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Una O'doherty

Ralph M. Steinman

Michael Peng

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# Dendritic Cells Freshly Isolated from Human Blood Express CD4 and Mature into Typical Immunostimulatory Dendritic Cells after Culture in Monocyte-conditioned Medium

By Una O'Doherty, Ralph M. Steinman, Michael Peng, Paul U. Cameron, Stuart Gezelter, Iris Kopeloff, William J. Swiggard, Melissa Pope, and Nina Bhardwaj

*From the Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, New York 10021*

## Summary

A procedure has been developed to isolate dendritic cells to a high degree of purity from fresh blood. Prior enrichment methods have relied upon an initial 1–2-d culture period. Purified fresh isolates lack the characteristic morphology, phenotype, and immunostimulatory function of dendritic cells. The purified cells have the appearance of medium sized lymphocytes and express substantial levels of CD4, but lack the T cell molecules CD3, CD8, and T cell receptor. When placed in culture, the cells mature in a manner resembling the previously described, cytokine-dependent maturation of epidermal dendritic cells (Langerhans cells). The cells enlarge and exhibit many cell processes, express much higher levels of major histocompatibility complex class II and a panel of accessory molecules for T cell activation, and become potent stimulators of the mixed leukocyte reaction. Among the many changes during this maturation process are a fall in CD4 and the appearance of high levels of B7/BB1, the costimulator for enhanced interleukin 2 production in T cells. These changes are not associated with cell proliferation, but are dependent upon the addition of monocyte-conditioned medium. We suggest that the freshly isolated CD4-positive blood dendritic cells are recent migrants from the bone marrow, and that subsequent maturation of the lineage occurs in tissues in situ upon appropriate exposure to cytokines.

**B**lood is the most accessible tissue for the study of dendritic cells (DCs)<sup>1</sup> in humans. As in other tissues and species, the isolated DCs have a group of distinctive features, many of which relate to the cell surface (1, 2). These include an unusual cell shape and motility, a distinct repertoire of surface molecules such as abundant MHC products and adhesion/costimulatory molecules, and strong APC function in T cell-dependent immunity. Prior methods for isolating DCs from blood began with a 1–2-d culture period of T-depleted blood mononuclear cells. During this time, dendritic cells acquired a low buoyant density (3) and little or no adherence to plastic (4), features that aided their separation from other non-T cells.

Cells with the typical morphology of DCs are not readily recognized in fresh blood, a fact emphasized by Thomas et al. (5). Moreover, it is known that certain populations of freshly isolated DCs exist in a less immunostimulatory or immature state. The best studied example is the epidermal Langerhans cell, which undergoes a series of changes when placed into culture (6–13). The cells become larger and more dendritic in shape, synthesize large amounts of MHC products and accessory molecules, and develop strong T cell binding and stimulatory function. Analogous changes have been observed in DCs cultured from spleen (14) and lung (15). Maturation in each case is driven by cytokines, particularly GM-CSF (7, 14, 15).

Using recently described cytofluorographic criteria for identifying DCs (1), we set out to isolate these APCs from fresh blood. The topic is of further interest because of recent findings that DCs are able to carry HIV-1 to antigen-responsive T cells and induce extensive lymphocyte destruction (16). Here, we describe a method for purifying DCs from fresh blood. We find that the cells express high levels of CD4 and are immunologically immature in several respects. When placed

A preliminary report of this work has been presented in abstract form at the Second International Symposium on Dendritic Cells in Fundamental and Clinical Immunology, Amsterdam, the Netherlands, June 21–25, 1992.

<sup>1</sup>Abbreviation used in this paper: DC, dendritic cell.

in culture, the DCs undergo profound changes in cell shape, surface antigen phenotype, and immunostimulatory function, and these events require a monocyte-conditioned medium.

## Materials and Methods

**Culture Medium.** H10 contains 10% heat-inactivated human serum (6102; Biocell, Rancho Dominguez, CA), 20  $\mu\text{g}/\text{ml}$  gentamicin (Gibco Laboratories, Grand Island, NY), and 10 mM HEPES in RPMI 1640 (Gibco Laboratories).

**DC Purification from Fresh Blood.** Buffy coats from units of blood were purchased from the New York Blood Center. Mononuclear cells were isolated by flotation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). T cells were depleted by rosetting with neuraminidase (Calbiochem-Behring Corp., La Jolla, CA)-treated sheep erythrocytes (Cocalico, Reamstown, PA). For convenience, when blood was obtained late in the day, the T-depleted mononuclear cells ( $\text{Er}^-$ ) were left overnight on ice. This did not change the results. Monocytes were removed from the  $\text{Er}^-$  by adherence to human gamma globulin (Calbiochem-Behring Corp.)-coated petri dishes (Falcon Labware, Oxnard, CA) (3). The remaining  $\text{Er}^- \text{FcR}^-$  cells were mixed with a cocktail of mAbs against CD3 (leu 4; Becton Dickinson & Co., Mountain View, CA), CD21 (HB135; American Type Culture Collection, Rockville, MD), and CD16 (3G8; kind gift of J. Unkeless [17]) to label T, B, and NK cells, respectively. After a 0.5-h incubation on ice, unbound mAbs were washed away with  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free medium ( $1\times$  Hanks [Gibco Laboratories] + 1% BSA + 1 mM EDTA), and the cells were panned by adherence to goat anti-mouse IgG (Cappel Laboratories, West Chester, PA)-coated petri dishes. Nonadherent cells were stained with PE-labeled mAbs to monocytes, B cells, and NK cells (CD14, CD19, and CD56, respectively) and fluorescein-labeled anti-HLA-DR (Becton Dickinson & Co.), and washed in  $\text{Ca}^{2+}$ -free medium. Cells that were PE cocktail negative but DR positive were then purified on a FACStar Plus<sup>®</sup> cell sorter (Becton Dickinson & Co.). The FITC-anti-HLA-DR did not inhibit DC function under the conditions used here (data not shown). For phenotyping studies with indirect antibodies (below), we omitted the FITC-anti-HLA-DR.

**DC Purification from Cultured Blood.** Day 2 DCs were prepared by culturing the  $\text{Er}^-$  fraction for 36 h in H10 on tissue culture dishes (Falcon Labware) before continuing with the purification procedure outlined above. Alternatively, a prior method (1) using metrizamide density gradients was used.

**Techniques Used to Decrease Aggregates.** To obtain high yields using a cell sorter, aggregation in the presorted fraction must be minimized. During the last steps of the purification, it was critical to wash cells in  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free medium. Also, if SRBCs persisted in the  $\text{Er}^-$  fraction, we used  $\text{NH}_4\text{Cl}$  to lyse the sheep cells. This can lead to some death and aggregation, but 5  $\mu\text{g}/\text{ml}$  DNAase (Boehringer Mannheim Biochemicals, Indianapolis, IN) disrupted many of the aggregates.

