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Dendritic cell ontogeny: A human dendritic cell lineage of myeloid origin

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ABSTRACT **Dendritic cells (DC) have been thought to represent a family of closely related cells with similar functions and developmental pathways. The best-characterized precursors are the epidermal Langerhans cells, which migrate to lymphoid organs and become activated DC in response to inflammatory stimuli. Here, we demonstrate that a large subset of DC in the T cell-dependent areas of human lymphoid organs are nonactivated cells and belong to a separate lineage that can be identified by high levels of the interleukin 3 receptor α chain (IL-3Rα^{hi}). The CD34$^+$IL-3Rα^{hi} DC progenitors are of myeloid origin and are distinct from those that give rise to Langerhans cells *in vitro*. The IL-3Rα^{hi} DC furthermore appear to migrate to lymphoid organs independently of inflammatory stimuli or foreign antigens. Thus, DC are heterogeneous with regard to function and ontogeny.**

Dendritic cells (DC) in lymphoid organs are potent antigen-presenting cells, which play an important role in the initiation of immune responses (1). Studies showing that epidermal Langerhans cells are precursors of DC have suggested that the unique role of DC as "natures adjuvant" is linked to their developmental pathway. Langerhans cells reside in the epidermis where the cells are capable of antigen uptake but have low ability for antigen presentation (2). In response to inflammatory signals, the cells migrate rapidly to lymphoid tissues and differentiate into mature, activated DC with potent ability for stimulation of T cells (3–6). Cells with characteristics of DC precursors have also been found in other tissues (7), and such cells also migrate in response to inflammatory mediators (6, 8). Thus, DC in lymphoid organs have been widely considered to represent the end stage of a stepwise differentiation and migration process, which is completed during inflammation and serves to initiate immune responses (9–12).

Presently, most of the knowledge about the developmental pathway of DC is based on results obtained by cell culture. Cells with characteristics of Langerhans cells and DC can be generated *in vitro* by culture of CD34$^+$ cells in the presence of granulocyte/macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor α (TNF-α) (13–19). Results from studies of *in vitro* colony formation have further indicated that the GM-CSF/TNF-α-responsive progenitors represent a separate DC colony forming cell (15). These and other observations have supported the view that DC are a family of closely related cells that constitute a distinct "DC lineage." However, progenitors committed to become DC have not yet been identified directly in bone marrow. The interpretation of results obtained by colony assays and cell culture is furthermore complicated by the fact that populations of lymphoid progenitors, granulomonocytic progenitors and peripheral blood monocytes also assume characteristics of DC *in vitro* (14, 18–22).

Primitive hematopoietic progenitors and cells committed to become lymphocytes, monocytes, granulocytes, and erythroid cells can be identified as discrete populations of freshly isolated CD34$^+$ bone marrow cells using specific cell surface markers (23–29). Similar characterization of DC progenitors has been difficult due to the lack of selective markers that identify the cells at an early stage of differentiation. In the present study, however, we demonstrate that antibodies to the interleukin 3 receptor α chain (IL-3Rα) selectively react with a large subset of DC in lymphoid organs and identify their precursors in blood and bone marrow. The CD34$^+$IL-3Rα^{hi} progenitors are of myeloid origin but committed to become DC and distinct from those that give rise to Langerhans cells. Unlike Langerhans cells, IL-3Rα^{hi} DC home to lymphoid tissue independently of inflammation or stimulation with foreign antigens.

MATERIALS AND METHODS

Tissue. Tissue from aborted fetuses of gestational age 19–21 weeks was obtained from Advanced Bioscience Resources (Alameda, CA), a nonprofit organization which provides tissue in compliance with state and federal laws. Blood donor buffy coats were obtained from the Stanford Blood Bank (Stanford, CA). Tonsils and adult lymph nodes were obtained from the tissue acquisition service and the clinical flow cytometry laboratory, Department of Pathology, University of Texas Southwestern Medical Center (Dallas).

Cell Preparation. Mononuclear cell suspensions were obtained by Lymphoprep gradient centrifugation (Nycomed, Oslo). Antibody-free CD3$^+$CD4$^+$ T cells were isolated to 99% purity from peripheral blood mononuclear cells (PBMC) using CD4 Dynabeads in combination with Detachabead reagent (Dynal, Oslo) after depletion of myeloid cells (CD14$^+$ and CD36$^+$) by Dynabeads (Dynal). Fetal bone marrow CD34$^+$ cells were isolated by positive immunomagnetic selection (Miltenyi Biotech, Auburn, CA), as described (29). Where noted, subsets of immunostained cells were sorted using a FACSVantage flow cytometer (Becton Dickinson).

