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Circulating CD2⁺ Monocytes Are Dendritic Cells

Keith Crawford,†‡ Dana Gabuzda,†§ Vassilios Pantazopoulos,* Jianhua Xu,** Chris Clement,†‡ Ellis Reinherz,‡¶ and Chester A. Alper‡†*†

Low levels of CD2 have been described on subsets of monocytes, macrophages, and dendritic cells. CD2 is expressed on about one-third of circulating monocytes, at levels one-half log lower than on T or NK cells, representing 2–4% of PBMC. FACS analysis of CD2⁺ and CD2⁻ monocytes revealed no significant difference in the expression of adhesion molecules (CD11a/b/c), class II Ags (HLA-DR, -DQ, -DP), myeloid Ags (CD13, CD14, CD33), or costimulatory molecules (CD80, CD86). Freshly isolated CD2⁺ and CD2⁻ monocytes were morphologically indistinguishable by phase contrast microscopy. However, scanning electron microscopy revealed large prominent ruffles on CD2⁺ monocytes in contrast to small knob-like projections on CD2⁻ monocytes. After 2 days of culture, the CD2⁺ monocytes largely lost CD14 expression and developed distinct dendrites, whereas the CD2⁻ monocytes retained surface CD14 and remained round or oval. Freshly isolated CD2⁺ monocytes were more potent inducers of the allogeneic MLR and more efficiently induced proliferation of naive T cells in the presence of HIV-1 gp120 than did CD2⁻ monocytes. After culture in the presence of GM-CSF and IL-4, CD2⁺ monocytes were up to 40-fold more potent than monocyte-derived dendritic cells or CD2⁻ monocytes at inducing allogeneic T cell proliferation. These findings suggest that circulating CD2⁺ and CD2⁻ monocytes are dendritic cells and the precursors of macrophages, respectively. Thus, dendritic cells are far more abundant in the blood than previously thought, and they and precursors of macrophages exist in the circulation as phenotypically, morphologically, and functionally distinct monocyte populations. The Journal of Immunology, 1999, 163: 5920–5928.

Dendritic cells (DC) are a distinct population of APC that are intimately involved in initiation of innate and adaptive immunity (1, 2). Steinman and Cohn (3) first described these adherent cells, distinguishable after 2 days in culture from monocytes (Mo) by their irregular shape and ability to generate pseudopodia. When freshly isolated from the circulation, DC efficiently phagocytose nominal Ags but are poor initiators of T cell activation (1–5). However, after prolonged culture, the phagocytic efficiency of DC decreases, while their ability to induce potent allogeneic and Ag-specific responses increases (6, 7). DC can activate both naive and memory T cells (8, 9), while Mo are capable only of activating memory T cells (1, 5). Mo possess other functional properties (e.g., tissue remodeling, complement protein production, and nonspecific bystander killing) that further distinguish them from DC (10–12).

Methods to investigate DC biology have been hindered by poor yields. These methods principally involved DC isolation by negative selection of specific cell-surface Ags present on other leukocytes but absent from DC (13–16). In addition, earlier methods incorporated some form of density-gradient separation (e.g., Metrizamide, Percoll) and short-term tissue culture. The DC yields of these methods were extremely low, representing ~0.1% of PBMC from blood and bone marrow. With reports that Mo are precursors of both DC and macrophages (Mφ) (17–19), the problem of low yield has been overcome. Most contemporary studies rely on Mo-derived DC (MDDC) as the primary source of DC (19, 20), while others generate DC from CD34-expressing bone marrow progenitors (2). MDDC are generated by 7 days of culture of Mo in recombinant human (rh) GM-CSF and rhIL-4 as described by Sallusto and Lanzavecchia (17). This method gives rise to immature DC expressing high levels of HLA-DR and CD86 and low to no CD14 or CD83. Maturation of immature DC population to efficient APCs is induced by additional culture with IL-1β, LPS, TNF-α, PHA, or calcium ionophore (19, 21). Although this method yields more DC than previous methods, the necessity for prolonged culture in the presence of cytokines raises concerns that MDDC may not be phenotypically or functionally similar to their counterparts in vivo (22, 23).

