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Inflammatory Spleen Monocytes Can Upregulate CD11c Expression Without Converting into Dendritic Cells

Scott B. Drutman, Julia C. Kendall, and E. Sergio Trombetta

Monocytes can differentiate into various cell types with unique specializations depending on their environment. Under certain inflammatory conditions, monocytes upregulate expression of the dendritic cell marker CD11c together with MHC and costimulatory molecules. These phenotypic changes indicate monocyte differentiation into a specialized subset of dendritic cells (DCs), often referred to as monocyte-derived DCs or inflammatory DCs (iDCs), considered important mediators of immune responses under inflammatory conditions triggered by infection or vaccination. To characterize the relative contribution of cDCs and iDCs under conditions that induce strong immunity to coadministered Ags, we analyzed the behavior of spleen monocytes in response to anti-CD40 treatment. We found that under sterile inflammation in mice triggered by CD40 ligation, spleen monocytes can rapidly and uniformly exhibit signs of activation, including a surface phenotype typically associated with their conversion into DCs. These inflammatory monocytes remain closely related to their monocyctic lineage, preserving expression of CD11c, scavenging function, tissue distribution and poor capacity for Ag presentation characteristic of their monocyte precursors. In addition, 3–4 d after delivery of the inflammatory stimuli, these cells reverted to a monocyte-associated phenotype typical of the steady state. These findings indicate that, in response to anti-CD40 treatment, spleen monocytes are activated and express certain DC surface markers without acquiring functional characteristics associated with DCs. The Journal of Immunology, 2012, 188: 3603–3610.

The functional specializations of dendritic cells (DCs) and monocytes–macrophages have been a topic of much investigation, with recent focus on their developmental lineages as a way of understanding the relationships between these two cell types. Under steady state conditions, monocytes act as versatile cells that can convert into a variety of tissue-resident and lymphoid organ macrophage subsets. Under these same conditions, conventional DCs (cDCs) derive from a specialized precursor that shares a common progenitor to, but is distinct from, monocytes (1, 2). This lineage separation is paralleled by a divergence of functional specializations. The monocyte–macrophage lineage is specialized for robust Ag scavenging and secretion of inflammatory cytokines, but their capacity to convert internalized Ag into peptide–MHC complexes is poor. In contrast, cDCs are specialized for the efficient conversion of small amounts of captured Ag into peptide–MHC complexes, migration to T cell zones, and initiation of T cell responses (3, 4).

During inflammation, the plasticity of monocytes may also extend to the formation of certain subsets of dendritic cells (DCs), making it difficult to distinguish between these two lineages. Monocyte-derived DCs include TNF/iNOS-producing DCs and other inflammatory DCs (iDCs) described under microbial infection or adjuvant-induced peritonitis (1, 5–8). Monocyte-derived iDCs are characterized as DCs based on the expression of surface markers characteristic of cDCs in the spleen and lymph nodes, namely high surface expression of CD11c, as well as MHC-II and costimulatory molecules. Some iDCs were found to be dispensable for Ag presentation and T cell priming (9), whereas other iDCs were proposed to contribute to T cell stimulation (8, 10–12). Given the variety in inflammatory settings under which these iDCs arise, it is unclear whether the various iDCs reported represent related populations with common functional properties or they encompass a spectrum of different monocyte-derived cell types.

Because most studies describing conversion of monocytes into iDCs rely on processes that last several days or weeks, we sought to evaluate the conversion of monocytes into iDCs in vivo under conditions that induce potent Ag-specific immunity. We studied the response of mice to anti-CD40 treatment, which has proven efficacy to prime effective T cell responses in experimental animals (13–27) and has shown significant clinical potential (28–30). We found that induction of systemic inflammation in mice with an activating Ab against CD40 uniformly induced surface CD11c expression on Ly6C<sup>hi</sup> monocytes. These cells also expressed MHC class II (MHC-II) and costimulatory molecules typically associated with DC-like phenotypes ascribed to iDCs. However, these Ly6<sup>hi</sup>CD11c<sup>hi</sup> monocyte-derived iDCs share functional properties with their Ly6C<sup>hi</sup>CD11c<sup>neg</sup> precursors, not with Ly6C<sup>neg</sup>CD11c<sup>hi</sup> cDCs. In addition, this phenotypic change accompanies an increase in endocytic capacity, highlighting their activated monocyte phenotype. After 3–4 d, this Ly6C<sup>hi</sup>CD11c<sup>hi</sup> monocyte-derived population reverts to a surface phenotype characteristic of monocytes, further supporting the continuity of the DC-independent lineage.