Immunophenotypic Analysis. Multicolor immunofluorescence staining and analysis was performed by standard methods (see ref. 29). Primary or secondary antibodies were conjugated to biotin, fluorescein isothiocyanate (FITC), phycoerythrin (PE),

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Abbreviations: DC, dendritic cell; IL-3Rα, interleukin 3 receptor α chain; GM-CSF, granulocyte/macrophage colony-stimulating factor; G-CSF, granulocyte CSF; M-CSF, macrophage CSF; M-CSFR, M-CSF receptor; TNF-α, tumor necrosis factor α; PBMC, peripheral blood mononuclear cells; FITC, fluorescein isothiocyanate; PE, phycoerythrin; FACS, fluorescence-activated cell sorter.

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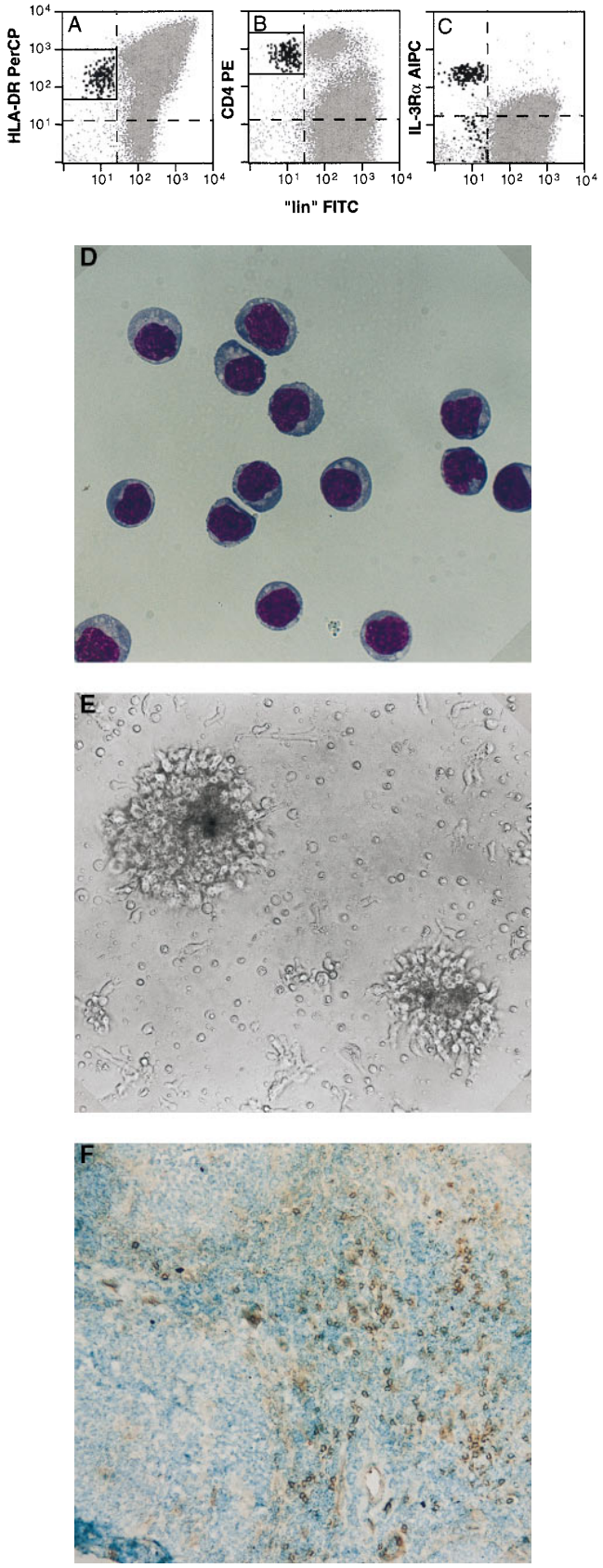

FIG. 1. Antibodies to IL-3Rα selectively stain DC in extrafollicular regions of human tonsils. (*A–C*) Tonsillar mononuclear cells were stained with anti-HLA-DR peridinin-chlorophyll protein (PerCP), CD4 PE, anti-IL-3Rα biotin + Streptavidin allo-phycocyanin (ALPC), and a mixture of FITC-conjugated lineage markers ("lin") for lymphocytes and monocytes (CD3, CD14, CD16, CD19, CD20, CD56, and goat-anti-human IgM). The cells were analyzed by four-color flow cytometry. Dendritic cells were identified as HLA-DR$^+$CD4$^+$lin$^-$—i.e., cells that simultaneously satisfy the criteria of the box regions in *A* and *B*, and are represented by large black dots. Dashed lines represent isotype control levels. (*D*) Wright–Giemsa staining of a cytocentrifuge slide of freshly FACS-sorted tonsillar HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells. (×600.) (*E*) FACS-sorted tonsillar HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells were cultured for 24 h with GM-CSF and IL-3 and photographed *in situ*. (×400.) (*F*) A frozen section of tonsillar tissue was stained with anti-IL-3Rα, biotinylated anti-mouse IgG and streptavidin peroxidase. (×100). Staining was visualized by diaminobenzidine and hydrogen-peroxide, and the section was counterstained with methylene blue.