**Viability Studies.** Purified day 0 DCs were cultured in H10 supplemented with various purified cytokines or conditioned media. DCs were plated at  $10^6/\text{ml}$ , 200  $\mu\text{l}$  per flat-bottomed microtest well in 96-well plates (Corning, Corning, NY). After 36 h of culture, cells were collected and counted by trypan blue (Sigma Chemical Co., St. Louis, MO) exclusion. Monocyte-conditioned medium was prepared by culturing plastic-, or human gamma globulin-, adherent cells (monocytes) for 24 h and harvesting the culture supernatant. Alternatively, T cell-depleted ( $\text{Er}^-$ )-conditioned medium was prepared by culturing these cells in H10 for 24 h at

$5 \times 10^6/\text{ml}$ . Conditioned media were stored in aliquots at  $-20^\circ\text{C}$ . Two purified cytokines were tested: GM-CSF ( $10^8$  U/mg; Kirin, Maebashi, Japan) and TNF- $\alpha$  ( $2 \times 10^7$  U/mg; Genzyme, Cambridge, MA). Lymphocult<sup>®</sup> (811020; Biotest Diagnostic, Denver, NJ) is a supernatant from PHA-stimulated blood mononuclear cells from which the PHA has been selectively removed.

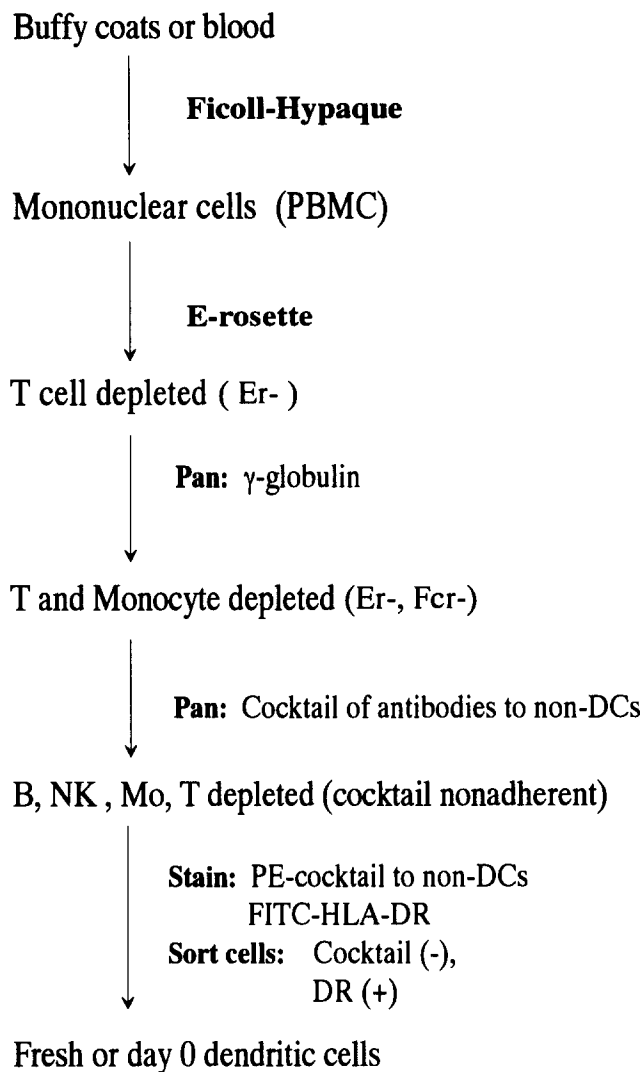
**Cytospin Preparations.** These were made in a cyto centrifuge (Shandon Southern Instruments Inc., Sewickley, PA), loading  $2 \times 10^4$  cells/slide. Slides were stored with desiccant at  $-20^\circ\text{C}$  before fixation in acetone and staining with anti-HLA-DR or anti-HLA-A,B,C (HB95; ATCC), followed by biotin goat anti-mouse Ig (4753; Boehringer Mannheim Biochemicals), avidin-biotin/horse-radish peroxidase (PK4000; Vector Laboratories, Burlingame, CA), and diaminobenzidine (04008; Polysciences, Warrington, PA).

**Phenotyping with mAbs.** A panel of mAbs was used to characterize day 0 DCs, day 2 DCs, and day 0 DCs cultured for 36 h in  $\text{Er}^-$ -conditioned media (see Results and Table 1). When an indirect immunolabeling method (mAb followed by anti-mouse Ig) was needed as in Fig. 5, it was necessary to modify the procedure shown in Fig. 1 so that the sorted DCs would not be labeled with anti-HLA-DR. The cocktail of mAbs used for panning were PE labeled and included reagents to CD3, CD14, CD16, and CD19. The larger cocktail-negative cells were selected using a FACStar Plus<sup>®</sup> (Becton Dickinson & Co.) cell sorter. Aliquots of the sorted cocktail-negative cells were stained with primary mouse mAb followed by PE goat anti-mouse IgG. Cells were quenched with 100  $\mu\text{g}/\text{ml}$  mouse IgG (015000003; Jackson, Westgrove, PA) and then stained with FITC-anti-HLA-DR. DR-positive cells, which accounted for  $>90\%$  of sorted cells, were then analyzed for PE stain on a FACScan<sup>®</sup> (Becton Dickinson & Co.).

**Mixed Leukocyte Reactions.** DCs were irradiated with 1,500 rad of  $^{137}\text{Cs}$  gamma radiation and added in graded doses to  $2 \times 10^5$  purified allogeneic T cells in 96-well flat-bottomed microtest plates. Allogeneic T cells were prepared by removing contaminating non-T cells in E-rosette-positive populations by gamma globulin panning followed by panning with anti-MHC class II mAb. [ $^3\text{H}$ ]Thymidine incorporation was measured at 120–132 h (6 Ci/mmol, 4  $\mu\text{Ci}/\text{ml}$  final).

## Results

A prior study (1) established that flow cytometry was useful in identifying dendritic cells in cultured human blood, and in developing an enrichment method. The DCs were identified in terms of their large size, absence of critical markers of other cell types ("null" phenotype), and expression of MHC class II. The non-DCs in cultured blood comprise at least 99% of the cells and could be removed by successive depletion of T cells, monocytes, and both B and NK cells. This was accomplished by E-rosetting, gamma globulin panning, and sedimentation on metrizamide columns. When we tried to repeat this method on fresh blood, we encountered a number of difficulties. Notable among these, we could not readily identify DCs that were typical in size and shape, nor could we use metrizamide columns to enrich "candidate" DCs, defining as candidates those cells that were negative for T, B, NK, and monocyte markers, but positive for HLA-DR. During the course of developing a method to purify DCs from fresh blood, we found that the cells expressed substantial levels of CD4, and only acquired the features of typical DCs after a period of maturation in culture.