Materials and Methods

**Mice**

C57BL/6 (B6), OT-I/RAG1 (OT-I), OT-II<sup>h2</sup>a/RAG1 (OT-II), and B6.SJL (CD45.1) mice were obtained from Taconic Farms. B6.129P2-Cd40<sup>tm1Kik</sup> (CD45.1) mice were obtained from Taconic Farms. B6.129P2-Cd40<sup>tm1Kik</sup> (CD45.1) mice were obtained from Taconic Farms.
CD40 KO) were obtained from The Jackson Laboratory. Mice were housed under specific-pathogen–free conditions and maintained in compliance with institutional and federal regulatory guidelines. anti-CD40 mAb-mediated inflammation was achieved by i.p. injection of 100 μg FGK4.5 mAb (Bio X Cell) or clone IC10 (LEAF grade, BioLegend). Rat IgG2a isotype control (LEAF grade, BioLegend) was used for control injections. Each injection of Ab (used to induce inflammation) or Ag (to study endocytosis or Ag presentation to T cells), contained undetectable levels of endotoxin, lower than 0.126 EU (∼13 pg) based on LAL test (Cambrex).

**Cells**

Unless otherwise specified, all cells were washed and resuspended in PBE (PBS with 0.5% BSA, endotoxin free; Equitech-Bio; 1 mM EDTA). Splenocytes were digested with Liberase Blendzyme 2 (Roche Diagnostics) for 15 min in PBS at 21˚C, passed through a 40-μm cell strainer, treated with ACK Buff (Lonza) to remove red cells, and resuspended in PBE. For purification of cells for in vitro Ag presentation experiments or for transfer experiments, splenocytes were first enriched by magnetic negative depletion with biotinylated Abs against CD19 (MB19.1), CD3 (145-2C11), NK1.1 (PK136), Ly-6G(1A8), and erythroid cell marker (TER-119) Abs (eBioscience or BioLegend), followed by enrichment using the EasySep Biotin Selection Kit (StemCell Technologies). Cells were subsequently sorted on a Dako MoFlo. Postsort analysis confirmed purity of >96% and viability of >95%. OT-I CD8+ or OT-II CD4+ T cells were isolated from the lymph nodes and spleens of OT-I/RAG1 knockout (KO) or OT-II/RAG1 KO mice by disruption through a 40-μm cell strainer, followed by negative selection using mouse CD8+ T cell or mouse CD4+ T cell enrichment kit, respectively (StemCell Technologies). Enriched T cells were pulsed with 0.5 mM CFSE (Invitrogen) for 5 min, washed twice, and resuspended in complete RPMI 1640.

**Monocyte transfer experiments**

Ly6C<sup>Hi</sup> monocytes (as identified in Fig. 1A) were purified from spleens of CD45.1 mice by cell sorting. Purified cells (5.0 x 10<sup>5</sup>) were injected i.v. into mice that were infected either with 100 μg anti-CD40 mAb or with control IgG or PBS as negative controls 5 min after cell transfer. At various time points after transfer, spleens were analyzed by flow cytometry, and the phenotypes of the endogenous (CD45.2) and transferred (CD45.1) cells were assessed.