Table 1. Expression of surface molecules on HLA-DR$^+$lin$^-$IL-3Rα^{hi}DC from mononuclear tonsillar cells, PBMC, and fetal lymph node (LN) cells, and on CD34$^+$IL-3Rα^{hi} cells from fetal bone marrow (BM)

	Tonsil	Fetal LN	PBMC	Fetal BM
CD1a	−	−	−	−
CD3	−	−	−	−
CD4	++/+++	++/+++	++	++
CD5	−	−	−	−
CD11b	−	−	−	−
CD11c	−	−	−	−
CD13	−/+	+	−/+	+
CD14	−	−	−	−
CD15	−	ND	−	−
CD16	−	−	−	−
CD19	−	−	−	−
CD20	−	−	−	−
CD32	+	+	+	+
CD33	+	+(+)	+(+)	+/++
CD34	−/+	+	−/+	++/+++
CD36	++/+++*	++/+++	++/+++	++/++++
CD40	++	+	++	+
CD45RA	++(+)	++(+)	++(+)	++
CD45RO	−	−	−	−
CD54	++/+++	ND	++/+++	+/++
CD56	−	−	−	−
CD58	+	ND	+/++	+/++
CD62L	−	+	+/++	+++
CD64	−	−	−	−
CD80	−	−	−	−
CD86	+	ND	+	+(+)
HLA-DR	++	++	++	++
HLA-DQ	−	ND	−/+	−

Cells were stained as described in legends to Figs. 1, 3, and 4. Mean fluorescence intensity (MFI) levels for the IL-3Rα^{hi} populations are expressed as −, indicating MFI in the first decade on a four log scale, which corresponds to isotype control levels. The +, ++, and +++ indicate MFI in the second, third, and fourth decades, respectively. A/sign means that MFI is on the border between two decades. Parenthesis means that the MFI is in the upper end of a decade. ND, not determined. The data are representative of at least three experiments.

*In one out of three experiments tonsillar HLA-DR$^+$lin$^-$IL-3Rα^{hi}DC were negative for CD36.

peridinin-chlorophyll protein, or allo-phycocyanin. Antibodies and streptavidin conjugates were from Becton Dickinson, except CD40, CD86 PE, anti-IL-3Rα biotin, and anti-IL-3Rα PE (PharMingen); CD64 (Meda Rex, West Lebanon, NH); anti-M-CSFR (Santa Cruz Biotechnology); donkey anti-rat IgG PE, goat-anti-mouse IgG PE, and goat-anti-human IgM FITC (Jackson ImmunoResearch). Cells were analyzed using a FACSCalibur flow cytometer (Becton Dickinson).

Media and Cytokines. Except where specifically noted, cells were cultured in Yssel's medium (30) supplemented with heat-inactivated 10% pooled human AB$^+$ serum and 10% fetal

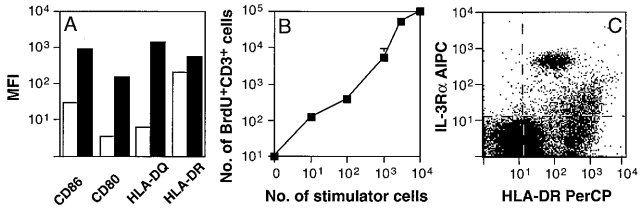

FIG. 2. IL-3Rα^{hi} DC are immature and appear in lymphoid organs independently of stimuli that cause up-regulation of major histocompatibility complex class II and costimulatory molecules. (*A*) Mononuclear cells from tonsil were stained with anti-HLA-DR, anti-IL-3Rα and lineage markers, and either CD86, CD80, HLA-DQ, or isotype control mAbs before (open bars) and after (filled bars) a 16-h incubation at 37°C in Yssel's medium (30). The HLA-DR$^+$lin$^-$IL-3Rα^{hi} population was analyzed for mean fluorescence intensity (MFI) staining with the markers indicated on the figure, and the bars represent MFI after isotype control levels were subtracted. (*B*) T cells (10^5) were cocultured with indicated numbers of allogeneic IL-3Rα^{hi}lin$^-$HLA-DR$^+$ cells from tonsil (stimulator cells). T cell proliferation was measured as the total number of CD3$^+$ BrdU$^+$ cells per well at day 6 of coculture. (*C*) Fetal lymph node cells were stained with anti-HLA-DR, anti-IL-3Rα, and lineage markers (data not shown) and analyzed by flow cytometry as described in Fig. 1 *A–C*. Data are representative of three experiments.

bovine serum. Recombinant human cytokines were used as noted at the following concentrations: GM-CSF (10 ng/ml), IL-3 (10 ng/ml), IL-6 (500 units/ml), IL-7 (50 ng/ml) (all from Collaborative Biomedical Products, Bedford, MA), erythropoietin (2.5 units/ml; CILAG, Schaffhausen, Switzerland), stem cell factor (40 ng/ml; Peprotech, Rocky Hill, NJ), granulocyte-CSF (G-CSF, 50 ng/ml, Amgen Biologicals), and macrophage-CSF (M-CSF, 10 ng/ml; R & D Systems).