Our study stemmed from the observation that SRBC-enriched T cell cultures contain large numbers of contaminating cells with DC morphology and immunofluorescence staining characteristics (24). Because SRBC bind to CD2, and 5–50% of circulating Mo are CD2⁺ (25), we explored the possibility that CD2 is a marker for DC in blood. This costimulatory molecule is a 40- to 60-kDa glycoprotein of the Ig superfamilly (26). CD2 is principally expressed by T and NK cells (26, 27), but low expression has also been reported on subsets of thymic B cells, DC, Mo, and Mφ (1, 25, 28). CD2 has three regions defined as T11₁, T11₂, and T11₃ (25). mAbs that bind to the T11₁ region inhibit binding of its physiological ligand, CD58 (LFA-3), on SRBC, while mAbs binding to the T11₂ and T11₃ regions do not interfere with binding. Ligation of CD2 with mAb pairs activates T and NK cells through the same pathway as the natural ligand CD58 (26, 27).

Our results show that CD2 is present on approximately one-third of CD14⁺, CD11c⁺ PBMC and that this CD2-expressing population is phenotypically, morphologically, and functionally different from...
the CD2\(^+\) CD14\(\text{high}\) population. Furthermore, our studies suggest that CD2\(^+\) CD14\(\text{high}\) and CD2\(^-\) CD14\(\text{high}\) PBMC represent DC and precursor Mo (pMo), respectively, in the circulation. Thus, we provide evidence that DC exist at a much higher frequency in the blood than previously considered and that they can be isolated from the PBMC without prolonged culture and without the addition of cytokines.

**Materials and Methods**

**Isolation of Mo from PBMC**

PBMC were isolated from buffy coats from healthy volunteers (Transfusion Therapy, Children’s Hospital, Boston, MA) by separation on Ficoll (Pharmacia, Piscataway, NJ) gradients, washed twice, and resuspended at 5 \times 10^8 cells/ml. This PBMC-enriched population was layered over a 14.5% w/v discontinuous Metrizamide (Sigma, St. Louis, MO) gradient (13) and centrifuged (Sorvall RT 6000, DuPont, Wilmington, DE) at 650 \times g for 10 min to separate the PBMC into low (Mo) and high (T, B, and NK cells) density fractions.

The CD2\(^-\) Mo were isolated from the low-density cells by first removing contaminating T, B, and NK cells with anti-CD3, anti-CD19, and anti-CD56 immunomagnetic beads (Miltenyi Biotech, Heidelberg, Germany). The remaining cell population was \(>95\%\) CD14\(\text{high}\) by flow cytometry. These cells were incubated with a 1:100 dilution of mouse mAb (in ascitic fluid) to human CD2 (10d2-4C1 (anti-T112); Dana-Farber Cancer Institute, Boston, MA) (26) for 30 min at 4°C, washed, and incubated with goat anti-mouse IgG magnetic beads (Miltenyi Biotech). Following incubation, the preparation was passed through a magnetic column according to the manufacturer’s instructions. The magnetic column retained the CD2\(^+\) cells, which were \(>96\%\) pure, while the CD2\(^-\) cells were \(>95\%\) pure by flow cytometry with anti-CD2 and anti-CD14. A blocking buffer containing 10\% v/v heat-inactivated pooled human serum (PHS) (Nabi, Boca Raton, FL) and human IgG (50 mg/ml; Immuno AG, Vienna, Austria) in HBSS without magnesium and calcium (Cellgro; Fisher Scientific, Pittsburgh, PA) was used to prevent nonspecific mAb binding during each stage of isolation or flow cytometric analysis. For morphologic and functional studies of freshly isolated, noncytokine-incubated CD2\(^+\) and CD2\(^-\) Mo, we used culture medium (CM) containing RPMI 1640 (Cellgro) supplemented with 10\% heat-inactivated PHS, 20 \mu g/ml gentamicin, 100 \mu U/ml penicillin, and 100 \mu g/ml streptomycin (Life Technologies, Gaithersburg, MD).