**Figure 1.** DC purification from fresh or cultured white blood cells. (*Cocktail*) Mixture of mAbs to non-DCs, e.g., to CD3, CD16, and CD21. (*PE-cocktail*) Mixture of PE-conjugated mAbs to independent antigens, e.g., CD14, CD19, and CD56.

**Isolation Procedure to Prepare Fresh and Cultured DCs.** The procedure (Fig. 1) began by depleting T cells and monocytes using E-rosetting and adherence to gamma globulin-coated petri dishes, respectively. Then, panning and two-color cell sorting were used to deplete residual non-DCs. The panning step replaced the metrizamide flotation step of the prior procedure, since fresh DCs do not have a low buoyant density (3). The enrichment of candidate DCs was monitored by cytofluorography (Fig. 2). We looked for enrichment of cells that were HLA-DR positive but “null,” i.e., lacking markers of other leukocyte lineages. The candidate DCs in Fig. 2 are in the box that is PE cocktail negative and FITC-HLA-DR positive. These cells were not detectable in the T cell-enriched  $Er^+$  fraction and were just detectable (2–4%) in the  $Er^-$  fraction. 2–7% of the  $Er^-FcR^-$  were DCs. When the

$Er^-FcR^-$  cells were coated with a group of mAbs to CD3, CD16, and CD21 and panned on goat anti-mouse-coated petri dishes, the nonadherent cells were 9–27% candidate DCs. This percentage was sufficient to permit a final purification by two-color cell sorting, using an independent panel of PE-conjugated mAbs to label non-DCs (anti-CD14, 19, 56) and FITC-anti-HLA-DR. The sorted cells were 98% pure. Although smaller than day 2 DC, the sorted cells were slightly larger than resting lymphocytes (Fig. 3).

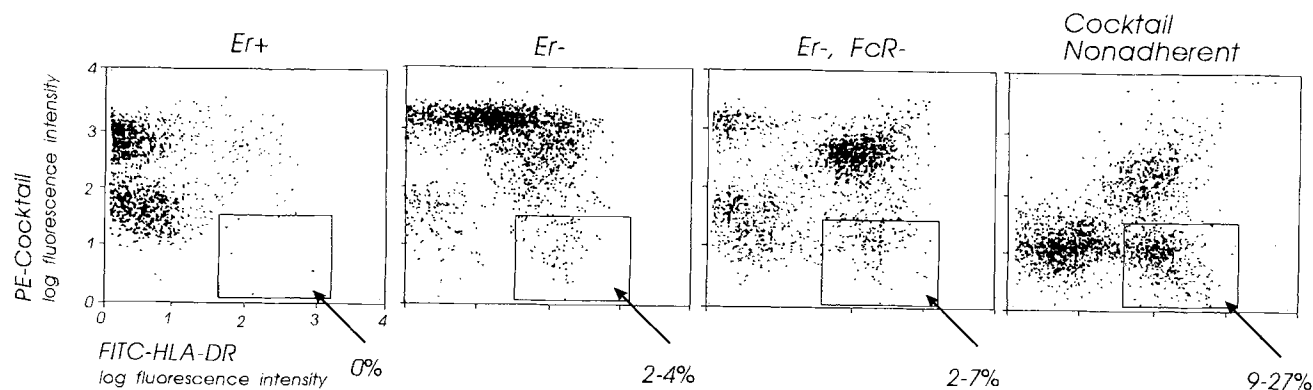
**Fresh DCs Require Monocyte-conditioned Medium to Remain Viable and Develop a Characteristic Morphology.** The candidate DCs that were freshly isolated on day 0 did not have the stellate shape of cultured DCs. When cultured in standard H10 medium, most of the cells died within 36 h. However, viability was maintained at higher levels with several conditioned media (Fig. 4). Of the media tested,  $Er^-$  or monocyte-conditioned media supported viability the best. Individual cytokines were not very active (data not shown), although GM-CSF resulted in improved viability (>30%) over H10 alone.

In cyto-spin preparations, the day 0 DCs had the appearance of medium-sized lymphocytes, lacked processes, and expressed intermediate levels of HLA-DR (Fig. 5). During the first few hours of culture in  $Er^-$  or monocyte-conditioned media, the cells were round and dispersed, but by 12 h aggregates formed. After 36 h, cytoplasmic processes or veils extended from the surfaces of the aggregates. When the cells were cultured in H10, some aggregates formed, but not all DCs entered the aggregates, in contrast to the uniform aggregation of DCs exposed to  $ER^-$ -conditioned medium. In H10, cytoplasmic processes did not develop to the same extent as with the conditioned medium. After culture in  $Er^-$  or monocyte-conditioned medium for 36 h, the cells were larger, had many processes, and expressed high levels of HLA-DR, like day 2 DCs. Both class I and II MHC products were noted in a high concentration in the perinuclear region of the cultured DCs (Fig. 5).

Fresh blood DCs did not appear to proliferate, since all nuclei contained a 2n (diploid) level of DNA with propidium iodide staining (data not shown).

**CD4 Levels on Fresh and Cultured DCs (Fig. 6).** On staining sorted day 0 and day 2 DCs, we were surprised to find that the former had relatively high levels of CD4. The levels were much higher than on plastic adherent monocytes (prepared by adhering fresh  $Er^-$  cells to tissue culture dishes for 1–2 h) and approached the levels on CD4-positive T cells. Although DCs shared CD4 with T cells, there was no CD3, TCR, or CD8 (below). Culturing day 0 DCs for 36 h in Lymphocult or 10% human serum (data not shown) resulted in a slight decrease in CD4 expression. However, if the cells were cultured in  $Er^-$  or monocyte-conditioned medium, CD4 expression was markedly decreased.

**Cell Surface Phenotype before and after Culture (Fig. 7 and Table 1).** Fresh DCs lacked several markers that are typical of monocytes, B cells, and NK cells (Table 1). The MHC-related molecules (HLA-DR, -DQ, -DP, class I, and invariant chain) were expressed at moderate levels on day 0 and up-



**Figure 2.** Monitoring the DC purification procedure. Two-color staining was performed on aliquots of each cell fraction. Dot plots of FITC-anti-HLA-DR vs. PE-cocktail (CD3, CD14, CD19, CD56) are shown. In this experiment, we panned the  $Er^- FcR^-$  with a cocktail of mAbs to CD3, 16, and 21. The percentage of DCs (cocktail negative, but DR positive) are listed beside the boxed cells (averages of four experiments). Candidate DCs were never detected in the  $Er^+$  fraction, but are always found in the  $Er^-$  fraction and greatly increase in frequency in the cocktail nonadherent cells. The double-negative cells seen in the cocktail nonadherents of this figure and Fig. 3 are T cells. These cells are not stained since we panned them with an unlabeled anti-(CD3), which prevented PE-anti-CD3 from binding. Labeling the T cells with a PE-labeled antibody is not necessary since these cells are HLA-DR negative; however, the T cells can be tagged with TCR-PE.

regulated some 10-fold upon culture to the levels found on DCs purified from cultured mononuclear cells. Many adhesion and costimulatory molecules (B7/BB1, LFA-3 [CD58], ICAM-1 [CD54], CD5, and CD40) were also expressed at higher levels after culture. B7/BB1, a costimulator molecule that enhances IL-2 production (18, 19), was not expressed on day 0 DCs.  $Fc\gamma RII$  (CD32) and  $Fc\gamma RI$  (CD64) were expressed weakly on day 0 DCs but were absent after culture. Most fresh DCs, like naive T cells, were CD45RA positive, CD45RO negative, and IL-2R (CD25) negative. Upon culture, a more activated phenotype developed, including expression of CD25.