Cell Cultures. IL-3Rα^{hi}lin$^-$HLA-DR$^+$ cells from tonsil and PBMC and CD14^{hi} monocytes from PBMC were sorted by fluorescence-activated cell sorter (FACS) and cultured for 36 h before mixing with T cells to allow maturation of DC precursors. GM-CSF and IL-3 were added to IL-3Rα^{hi}lin$^-$HLA-DR$^+$ cells to enhance survival. Stimulator cells were washed twice before coculture with CD4$^+$ T cells (10^5 per well) in flat-bottomed 96-well plates for 6 days. Bromodeoxyuridine (BrdU; Sigma) (50 μM) was added 12 h before harvest latex particles (10^5 per well) were added immediately prior to harvest as a reference for cell counts (29). The cells were stained with CD3 PE, followed by fixation, permeabilization, staining with anti-BrdU FITC, and flow cytometric analysis, as described (29). Cocultures with stimulator cells derived by culture of fetal bone marrow cells were pulsed with 1 μCi (1 Ci = 37 GBq) [^{3}H]thymidine for 8 h before collecting and counting.

RESULTS AND DISCUSSION

IL-3Rα Is a Selective Marker for a Large Subset of DC in T Cell-Rich Zones of Human Peripheral Lymphoid Organs. Human tonsils contain DC that can be identified as cells that lack lineage markers for monocytes and lymphocytes (lin$^-$) and are positive for HLA-DR and CD4 (HLA-DR$^+$CD4$^+$lin$^-$) (Fig. 1 *A* and *B*) (31, 32). In an attempt to identify DC-selective markers, we screened antibodies to leukocyte differentiation antigens for selective reactivity with this population. Antibodies to IL-3Rα reacted strongly with more than 85% of HLA-DR$^+$CD4$^+$lin$^-$ cells (0.32–0.37% of tonsillar mononuclear cells, $n = 3$), but weakly with most other cells ($n = 3$) (Fig. 1*C*). The staining was sufficiently specific to allow a 200-fold enrichment of HLA-DR$^+$CD4$^+$lin$^-$ cells with a single positive immuno-magnetic selection ($n = 2$). HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells were also found in adult cervical, axillar, intramammary, mesenteric, and femoral lymph nodes. The frequency ranged from 0.1%–1.8% (average 0.5%, $n = 9$). In all cases, cells staining brightly with the anti-IL-3Rα were found within the HLA-DR$^+$lin$^-$ population and constituted the majority of these cells (on average 60.2 ± 11.2%, $n = 9$).

Freshly sorted HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells showed an immature morphology without cytoplasmic protrusions (Fig. 1*D*). The majority of the cells died rapidly in culture, but could be partially rescued by the addition of cytokines (GM-CSF and IL-3). Under these conditions, the cells rapidly formed large aggregates of cells as previously reported for tonsillar DC (32) (Fig. 1*E*). After 3–5 days the cells were more dispersed and showed multiple long processes characteristic of DC (data not shown).

IL-3Rα^{hi} cells were found almost exclusively in the T cell-rich extra-follicular regions of the tonsil (Fig. 1*F*). The localization as well as the the cytokine requirements and phenotype of the HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells (Table 1) suggest that they are identical to the "plasmacytoid T cell" DC that were recently characterized by Grouard *et al.* (33).

IL-3Rα^{hi} DC Are Immature and Appear in Lymphoid Organs Independently of Stimuli That Cause Up-Regulation of Major Histocompatibility Complex (MHC) Class II and Costimulatory Molecules. Originally, it was concluded that DC in lymphoid tissues such as tonsils are activated, mature antigen-presenting cells, because isolated cells expressed high levels of MHC class II and costimulatory molecules (31, 32). However, in those studies the cells were isolated after 1–2 days of cell culture. As shown in Fig. 2*A* and Table 1, HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells in fresh preparations of tonsillar mononuclear cells expressed low levels of CD80 (B7.1), CD86 (B7.2), and HLA-DQ. Overnight culture of unseparated cells in the absence of cytokines was sufficient to induce the mature phenotype (Fig. 2*A*). Sorted HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells that were allowed to mature in culture for 36 h and kept viable with GM-CSF and IL-3 were potent stimulators of allogeneic CD4$^+$ T cells (Fig. 2*B*). These results are surprising in view of the hypothesis that DC migrate to lymphoid organs in response to signals that lead to cell activation. However, these data are in agreement with those of Grouard *et al.* (33), who also studied freshly isolated cells, and with previous reports