**Generation of MDDC**

MDDC were generated as described by Sallusto and Lanzavecchia (17). In brief, Mo were obtained as described above. This enriched population, \(>95\%\) CD14\(\text{high}\), was subsequently cultured for 7 days in the presence of rhGM-CSF and rHL-4 at 500 U/ml and 250 U/ml, respectively. Every 2 days, 50\% of the CM was removed and replaced with fresh rhGM-CSF/IL-4 medium.

**Isolation and activation of naive CD4 T cells**

Naive CD4 T cells were isolated from the high-density PBMC fraction of healthy HIV-1-seronegative donors by negative selection, according to the manufacturer’s instructions, with immunomagnetic beads (Miltenyi Biotech) specific for CD8, CD14, CD19, CD56, and CD45RO Ags. The enriched population was \(>95\%\) pure for CD4/CD45RA-expressing T cells as determined by FACScan analysis. Allreactive MLR and Ag-specific T cell proliferation assays were performed with naive CD4 T cells obtained as described above and resuspended in CM at 10\(^6\) cells/ml. Autologous T cells (10\(^6\)/well) were cultured with freshly isolated CD2\(^+\) or CD2\(^-\) Mo (5 \times 10\(^3\)) in 96-well U-bottom plates (Falcon; Fisher Scientific) in the absence or presence of 10 \mu g/ml recombinant HIV-1 gp120 (HIV-1, catalog no. 1061; Immunodiagnostics, Bedford, MA). Allogeneic T cells (1 \times 10\(^4\)) were cultured with freshly isolated CD2\(^+\) Mo, CD2\(^-\) Mo, and total Mo (1 to 3 \times 10\(^3\)) or GM-CSF and IL-4 precultured CD2\(^+\) Mo, CD2\(^-\) Mo, or MDDC (5 \times 10\(^2\) to 2 \times 10\(^3\)) in 96-well U-bottom tissue culture plates (Falcon; Fisher Scientific). Following incubation at 37°C in humidified 5\% CO\(_2\) for 6 days, alloreactive and autologous cocultures were then pulsed with 1 \mu Ci (37kBq) \[^{3}H\]thymidine (NEN-DuPont, Boston, MA) for an additional 18 h and harvested on a UniFilter-96 (Packard Instrument, Meriden, CT). The DNA-associated radioactivity was measured by scintillation counting (TopCount microplate scintillation counter; Packard Instruments, Meriden, CT).

**CD2\(^+\) and CD2\(^-\) Mo morphology**

The morphology of the freshly isolated or matured CD2\(^+\) and CD2\(^-\) Mo was assessed by scanning electron or phase contrast light microscopy. Freshly isolated cells were suspended in 10\% PHS in PBS at a concentration of 2 \times 10\(^6\) cells/ml, and 20 \mu l (4 \times 10\(^5\)) cells were added to polylysine-coated cover slips and incubated at 37°C in 7% CO\(_2\) for 30 min before fixation in 1.25\% glutaraldehyde in 150 mM sodium cacodylate buffer at pH 7.2. The samples were postfixed with 1\% OsO\(_4\), dehydrated, embedded in Epon, and analyzed by the Core Electron Microscopy Facility at Dana-Farber Cancer Institute with a Philips EM 300 electron microscope. Phase contrast photomicrographs were taken of CD2\(^+\) and CD2\(^-\) Mo plated (2 \times 10\(^5\)) cells/ml in 6-well tissue culture plates and cultured for 36–48 h.

**Flow cytometry**

The following directly conjugated mAbs were used: anti-HLA-A, B, C (B9.12.1; Immunotech, Westbrook, ME); anti-HLA-DR (B8.12.2; Immunotech); anti-HLA-DQ (Leu-10; Becton Dickinson, San Jose, CA); anti-CD2 (SF13P2H9 (T11.1); Coulter, Miami, FL); Leu 5b (Becton Dickinson); and 39C1.5 (Immunotech); anti-CD13 (Leu-M7; Becton Dickinson); anti-CD14 (MY4; Coulter and TUK4; Caltag, Burlingame, CA); anti-CD33 (Leu-M9; Becton Dickinson); anti-CD19 (J4.119; Immunotech); anti-CD56 (84H10; Immunotech); anti-Thy-1.2 (5a-8; Immunotech); and anti-TCR\(\beta\) (BMA031; Immunotech). To increase the staining intensity of CD2 of Mo in some analyses, biotinylated anti-CD2 (T11.1; Coulter) and strepavidin PE (Becton Dickinson) was used. Matched isotype controls IgG1-FITC (Immunotech) or IgG1-biotin (Immunotech), and IgG2a-PE (Immunotech) were used for appropriate studies, and limits of negativity for each histogram reflect the quadrant boundary for the isotype controls. The samples were washed, fixed in 1\% paraformaldehyde (Sigma), and analyzed on FACScan (Becton Dickinson).

