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Development of CD8α-Positive Dendritic Cells from a Common Myeloid Progenitor

David Traver,[1]* Koichi Akashi,[2]* Markus Manz,[1]*† Miriam Merad,[1] Toshihiro Miyamoto,[1] Edgar G. Engleman,[1] Irving L. Weissman[1]

Dendritic cells (DCs) are critical in both initiating adaptive immune responses and maintaining tolerance to self antigens. These apparently contradictory roles have been suggested to depend on different subsets of DCs that arise from either myeloid or lymphoid hematopoietic origins, respectively. Although DC expression of CD8α is attributed to a lymphoid origin, here we show that both CD8α$^+$ and CD8α$^-$ DCs can arise from clonogenic common myeloid progenitors in both thymus and spleen. Thus, expression of CD8α is not indicative of a lymphoid origin, and phenotypic and functional differences among DC subsets are likely to reflect maturation status rather than ontogeny.

Dendritic cells (DCs) are specialized antigen-presenting cells that both orchestrate specific immune responses (*1*, *2*) and delete potentially autoreactive T cells (*3*, *4*). These seemingly contradictory functions have been suggested to result from the actions of different DC subsets (*5*, *6*). Recently, murine DCs have been subdivided into at least two populations, myeloid and lymphoid, on the basis of anatomic localization (*7*, *8*), transplantation experiments (*3*), and cell surface phenotypes (*8*, *9*). DCs bearing the CD11c$^+$ MHCII$^+$ Mac-1$^+$ CD8α$^-$ cell surface markers (hereafter referred to as CD8α$^-$ DCs) can efficiently derive from myeloid precursors (*10*). Alternatively, DCs bearing the CD11c$^+$ MHCII$^+$ Mac-1$^-$ CD8α$^+$ cell surface phenotype (hereafter referred to as CD8α$^+$ DCs) can arise at low frequencies from thymic T cell progenitors (*3*). It has been proposed that myeloid DCs direct adaptive immune responses whereas lymphoid DCs eliminate self-reactive lymphocytes, suggesting that DC ontogeny underlies these largely nonoverlapping functions (*5*).

Both clonogenic common lymphoid progenitors [CLPs, ~0.02% of whole bone marrow (BM)] (*11*) and clonogenic common myeloid progenitors (CMPs, ~0.2% of whole BM) (*12*) have been isolated and respectively give rise to each of the major branches of the hematopoietic system (*13*). The transplantation of 2×10^4 CMPs (contained within 1×10^7 BM cells) into CD45 congenic mice (Fig. 1A) showed abundant DC reconstitution of recipient spleens, lymph nodes (LNs), and thymi. Recipient spleens showed an average of 1×10^4 CD8α$^-$ DCs and 8×10^4 CD8α$^+$ DCs at 17 days after transplantation (Fig. 1, B and C), the numerical peak for CMP-derived DCs. Recipient thymi contained an average of 2×10^3 CMP-derived DCs, all of which were CD8α$^+$ (Fig. 2E).

CMP-derived CD8α$^+$ DCs expressed the costimulatory markers characteristic of active DCs, including CD40, CD80, CD86, and Dec205 (Fig. 2, A to D). CMP-derived