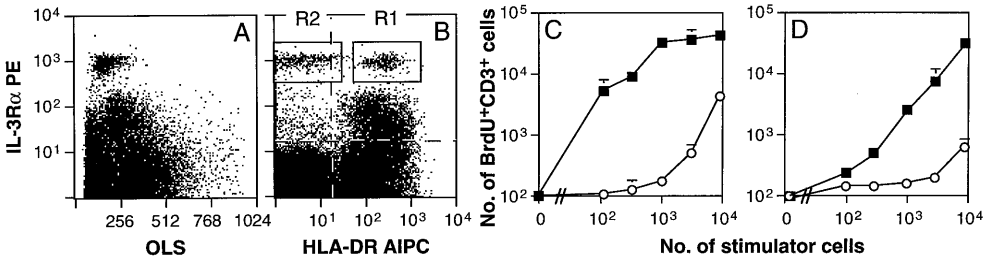

FIG. 3. HLA-DR$^+$lin$^-$IL-3Rα^{hi} DC are present in peripheral blood. (*A* and *B*) PBMC were stained with anti-HLA-DR, anti-IL-3Rα, and lineage markers (not shown) and analyzed by flow cytometry as described in Fig. 1 *A–C*. R1 and R2 in *B* represent regions used to sort IL-3Rα^{hi} blood cells that were positive or negative for HLA-DR, respectively. (*C* and *D*) HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells sorted from blood according to R1 in *B* were first cultured separately with IL-3 and GM-CSF for 36 h and then incubated with allogeneic (*C*) or autologous (*D*) CD4$^+$ T cells. T cell proliferation was measured as total number of CD3$^+$BrdU$^+$ cells per well at day 6 of coculture with indicated numbers of IL-3Rα^{hi}lin$^-$HLA-DR$^+$ cells (■) or CD14^{hi} monocytes (○) from the same donor (stimulator cells). Individual displays show data that are representative of three experiments. OLS, ortogonal light scatter.

