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J Immunol 2015; 195:1774-1781; Prepublished online 15 July 2015;
doi: 10.4049/jimmunol.1500522
http://www.jimmunol.org/content/195/4/1774

Supplementary Material  http://www.jimmunol.org/content/suppl/2015/07/15/jimmunol.150052

2.DCSupplemental

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Differentiation Kinetics of Blood Monocytes and Dendritic Cells in Macaques: Insights to Understanding Human Myeloid Cell Development

Chie Sugimoto, Atsuhiko Hasegawa, Yohei Saito, Yayoi Fukuyo, Andrew A. Lackner, Woong-Ki Kim, Elizabeth S. Didier, and Marcelo J. Kuroda

Monocyte and dendritic cell (DC) development was evaluated using in vivo BrdU pulse-chase analyses in rhesus macaques, and phenotype analyses of these cells in blood also were assessed by immunostaining and flow cytometry for comparisons among rhesus, cynomolgus, and pigtail macaques, as well as African green monkeys and humans. The nonhuman primate species and humans have three subsets of monocytes, CD14⁺CD16², CD14⁺CD16⁺, and CD14⁻CD16⁺ cells, which correspond to classical, intermediate, and nonclassical monocytes, respectively. In addition, there exist presently two subsets of DC, BDCA-1⁺ myeloid DC and CD123⁺ plasmacytoid DC, that were first confirmed in rhesus macaque blood. Following BrdU inoculation, labeled cells first appeared in CD14⁺CD16⁻ monocytes, then in CD14⁺CD16⁺ cells, and finally in CD14⁺CD16² cells, thus defining different stages of monocyte maturation. A fraction of the classical CD14⁺CD16⁻ monocytes gradually expressed CD16² to become CD14⁺CD16² cells and subsequently matured into the nonclassical CD14⁺CD16² cell subset. The differentiation kinetics of BDCA-1⁺ myeloid DC and CD123⁺ plasmacytoid DC were distinct from the monocyte subsets, indicating differences in their myeloid cell origins. Results from studies utilizing nonhuman primates provide valuable information about pathogenesis mechanisms and intervention strategies in humans.

Received for publication March 3, 2015. Accepted for publication June 16, 2015.

This work was supported by National Institutes of Health Grants AI087302, AI091501, AI097059, AI1110163, AI1116198, and HL125054 (to M.J.K.) and P50OD01104 (to Tulane National Primate Research Center). This work was also supported by a grant from Virginia’s Commonwealth Health Research Board (11-09 to W.-K.K.), as well as AIDS research grants from the Health Sciences Research Grants and from the Ministry of Health, Labor, and Welfare of Japan.

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The online version of this article contains supplemental material.

Abbreviations used in this article: BDCA, blood DC Ag; cART, combination anti-retrovirus therapy; DC, dendritic cell; EdU, 5-ethynyl-2'-deoxyuridine; mDC, myeloid DC; NHP, nonhuman primate; pDC, plasmacytoid DC.

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Nonhuman primates (NHP) are genetically and physiologically closely related to humans, and thus serve as valuable models of human diseases and immune responses (13). An added advantage is that many Abs to human monocytes, macrophages, and DC exhibit cross-reactivity to these cells from rhesus macaques (14, 15). In earlier studies, we successfully demonstrated that in vivo BrdU pulse-chase experiments could be applied to monitor changes in the turnover rates of blood monocytes during viral and bacterial infections in rhesus macaques that were predictive for disease outcomes (16, 17). BrdU, a thymidine analog, incorporates into hematopoietic progenitor cells possessing proliferating capacity in bone marrow, and thus can be used as a tool to characterize differentiation of myeloid lineage cells in vivo. The purpose of this study therefore was to characterize the phenotype, turnover, and differentiation of monocytes and DC in rhesus macaques to expand our knowledge of the biology of myeloid cell development in humans. Such information is difficult to monitor longitudinally in humans, and the results from studying rhesus macaques will contribute to better understanding mechanisms of disease pathogenesis and development of immune responses produced by monocytes, macrophages, and DC in humans.