**Endocytosis assays**

Soluble GFP protein was prepared as described previously (31). The construct in pET-28 vector (Novagen) in BL21 Escherichia coli (Novagen) was grown in TB media (Invitrogen) at 37˚C to a density of ∼0.1 absorbance units at 600 nm, then at 24˚C for 16 h with 1 mM isopropyl β-D-thiogalactoside (Sigma). Cells were lysed with lysozyme, sonication, and freeze–thaw cycles, and the his-tagged protein was affinity purified on Ni-Sepharose (Pharmacia). The resulting protein was further purified by ion exchange with Q-Sepharose (Pharmacia). The resulting protein had <1.26 EU/mg of endotoxin (<∼125 pg/mg) by LAL test (Cambrex). For in vivo soluble Ag endocytosis assays, 2 mg GFP was injected i.v., and 30 min later splenocytes were collected and analyzed for Ag capture as compared with a similarly treated mouse not injected with Ag. Endotoxin-free OVA (prepared as described below for Ag-presentation assays) at 10 mg/ml in PBS was labeled using fluorescein-5-isothiocyanate (Invitrogen) according to the manufacturer’s instructions. Excess unincorporated fluorescein was removed over a Sephade G-25 column (GE Healthcare) followed by several buffer exchanges with PBS using an Amicon Ultra-4 centrifugal filter device (Millipore) and filtration over a Polymixin-B-Sepharose column (Pierce). For endocytosis assays, mice were injected with 900 μg FITC-OVA, and 20 min later spleens were analyzed by flow cytometry to detect FITC-OVA uptake. Fluorescent particulate Ag was prepared by incubating 1.8 μm streptavidin-coated polystyrene microspheres (Spherotech) at 2.8 x 10<sup>9</sup>/ml with 10 μg/ml Fluorescein-biotin (Invitrogen) in PBS for 30 min and washed in PBE. For 2-μm particle uptake assays, 1.4 x 10<sup>9</sup> particles were injected i.v. and 60 min later splenocytes were collected and analyzed for Ag capture. Cells were also stained with PE-conjugated anti-fluorescein (Invitrogen) to distinguish cells that completely internalized particles (fluorescein positive, PE negative), from cells with particles stuck to their surface (fluorescein positive, PE positive). For 5-μm particle endocytosis assays, 0.8 x 10<sup>9</sup> Fluoresbrite YG carboxylate microspheres (Polysciences) were
were analyzed by flow cytometry.

**Ag-presentation assays**

OVA protein (grade IV; Sigma) was purified to remove any potential endotoxin contamination by ion exchange using Q-Sepharose (Pharmacia) as described previously (31). To assay presentation of Ag captured by cells in vivo, 1.0 mg OVA protein was injected i.v. into mice, and 30 min later cells were purified by cell sorting as described above. Various numbers of APCs were cocultured with 50,000 CFSE-labeled OT-I CD8^+ T cells or OT-II CD4^+ T cells in U-bottom 96-well plates. Sixty hours later, T cell proliferation was assessed using flow cytometry to measure the dilution of CFSE accompanying each T cell division. Cells were cultured in RPMI 1640 (Life Technologies) with 10% heat-inactivated FBS (Invitrogen), 100 units/ml penicillin, 100 μg/ml streptomycin (Life Technologies), nonessential amino acids, 110 μg/ml sodium-pyruvate, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin (Life Technologies), and 100 μM mercaptoethanol (Sigma) in a 5% CO_2 37˚C incubator.

**Flow cytometry**

Cells were preincubated with 10 μg/ml 2.4G2 mAb (Bio X Cell) for 15 min at 4˚C in PBE, incubated with mAb conjugates for 30 min at 4˚C, washed in PBE, and resuspended in PBE with 0.5 μg/ml 7-aminoactinomycin-D (Invitrogen) 10 min before analysis. Data were collected on a FACS Canto (BD Biosciences) and analyzed with FlowJo software (TreeStar). Mean ± SD of multiple experiments was calculated using Prism software (GraphPad Software) Abs: TCR-β (H57-597), CD4 (12/2F1), CD8 (53-6.7), CD45.1 (A20), CD11b (M1/70), CD11c (N418), Ly-6C (HK1.4), CD115 (AFS98), F4/80 (BMS), Mac-3 (M3/84), CD14 (Sa14-2), Mac-2 (m3/38), CD43 (1B11), CD45.1 (A20), CD45.2 (104), CD8α (53-6.7), CD4 (GK1.5), CD86 (GL-1), CD80 (16-10A1), IA/E (M5/114.15.2), H2-Kb (AF6-85.5), CD40 (HM40-3), and isotype control in corresponding fluors (mouse IgG2a, Rat IgG1, Rat IgG2a, Rat IgG2b, Armenian Hamster IgG, Armenian Hamster IgM) were purchased from eBioscience or BioLegend.