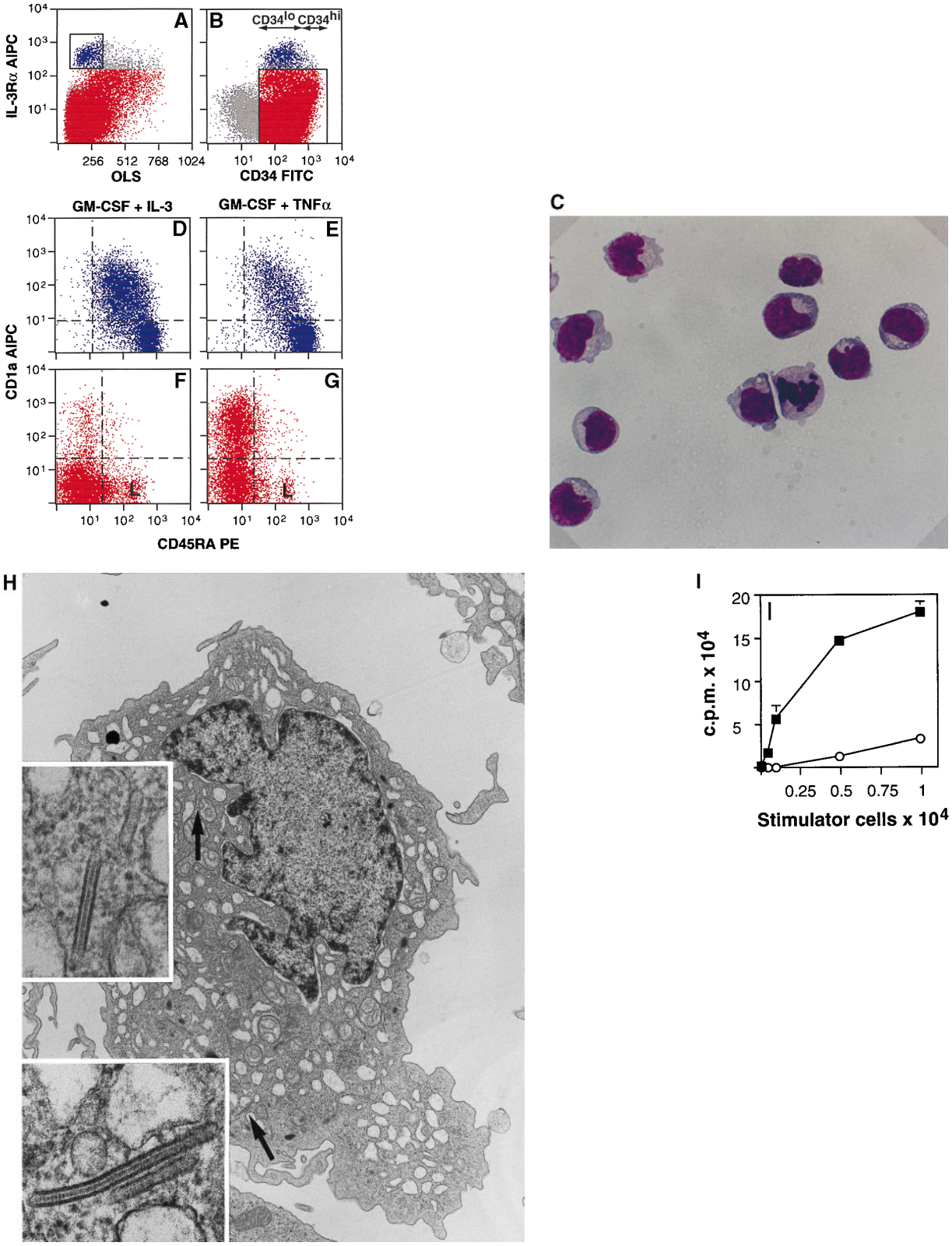

FIG. 4. Proliferating progenitors for IL-3Rα^{hi} DC are found as a discrete CD34$^+$IL-3Rα^{hi} population that is distinct from the cells that give rise to Langerhans cells in response to GM-CSF and TNF-α. (*A* and *B*) Isolated CD34$^+$ fetal bone marrow cells were stained with CD34 and anti-IL-3Rα. The cells were analyzed by flow cytometry as described in Fig. 1 *A–C*. The CD34$^+$IL-3Rα^{hi} population was defined according to the region in *A* (blue dots). CD34$^+$IL-3Rα^{lo} cells were defined according to the region in *B* (red dots). OLS, ortogonal light scatter. (*C*) Freshly sorted Wright–Giemsa-stained CD34$^+$IL-3Rα^{hi} cells display mitotic figures. (×600.) (*D–G*) CD34$^+$IL-3Rα^{hi} cells (blue) and CD34$^+$IL-3Rα^{lo} cells (red) were sorted according to the regions in *A* and *B*, respectively, and cultured in the presence of indicated cytokines, stained with CD1a and CD45RA after 5 days of culture, and analyzed by FACS. L = B lymphoid cells staining brightly with CD19 (data not shown). None of the cultured CD34$^+$IL-3Rα^{hi} cells (*D* and *E*) stained positively with CD19 (data not shown). Dashed lines indicate isotype control levels. (*H*) Transmission electron microscograph (×5,000) of a CD1a$^+$ cell sorted from CD34$^+$IL-3Rα^{lo} cells cultured with GM-CSF and TNF-α, as described in *G*. The arrows point to areas containing Birbeck granules, (shown in *Insets*, ×45,000). (*I*) CD4$^+$ T cells (10^5) were cocultured with indicated numbers of

showing that DC in murine spleens were immature immediately after isolation and differentiated rapidly *in vitro* (34, 35).

The nonactivated phenotype of DC in tonsils may seem like a paradox since tonsils are typically removed after repeated inflammations. One can further not exclude the possibility that the isolated cells had migrated to lymphoid tissue in response to inflammation, but not yet assumed the activated phenotype. To determine whether the presence of HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells in lymphoid tissue depends on previous exposure to foreign antigens or inflammatory stimuli, we examined whether the cells could be found in fetal lymph nodes. Presumably, these lymph nodes drain sterile, noninflamed tissues. As shown in Fig. 2*C*, HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells were present at high frequencies in the fetal lymph nodes ($2.6 \pm 1.1\%, n = 3$). These cells were more frequent than cells expressing high levels of myeloid markers, such as CD13 and CD33 ($1.5 \pm 0.2\%$, $n = 3$). The HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells in fetal lymph nodes expressed the same combination of markers as those in tonsils (Table 1). This cell type is therefore most likely capable of migrating to lymphoid tissue independently of inflammation and foreign antigens and without initiating immune responses.

IL-3Rα^{hi} DC Are Present in Peripheral Blood. HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells were readily detectable as a population with low orthogonal light scatter in blood from adult donors, and constituted $0.47 \pm 0.14\%$ ($n = 8$) of PBMC (Fig. 3 *A* and *B*, R1). A second population of IL-3Rα^{hi} cells was present among PBMC, but these cells were HLA-DR$^-$ (Fig. 3*B*, R2) and were found to be basophilic granulocytes (data not shown). The HLA-DR$^+$lin$^-$IL-3Rα^{hi} blood cells expressed the same combination of markers and had similar morphology as shown for the tonsillar counterparts (Table 1 and data not shown). However, an interesting difference was that whereas tonsillar DC did not express the lymph node homing molecule L-selectin (CD62L), the cells from PBMC were positive for this marker (Table 1).

Whereas multiple cell types can stimulate allogeneic T cells, DC are characterized by their higher potency relative to other antigen-presenting cells (13, 15, 21, 31, 33, 36–39). The cells also induce proliferation of autologous T cells *in vitro* (37). The data in Fig. 3 *C* and *D* demonstrate that HLA-DR$^+$lin$^-$IL-3Rα^{hi} blood cells were up to 100-fold more potent than monocytes in stimulating both allogeneic and autologous T cells. Cells with similar characteristics have previously been identified in blood and were referred to as CD11c$^-$ DC or CD33^{dim}CD14$^-$CD16$^-$ DC (36, 37).