Materials and Methods

Nonhuman primates

Rhesus macaques (Macaca mulatta), cynomolgus macaques (Macaca fascicularis), pigtail macaques (Macaca nemestrina), and African green monkeys (Chlorocebus sabaeus) were housed at the Tulane National Primate Research Center and were sampled for blood samples used in this study. In addition, blood was obtained from a set of rhesus macaques infected with 300 tissue culture-infective dose50 units of SIVmac239 i.v. and treated with s.c. inoculation of combination antiretrovirus therapy (cART) that comprised 20 mg/kg tenofovir disoproxil fumarate once daily and 40 mg/kg of 60 mg/kg body weight, and EDTA anticoagulated blood samples were collected at varying times after administration, as indicated in Results, to measure cell turnover rate was determined by Ab staining and flow cytometry. Blood from cynomolgus macaques, pigtail macaques, and African green monkeys was only used for phenotype analyses in this study.

Blood specimens and BrdU administration to measure cell turnover

Rhesus macaques were injected with the thymidine analog, BrdU, i.v. at a dose of 60 mg/kg body weight, and EDTA anticoagulated blood samples were collected at varying times after administration, as indicated in Results, to measure cell turnover rate was determined by Ab staining and flow cytometry. Blood from cynomolgus macaques, pigtail macaques, and African green monkeys was only used for phenotype analyses in this study.

Flow cytometry

Blood specimens collected in EDTA anticoagulant were washed once with at least 5 vol PBS containing 2% FBS in PBS (2% FBS-PBS) and resuspended in the original volume of 2% FBS-PBS. Fresh blood specimens were stained for the evaluations in this study. There was negligible nonspecific background staining, so the live/dead marker stain was not used in this work. Two hundred microliters of washed blood were stained for expression of surface markers by incubation with Abs for 20 min at room temperature (see Table I for list of Abs). RBCs were lysed with 1× FACS lysis solution (BD Biosciences) and the remaining cells were permeabilized and stained for 20 min, Perm/Wash buffer (BD Biosciences) supplemented with 10% DMSO to boost permeabilization for 10 min (18), and Cytofix/Cytoperm (BD Biosciences) for 5 min. For analysis of BrdU incorporation, cells were incubated with anti-BrdU Ab for 20 min at room temperature after 1-h incubation at 37°C with DNase I (Roche Diagnostics). After washing, all sets of cells were analyzed for cell surface phenotype using FlowJo software version 9.6 (Tree Star). Live/Dead marker was not used in this study.

Ag-specific cell proliferation assay

PBMC were enriched by Ficoll gradient centrifugation of blood obtained from rhesus macaques chronically infected with SIVmac239 and undergoing cART. These animals that controlled plasma viral loads 1000 copies/ml and exhibited normal monocyte turnover and phenotype (data not shown) were selected for this study because they expressed SIV-specific cell proliferation and immune responses. Monocytes, DC subsets, and CD3+ T cells were sorted by flow cytometry using the BD FACS Aria (Fig. 1). Each monocyte and DC population was incubated for 2 h with SIVmac251 Gag PR55 protein (National Institutes of Health AIDS Research and Reference Reagent Program) for stimulating Ag-specific CD3+ T cell proliferation ex vivo. After washing with RPMI 1640 supplemented with 10% FBS, 10,000, 5,000, and 2,500 cells of Gag-pulsed monocytes or DC subsets were cultured with 1 × 106 CD3+ T cells in a 96-well U-bottom culture plate for 4.5 d. Another thymidine analog, 5-ethyl-2′-deoxyuridine (EdU), was added at a final concentration of 10 μM during the last 18 h of the culture, and cell proliferation was detected as a function of EdU incorporation using the Click-iT EdU Flow Cytometry Assay Kit (Life Technologies). Results were acquired using a FACSCanto II flow cytometer (BD Biosciences), and data were analyzed by FlowJo software.

