Use of interleukin 7 receptor-α knockout donor cells demonstrates the lymphoid independence of dendritic cells

Satoshi Takeuchi and Stephen I. Katz

The precise lineage of dendritic cells (DCs), including skin Langerhans cells (LCs), is unclear. Interleukin 7 (IL-7) and its receptor (IL-7Rα) are known to mediate lymphopoiesis, and IL-7 is also known to be essential for the generation of DCs from lymphoid-committed precursors in vitro. Thus, to determine the developmental lymphoid (or IL-7Rα) dependency of various DCs and to examine the importance of IL-7/IL-7Rα for DC development in vivo, we used IL-7Rα knockout (KO) donor cells to reconstitute DCs/LCs in sublethally irradiated recipients and compared the results to those obtained using wild-type (WT) donor cells. We found that lymphoid lineage cells (except natural killer [NK] cells), including thymocytes, were less efficiently reconstituted by IL-7Rα KO donor cells, whereas myeloid lineage cells and DCs/LCs were equally well reconstituted by both the IL-7Rα KO and WT donor cells. Overall, we conclude that IL-7Rα is not required for the development of DCs/LC in vivo.

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Study design

All monoclonal antibodies (mAbs) were purchased from BD Biosciences PharMingen (San Diego, CA) except for the anti–PDCA-1 antibody, which was purchased from Miltenyi Biotec (Auburn, CA). Propidium iodide (PI), deoxyribonuclease I (DNase I), ammonium thiocyanate, and olive oil were from Sigma-Aldrich (St Louis, MO); trinitrochlorobenzene (TNCB) was from Polyscience (Warrington, PA); 1 × phosphate-buffered saline (PBS) and 1 × Hanks balanced salt solution (HBSS) were from Invitrogen (Grand Island, NY); fetal bovine serum (FBS) was from Gemini Bio-Products (Woodland, CA); trypsin was from USB (Cleveland, OH); and ACK lysing buffer and 0.5 M EDTA were from Quality Biological (Gaithersburg, MD).

Animals and BM reconstitution protocol

IL-7Rα KO mice (C57BL/6 background: CD45.2) were purchased from the Jackson Laboratory (Bar Harbor, ME) and C57BL/6 (WT control: CD45.2) and B6-Ly5.2/Cr (recipient: CD45.1) mice from the NCI/Frederick Cancer Research and Development Center (FCRDC; Frederick, MD). Recipient mice were x-irradiated at a dose of 7.6 Gy to 24 hours prior to intravenous transfer of 1 × 10⁶ BM cells/recipient. One ear of each recipient mouse was painted with 20 μL of a 1.5% TNCB solution (in 4:1 of acetone-olive oil) immediately after cell transfer to enhance EN granzyme. The other ear was left unpainted. Recipient BM, thymi, spleens, peripheral lymph nodes (pLNs); mixture of axillary and superficial inguinal LNs, hereafter called pLNs), and epidermis were assessed for chimerism of various cell types 4 and 8 weeks after reconstitution with IL-7Rα KO or WT donor BM cells.
and found to be reconstituted by IL-7Rα KO donor cells as well as by WT donor cells (Supplemental Figure S3).

IL-7Rα KO donor cells reconstitute LCs but not other epidermal leukocytes

IL-7Rα KO donor cells were able to give rise to LCs at 4 weeks after reconstitution as demonstrated in epidermal sheets prepared from irritated (TNCB-painted) ears (Figure 2A). No significant difference was found when donor cells came from IL-7Rα KO rather than from WT mice (Figure 2B and Supplemental Figure S3). In accordance with previous reports,9,20 epidermal γδ T cells were not reconstituted by either IL-7Rα KO or WT donor BM, even in irritated ears (Figure 2C). The major γδ T-cell population seen in irritated ears was of donor origin when WT donor cells were used and was of host (also WT) origin when IL-7Rα KO donor cells were used (Figure 2D). These γδ T cells are known to populate the epidermis of irradiated recipient mice,21,22 although usually at a later time period after reconstitution.