Precursors of IL-3Rα^{hi} DC Are Found Among CD34$^+$ Bone Marrow Cells and Are Distinct from the Cells That Give Rise to Langerhans Cells in Response to GM-CSF and TNF-α. IL-3Rα^{hi} cells were readily identified as a distinct subset of CD34$^+$ fetal bone marrow cells with low orthogonal light scatter ($3.1 \pm 0.9\%$, $n = 5$) (Fig. 4 *A* and *B*). The majority of the CD34$^+$IL-3Rα^{hi} cells were within the CD34^{lo} subset, which contains lineage-committed progenitors (Fig. 4*B*) (23). The CD34$^+$IL-3Rα^{hi} cells had morphology and immunophenotype similar to IL-3Rα^{hi}lin$^-$HLA-DR$^+$ cells in blood and tonsil, but appeared more immature, and mitotic figures were frequently observed (Fig. 4*C* and Table 1).

Previous studies have demonstrated that DC and Langerhans cells can be derived by culture of CD34$^+$ cells with GM-CSF and TNF-α (13–19). The subset of CD34$^+$ cells that contains these TNF-α-dependent progenitors has not yet been identified. We therefore examined whether they were identical to the CD34$^+$IL-3Rα^{hi} cells. The sorted IL-3Rα^{hi} population (Fig. 4*A*) formed aggregates of cells with DC morphology during culture with either GM-CSF and IL-3 or GM-CSF and TNF-α (data not shown), and after 5 days the cells were positive for CD1a (Fig. 4 *D* and *E*). However, differentiation into DC occurred independently of TNF-α, and the CD1a$^+$ cells co-expressed CD45RA, which is absent from Langerhans cells (39) (Fig. 4 *D* and *E*). Tonsillar HLA-DR$^+$lin$^-$IL-3Rα^{hi} cells also obtained a CD1a$^+$CD45RA$^+$ phenotype when cultured under the same conditions (data not shown). In contrast, sorted CD34$^+$ cells with low levels of the IL-3Rα (Fig. 4*B*) gave rise to few CD1a$^+$ cells when cultured with GM-CSF and IL-3, but large numbers of CD45RA$^-$CD1a$^+$ cells in the presence of GM-CSF and TNF-α (Fig. 4 *F* and *G*). Consistent with a phenotype of Langerhans cells, 30–40% of these CD45RA$^-$CD1a$^+$ cells contained Birbeck granules (Fig. 4*H*). The frequency of Birbeck granule-positive cells among CD1a$^+$ cultured CD34$^+$IL-3Rα^{hi} cells was less than 4%, and may therefore reflect contamination of sorting gates. Finally, CD34$^+$IL-3Rα^{hi} cells cultured with GM-CSF and IL-3 were potent stimulators of allogeneic CD4$^+$ T cells, and more potent than CD14$^+$ macrophages generated by culture of CD34$^+$M-CSFR$^+$ cells from the same donor in M-CSF (Fig. 4*I*).

IL-3Rα^{hi} DC Are of Myeloid Origin. The distribution of M-CSF receptor (M-CSFR) and IL-3Rα among CD34$^+$ cells suggested that CD34$^+$IL-3Rα^{hi} cells may derive from cells in the M-CSFR^{hi} population, which down-regulate the M-CSFR as they up-regulate the IL-3Rα (arrow in Fig. 5*A*). Because M-CSFR expression on CD34$^+$ cells is restricted to granulomonocytic progenitors, this would indicate that IL-3Rα^{hi} cells belong to the granulomonocytic lineage (29). To test this possibility, immature progenitors (i.e., CD34^{hi}, see Fig. 4*B*) with high levels of M-CSFR and low levels of IL-3Rα (region in Fig. 5*A*) were sorted and cultured. After 60 h of culture two populations of cells that had downmodulated the M-CSFR were observed, with high and low levels of IL-3Rα, respectively (Fig. 5*B*). These cells were sorted and cultured for 5 additional days in medium supplemented with GM-CSF and IL-3. At this stage, the cultures from the IL-3Rα^{lo} population contained CD15$^+$ granulocytic cells (Fig. 5*C*) whereas cultures from the IL-3Rα^{hi} population contained CD1a$^+$ cells with DC morphology (Fig. 5*D* and data not shown). The latter cells induced strong proliferation of allogeneic T cells compared with equal numbers of CD14$^+$ macrophages generated by culture of CD34$^+$M-CSFR$^+$ cells for 5 days with M-CSF (29) ($n = 2$, data not shown). Addition of TNF-α to the medium did not increase the number of CD34$^+$IL-3Rα^{hi} cells generated from M-CSFR^{hi} myeloid progenitors, further suggesting that the cells are distinct from the TNF-α-dependent Langerhans cell progenitors (data not shown).