Statistical analyses

Mann–Whitney rank test was used to compare the mean difference between groups using GraphPad Prism version 5.0f for Mac (GraphPad Software, San Diego, CA). The p value <0.05 was considered statistically significant.

Results

Blood monocyte and DC subpopulation phenotypes are similar in rhesus macaques and humans

Blood monocytes and DC subsets from rhesus macaques and humans were evaluated by multicolor flow cytometry using previously described panels ofAbs to phenotypic markers (3, 14, 15) and as shown in Table I and Fig. 1. Because monocytes and DC are considered myeloid lineage cells, HLA-DR–positive and lymphocyte/NK marker-negative cells were gated to further characterize monocytes and DC. Although CD56 is a common NK marker in humans, it also is expressed on monocytes in rhesus macaques (19). Thus, CD3 was used instead of CD56 to discriminate NK cells in rhesus macaques. NKG2A or NKp46 was not applied to further exclude NK cells because most NKG2A-positive cells are HLA-DR–positive or CD8 positive. Therefore, these HLA-DR–positive and lymphocyte/NK lineage marker-negative cells contained negligible numbers of NK cells (data not shown). As reported previously, four distinct populations of cells could be distinguished based on CD14 and CD16 expression on HLA-DR–positive, lymphocyte/NK lineage marker-negative cells in both humans and rhesus macaques (7, 20) (Fig. 1). Of these four populations, three were identified as monocyte subsets.

Table I. Abs used in this study

<table>
<thead>
<tr>
<th>Markers</th>
<th>Clone</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>CD1c (BDCA-1)</td>
<td>AD5-8E7</td>
<td>Miltenyi Biotec</td>
</tr>
<tr>
<td>CD3</td>
<td>SP34-2</td>
<td>BD Biosciences</td>
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<td>CD8</td>
<td>SK2</td>
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<td>S-HCL-3</td>
<td>BD Biosciences</td>
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<td>B9E9</td>
<td>Beckman Coulter</td>
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<td>MB4-6D6</td>
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<td>CD56</td>
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<tr>
<td>CD123</td>
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<td>Mac2-158</td>
<td>Trillium</td>
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<tr>
<td>HLA-DR</td>
<td>L243</td>
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Downloaded from http://www.jimmunol.org/ by guest on April 20, 2017
corresponding to classical (CD14+CD16-), intermediate (CD14+CD16+), and nonclassical (CD14-CD16+) monocytes.

Blood DC populations are commonly identified from within the population of cells that are positive for HLA-DR and negative for lymphocyte/NK/monocyte markers. MacDonald et al. (12) described four distinct sets of DC populations that were identified in humans as CD11c+CD16+, CD11c+BDCA-1+, CD11c+CD141+, and CD11c+CD123+. In rhesus macaques, however, three populations of cells expressing DC markers are identified by Autissier et al. (14) as CD11c+CD16+, CD11cdimBDCA-1+, and CD11c+CD123+, but not CD141+ DC. Using this reported gating strategy, the CD14+CD16+ monocytes and CD11c+CD16+ DC populations completely overlapped and identified the same cell subsets in humans and rhesus macaques (Supplemental Figs. 1, 2). The CD16+ population previously identified as a DC subset is recently classified instead as a subset of monocytes (3). Therefore, Abs to BDCA-1, CD141, and CD123 markers were used to identify three DC subsets in humans (BDCA-1+, CD141+, and CD123+ DC) (Fig. 1A), and only two DC subsets have been identified in rhesus macaques due to the lack of a reliable rhesus CD141-specific Ab (Fig. 1B, Table II). By careful analysis of the three distinct DC subsets in human blood, we observed a CD141+ DC subset that