**RT-PCR**

Total RNA was isolated using ULTRASPEC (Biotec Laboratories, Houston, TX), and cDNA was synthesized by reverse transcription from mRNA.
using oligo dT_{12-18} base primers and Moloney murine leukemia virus reverse transcriptase (Life Technologies). PCR reactions were performed in a 24-μl reaction volume containing 2.4 μl of 10× PCR reaction buffer, 0.75 U Ampli-Tag Gold DNA polymerase (Perkin-Elmer, Norwalk, CT), 1 μl dNTP (Life Technologies), and 0.5 μl each of 5' and 3' amplification primers. cDNA from PBMC, T cells, and EBV-transformed B cells contained 5 ng of reverse transcriptase product; that from CD2− Mo contained 25 ng reverse transcriptase product. The primers used were: CD2, 5'-GCAACT CTG GOCGGATGATCAGGA-3' and 5'-GAGGCCTGTGCTGAACAGGGT-3' (Genosys Biotechnologies, Woodlands, TX); TCRζ, 5'-CAAGATGAAGGT GGAAGGCGC-3' and 5'-AATCCCGTGCTTGTAGCA-3' (a gift from Dr. J. Lieberman); CD14, 5'-AGCACCTTCCAGACCTGT-3' and 5'-CAGCACACGGTGCTAGGC-3' (Genosys Biotechnologies); GAPDH, 5'-GAAGGTGAAGGTCGGAGTC-3' and 5'-CAAATGTGTCTCATGGAT GACC-3' (Genosys Biotechnologies). All primers were run for 30 cycles at 95°C for 1 min, 56°C for 1 min, and 72°C for 1 min.

**Statistical analyses**

Data are presented as mean value ± SEM. Paired and unpaired Student’s t tests were used for analysis of statistical significance. Values of p < 0.05 were considered statistically significant.

**Results**

**Immunofluorescence analysis of Mo**

The PBMC were first studied by two-color immunofluorescence analysis for the expression of CD2, CD14, and TCRβ Ags (Fig. 1). Sixty percent of the PBMC expressed the TCR, and smaller percentages expressed CD14 or neither of the Ags (Fig. 1A). The population expressing the TCR represents the T cells; the population expressing CD14 represents Mo; the double negative population represents B cells and NK cells collectively. When the PBMC were stained with anti-CD2 and anti-CD14 mAbs, CD2 expression on Mo appeared as a continuum. Nevertheless, four populations were evident (Fig. 1B): CD2+CD14−, CD2−CD14high, CD2+CD14high, and CD2−CD14−. The CD2+CD14high population constituted approximately one-third of the total CD14high PBMC, and the level of CD2 expression was one-half log lower in intensity than on T cells.

CD2 expression on the purified Mo was compared with the expression of other classical leukocyte Ags. Two-color analysis revealed no discernible differences between the CD2+ and CD2− Mo with respect to the percent expression of CD13, CD14, or CD33 (Fig. 2, A–C). Although there was some variation in the mean fluorescence intensity from sample to sample of CD13, the percentages of CD2+ cells among the Mo expressing CD13, CD14, or CD33 were the same at 33%, 30%, and 32%, respectively. Two-color analysis of MHC Ags (Fig. 2, D–F) showed that >99% of the Mo expressed MHC class I Ags (with some variation in mean fluorescence intensity). In contrast, class II Ags were found on a lower percentage. HLA-DR was expressed on 73% of the Mo, while HLA-DQ was expressed on <3%. However, no significant difference was seen in the percentage positive or level of HLA-DR or HLA-DQ expression on CD2+ and CD2− Mo.