**Intracellular TNF-α**

Mice were injected with 100 μg anti-CD40 mAb i.p., and 40 h later splenocytes were isolated and cultured in complete RPMI 1640 in the presence of media alone, 200 ng/ml LPS (from *Salmonella typhimurium*; Sigma), 5 μg/ml anti-CD40 mAb, or 5 × 10^7/ml heat-killed *Listeria* (strain 10402S), and 5 μg/ml brefeldin A (Invitrogen). Sixty minutes later, cells were first stained for surface markers (see flow cytometry above) and then for intracellular TNF-α using Cytofix/Cytoperm (BD Biosciences) according to the manufacturer’s instructions. TNF-α was detected using biotin–anti-TNF-α (MP6-XT22; BioLegend), followed by streptavidin-PE (Invitrogen). Biotin-rat IgG (BioLegend) was used as an isotype control.

**Confocal microscopy**

Spleens were immersed in OCT media (Tissue-Tek) and frozen in an isopentane-liquid nitrogen bath; 10-μm cryosections were cut in acetone for 5 min at −20˚C. All subsequent steps were performed at room temperature. Sections were dried for 1 h, rehydrated in PBS for 10 min, and blocked with 5% goat serum and 5% newborn calf serum, and 5% rabbit anti-CD40 mAb, or 5 × 10^7/ml heat-killed *Listeria* (strain 10402S), and 5 μg/ml brefeldin A (Invitrogen). Sixty minutes later, cells were first stained for surface markers (see flow cytometry above) and then for intracellular TNF-α using Cytofix/Cytoperm (BD Biosciences) according to the manufacturer’s instructions. TNF-α was detected using biotin–anti-TNF-α (MP6-XT22; BioLegend), followed by streptavidin-PE (Invitrogen). Biotin-rat IgG (BioLegend) was used as an isotype control.
gated CD11c (N418; BioLegend) for 1 h, and monocytes were stained with 0.6 μg/ml FITC-conjugated anti-Ly6C (HK1.4; Biolegend) for 1 h followed by 2 μg/ml Alexa488-conjugated goat anti-fluorescein (Invitrogen) for 1 h. Control stains were performed with normal rabbit serum or isotype controls labeled with Alexa647 or FITC. Sections were mounted with Prolong Gold (Invitrogen) and imaged with a Zeiss Plan Apochromat 10× 0.45NA objective on a Zeiss LSM510 microscope.

**Results**

**Ly6C^Hi-CD11c^Hi** monocytes accumulate in the spleen of mice after treatment with anti-CD40 mAb

We focused on the two major populations of monocytes in the spleen: Ly6C^Hi-CD11c^Neg monocytes and Ly6C^Neg-CD11c^Low monocytes (Fig. 1A). Unlike monocytes, cDCs are Ly6C^Neg and express high levels of CD11c, a marker that is typically used to identify these cells (Fig. 1A). To analyze the behavior of monocytes and the potential formation of iDCs in vivo under conditions that induce strong Ag-specific immunity, we studied mice treated with an agonistic Ab against CD40, which has proven efficacy to prime effective T cell responses in experimental animals (13–27).

We found that induction of systemic inflammation in mice with an anti-CD40 mAb induced CD11c expression on Ly6CHi monocytes in the spleen, reaching levels comparable to cDCs in the same mouse within 40 h (Fig. 1B, 1C). The same induction of CD11c expression on Ly6C^Hi monocytes was obtained with two different clones.