### Table II. Phenotype comparison of monocyte and DC subsets from human and rhesus macaque blood

<table>
<thead>
<tr>
<th>Human Monocye DC</th>
<th>Human Monocye DC</th>
</tr>
</thead>
<tbody>
<tr>
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<td>CD14+CD16-</td>
</tr>
<tr>
<td>CD14+CD16+</td>
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</tr>
<tr>
<td>CD14-CD16+</td>
<td>CD14-CD16+</td>
</tr>
<tr>
<td>CD123+ pDC</td>
<td>CD123+ pDC</td>
</tr>
<tr>
<td>BDCA-1+ mDC</td>
<td>BDCA-1+ mDC</td>
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</table>

<table>
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<th>Rhesus Macaque Monocye DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14+CD16-</td>
<td>CD14+CD16-</td>
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<tr>
<td>CD14+CD16+</td>
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</tr>
<tr>
<td>CD14-CD16+</td>
<td>CD14-CD16+</td>
</tr>
<tr>
<td>CD123+ pDC</td>
<td>CD123+ pDC</td>
</tr>
<tr>
<td>BDCA-1+ mDC</td>
<td>BDCA-1+ mDC</td>
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</tbody>
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**FIGURE 1.** Phenotyping of blood monocyte and DC subsets in humans (A) and rhesus macaques (B). EDTA-treated blood samples were stained with Abs shown in Table I and analyzed by 11-color flow cytometry. (A) In HLA-DR+CD3+CD20-CD56- populations, human monocyte and DC subsets were gated and divided into four populations by CD14 and CD16 expression, as follows: (Aa) CD14+CD16- monocytes, (Ab) CD14+CD16+ monocytes, (Ac) CD14+CD16- monocytes, and a CD14+CD16- population that was further divided into (Ad) CD123+ pDC and (Ae) BDCA-1+ mDC. In addition, a CD141+ mDC (Af) was identified. (B) To analyze rhesus monocyte and DC subsets, HLA-DR+CD3+CD20-CD8- cell populations were similarly gated and further divided, as described in (A), with the exception that Ab to human CD141 (BDCA-3) did not cross-react to, or detect this marker on, rhesus macaque cells. The populations of cells identified included the following: (Bb) CD14+CD16+ monocytes, (Bc) CD11c+CD16+ monocytes, (Bd) CD14+CD16+ monocytes, (Be) CD11c+CD16- monocytes, (Af) CD141+ mDC, and (Be) BDCA-1+ mDC.
expressed CD11c, but was negative for CD16 and BDCA-1 and did not overlap with any of the other positive subsets of DC (Fig. 1A).

The rhesus cell subset that was negative for CD16 and BDCA-1 within the HLA-DR+ population, but lineage marker-negative cell fraction of cells (i.e., negative for CD3, CD20, CD8, CD16, and CD14) may include the rhesus homologous CD141+ cells. Therefore, the CD123+BDCA-1+ fraction of rhesus cells and the equivalent fraction from human samples were compared with assessed proportions of monocyte and DC subsets (Fig. 2). In both humans and rhesus macaques, similar patterns were observed. Over 70% of the HLA-DR+ lymphocyte-lineage marker-negative cells were CD14+ monocytes, and <10% of the cells comprised a subset of DCs. Statistically significant differences in the mean percentage of HLA-DR+ lymphocyte lineage-negative subsets, however, were observed between human and rhesus macaque blood DC subsets. Specifically, levels of BDCA-1+ DC and BDCA-1-CD123+ DC cell populations were higher in rhesus macaques, whereas CD123+ pDC numbers were lower in rhesus macaques compared with humans. No significant difference was observed in the monocyte subset levels between humans and rhesus macaques (Fig. 2A).