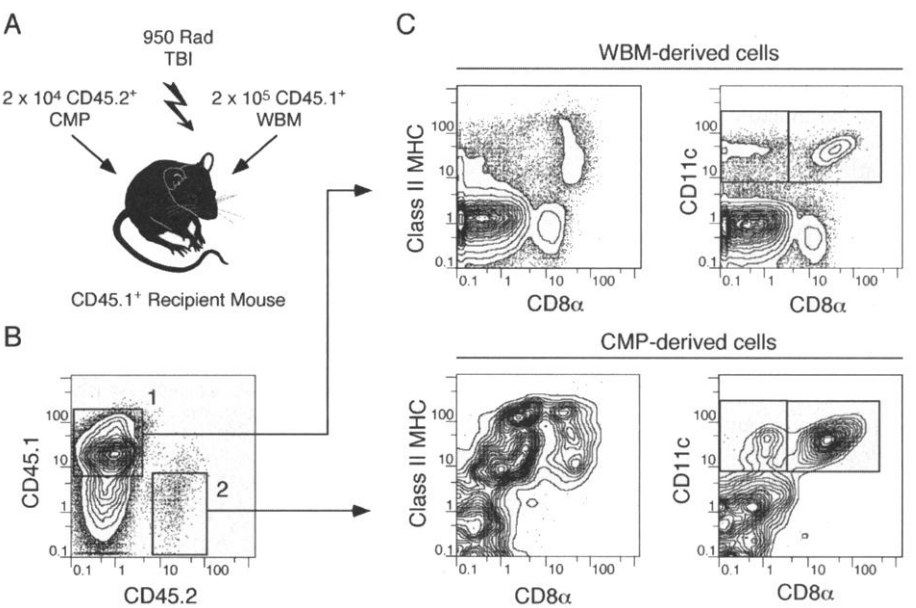

Fig. 1. Common myeloid progenitors (CMPs) give rise to CD8α$^+$ DCs. (**A**) CMPs were stained, sorted, and competitively transplanted into lethally irradiated CD45 congenic animals along with host-type whole bone marrow (WBM) as described (*12*). (**B**) CD45 expression was used to gate on host-type (CD45.1) or donor-type (CD45.2) splenocytes at 17 days after transplantation. (**C**) Analysis of DC phenotype arising from WBM (gate 1) or CMPs (gate 2).

[1]Department of Pathology and Department of Developmental Biology, Stanford University, Stanford, CA 94305, USA. [2]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02115, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: manz@stanford.edu

CD8α$^-$ DCs showed similar profiles. CD8α$^-$ DCs, however, showed prominent expression of Mac-1, whereas CD8α$^+$ DCs were Mac-1$^-$ (Fig. 2F). For morphological examination, splenic CMP-derived CD8α$^-$ and CD8α$^+$ DCs were cultured overnight (*14*) and then cytocentrifuged. Both CD8α$^+$ (Fig. 2G) and CD8α$^-$ subclasses displayed the nuclear, cytoplasmic, and stellate characteristics of DCs (*8*).

DCs can process and present antigens acquired from engulfed apoptotic cells (*15*). We were thus concerned that the CD8α staining on CMP-derived DCs might have been acquired. To examine this possibility, we transplanted wild-type (WT) CMPs into CD8-deficient mice (*16*). DCs derived from endogenous host progenitors did not express CD8α, whereas DCs derived from transplanted WT CMPs showed both CD8α$^-$ and CD8α$^+$ subsets (Fig. 3A). Accordingly, CD8α$^+$ but not CD8α$^-$ DCs sorted from WT mice showed transcription of the CD8α gene (Fig. 3B). Neither DC subset was contaminated by T cells, as evidenced by lack of CD3ε expression (Fig. 3B).

Functionally, CMP-derived CD8α$^+$ DCs appeared normal, because they were able to stimulate antigen-specific T cells, produce stimulatory cytokines, and initiate the mixed leukocyte reaction (MLR) (*17*). In addition, they expressed molecules preferentially expressed by DCs such as interleukin-12 (IL-12) (*2*, *17*), Epstein-Barr virus–induced molecule 1 ligand chemokine (ELC) (*18*), and the class II activator (CIITA) protein (*19*) (Fig. 3B).

The absence of CD8α$^-$ and the presence of CD8α$^+$ DCs in *RelB* (*20*) and *PU.1* (*21*) knockout mice has been taken as evidence to support the lymphoid origins of DCs and has led to the proposal that these gene functions are required for the development of myeloid but not lymphoid DCs (*20*, *21*). We found, however, that *RelB* and *PU.1* were expressed in both CD8α$^+$ and CD8α$^-$ DCs (Fig. 3B). Additionally, a different *PU.1* knockout mouse lacks all DC subsets (*22*). Although DCs are required for the efficient production of effector lymphocytes, lymphocyte deficiencies likewise perturb normal DC development (*23*, *24*), suggesting that environmental defects in mutant mice may be responsible for altered DC phenotypes. The results presented here confirm the thesis that the most direct test of lineage relationships is the isolation and transfer of marked, clonal lineage–restricted progenitors, because progenitor/progeny relationships are rarely resolved from targeted deletions (*25*).