Because Mo and MΦ have greater autofluorescence than other leukocytes and our results did not show a clear separation of the CD2+ and CD2− Mo among CD14high cells, we cultured the PBMC in CM without added cytokines for 48 h before isolating the Mo. When the expression of HLA-DR was compared with that of CD2, the staining pattern was similar to that of the freshly isolated Mo with no point of clear separation (Fig. 3B). However, with respect to CD14 and CD2 expression, there was evidence of two distinct populations; CD2−CD14high and CD2+CD14− (Fig. 3C). These findings indicate that CD2− Mo lose CD4 expression after 2 days of culture and CD2− Mo do not and confirm that the limits set from the isotype controls were appropriate. The loss of CD14 by CD2− but not CD2+ Mo was also seen in the separated, incubated populations (data not shown).

**Characterization of CD2 on human Mo**

To further characterize CD2 on Mo, we compared T111, Leu 5b, and 39Cl.5 CD2 epitopes on T cells with their presence on Mo. We used the TUK4 mAb to CD14 to identify Mo. Each of the three CD2 epitopes was present on 79% of the T cells (Fig. 4, A–C). In

**FIGURE 2.** Comparison of lineage-specific Ags on Mo-enriched cells. Mo, depleted of T, B, and NK cells by immunomagnetic beads, were stained with fluorescent anti-CD2 (T11) and anti-CD13 (A), anti-CD14 (MY4) (B), anti-CD33 (C), anti-HLA-ABC (O), anti-HLA-DR (E), or anti-HLA-DQ (F) mAbs. Results are displayed as dot plots. All studies were performed with isotype controls, and negativity limits were set according to their location. Results obtained are representative of at least three independent studies.
contrast, T111, Leu 5b, and 39C1.5 epitopes were present on 35% (mean = 32.5% ± 0.35%, n = 4), 24% (mean = 20% ± 3.5%, n = 2), and 18% (mean = 13.5% ± 3%, n = 2) of the Mo, respectively (Fig. 4, D–F). To exclude the possibility that CD2 detection on Mo was due to nonspecific binding of the anti-CD2 by Mo, competitive-inhibition analysis was performed. We used the T111 and T112 mAbs, which bind to the same percentage of Mo (not shown), but recognize different epitopes on CD2 (24), and an

**FIGURE 3.** HLA-DR, CD2, and CD14 expression on cultured Mo. PBMC were cultured for 48 h and depleted of T, B, and NK cells by immunomagnetic bead selection. The remaining population was stained with anti-CD2-FITC (B and C) and either anti-HLA-DR-PE (B) or anti-CD14-PE (C). The myeloid population was analyzed and FITC- and PE-conjugated isotype controls were used to define quadrants (A). Results are representative of three independent studies.

**FIGURE 4.** CD2 expression on a subpopulation of Mo. Freshly isolated Mo were assessed for the presence of T111, Leu 5b, and 39C1.5 epitopes of CD2. T cells (A–C) and Mo (D–F) were stained with anti-CD14-PE (TUK4) (A–F), and anti-T111-FITC (A and D), anti-Leu 5b-FITC (B and E), or anti-39C1.5-FITC (C and F) mAbs. Specific binding of CD2 mAbs was further investigated by competitive inhibition analysis. Anti-T111-selected Mo were stained with anti-T111-PE (G–I) following preincubation with no mAb (G) or preincubation with unlabeled anti-T111 (H) or unlabeled irrelevant mAb, anti-Thy-1 (I). Results are representative of at least three experiments.
irrelevant Ab of the same IgG2a isotype, anti-Thy-1, to confirm binding specificity. The CD2\(^+\) Mo were selected with anti-T11\(_2\) and stained with anti-T11\(_1\)-PE (Fig. 4G) or preincubated with unlabeled anti-T11\(_1\) (Fig. 4H) or anti-Thy-1 (Fig. 4I) and then stained with anti-T11\(_1\)-PE. Loss of immunofluorescent staining occurred on T11\(_1\)-selected cells preincubated with unlabeled anti-T11\(_1\), but not on T11\(_1\)-selected cells preincubated with unlabeled anti-Thy-1. Thus, CD2 positivity represents specific binding to CD2 on a subpopulation of Mo.