Comparative expression of CD11c on monocytes and DC in human and nonhuman primate blood

CD11c is considered a mDC marker in humans and mice and also has been widely used to identify mDC in rhesus macaques (3, 12, 21). After detailed characterization of the different DC subsets shown in Fig. 1 and Supplemental Fig. 1, it became apparent that myeloid DC subsets, as well as monocyte subsets, were positive for CD11c expression in humans, whereas only monocytes expressing CD16 (nonclassical monocytes) were positive for CD11c expression in rhesus macaques. Thus, relatively few DC were included within the CD11c+ blood cell fraction in rhesus macaques. Because anti-human Abs are generally used for NHP samples analyzed in these studies, the differences in staining patterns could result from differences in levels of cross-reactivity. Therefore, we tested rhesus macaque blood specimens with two different mAb clones (S-HCL-3 and 3.9) for detecting CD11c and observed that cells incubated with clone 3.9 did not exhibit staining by S-HCL-3 in rhesus macaques. Over 70% of the HLA-DR+ lymphocyte lineage-negative cells were CD14+ monocytes, and <10% of the cells comprised a subset of DCs. Statistically significant differences in the mean percentage of HLA-DR+ lymphocyte lineage-negative subsets, however, were observed between human and rhesus macaque blood DC subsets. Specifically, levels of BDCA-1+ DC and BDCA-1-CD123+ DC cell populations were higher in rhesus macaques, whereas CD123+ pDC numbers were lower in rhesus macaques compared with humans. No significant difference was observed in the monocyte subset levels between humans and rhesus macaques (Fig. 2A).

Nonclassical CD14−CD16+ monocytes differentiate from classical CD14+CD16− monocytes that originated from bone marrow

Incorporation of BrdU following injection has been used to successfully track the fate of CD14+ (classical monocytes) in rhesus macaques (16). To further investigate the origin and differentiation of the monocyte and DC subsets, blood samples were collected from four rhesus macaques between days 0 and 21 after BrdU injection to follow the kinetics of BrdU incorporation by myeloid cells using flow cytometry (Fig. 5). BrdU-positive cells appeared as early as day 1 in the CD14−CD16+ cell fraction, and then peaked between days 2 and 4 primarily in the CD14+CD16+ cells (Fig. 5B). BrdU incorporation was subsequently detected in the CD14+CD16+ cell fraction on day 3 and then in the CD14+CD16− monocytes on day 7 (Fig. 5B). The percentage of BrdU-positive...
cells gradually decreased after day 7 until no BrdU staining cells were observed 21 d after injection (Fig. 5B).

We also followed the kinetics of BrdU incorporation into each subset of monocytes and DCs based on the gating strategy described in Fig. 1, and the results chronicle the development of each cell subset after emigration from the bone marrow to blood and subsequent transition to tissues (Fig. 6). The initial peak in BrdU incorporation occurred in CD14+CD16– classical monocytes within 2 d. Then CD14+CD16– intermediate monocytes were observed to preferentially stain for BrdU incorporation within 4 d, and finally, CD14–CD16+ nonclassical monocytes exhibited peak BrdU uptake ~10 d after BrdU injection (Fig. 6A). At that time, no BrdU staining was observed in the classical or intermediate monocyte subsets (Fig. 6). These results indicate that CD14+CD16– classical monocytes are continuously generated from bone marrow and that there is a steady-state differentiation from intermediate to nonclassical monocytes.

In contrast to the monocyte subsets, two of the DC subsets identified in rhesus macaques, BDCA-1+ mDC and CD123+ pDC, showed distinct kinetics of BrdU incorporation (Fig. 6). The BDCA-1+ mDC showed the fastest turnover and exhibited peak BrdU labeling within the first 24 h. BrdU incorporation peaked after 4 d in the CD123+ pDC subset, and the decline or loss of BrdU occurred more slowly than the decline observed for the other monocyte or DC subsets (Fig. 6). These results imply that the two DC subpopulations of cells originate from distinct cells in the bone marrow and develop independently from monocyte differentiation (Fig. 7).