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**FIGURE 3.** Ly6C^Hi-CD11c^Hi cells arise from monocytes in vivo. (A) Mice were injected with 1.0-μm YG beads, and 1 h later cells that had internalized beads (mostly monocytes) were analyzed and overlaid onto all cells. (B) Mice were injected with 1.0-μm YG beads and then either control-treated or injected with anti-CD40 mAb. To track the fate of the cells that had internalized beads, 24 or 40 h later, the phenotype of the bead-containing cells was analyzed and overlaid onto all the cells. (C) Similar to (A), but cells were examined 24 h after bead injection. (D) Similar to (B), but mice were injected with anti-CD40 24 h after injection of beads. (E) Spleen Ly6CHi monocytes were sorted to purity from CD45.1 mice using the gates shown in Fig. 1. (F) Approximately 5 × 10⁵ purified monocytes as shown in (E) were injected into CD45.2 mice that were either control treated, or immediately injected with anti-CD40 mAb. Twenty-four or 40 h later, the phenotype of the endogenous and transferred spleen monocytes was analyzed by flow cytometry. Data are representative of three experiments, two mice per group. The numbers inside plots represent the percentage of cells in the adjacent gate.
of agonistic anti-CD40 mAb, whereas this conversion did not occur in mice that lacked CD40 (Supplemental Fig. 1A).

These Ly6CHi-CD11cHi cells are also apparent in the CD11b versus CD11c plots commonly used to detect the appearance of iDCs (Supplemental Fig. 1B, 1C). This Ly6CHi-CD11cHi-CD11bHi phenotype is typical of iDCs described under various inflammatory stimuli. In mice treated with anti-CD40 for 40 h, we could identify four populations for further comparison: Ly6CHi monocytes, Ly6Cneg monocytes, Ly6CHi-CD11cHi cells, and CD11b+ cDCs (Fig. 1C). A comparison of these four populations shows that Ly6CHi-CD11cHi cells and CD11b+ cDCs express similar levels of CD11c, whereas Ly6Cneg monocytes express low levels of CD11c and Ly6CHi monocytes do not express CD11c (Supplemental Fig. 1D). Although similar populations of Ly6CHi-CD11cHi cells could be detected in other organs after anti-CD40 treatment (Supplemental Fig. 1E) we focused our analysis on spleen-derived monocytes because of the abundance of these cells and to be able to establish a direct comparison with the well-characterized cDCs from that organ.

In addition to high levels of CD11c, these Ly6CHi-CD11cHi cells also expressed MHC-II and costimulatory molecules at levels similar to cDCs from the spleen of the same mouse, which is another surface phenotype typical of iDCs (Fig. 2A). Although these Ly6CHi-CD11cHi cells shared some surface characteristics with cDCs, the expression levels of other markers such as F4/80, Mac-2, Mc-3, CD14, and CD115 remained closer to their Ly6CHi monocyte precursors than to cDCs (Fig. 2A). The Ly6Cneg monocytes in the spleen of the same animals retained lower MHC-II expression under the same conditions (Fig. 2B). These results show that treatment of mice with anti-CD40 mAb induces the appearance of a population of Ly6CHi-CD11cHi cells that shares surface markers with both cDCs and monocytes. For simplicity we will continue to refer to these monocyte-derived Ly6CHi-CD11cHi cells as iDCs, although this population likely differs from previously described inflammatory monocyte-derived iDCs, as shown below.