Thymus Contains Small Numbers of IL-3Rα^{hi} DC That Express Low Levels of CD34. Earlier studies have demonstrated that some DC may share the differentiation pathway of the T cell lineage and that DC are generated by progenitors in the thymus (18, 19, 40). We therefore investigated whether IL-3Rα^{hi} DC were present in the thymus. The results showed that less than 0.1% of the total thymic cell population were IL-3Rα^{hi} DC and that the IL-3Rα^{hi} cells constituted only $15 \pm 2\%$ ($n = 3$) of HLA-DR$^+$lin$^-$ subset. This may indicate that other DC populations than the IL-3Rα^{hi} cells are predominant in thymus. In addition, the IL-3Rα^{hi} DC in thymus expressed low levels of CD34 compared with bone marrow IL-3Rα^{hi} cells from the same donor (data not shown). Similar low levels of CD34 were found on the cells in fetal lymph nodes (Table 1). It therefore seems likely that the few IL-3Rα^{hi} DC in the thymus represent cells that have migrated from bone marrow and are distinct from thymic DC progenitors.

IL-3Rα^{hi} DC Constitute a Separate Lineage of DC That Follow a Differentiation Pathway Distinct from Langerhans Cells. The data presented here demonstrate that a subset of DC in human

allogeneic DC (■) or macrophages (○) from the same donor. The DC were generated by culturing sorted CD34$^+$IL-3Rα^{hi} cells for 5 days with GM-CSF and IL-3 (■). The macrophages were generated by culture of CD34$^+$M-CSFR$^+$ cells for 5 days with M-CSF and purified by FACS sorting of CD14$^+$ cells. T cell proliferation was measured as incorporation of [^{3}H]thymidine at day 6 of coculture. c.p.m; counts per minute. Data are representative of three experiments.

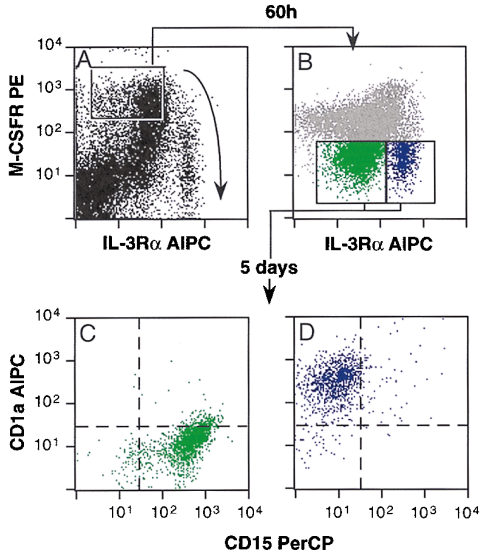

FIG. 5. IL-3Rα^{hi} DC follow the myeloid differentiation pathway to the branching point of the granulocytic and monocytic lineages. (*A*) CD34$^+$ cells were incubated for 12 h in serum-free medium (25) to allow up-regulation of the M-CSFR, and stained with CD34, anti-M-CSFR, and anti-IL-3Rα. The arrow shows the suggested differentiation pathway of CD34$^+$IL-3Rα^{hi} cells. The region shows sorting criteria for M-CSFR^{hi}/IL-3Rα^{lo} cells. An additional gate was set to include only CD34^{hi} cells, shown in Fig. 4*B*, to restrict the sort to immature progenitors (29). (*B*) After a 60-h culture of CD34^{hi}M-CSFR$^+$ cells in serum-free medium (25) containing stem cell factor, G-CSF, GM-CSF, IL-3, and IL-6, the cells were stained with anti-M-CSFR and anti-IL-3Rα. The regions indicate criteria for sorting of the two populations that had downmodulated the M-CSFR during the culture period. (*C* and *D*) After a 5-day secondary culture of IL-3Rα^{lo}M-CSFR^{lo} cells (green) and IL-3Rα^{hi}M-CSFR^{lo} cells (blue) with IL-3 and GM-CSF, the cells were stained with CD1a and CD15 and analyzed by FACS. The small subset of CD15^{lo} cells in *C* represent basophilic granulocytes (27, 29).

lymphoid organs and blood represent a separate lineage of cells. The cells can be readily identified and isolated on the basis of their high levels of the IL-3Rα. Their precursors in the bone marrow appear to follow the myeloid differentiation pathway to the branching point of the granulocytic and monocytic lineages. Progenitors that have committed to this DC lineage are distinct from those that give rise to Langerhans cells when cultured in the presence of GM-CSF and TNF-α. Their progeny furthermore lack several characteristics of Langerhans cells that were previously considered common to DC. Unlike Langerhans cells, the IL-3Rα^{hi} DC appear to undergo little differentiation during transit from the bone marrow to lymphoid organs. The IL-3Rα^{hi} DC further seem capable of migrating to lymphoid tissue independently of inflammatory stimuli. The results concur with previous reports showing that some DC spend less than 24 h in transit from the progenitor pool to lymphoid tissue and that DC turnover occurs constantly during steady state conditions (1, 41–43). Thus, the DC system is constituted by multiple cell types with distinct developmental pathways and functional properties. A large proportion of the cells may enter lymphoid tissue without inducing immune responses.

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