**Discussion**

Nonhuman primates are physiologically similar to humans and thus serve as important animal models to better understand the human immune system. In this study, we compared the phenotype of blood monocytes and DC in nonhuman primates, particularly in rhesus macaques, with the phenotype of equivalent cells in human blood. Using our results and recommendations of the Nomenclature Committee of the International Union of Immunological Societies, we attempted to develop concordant classifications (3) that can be assigned to the cell subpopulations in rhesus macaques and determined the proportions of monocyte and DC subsets that reside in the HLA-DR+–positive and lymphocyte (T, B, and NK cells) lineage-negative cell fractions (Figs. 1, 2). We confirmed that the three subsets of monocytes, CD14+CD16+, CD14+CD16–, and CD14–CD16–, corresponding to classical, intermediate, and nonclassical monocytes, respectively, and two subsets of DC, BDCA-1+ mDC and CD123+ pDC, described for humans, were also represented in rhesus blood (Fig. 1). Despite the lack of clearly identifiable CD141+ mDC in rhesus blood, our overall results are consistent with previous reports (4, 14, 15).

The results in this study further examined additional relationships between human and rhesus macaque blood myeloid cell phenotypes (Fig. 1). For example, mDC in rhesus macaques have been analyzed by the use of the CD11c marker on HLA-DR+ lymphocyte lineage (CD3, CD20, CD8, and CD14) marker-negative cells (Supplemental Fig. 2). This conventional mDC population includes a large proportion of nonclassical monocytes defined by the expression of CD16. This is most likely attributed to the differences between humans and nonhuman primates regarding expression of CD11c (Fig. 3, Supplemental Fig. 2). CD11c was expressed only by CD16+ monocyte populations, including CD14+CD16+ and CD14+CD16– monocytes in NHP, whereas, in humans, all of the monocyte and DC subsets, except for the CD123+ pDC, express CD11c. This may have led to the belief that CD11c+ HLA-DR+ lymphocyte lineage (CD3, CD20, CD8, and CD14) marker-negative cells in nonhuman primate blood include
A mDC subset. Furthermore, the Ag presentation assay comparing the monocyte and DC subsets in rhesus macaque blood. (A) Eleven healthy normal adult rhesus macaques were administrated BrdU i.v., and blood was drawn and analyzed at indicated time points. The percentage of BrdU+ cells within each of the monocyte subsets (CD14+CD16+, CD14+CD16−, and CD14−CD16+ monocytes) and two DC subsets (BDCA-1+ mDC and CD123+ pDC), as well as the non–BDCA-1 and CD123 populations identified in Fig. 1B was plotted as a percentage of the HLA-DR+ lymphocyte lineage-negative cells. (B) Mean values (% ± SD) of BrdU incorporation by the monocyte (left panel) and DC plus BDCA-1− and CD123+ populations (right panel) were plotted from the 11 animals.

FIGURE 6. Kinetics of BrdU incorporation by each monocyte and DC population in rhesus macaque blood. (A) Eleven healthy normal adult rhesus macaques were administrated BrdU i.v., and blood was drawn and analyzed at indicated time points. The percentage of BrdU+ cells within each of the monocyte subsets (CD14+CD16+, CD14+CD16−, and CD14−CD16+ monocytes) and two DC subsets (BDCA-1+ mDC and CD123+ pDC), as well as the non–BDCA-1 and CD123 populations identified in Fig. 1B was plotted as a percentage of the HLA-DR+ lymphocyte lineage-negative cells. (B) Mean values (% ± SD) of BrdU incorporation by the monocyte (left panel) and DC plus BDCA-1− and CD123+ populations (right panel) were plotted from the 11 animals.

These results imply that additional DC subsets homologous to the CD14+ DC in human blood exist in nonhuman primate blood (Fig. 7).