Spleen Ly6CHi-CD11cHi cells derive from monocytes

The appearance of Ly6CHi cells expressing high levels of CD11c following injection of anti-CD40 suggested that Ly6CHi-CD11cHi cells were the result of upregulation of CD11c on Ly6CHi-CD11cNeg monocytes in the spleen. However, these results could not exclude the possibility that the Ly6CHi-CD11cNeg population was disappearing and simultaneously replaced by unrelated populations with increasingly higher levels of CD11c. To distinguish between these alternatives, we used a bead-labeling protocol for tracking monocytes in situ (32–34). One hour after injection, beads were associated primarily with Ly6CHi and Ly6Cneg monocytes, and to a lesser degree with DCs (Fig. 3A, Supplemental Fig. 2). When control-treated mice (uninflamed) were injected i.v. with FITC-ova [instead of GFP (solid line)], a FITC-ova protein fluorescence signal of cells from a mouse injected with GFP (solid line) compared to a control mouse is shown in each panel, ±SD. (E) Similar to (A), but 5.0-μm particles were used. (F) Similar to (A) and (B), but mice were injected with soluble GFP protein. After 30 min, GFP capture was analyzed by comparing the fluorescence signal of cells from a mouse injected with GFP (solid line) compared to a control mouse is shown in each panel, ±SD. (B) The same experimental approach as in (C), but mice were injected i.v. with FITC-ova [instead of GFP as used in (C)]. Data are representative of three experiments, three mice per group. Results are expressed as mean ± SD from the mean.
To evaluate the role of CD40 on the responding splenic monocytes, we prepared mixed bone-marrow chimeras between wild type and CD40−/− mice (Supplemental Fig. 3). We found that ligation of CD40 on bone marrow-derived cells is required for the induction of Ly6CHiCD11cHi cells, and the formation of this population is further enhanced when CD40 is also expressed on somatic cells (Supplemental Fig. 3A). In addition, wild type Ly6CHi monocytes transferred into CD40−/− recipients could not be induced to form Ly6CHiCD11cHi cells, indicating that CD40 ligation on the monocytes alone is insufficient, but a variety of cell types contribute to the inflammatory response to anti-CD40 (Supplemental Fig. 3B).

Ly6CHiCD11cHi cells functionally resemble activated monocytes

The apparent activation of Ly6CHi monocytes in mice treated with anti-CD40 led to a surface phenotype with similarities to both cDCs and their Ly6CHi monocyte precursors; therefore, we wanted to determine whether these iDCs exhibit functional characteristics of cDCs. To this end, we compared the endocytic capacity and Ag-presenting capacities of Ly6CHi monocytes, iDCs (Ly6CHiCD11cHi), and cDCs, all of them isolated from the same mice treated with anti-CD40. To examine their phagocytic capacity in

![Figure 5](http://www.jimmunol.org/)

**Figure 5.** Ly6CHiCD11cHi cells retain functional properties of monocytes. (A) Despite a large capacity for Ag capture, Ly6CHiCD11cHi cells do not present the internalized Ag to T cells, a feature similar to monocytes but in contrast to dendritic cells. Mice were treated with anti-CD40 and 40 h later injected i.v. with 1 mg OVA protein; 30 min after OVA injection, splenocytes were harvested and Ly6CHi monocytes, iDCs (Ly6CHiCD11cHi), and cDCs, all of them isolated from the same mice treated with anti-CD40. To examine their phagocytic capacity in

![Figure 6](http://www.jimmunol.org/)

**Figure 6.** Similar to Ly6CHi monocytes, Ly6CHiCD11cHi cells ultimately differentiate into Ly6CNeg monocytes. (A) The disappearance of Ly6CHiCD11cHi cells 90 h after anti-CD40 treatment correlates with an increase in Ly6CNeg monocytes. (B) Similar to Fig. 3F, purified CD45.1 monocytes were injected into CD45.2 mice, which were immediately injected with anti-CD40 mAb; 90 h later, the phenotype of the endogenous and transferred spleen monocytes was analyzed by flow cytometry. (C) Similar to Fig. 3B, mice were injected with 1.0-μm YG beads and then immediately injected with anti-CD40 mAb; 90 h later the phenotype of the bead-containing cells was analyzed and overlaid over all the cells. Data are representative of three experiments, two mice per group.
In agreement with their high scavenging and poor Ag presentation activities, Ly6C<sup>H</sup>-CD11c<sup>H</sup> monocyte-derived Ags were found predominantly in the red pulp of the spleen of inflamed mice, but they were essentially absent from T cell areas, in contrast with cDCs (Fig. 5B). Finally, we examined the ability of each population to produce inflammatory cytokine TNF-α after further stimulation. Ly6C<sup>H</sup>-CD11c<sup>H</sup> cells, cDCs, and resident spleen monocytes all had the capacity to produce TNF-α upon re-stimulation in vitro (Supplemental Fig. 4). In conclusion, although the surface phenotype of Ly6C<sup>H</sup>-CD11c<sup>H</sup> monocyte-derived cells shares some markers with cDCs, they share other surface markers, similar scavenging, and poor Ag-presenting characteristics of their monocyte precursors, and they consequently do not appear to acquire functional properties associated with cDCs.