The best evidence for a lymphoid pathway of DC development thus comes from transplantation experiments with lymphoid-restricted progenitors. Early thymocyte progenitors can give rise to normal ratios of T cells and DCs when transplanted back into irradiated thymi (*3*). To compare the DC reconstitution potentials of CMPs and CLPs, the earliest lymphoid precursor in mouse BM, we performed several transplants of each population into CD45 congenic recipients. Transplantation of 1 × 10^3 to 2 × 10^3 CLPs (contained within 1 × 10^7 BM cells) resulted in the generation of ~2 × 10^7 splenic B and T lymphocytes at 3 to 5 weeks after injection (*11*), but not in detectable numbers of DCs above background. Higher dose CLP transplants, however, gave rise to substantial numbers of both CD8α$^+$ and CD8α$^-$ DCs in the spleen, and CD8α$^+$ DCs in the thymus. In one such experiment, we transplanted 7 × 10^3 CLPs from CD45.1 C57Bl/Ka donors along with 20 × 10^3 CMPs from CD45.2 C57Bl/Ka donors and 450 hematopoietic

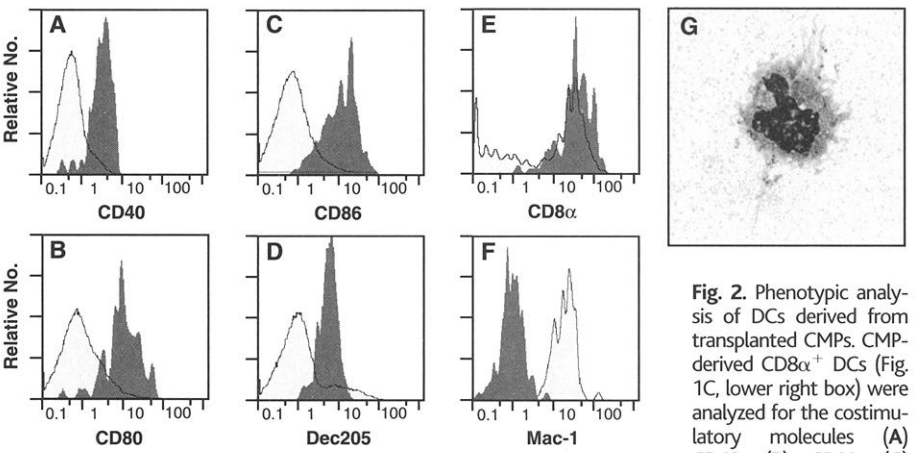

Fig. 2. Phenotypic analysis of DCs derived from transplanted CMPs. CMP-derived CD8α$^+$ DCs (Fig. 1C, lower right box) were analyzed for the costimulatory molecules (**A**) CD40, (**B**) CD80, (**C**) CD86, and (**D**) Dec205, which has previously been shown to be preferentially expressed on the CD8α$^+$ DC subset (*5*). CD8α$^+$ DCs were uniformly positive for all markers tested (filled histograms); stainings were compared to whole splenocytes as control (open histograms). (**E**) All CMP-derived thymic DCs expressed high levels of CD8α (filled histogram), whereas CMP-derived splenic DCs showed both CD8α$^+$ and CD8α$^-$ subsets (open histogram). (**F**) CD8α$^+$ DCs were found to be Mac-1$^{-/lo}$ (filled histogram), whereas CD8α$^-$ DCs were Mac-1$^+$ (open histogram). (**G**) CD8α$^+$ DCs derived from transplanted CMPs were double-sorted to purity and placed overnight in cultures containing GM-CSF (*14*). Cells were then cytocentrifuged and stained for morphology as described (*12*).