Expression of certain molecules did not change with culture. CD11a, CD18, CD29, CD43, CD45, and CD72 were all present, while CD70 was absent on all three kinds of DCs.

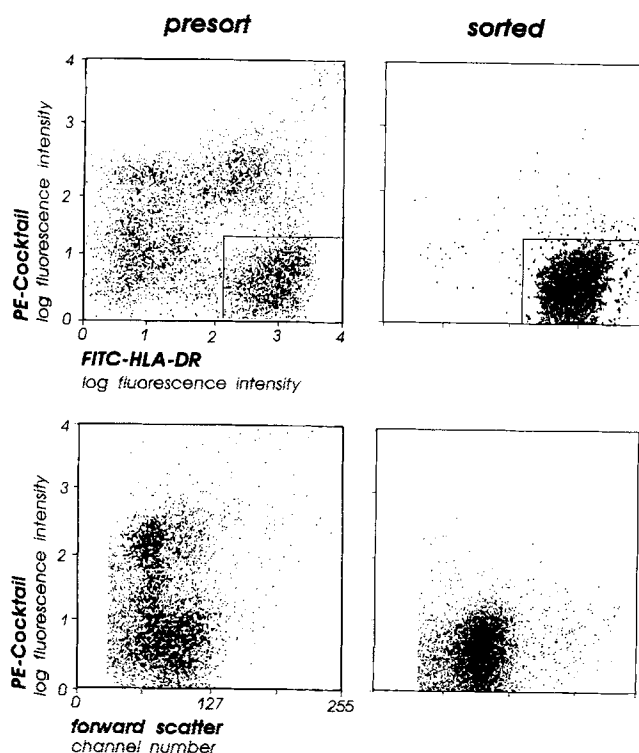
In general, day 0 DCs cultured in  $Er^-$ -conditioned medium resembled prior day 2 isolates except for two antigens, CD45RA and CD11c. When purified DCs were cultured in conditioned medium, the phenotype remained CD45RA<sup>+</sup> and CD11c<sup>-</sup>, whereas when DCs were purified after culture, the phenotype was CD45RA<sup>-</sup> and CD11c<sup>+</sup>.

There was a small subset of fresh DCs that expressed CD11c, CD45RO, and CD5. This phenotype was intermediate between the majority of fresh and cultured DCs and implies that there is an additional, more activated population of DCs circulating in the blood. A small fraction of fresh DCs also expressed CD2 and CD7, but these antigens were not present on cultured DCs.

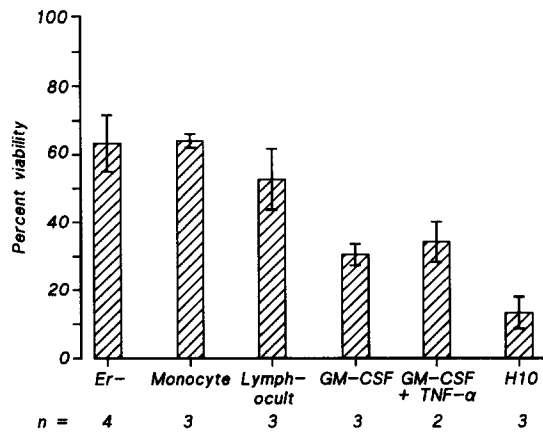
**Enhanced T Cell-stimulating Function after Maturation (Figs. 8 and 9).** Fresh DCs were poor stimulators of the allogeneic MLR, but after culture in conditioned medium, the APCs had become as potent stimulators as day 2 DCs. In dose-response studies, stimulatory activity increased 10–30-fold after culture. The MLRs induced by day 0 DCs were pulsed with [<sup>3</sup>H]TdR at several time points (120, 144, and 156 h), in case a longer culture period was needed to detect T cell stimula-

tion, but little (less than threefold) to no increase in proliferation was seen at these later times (data not shown).

Many studies have used fresh blood mononuclear cells to stimulate allogeneic T cells. Initially, this appeared to conflict



**Figure 3.** FACS analysis of presorted and sorted cells during the isolation of fresh DCs. (Top left) Presorted cells stained with PE-cocktail and FITC-anti-HLA-DR. The boxed cells represent the cells selected by the sorter. After sorting (top right), 98% of the cells were within the box. On the bottom are PE-cocktail vs. forward scatter plots of presorted (left) and sorted cells (right) to demonstrate that DCs have a slightly higher forward scatter relative to lymphocytes in the presort population.

