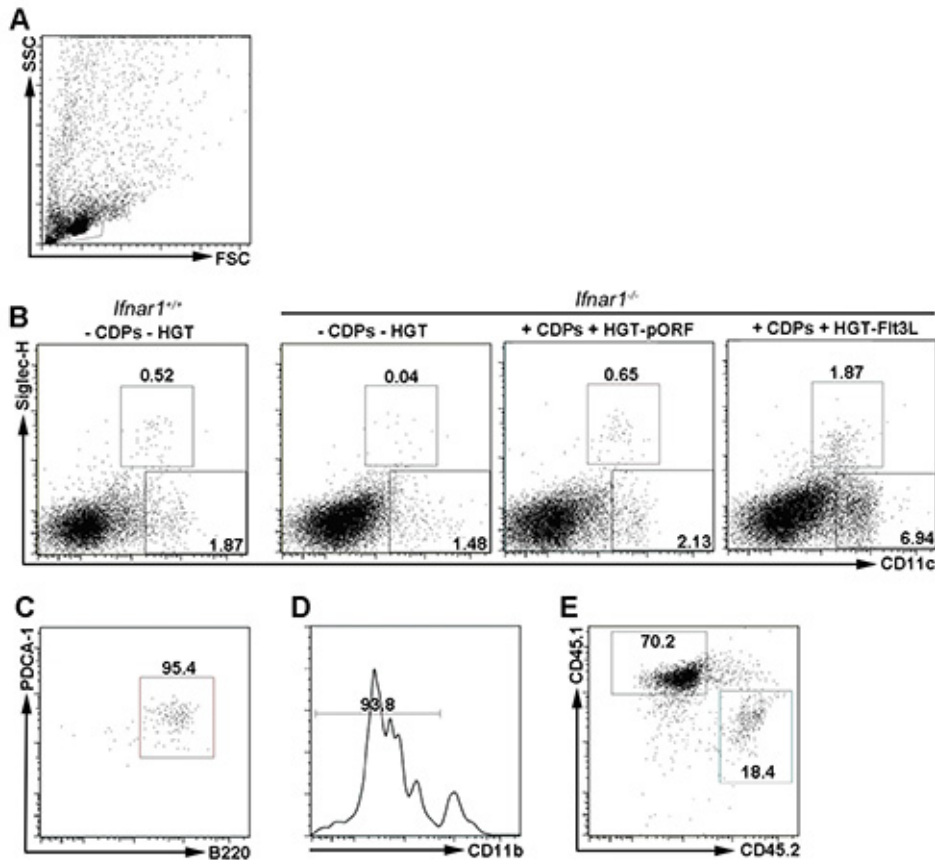


Figure 2. Analysis of PP pDC in *Ifnar1*^{-/-} mice upon Flt3L HGT and adoptive transfer of CDPs. *Ifnar1*^{-/-} mice were injected intravenously with 5 µg of plasmid encoding Flt3L or an empty vector (pORF) by HGT. Two days later, 10⁵ FACS-purified CDPs were adoptively transferred via tail vein injection. Seven days post CDP transfer, PPs were collected and analyzed for pDC amounts (**A**, **B**). CD11c⁺ Siglec-H⁺ pDCs in mice that received CDPs + Flt3L HGT were further analyzed for PDCA-1, B220 (**C**), CD11b (**D**), CD45.1 and CD45.2 (**E**) expression. The expression pattern of PDCA-1, B220 and CD11b was similar in pDCs in all 3 groups (data not shown). [Please click here to view a larger version of this figure.](#)

Discussion

The adoptive transfer technique described herein assessed the contribution of CDPs to the PP pDC population in recipient mice that are deficient in PP pDCs (e.g. *Ifnar1*^{-/-} mice). In future experiments, it will be important to evaluate the potential of other progenitor subsets in generating PP pDCs, in particular whether PP pDCs derive from Flt3⁺ CLPs. This question is significant since it remains unclear why PP pDCs are uniquely sensitive to IFNAR-STAT1 signals for PP accrual⁵, and if PP pDCs follow similar developmental cues as pDCs in other organs.