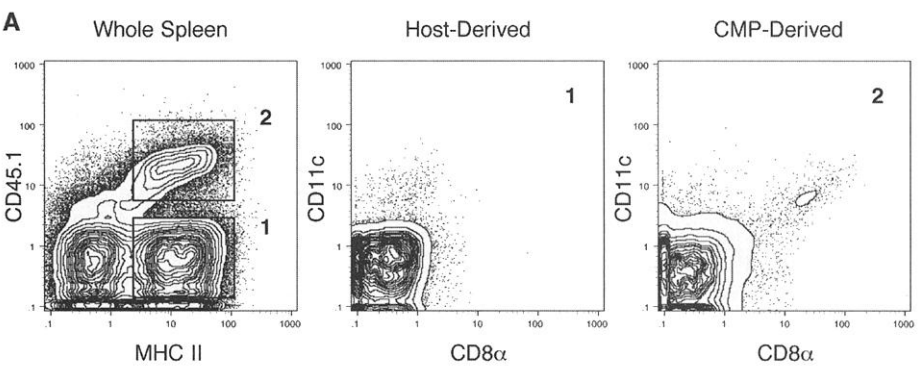

Fig. 3. CD8α is autonomously expressed by CMP-derived DCs. (**A**) CMPs were sorted from WT CD45.1$^+$ BM and transplanted into sublethally irradiated CD8$^{-/-}$ mice (*16*). Host-derived, CD45.1$^-$ cells (gate 1) did not express CD8α (middle), whereas the majority of CMP-derived (gate 2) CD45.1$^+$ MHCII$^+$ CD11c$^+$ DCs were CD8α positive (right). DC profiles are shown from unenriched, whole spleen. (**B**) Transcriptional profile of CMP-derived DCs. CD8α$^+$ DCs derived from transplanted CMPs expressed the *CD8α* gene, whereas CMP-derived CD8α$^-$ DCs did not. Neither population expressed the T cell–specific *CD3ε* gene. Both populations expressed the *CIITA*, *ELC*, *RelB*, and *PU.1* genes. Reverse transcriptase–polymerase chain reaction conditions were as described (*12*, *18*–*20*, *28*).