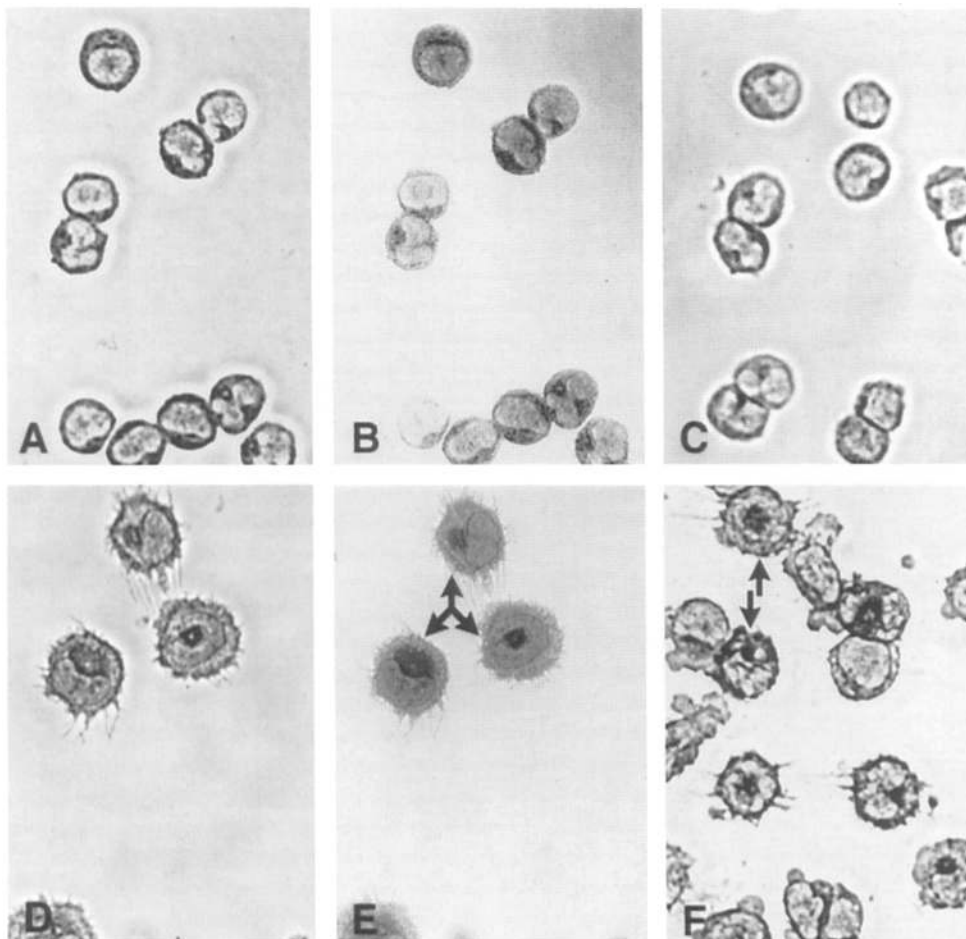


**Figure 4.** Viability of day 0 DCs after culturing for 36 h in conditioned media or cytokine-supplemented media. (n) Number of experiments that were averaged to give the percent viability. The supplements were: Er<sup>-</sup>, 50% Er<sup>-</sup>-conditioned medium; monocyte, 50% monocyte-conditioned medium; Lymphocult, 30% Lymphocult; GM-CSF, 490 ng/ml; and GM-CSF + TNF- $\alpha$ , 3 ng/ml.

with our data, where fresh DCs were poor stimulators of foreign T cells, until we realized that other leukocytes could be providing cytokines that DCs needed for maturation into potent stimulators. We wondered whether adding Er<sup>-</sup>-conditioned medium directly to an allo-MLR would enhance the immunostimulatory capacity of fresh DCs. We prepared highly enriched sorted populations, irradiated them, and added the APCs in graded doses to purified allogeneic T cells in control medium or medium supplemented with 40% Er<sup>-</sup>-conditioned medium. The latter enhanced stimulatory activity almost 10-fold (Fig. 9).

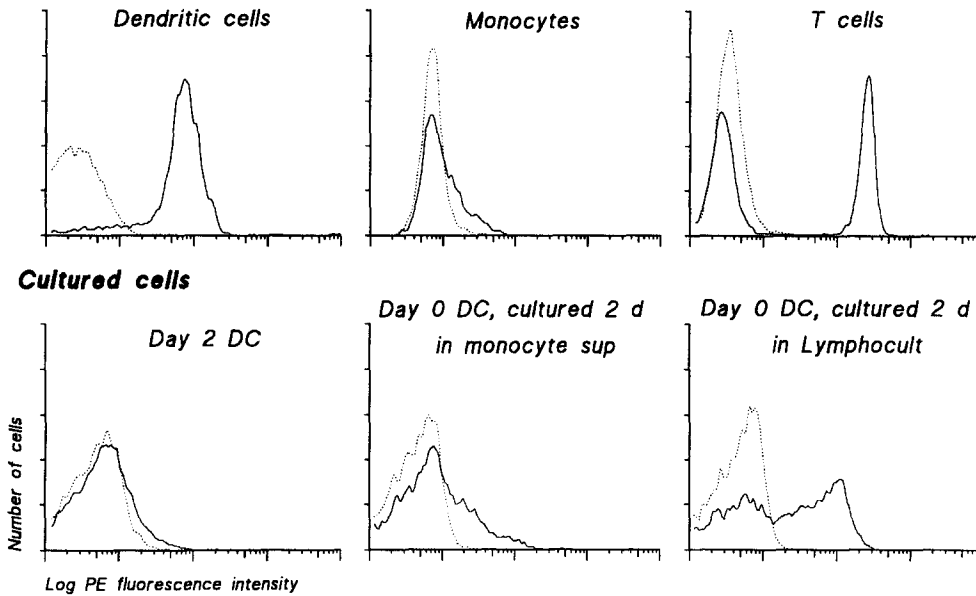
## Discussion

To study the induction of T cell-dependent immune responses in several clinical contexts, it would be of value to use human blood dendritic cells (20). DCs are potent APCs and capable of inducing primary immune responses in tissue culture and whole animal models (21). Blood has been one of the many tissues that has been used as a source of DCs



**Figure 5.** Cytospin morphology of fresh (day 0) DCs and day 0 DCs cultured for 36 h in Er<sup>-</sup>-conditioned medium. DCs were purified as in Fig. 1 except FITC-anti-HLA-DR was not added. DCs were selected by sorting for PE-cocktail negatives. (A) Fresh DCs, MHC class II stain, phase contrast; (B) fresh DCs, MHC class II stain, brightfield to show the brown reaction product in the cytoplasm; (C) fresh DCs, MHC class I stain, phase contrast; (D) DCs cultured in monocyte-conditioned medium, MHC class II stain, phase contrast; (E) cultured DCs, MHC class II, bright field; (F) cultured DCs, MHC class I stain, phase contrast. Arrows point to a concentration of MHC antigens in the perinuclear areas of the DCs.

## Fresh cells



**Figure 6.** CD4 is expressed by freshly isolated human DCs. DCs were purified as in Fig. 1 and stained with PE-anti-CD4 (mAb leu 3a) immediately or after culture in either monocyte-conditioned medium or Lymphocult for 36 h. For comparison, monocytes (prepared by adhering fresh  $E_r^-$  for 1–2 h on tissue culture dishes), T cells ( $E_r^+$  cells), and day 2 DCs are also stained with PE-anti-CD4. The dotted lines are isotype-matched controls and the solid lines are obtained with PE-anti-CD4.

in humans, the others being tonsil (22), skin (9), rheumatoid synovial effusions (23–25), thymus (26), gut (27), and lung (28). In blood, as in other tissues, DCs are distinguished by strong MLR stimulatory activity and an absence of typical monocyte and lymphocyte markers (1, 29, 30). Subsequent studies have used human blood to probe other aspects of DC function, including the expression of adhesion and costimulator molecules (31), the immunoselection of antigen-reactive T cells for cloning procedures (32, 33), and the presentation of human pathogens and superantigens (33, 34). However in all prior work, a culture period of 1–2 d has been part of the purification protocol. We have worked out a method for reliably enriching these APCs from fresh blood. The purified DCs prove to be immature in several respects and require supplementation with cytokines to develop further. Our findings are discussed from several perspectives.