In theory, this method might also be performed in mice that lack pDC populations in all organs. For example, mice with deficiency in the transcription factor E2-2, the pDC "master regulator" (i.e. *Tcf4*^{-/-} mice), show striking pDC depletion¹⁹. However, *Tcf4*^{-/-} mice are embryonic lethal, and thus *Tcf4*^{-/-} bone marrow chimeric mice would need to be used as recipients for adoptive transfer experiments. However, it remains unknown whether PP pDCs are sensitive to irradiation and would be effectively depleted in *Tcf4*^{-/-} chimeras. Moreover, lethal irradiation has wide effects on the hematopoietic system and supporting stromal populations that might impact PP pDC reconstitution. Thus, the use of *Ifnar1*^{-/-} mice as recipients to assess the capability of adoptively transferred progenitor subsets to generate PP pDCs might be preferred to *Tcf4*^{-/-} chimeras. *Stat1*^{-/-} mice also show a striking reduction in PP pDCs and could be employed as recipients for adoptive transfer studies to study PP pDC developmental origins⁵. A caveat to the use of *Ifnar1*^{-/-} or *Stat1*^{-/-} mice is their immunodeficient status, which could impact PP pDC reconstitution or function in unknown ways. Thus, independent approaches to assess PP pDC developmental origins would enhance confidence in data interpretation.

Technically, it should be noted that the CDP population is found at a very low frequency in total bone marrow, thus depletion of lineage marker-positive cells by magnetic bead-mediated column chromatography prior to FACS is important for efficient purification of the progenitor cells by FACS. This depletion step enriches lineage-negative cells, resulting in decreased FACS time (and cost) for progenitor purification. The depleting procedure described herein utilizes a mixture of lineage-specific antibodies that is combined for each experiment. Commercial lineage antibody cocktails are also available and can be used for removal of lineage-positive cells, in place of the mixture that we describe. The advantage of the described approach is its flexibility in being adaptable for different depletion purposes, by adjusting the antibodies that are present in the mixture.

In the preparation of single cell suspensions from PPs, it is important to remove as much of the intestinal tissue surrounding the PPs as possible. This can be accomplished by careful dissection of the PPs. Sufficient digestion of PPs with collagenase is a key step to release leukocytes from the intestinal tissue. The Percoll density-gradient centrifugation technique described is a preferred method to enrich leukocytes from digested PP

samples. In addition, it is important to note that the pDC marker protein PDCA-1 may be regulated by type I IFN and other stimuli²⁰. Therefore, Siglec-H is preferred as a pDC marker for studies involving cytokine manipulation.

The use of HGT has a clear advantage in terms of being highly cost effective. While Flt3L HGT was utilized in this study, the ability of other cytokines or soluble factors to regulate PP pDCs could be tested using HGT, in the absence or presence of adoptive cell transfer of hematopoietic progenitors. However, HGT results in sustained production of cytokines from the transferred plasmid versus the more transient increases observed during recombinant cytokine injection, which depend on cytokine half-life^{5,13}. The extended elevation of circulating cytokine may not reflect physiological amounts achieved during emergency hematopoietic or infection responses. This caveat should be kept in mind during experimental planning stages and assessment of data.

In future work, selective manipulation of the PP pDC population, as described herein, may aid in addressing PP pDC function. PP pDCs are conditioned by mediators present in the mucosal environment and are deficient in type I IFN production upon TLR activation^{4,5}. Analysis of PP pDCs reconstituted within *Stat1*^{-/-} mice demonstrated these cells have a comparably low ability to induce type I IFN upon TLR9 triggering relative to PP pDCs arising naturally (not shown), indicating PP pDCs derived from transferred CDPs retain at least this property of natural PP pDCs. The reduced type I IFN production of PP pDCs contrasts with the robust type I IFN secretion of other pDC populations and raises a question regarding the functional role of PP pDCs. Moreover, PP pDCs resemble pDCs that develop in the presence of type I IFN, a pDC population that demonstrates efficient stimulation of IL-17-producing CD4⁺ T lymphocytes (Th17 cells)⁵. While Th17 cells are widely considered to be an inflammatory-inducing population, they have both protective and pathogenic roles in the gut²¹. Separately, pDCs have been reported to mediate systemic tolerance to orally administered antigen¹⁵. The role of PP pDCs in local and systemic immunity is of significant interest, as understanding this point may reveal new approaches to manipulate intestinal immune and inflammatory responses in disease therapy.

In conclusion, the method presented herein enables the assessment of the developmental potential of hematopoietic progenitor subsets for generating PP pDCs. This procedure provides mice with reconstituted PP pDCs. Thus, this approach may be used not only for evaluating PP pDC hematopoietic origins but also for understanding the contribution of PP pDCs to immune functions within the intestinal environment.

Disclosures

The authors declare no competing financial interests.

Acknowledgements

We thank Drs. Alex Gelbard and Willem Overwijk for advice on hydrodynamic gene transfer. This work was supported by grants from the NIH (AI098099, SSW), the MD Anderson Center for Cancer Epigenetics, the MD Anderson Center for Inflammation and Cancer (SSW), and the R.E. Bob Smith Education Fund (HSL).

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