In the current study, lymphoid lineage cells (except for NK cells), including thymocytes, are less efficiently reconstituted by IL-7Rα KO donor cells as compared to WT donor cells, whereas myeloid lineage cells are well reconstituted by both the KO and WT donor cells, thereby validating this model. In recipient spleen, peripheral LNs and thymus, both CD8α+ and CD11b+ DC subsets are well reconstituted by both KO and WT donor cells at both 4 and 8 weeks after reconstitution, suggesting that

Results and discussion

IL-7Rα KO cells reconstitute various DC subsets

To determine the extent of chimerism at 4 and 8 weeks by WT or IL-7Rα KO donor cells, flow cytometric analysis was performed using a combination of antibodies with donor (CD45.2) or host (CD45.1) cell markers (Supplemental Figure S1, available at the Blood website; click on the Supplemental Figures link at the top of the online article). All the myeloid lineage cells such as polymorphonuclear (PMN) cells or monocytes in BM and spleen were well reconstituted, whereas lymphoid lineage cells, such as T cells, B cells, thymocytes (except for natural killer [NK] cells and thymic precursor populations) were poorly reconstituted by IL-7Rα KO donors, as compared to those reconstituted by WT donor cells. Various splenic and LN-DC and BM-plasmacytoid DCs18 were examined using the DC marker CD11c along with CD11b, CD8α, or B220 mAbs (Supplemental Figure S2). IL-7Rα KO donor cells reconstituted these populations as well as did the WT donor cells (Figure 1).

In another set of experiments, CD8α+ or CD11b+ thymic DCs and pLN, splenic, and thymic-plasmacytoid DCs were also examined using a more specific anti-PDCA-1 mAb (Miltenyi Biotec) and found to be reconstituted by IL-7Rα KO donor cells as well as by WT donor cells (Supplemental Figure S3).

Statistical analysis

Mean values and standard deviation (SD) calculation, graph production, and statistical evaluation (Student t test with 2-tailed and 2 sample unequal distribution method) were performed using CA-Cricket Graph III version 1.5.3 (Computer Associates International, Islandia, NY) and Microsoft Excel X for Mac (Microsoft, Seattle, WA). P values of less than .05 were considered significant.

Preparation of single-cell suspensions from various lymphoid organs and skin, and of epidermal sheets

BM cells were collected by flushing mouse tibia and fibulae with HBSS media supplemented with 5% fetal calf serum (FCS). Spleen, thymus, or pLNs were mashed on a 100-μm mesh spleen filter, suspended in HBSS containing 5% FCS and treated with ACK lysing buffer. Epidermal-cell suspensions and epidermal sheets were prepared as previously described.17 All cell suspensions prepared from recipient BM, thymus, spleen, pLNs, or epidermis were washed with PBS with 5% FCS containing 2 mM EDTA once before use. For flow cytometric analysis, cells were stained with various antibodies for 20 minutes at 4°C after Fc blocking and were assessed using a Becton Dickinson FACS Calibur flow cytometer and analyzed using CELLQuest software version 3.3 (San Jose, CA).

Figure 1. Examination of lymphoid (or IL-7Rα) dependency of splenic and pLN DC subsets and BM-plasmacytoid DCs. There was no significant difference in the chimerism of any DC subset examined when recipient mice were reconstituted by WT and IL-7Rα KO (Δ)donor cells and were compared at both 4 and 8 weeks after reconstitution (n = 4 each).

Figure 2. Lack of IL-7Rα does not affect the engraftment of LCs but does affect engraftment of epidermal γδ T cells. (A) IL-7Rα KO donor cells gave rise to epidermal LCs (major histocompatibility complex class II [MHC II]+ and CD45.2+) seen among host-derived LCs (MHC II single positive). Green is MHC II; red, CD45.2 (donor marker). Original magnification, × 20 for all images. Images were visualized under a Nikon TE300 microscope equipped with a 20 × 0.75 objective lens (Nikon, Tokyo, Japan). A Coolscan Pro digital camera was used to capture images (Roper Scientific, Duluth, GA), and IPlab software (Scanalytics, Fairfax, VA) was used to process them. Adobe Photoshop 7.0 software (Adobe, San Jose, CA) was used for subsequent image processing. (B-D) There was no significant difference in the chimerism of epidermal LCs when WT- and IL-7Rα KO–reconstituted mice are compared at both 4 and 8 weeks after reconstitution (B). There was little to no reconstitution of γδ T cells (C). (D) The major source for engrafted epidermal γδ T cells was of donor origin when WT donor cells were used and of host origin when IL-7Rα KO donor cells were used (n = 4 each).
IL-7Rα is not required for the development of these DCs in these organs and indicating that these DC subsets are not lymphoid dependent.

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We also assessed the potential lymphoid (IL-7Rα) dependency of LCs. LCs are derived from BM and are renewed from lymphoid dependent. LC engraftment and the extent of LC chimerism did not differ when WT and IL-7Rα KO donor cells were compared, thus negating the possibility that LCs are lymphoid dependent.

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References

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