DC and T cells have been shown to form stable aggregates that persist in culture after enrichment (24). To rule out T cell contamination as a possible explanation for the presence of CD2 on the Mo, mRNA isolated from PBMC, CD2\(^+\) Mo, B cells, and T cells was analyzed by RT-PCR (Fig. 5). There was detectable CD2 mRNA in PBMC, T cells, and CD2\(^+\) Mo. In contrast, TCR mRNA was detectable only in the PBMC and T cell samples and not in the CD2\(^+\) Mo sample. GAPDH primers were used as an internal standard and showed that the same quantity of mRNA was present in all samples. Thus, the CD2 detected on CD2\(^+\) Mo was not due to T cell contamination.

**Immunofluorescence analysis of CD2\(^+\) and CD2\(^-\) Mo**

The preceding experiments demonstrated several distinct epitopes of CD2 on CD2\(^+\) Mo. Consequently, an isolation protocol was designed based on the unique properties of CD2 (see Materials and Methods). This protocol rapidly (4–5 h) produced >95% pure populations of CD2\(^+\) and CD2\(^-\) Mo without culturing, in a state similar to that in circulating blood. The CD2\(^-\) Mo obtained by this protocol were 25–36% (mean = 31% ± 1.64%, n = 6) of the purified Mo population, which correlates closely with the percentage of CD2\(^+\) Mo seen by immunofluorescence in the unseparated Mo (Fig. 2). Thus, the apparent continuum of CD2 expression on Mo could be resolved into distinct (but usually overlapping) CD2\(^+\) and CD2\(^-\) subsets. Two-color analysis of the freshly isolated CD2\(^+\) and CD2\(^-\) populations showed similar levels of CD14 (Fig. 6, B and F) and HLA-DR (Fig. 6, C and G), but no detectable levels of TCR or CD3 (Fig. 6, D and H), CD19, or CD56 (not shown). As in the unseparated Mo population (Fig. 2), the CD2\(^+\) and CD2\(^-\) populations expressed similar levels of CD13, CD33, and HLA-DQ (not shown). Additionally, there were no detectable differences in the expression of CD1a, CD11a/c, CD50, CD54, CD58, CD80, or CD86 (Table I). The CD83 Ag, which is expressed by cultured DC (21, 29–31), was not expressed on freshly isolated CD2\(^+\) or CD2\(^-\) Mo. After 2 days of culture, CD83 became detectable on CD2\(^+\) Mo, but this expression varied from donor to donor.

**Morphologic characteristics of enriched CD2\(^+\) and CD2\(^-\) Mo**

Previous studies have shown that after 36–48 h of culture, Mo are large, round or oval cells with an eccentric nucleus, while DC are large, irregularly shaped cells with dendritic-like projections (1). These morphologic features also distinguish the two populations.
This suggests that fresh or cytokine-incubated CD2\(^{+}\) stimulation (p), the CD2\(^{+}\) population increased to levels that were 10- and 40-fold greater than that of MDDC (Fig. 8, C). GM-CSF- and IL-4-cultured CD2\(^{+}\) Mo were purified as described in Materials and Methods, and either examined fresh by scanning electron microscopy (A–D) or after 2 days in culture by phase contrast microscopy (E and F). Original magnifications: A and B, \(\times 2600\); C and D, \(\times 9000\); E and F, \(\times 400\).

To determine the ability of the freshly isolated CD2\(^{+}\) Mo to process and present a nominal Ag, the CD2\(^{+}\) and CD2\(^{-}\) Mo were cultured with autologous naive CD4 T cells at 1:20 (APC-to-T cell ratio) in the presence and absence of HIV-1 gp120 (Fig. 8C). Both CD2\(^{+}\) and CD2\(^{-}\) Mo induced naive CD4 T cell activation with stimulatory indices of 41 and 6, respectively. However, the induction of naive CD4 T cell activation by CD2\(^{-}\) Mo was significantly different from control values (p < 0.02), while the induction by CD2\(^{+}\) Mo was not (p > 0.05). These findings further corroborate our hypothesis that CD2\(^{+}\) Mo are DC.