Ly6C<sup>H</sup>-CD11c<sup>H</sup> cells ultimately convert into Ly6C<sup>N</sup> monocytes

We considered the possibility that the conversion of Ly6C<sup>H</sup> monocytes to a DC-like phenotype could take longer, and we consequently followed the fate of these cells at later time points after delivery of anti-CD40 stimulation. We found that up to 5 d after injection of anti-CD40, a decrease in Ly6C<sup>H</sup>-CD11c<sup>H</sup> cells correlated with an increase in Ly6C<sup>N</sup>-CD11c<sup>N</sup> monocytes (Fig. 6A). To determine whether this reduction in the numbers of Ly6C<sup>H</sup>-CD11c<sup>H</sup> cells could indicate their further conversion into cDC-like population, we performed adoptive transfer experiments for longer periods, after Ly6C<sup>H</sup>-CD11c<sup>H</sup> cells had apparently disappeared. Interestingly, the transferred Ly6C<sup>H</sup> monocytes, which convert into Ly6C<sup>N</sup>-CD11c<sup>N</sup> cells after 24 to 40 h (Fig. 3F), subsequently converted into Ly6C<sup>N</sup>-CD11c<sup>N</sup> monocytes (Fig. 6B); this is similar to the fate of Ly6C<sup>H</sup> monocytes in the steady state (Fig. 3F) (34–36). In addition, when we mapped the fate of these cells using the bead tracking techniques as in Fig. 3B, we also found that the Ly6C<sup>N</sup>-CD11c<sup>N</sup> cells had converted into Ly6C<sup>N</sup> monocytes at later time points (Fig. 6C).

Discussion

Our results indicate that under sterile systemic inflammatory conditions induced with anti-CD40, Ly6C<sup>H</sup> monocytes induce CD11c expression together with other surface markers typically associated with their acquisition of DC-like phenotype. However, such monocyte-derived CD11c<sup>H</sup> cells retain many characteristics of monocytes, including several surface markers, a high endocytic capacity, and inefficient conversion of internalized Ags into peptide–MHC complexes for T cell stimulation. In addition, these cells follow the fate of their monocyte lineage, ultimately converting into Ly6C<sup>N</sup> monocytes. These findings demonstrate that the upregulation of CD11c by monocytes and the conversion to DCs can be distinct processes; therefore, under certain inflammatory conditions, the expression of CD11c, MHC-II, and costimulatory molecules is insufficient to identify a population with similar properties as cDCs. Although not observed in this study, the expression of CD11c on other cell types, such as NK cells (37–39), may also be subject to regulation under inflammatory conditions.

The results presented in this and previous studies have shown that the material scavenged by monocyte and some monocyte-derived iDCs is apparently not presented to T cells efficiently (3, 9, 40). Given the abundance of iDCs and their vigorous scavenging capacity, it is important to establish the fate of such scavenged material, which can be transferred to other cell types, including Ag-presenting cDCs, even as preformed pMHC complexes (3, 41–46).

In prior studies describing monocyte-derived DCs, it has not been easy to distinguish between fully converted monocytes and functional DCs (47, 48). In previously described models relying on peritonitis induced by long-term effects of emulsified adjuvants or microbial infections, it has been difficult to establish a direct precursor-product relationship between monocytes and cDCs without ruling out alterations in the properties of cDC or pre-DC progenitors (7, 48). A recent study has shown that components from Gram-negative bacteria such as LPS can induce the conversion of blood monocytes into Ag-presenting monocyte-derived DCs migrating to lymph nodes (8). Given that conversion of monocytes into Ag-presenting DCs does not appear to be complete under certain inflammatory conditions with similar components derived from Gram-negative bacteria (49) or under sterile inflammation as reported in this study, it will be important to define the conditions that induce the full conversion of monocytes into iDCs capable of T cell stimulation.

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Disclosures

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