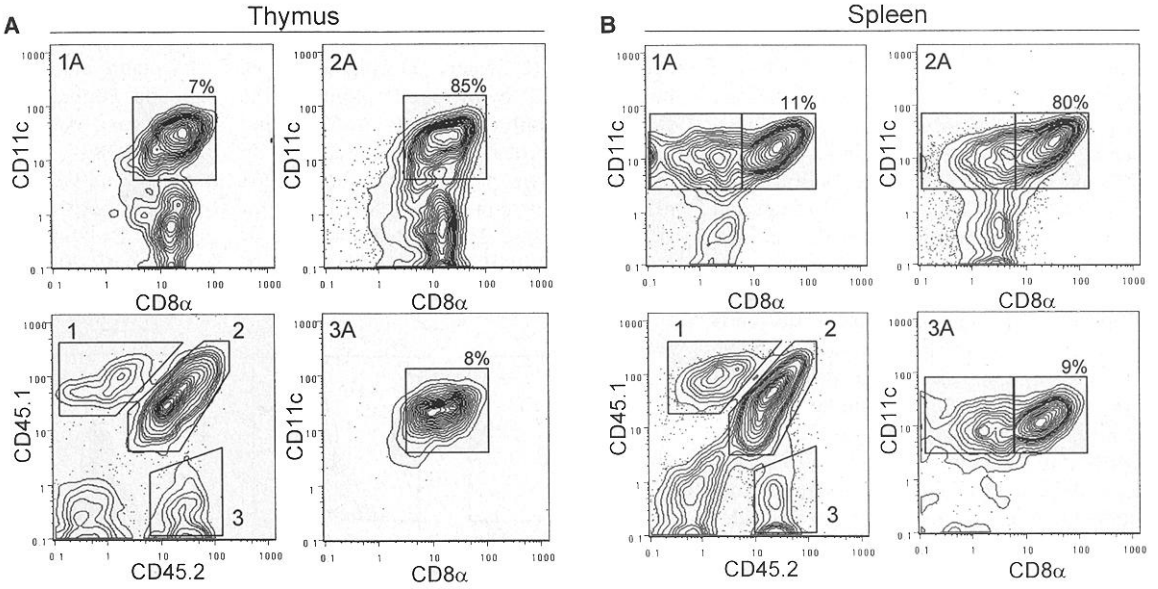

Fig. 4. Generation of thymic and splenic DCs from common myeloid and common lymphoid progenitors. CMPs ($CD45.2^+$) and CLPs ($CD45.1^+$) were stained, sorted, and competitively transplanted into lethally irradiated F_1 ($CD45.1^+ \times CD45.2^+$) animals along with host-type HSCs ($CD45.1^+ \times CD45.2^+$) (*13*). Lower left contour plots in (**A**) and (**B**) show $CD11c^+$-enriched (A) thymic and (B) splenic progeny of (1) 7×10^3 CLPs, (2) 450 host-type HSCs, and (3) 20×10^3 CMPs at 14 days after transplantation (*14*). Contour plots 1A, 2A, and 3A show DC phenotypes derived from (1) CLPs, (2) HSCs, or (3) CMPs, respectively. Percentages of total enriched DCs from each organ are given.

stem cells (HSCs) from (CD45.1 × CD45.2) F_1 C57Bl/Ka donors into lethally irradiated (CD45.1 × CD45.2) F_1 C57Bl/Ka hosts. In this experiment the mice were killed at 14 days, and $CD11c^+$-enriched populations of cells from the spleen and thymus were analyzed for donor origin (*14*). As shown in Fig. 4, about 85% of the $CD11c^+$ thymic cells were of HSC origin, about 7% from CLP donors, and about 8% from CMP donors. In the same mice, about 80% of the $CD11c^+$ spleen cells were of HSC origin, 11% of CLP origin, and 9% of CMP origin. Overall, on a per-cell basis, CLPs are roughly equivalent or slightly superior to CMPs in the formation of splenic DCs and are more effective in the generation of thymic DCs. However, CMPs are 10-fold more abundant than CLPs in whole BM. If DC development proceeds exclusively through either CMP or CLP stages, and if both CMPs and CLPs normally produce DCs at the rates measured by transplantation, then we calculate that the majority of all splenic DCs and about half of all thymic DCs should be derived from the myeloid lineage.

Although DCs can be derived from either myeloid or lymphoid precursors, our findings show that expression of CD8α is not a marker of lymphoid derivation, and that the majority of extrathymic DCs likely derive from a progenitor common to all myeloid lineages. A common progenitor for extrathymic DCs would thus imply that phenotypic and functional differences among these subsets are mandated by tissue microenvironments. In the thymus, DCs appear to derive equally from myeloid and lymphoid precursors. This finding may explain the ability of thymic DCs to develop in the complete absence of thymic T cell progenitors (*26*). Additionally, differing ontogenies of DCs may reflect functionally discrete roles and may explain the paradoxical ability of DCs to both activate specific immune responses and delete potentially autoreactive lymphocytes. Further resolution of these issues requires functional comparisons between authentic lymphoid and myeloid DCs.

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13. Thorough methodologies for staining and sorting CMPs, CLPs, and HSCs can be found at www.metazoa.com UPL 2001, UPL 2030, and UPL 2033, respectively.
14. DCs were isolated as described (*9*) from transplanted tissues and double-sorted to obtain pure splenic or thymic DCs for all functional assays. Splenic DCs were cultured overnight in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF, 10 ng/ml) (Peprotech). In some experiments (Fig. 4), DCs were positively selected with anti-CD11c (N418) MicroBeads (Miltenyi Biotec) over a magnetic column (MiniMACS, Miltenyi Biotec) according to the manufacturer's instructions.
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