**Discussion**

This study provides evidence that CD2\(^{+}\) CD14\(^{high}\) and CD2\(^{-}\) CD14\(^{high}\) PBMC represent DC and Mo, respectively. The DC were shown to be far more abundant in the peripheral blood than previously recognized (13–16, 32), constituting about one-third of the CD14\(^{high}\) cells (Fig. 1) and 2–4% of the total PBMC. Furthermore, we showed that CD2 is expressed on both freshly isolated and cultured DC and thus can be used as a means of identification and positive selection. The apparent continuum of CD2 expression on Mo is a result of different antigenic stimulation and, therefore, further studies are needed to identify the role of CD2 in Mo function.

Table 1. **Surface Ag expression of freshly isolated CD2\(^{+}\) and CD2\(^{-}\) Mo**

<table>
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<tr>
<th>Determinant</th>
<th>mAb Source</th>
<th>CD2(^{+}) Mo</th>
<th>CD2(^{-}) Mo</th>
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<tr>
<td>HLA-DQ</td>
<td>Leu-10 Becton Dickinson</td>
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<td>IT2.2 PharMingen</td>
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* Each (+) indicates an increase of 0.5 log in mean fluorescent intensity vs. isotype-matched controls.

*CD4 T cell activation by CD2\(^{+}\) Mo*

To test the capacity of CD2\(^{+}\) Mo to stimulate allogeneic and primary Ag-specific T cell responses, we investigated their ability to activate naive CD4 T cells in MLR and Ag-specific proliferation assays. First, we compared the ability of freshly isolated CD2\(^{+}\), CD2\(^{−}\), and total Mo to induce an alloreactive MLR (Fig. 8A). The freshly isolated CD2\(^{+}\) population induced 2- to 3-fold greater stimulation (p < 0.02) than the total or CD2\(^{−}\) population at various stimulator/responder ratios. Because of the recent introduction of MDDC as a source of DC, we compared the allogeneic T cell stimulation by GM-CSF- and IL-4-cultured CD2\(^{+}\) and CD2\(^{-}\) Mo to that of MDDC (Fig. 8B). GM-CSF- and IL-4-cultured CD2\(^{+}\) Mo, at low concentrations, were significantly more efficient than MDDC (p < 0.02) in naive T cell activation. However, at APC concentrations of 20%, the efficiency of naive T cell activation by MDDC and CD2\(^{-}\) Mo decreased, while that of the CD2\(^{+}\) Mo population increased to levels that were 10- and 40-fold greater (p < 0.0005) than that of CD2\(^{-}\) Mo and MDDC, respectively. This suggests that fresh or cytokine-incubated CD2\(^{+}\) Mo are much more potent stimulators of naive CD4 T cells than MDDC, particularly at higher concentrations, and further supports the hypothesis that the CD2\(^{+}\) Mo are DC.
induce proliferation of naive T cells in the presence of foreign Ag compared with CD2
Mo or MDDC. In contrast to CD2 expression, myeloid Ags (CD13, CD14, and CD33), MHC, or costimulatory and adhesion molecules were expressed at similar levels on freshly isolated DC and Mo. Therefore, these cell-surface Ags were not useful in distinguishing the two populations in the circulation. Collectively, these results suggest that what is thought to be a subset of circulating CD2
Mo consists, in fact, of DC and provide support for the concept that DC and Mo exist in the circulation as distinct populations.

Our results do not support the concept that DC exist as two populations, CD2
CD14 and CD2
CD14 (14, 16). Rather, they suggest that most peripheral blood DC are CD2
CD14
high. The discrepant results are likely to be explained by the use of different mAbs to detect CD2 (14, 16) and anti-CD14 mAbs or SRBC to deplete Mo, T, and NK cells (31, 33). Most studies of CD2 on DC have used non-T111 mAbs such as Leu 5b (13, 14, 16). However, our results suggest that the Leu 5b and 39C1.5 CD2 epitopes are detectable on only a fraction of DC (Fig. 4, E and F) that may vary from 10–70% (our unpublished observations). Furthermore, the failure of some studies to detect CD2 expression on DC presumably has been because of the one-half log lower expression of CD2 by freshly isolated DC compared with T cells (Fig. 4, A and D) or NK cells (our unpublished observations). Together, these findings suggest that isolation methods that enrich for HLA-DR
CD14
DC (1, 13, 14, 16) lose 90% of DC primarily because of the reagents used to deplete T cells, NK cells, and Mo. Although Weissman et al. demonstrated enrichment of T cells and DC with SRBC (24), which bind to CD2
(26), some studies have continued to use neuraminidase-treated SRBC or anti-CD2 mAbs to deplete T and NK cells (34, 35).