**The Purification Procedure.** To purify DCs we made several assumptions based on prior work. DCs would: (a) not rosette sheep erythrocytes (in contrast to T cells); (b) express little to no Fc receptors that bind to dishes coated with human Ig (in contrast to monocytes); (c) lack such differentiation antigens as CD3, CD14, CD16, CD19, CD21, and CD56 (in contrast to other lineages); but (d) express detectable levels of MHC class II molecules. After depleting non-DCs on the basis of the criteria above, we still required a final purification step by FACS<sup>®</sup>. Of considerable importance was the need for calcium-free media to prevent cell aggregation, and the use of cytofluorography to monitor DC enrichment and yields (Figs. 1 and 2).

Thomas et al. (5) have also approached the study of DCs in fresh blood, but using different methods from ours. For example, they designed other approaches to deplete monocytes by emphasizing the selection of  $CD33^+CD14^-$  cells while we depleted monocytes by adhering them to gamma

globulin-coated petri dishes. We have not had the opportunity to compare the two methods. However, the findings of Thomas et al. (5) are different from ours. In particular, the cytokine-dependent maturation process of fresh DCs did not seem to occur. Cytokines did not appear to be needed to maintain viability; the development of potent MLR stimulatory function was not observed; and the marked upregulation of the B7/BB1 antigen, except on a minority of cells, was not evident. It is possible that  $CD33^+CD14^-$  populations are less enriched in DCs than the population described here.

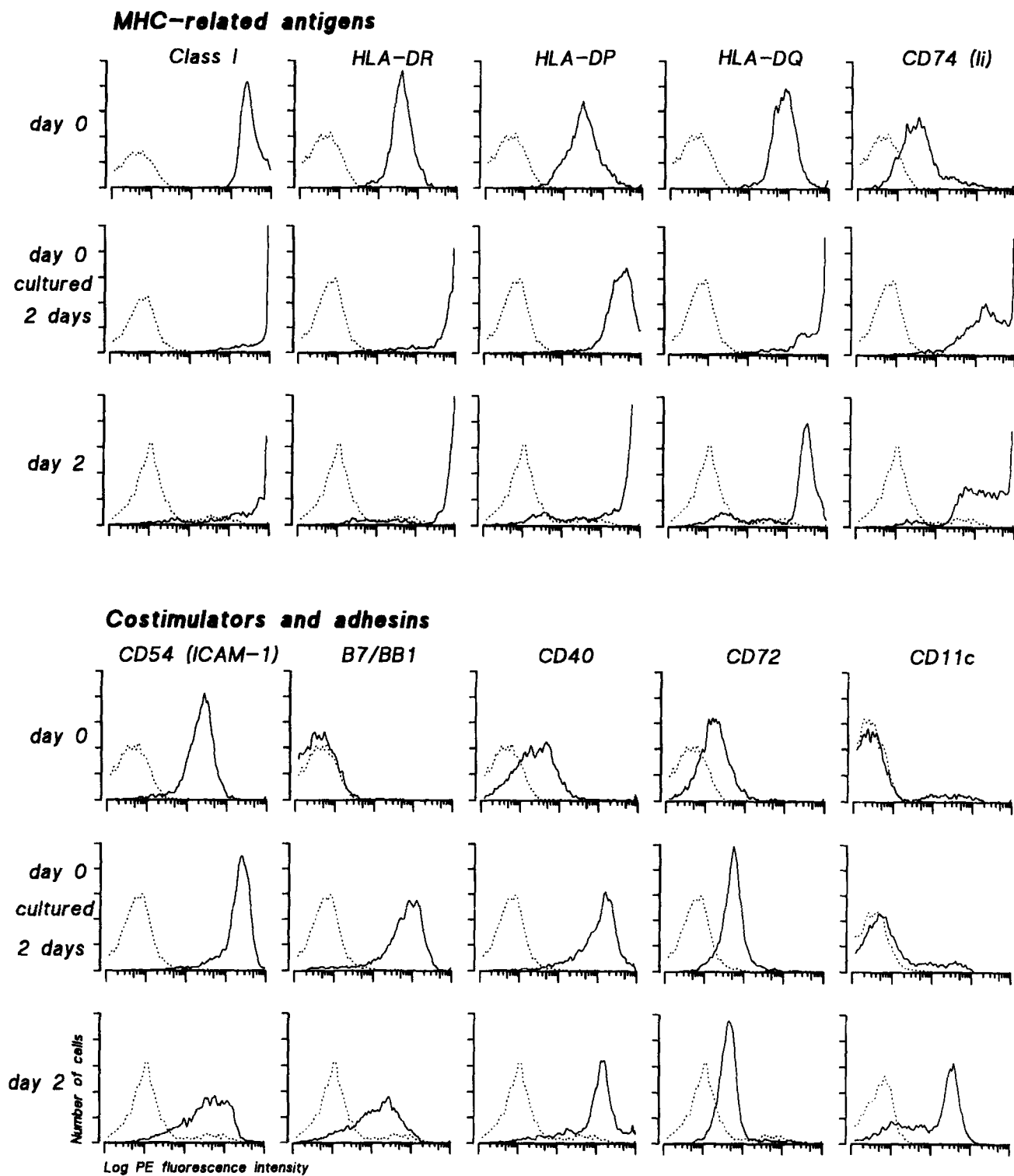
**The Maturation Phenomenon.** In the case of DCs in epidermis (6), and to some extent in spleen (14) and lung (15), there is information that the phenotype and function of freshly isolated DCs change markedly in culture. This phenomenon is termed “maturation” because it occurs in the absence of cell proliferation and entails the development of strong T cell stimulatory function (6, 7), as well as such associated features as expression and/or upregulation of adhesion and costimulatory molecules (9–13). When we tried to identify DCs in fresh blood, we found that the APCs were immature on several grounds: morphology (Fig. 5), i.e., an absence of cytoplasmic processes; cell surface composition, i.e., relatively low levels of MHC products and accessory molecules (Fig. 6); and function, i.e., weak stimulatory capacity in the allogeneic MLR (Fig. 8). The weak stimulatory function of fresh DCs is consistent with their low expression of accessory molecules in contrast to day 2 DCs (31). GM-CSF, a monocyte product, by itself did not fully replace the function of monocyte-conditioned medium. GM-CSF only partially improved viability, and many of the surviving cells still expressed CD4 (data not shown). The effects of GM-CSF on DCs from other tissues have been more marked (7). IL-6, another monocyte product, did not improve viability and did not enhance the effects of GM-CSF (data not shown). Fur-

**Table 1.** Summary of the Cell Surface Phenotypes of Day 0 DCs, Day 2 DCs, and Day 0 DCs Cultured in T Cell-depleted Conditioned Medium