BrdU, a thymidine analog, is incorporated during DNA synthesis at the S-phase of cell cycle and is considered a reliable marker of dividing cells (23–25). In vivo BrdU pulse-chase studies thus are useful for tracing the development, kinetics, trafficking, and turnover of replicating cells of the immune system in animal models (17, 26–29). Results presented in this work indicate that division of monocytes occurs at the hematopoietic stem cell-derived progenitor stage with myeloid-restricted differentiation potential in bone marrow, and that monocytes are then released into the circulation at the end of S-phase (30). Although less information has been reported about the development and maturation of blood DC, our results shown in Figs. 5 and 6 directly support previous findings that the CD14+CD16+ cells derived from a fraction of monocyte subsets were clearly distinct from the mDC subsets (22, 31). BrdU-positive CD14+CD16− monocytes were observed within the first 24 h and rapidly increased to peak levels at day 2, and BrdU uptake was then detected sequentially in CD14+CD16+ and then CD14−CD16+ fractions of cells. It is interesting to note that the BrdU+CD14+CD16− monocytes observed 24 h after BrdU injection expressed lower levels of CD14 Ag, suggesting that newly recruited monocytes from bone marrow gradually contribute to increasing the level of CD14 expression in the blood. These data also strongly suggest that nonclassical monocytes differentiate from CD14+CD16− (classical monocytes) by gradually expressing CD14+ to become CD14+CD16− (intermediate monocyte) cells and subsequently mature to the nonclassical CD14−CD16+ cell subset as they gradually exhibit decreased expression of CD14 protein while circulating in blood. Our studies using BrdU pulse-chase analyses in rhesus macaques thus directly demonstrated a link in the differentiation and turnover among the three monocyte subsets and corroborate previous studies linking the developmental relationship among three monocyte subsets using M-CSF treatment in humans and NHP (32, 33).

In vivo BrdU labeling of monocytes also has contributed to better understanding mechanisms of pathogenesis in infectious diseases (17, 26, 27, 29). Because BrdU is considered carcinogenic, it has only rarely been applied in human studies (34), so its use is more common in laboratory animals. For example, SIV infections in...
Recently, a novel distinct DC-restricted precursor that produces tors in the bone marrow are strongly committed to produce both monocyte and BDCA-1+ DC subsets. The common DC progenitor differentiated 6 d, indicating a slower cell turnover than exhibited by the increased during the first 4 d and slowly decreased over the sub-

2
into CD14
BDCA-1+ mDC exhibiting the highest turnover kinetics among precursors. Our data suggest, however, that monocyte subsets did differentiate into monocyte-derived DC with many characteristics from distinct progenitors. CD14 + monocytes are known to dif-

2
classical monocytes, and that a large proportion of these classical monocytes rapidly disappeared from the circulation to become tissue macrophages. A fraction of the classical monocytes differ in maturation stage and inflammatory response.

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2
analysis using bromodeoxyuridine: comparison of methods for analysis of total somatic cell transit time.


Acknowledgments

We thank Jason Dufour for veterinary support; Julie Bruhn and Calvin Lanclos for flow cytometry expertise; and Erin Haupt, Ashley Leach, Toni Penney, Deserie Waguespack, and Faith Schiro for technical support.

Disclosures

The authors have no financial conflicts of interest.


Supplemental Figure 1. CD11c, CD11b and CD163 expression on monocyte and DC subsets. The HLA-DR+ lymphocyte-lineage marker-negative cell population was analyzed for expression of CD14, CD16, CD11b, CD11c, and CD163. Panels A and B represent results from human blood and Panels C and D represent results from rhesus macaque blood. (A and C) Each monocyte subset is shown in different color: CD14+CD16- (red), CD14+CD16+ (green), and CD14−CD16+ (blue). (B and D) DC populations were shown as CD1c+ mDC (red) and CD123+ pDC (blue).
Supplemental Figure 2. Comparison of conventional gating strategies between monocytes and DC in blood of rhesus macaques. The HLA-DR+ lineage- (CD20/CD3/CD8) population was analyzed by two different strategies for monocytes (red arrows) or DC (blue arrow). The DC populations traditionally described as mDC (blue dots) and pDC shown (red dots) were identified as CD14-CD16+ and CD14-CD16- on monocyte gate, respectively (green arrows).