CD14 expression on DC but not on Mo is lost after 2 days of culture in either PBMC (Fig. 3) or in isolated populations (data not shown). Because cultured DC after incubation have characteristic morphology and are largely CD14
, whereas Mo largely retain CD14 expression, the concept arose that DC circulate as CD14
myeloid cells (14). Our studies suggest that the use of anti-CD14 mAbs to deplete Mo (13, 14, 16) not only depletes the majority of DC but leaves a heterogeneous population of CD14
Mo and DC (Fig. 4D).

The ultimate criteria for distinguishing DC from Mo/Mφ are the DCs unique morphology after culture, greater capacity to induce an alloreactive MLR (36), and the induction of Ag-specific proliferation of naive T cells (8). Morphologically, freshly purified Mo are indistinguishable from DC by light microscopy (37, 38). In this report, evidence is presented that CD2
Mo (DC) are morphologically distinguishable from CD2
Mo (pMφ).

In contrast to previous findings by others (14, 16) that freshly isolated DC require preculture in conditioned medium before a potent T cell response is generated, we observed full Ag-presenting capacity in freshly isolated DC. One possible explanation for the preculturing requirement of HLA-DR-sorted CD14
DC is to allow the recovery of APC function. APC function is inhibited by anti-HLA-DR mAbs, which can block HLA-DR-TCR interaction (39, 40) or inhibit the phagocytic stimuli required for efficient activation (41). Although we enriched by positive selection with anti-CD2 mAbs, our method does not appear to affect DC function.
Although CD2 expression on Mo/DC has been known for many years (13, 25), it was not until recently that emphasis has been placed on CD2-expressing subsets. Takamizawa et al. demonstrated that the CD2+ CD14+ and CD2− CD14− DC were functionally distinct populations (16). Both populations could induce allogenic T cell activation; however, only the CD2+ CD14− DC population had the ability to activate naïve T cells. This is consistent with our finding that the CD2+ population is able to initiate an HIV-1 gp120-specific T cell response. In addition to HIV-1 gp120, we have found that DC efficiently present other nominal Ags, such as hepatitis B surface Ag and keyhole limpet hemocyanin, to naïve T cells (data not shown).

The lower T cell stimulation induced by fresh or GM-CSF/IL-4-4-incipubated CD2− Mo at higher APC-to-T cell ratios suggests the presence of an inhibitory activity not present in CD2+ Mo. This activity would be consistent with the presence of classic Mo/Mf differentiation.

Recent studies by Randolph et al. (42) demonstrated that GM-CSF and IL-4 are not required for DC differentiation from Mo, suggesting that either transmigration across an endothelial barrier, prolonged culture, or adherence to collagen are sufficient to induce the DC phenotype. Once differentiated, the DC described by these authors are phenotypically similar to our cultured DC, expressing HLA-DR, CD83, and CD86, but no or low CD14. Our results are consistent with their findings, and suggest that prolonged culture on tissue culture plastic is sufficient to induce further DC differentiation.

Because endothelial cells do not secrete IL-4 or IL-13 (43), it appears likely that DC differentiation in vivo is different from the proposed GM-CSF and IL-4 or IL-13 models (17, 18). It may be that DC expansion from GM-CSF- and IL-4-cultured Mo does not induce the differentiation of DC from Mo, but instead induces activation of that fraction of DC already present in the starting CD14high population. Our results do not preclude the possibility that DC can arise from Mo, but rather suggest that the CD2+ CD14high Mo subpopulation consists of DC. The ability to isolate pure primary DC in high yield from the peripheral blood without cytokine stimulation has considerable importance for the study of human DC biology and will help to elucidate the potential role of these cells in graft-vs-host disease, in immune-mediated diseases, and for immunotherapy.

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