Antigen	Source	Day 0 DC	Day 0 DC, cultured 2 d	Day 2 DC
<b>Monocytes</b>				
CD14	ATCC (TIB 228)	-	-	-
<b>B cells</b>				
CD19	AMAC	-	-	-
CD20	Becton Dickinson & Co.	-	-	-
<b>NK cells</b>				
CD56	Becton Dickinson & Co.	-	-	-
<b>T cells</b>				
CD2	Dako Corp.	Most -, few ++	-	-
CD3	Becton Dickinson & Co.	-	-	-
CD4	"	+	±	±
CD5	"	Most -, few ++	Half -, half ++	Few -, most ++
CD7	Serotec	Most -, few ++	-	-
CD8	Becton Dickinson & Co.	-	-	-
TCR	"	-	-	-
<b>MHC</b>				
HLA-A,B,C	ATCC (HB95)	+++	++++	++++
HLA-DR	Becton Dickinson & Co.	++	+++	+++
HLA-DP	PL-15 (gift of Dr. S.Y. Yang, New York, NY)	++	+++	+++
HLA-DQ	ATCC, HB144	++	+++	+++
Ii CD74	BU45 (40) (gift of G. Johnson)	+	++ to +++	++ to +++
<b>Adhesins and costimulators</b>				
CD54	84H10 (41)	++	+++	++
CD58	ATCC (HB205)	+	++	++
B7/BB1	Becton Dickinson & Co.	-	++	+
CD11a	ATCC (HB202)	++	++	++
CD11b	Exalpa	-	-	-
CD11c	Becton Dickinson & Co.	Most -, few ++	Most -, few ++	Few -, most ++
CD18	IB4 (42)	++	++	++
CD29	A1A5 (43)	++	++	++
CD40	Biosource	± to +	++	++
CD72	Serotec	+	+	+
<b>Activation</b>				
CD45RA	Serotec	Few -, most +	Few -, most +	-
CD45RO	UCHL-2 (44)	Most -, few +	Most ±, few +	Few -, most +
CD25	Becton Dickinson & Co.	-	+	+
<b>Receptors</b>				
CD16	Becton Dickinson & Co.	-	-	-
CD32	AMAC	±	-	-
CD64	ATCC (HB9469)	±	-	-
<b>Miscellaneous</b>				
CD1a	Coulter	-	-	-
CD43	Becton Dickinson & Co.	++	++	++
CD45	ATCC (HB196)	++	++	++
CD70	Serotec	-	-	-

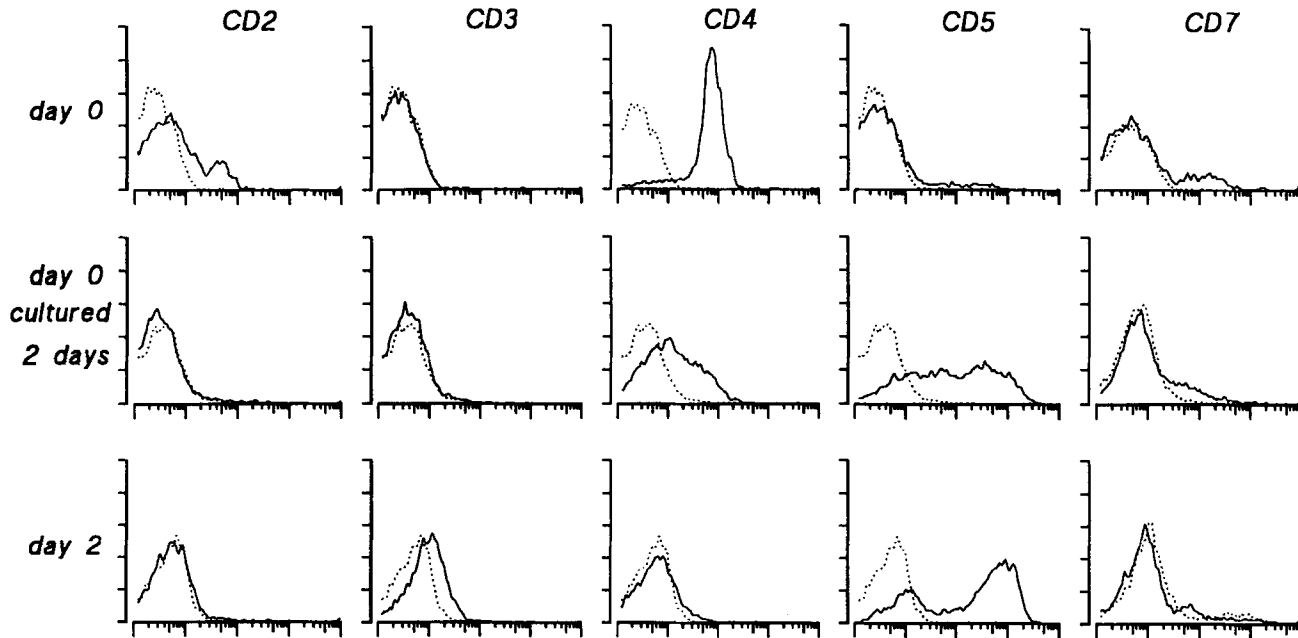
Each + corresponds to an increase in fluorescence intensity of one logarithm using the scale in Fig. 7.



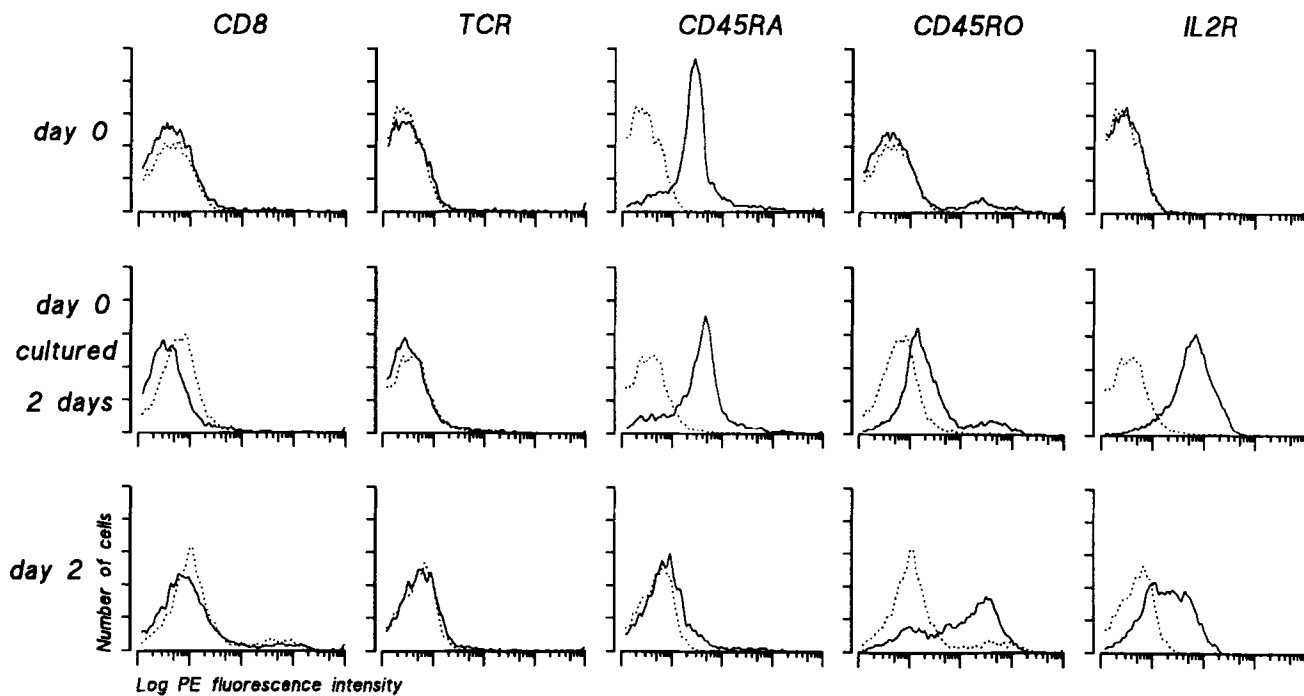


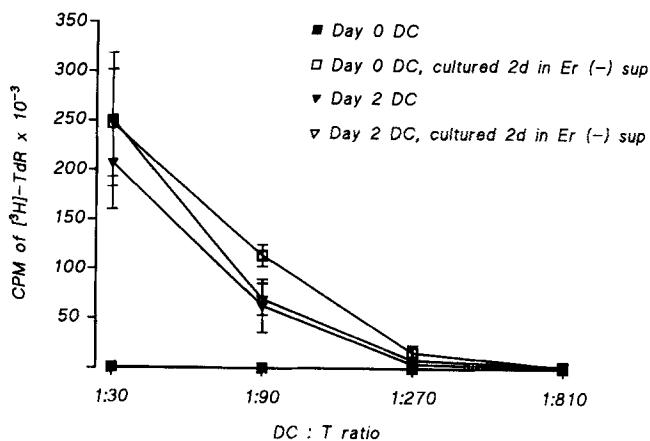
**Figure 7.** Cell surface phenotype of day 0 DCs, compared with day 2 DCs, and day 0 DCs cultured for 2 d in T cell-depleted conditioned medium. The antibodies are presented in groups, corresponding to MHC-related antigens, costimulators and adhesins, T cell antigens, and activation antigens. The dotted lines are isotype-matched nonreactive control mAbs. Staining with anti-class I and anti-class II mAbs of day 0 DCs cultured 2 d and day 2 DCs is offscale.

### T cell antigens

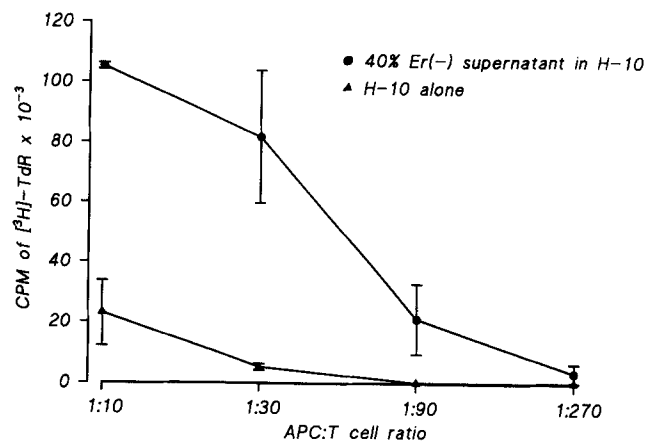


### Activation antigens





**Figure 8.** The MLR stimulatory capacity of day 0 and day 2 DCs, with and without prior culture of the APCs in conditioned medium from E-rosette-negative mononuclear cells. Graded doses of irradiated DCs were used to stimulate  $2 \times 10^5$  allogeneic T cells. Cells were pulsed with  $[^3\text{H}]\text{-TdR}$  for 12 h after 120 h of culture.



**Figure 9.** MLR stimulation by day 0 DCs in the presence and absence of 40%  $\text{Er}^-$ -conditioned medium added continuously to the MLR culture. Irradiated DCs were added at graded doses to  $2 \times 10^5$  purified allogeneic T cells as in Fig. 8.

ther study will be required to identify the factors that mediate the phenomenon described here. Nevertheless, our findings suggest that when APC function is evaluated in human blood, the stimulatory role of DCs is at least partly dependent upon cytokines being made by other cell types. Indeed, if  $\text{Er}^-$ - or monocyte-conditioned medium is added to the MLR when the stimulator cells are fresh DCs, the response rises dramatically (Fig. 9).

**The Origin and Destination of DCs in Fresh Blood.** From the phenotyping experiments, there appeared to be at least two populations of DCs in blood. The majority expressed the  $\text{CD45RA}^+/\text{RO}^-$  markers. We hypothesize these are immature DCs that have recently migrated from the bone marrow. A minor subpopulation expressed  $\text{CD5}$ ,  $\text{CD11c}$ , and  $\text{CD45RO}$ . These may represent a more mature population that had been exposed to cytokines after passage through other tissues. This is possible since DCs have been shown to migrate from cardiac allografts through the blood to spleen in mouse (35).

**CD4 Expression by Human Blood DCs.** CD4 is expressed

at low levels on human Langerhans cells (36) and on precursors in mouse marrow (37). CD4 is also detected on DCs that grow out from precursors in human umbilical cord blood in the presence of GM-CSF and  $\text{TNF-}\alpha$  (38).

The expression of CD4 that is described here may cast a new light on the potential contribution of DCs to the pathogenesis of AIDS. Expression of CD4 does not necessarily indicate that DCs are readily infected with HIV, since the levels of proviral DNA that have been detected in cultured DCs from HIV-1-infected individuals in a single study corresponds to less than <1 in 1,000 DCs (39). Prior work has shown that DCs, without being infected, can carry HIV to antigen-reactive T cells and lead to extensive T cell syncytium formation and death (16). The presence of CD4 on DCs, which should allow high affinity adsorption of HIV, dictates a reexamination of these issues in fresh isolates. In contrast to prior work on cultured DCs, might the CD4-positive DCs in fresh blood be more susceptible to infection with HIV-1 and/or be more efficient at trapping and carrying the virus to lymphoid and nonlymphoid organs?

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Address correspondence to Una O'Doherty, Laboratory of Cellular Physiology and Immunology, The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399. Paul Cameron's present address is Macfarlane Burnet Center, Fairfield Hospital, Fairfield, Victoria, Australia. Iris Kopeloff's present address is the Department of Dermatology, The New York University Medical Center, New York, NY.

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