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Relationships between Distinct Blood Monocyte Subsets and Migrating Intestinal Lymph Dendritic Cells In Vivo under Steady-State Conditions

Ulf Yrlid, Christopher D. Jenkins, and G. Gordon MacPherson

The origins of dendritic cells (DCs) are poorly understood. In inflammation, DCs can arise from blood monocytes (M$_{0}$s), but their steady-state origin may differ, as shown for Langerhans cells. Two main subsets of M$_{0}$s, defined by expression of different chemokine receptors, CCR2 and CX$_{3}$CR1, have been described in mice and humans. Recent studies have identified the inflammatory function of CCR2$^{\text{high}}$CX$_{3}$CR1$^{\text{low}}$ M$_{0}$s but have not defined unambiguously the origin and fate of CCR2$^{\text{low}}$CX$_{3}$CR1$^{\text{high}}$ cells. In this study, we show that rat M$_{0}$s can also be divided into CCR2$^{\text{high}}$CX$_{3}$CR1$^{\text{low}}$(CD43$^{\text{low}}$) and CCR2$^{\text{low}}$CX$_{3}$CR1$^{\text{high}}$(CD43$^{\text{high}}$) subsets with distinct migratory properties in vivo. Using whole body perfusion to obtain M$_{0}$s, including the marginating pool, we show by adoptive transfer that CD43$^{\text{low}}$ M$_{0}$s can differentiate into CD43$^{\text{high}}$ M$_{0}$s in blood without cell division. By adoptive transfer of blood M$_{0}$s followed by collection of pseudoafferent lymph, we show for the first time that a small proportion of intestinal lymph DCs are derived from CCR2$^{\text{low}}$CX$_{3}$CR1$^{\text{high}}$(CD43$^{\text{high}}$) blood M$_{0}$s and indicate that they may contribute to migratory intestinal DCs in vivo in the absence of inflammatory stimuli. The Journal of Immunology, 2006, 176: 4155–4162.

Phenotypic heterogeneity among blood monocytes (M$_{0}$s$^{2}$) exists in humans, mice, swine, and rats (1–4). In mice and humans, two major, functionally distinct subsets can be distinguished by expression of chemokine receptors and other markers (3, 4). One “inflammatory” subset, CCR2$^{\text{high}}$CX$_{3}$CR1$^{\text{int/low}}$, enters inflamed tissues in response to MCP-1 (5) while the other, CCR2$^{\text{low}}$CX$_{3}$CR1$^{\text{high}}$, migrates toward fractalkine (CX3CL1) rather than MCP-1 (6). Murine CCR2$^{\text{low}}$CX$_{3}$CR1$^{\text{int/low}}$ M$_{0}$s selectively express high levels of CD43 but low levels of Ly6C (7). In rats, M$_{0}$ subsets have been defined by CD43 expression (1) but the functions of these subsets and their relationship to their mouse and human counterparts are unknown. Moreover, the developmental relationships between M$_{0}$ subsets have not been fully defined in any species.

Dendritic cells (DCs) migrate constitutively from peripheral tissues to draining LNs (DLNs) in lymph and thus need continual replenishment. During inflammation, numbers of tissue DCs increase markedly, and at least some are derived from blood M$_{0}$s. The origins of steady-state (SS) DCs are much less clear; murine Langerhans cells arise from a self-replenishing peripheral pool in SS but from blood precursors during inflammation (8). It has been shown that after intracutaneous injection of latex beads CCR2$^{\text{high}}$ M$_{0}$s are recruited to murine skin (9). Most bead-containing cells remain locally and become macrophages but in addition MHC-I$^{\text{high}}$ latex$^{\text{+}}$ cells with DC characteristics can be recovered from the DLN (9–11). Beads are carried preferentially to the DLN by the small proportion of recruited CCR2$^{\text{high}}$CX$_{3}$CR1$^{\text{low}}$ M$_{0}$s that express CCR7 and CCR8 (9). In contrast, in the SS, CCR2$^{\text{low}}$CX$_{3}$CR1$^{\text{high}}$ but not CCR2$^{\text{high}}$CX$_{3}$CR1$^{\text{low}}$ blood M$_{0}$s enter lungs after i.v. transfer (3). Importantly, the fate of CCR2$^{\text{low}}$CX$_{3}$CR1$^{\text{high}}$ M$_{0}$s in tissues remains uncertain.

In this study, we examine the origins and fates of M$_{0}$s using approaches available in rats but not in mice or humans. To maximize blood M$_{0}$s recovery, including the sizeable marginating pool (not recovered by bleeding), we used vascular perfusion (12). To trace the fate M$_{0}$s in blood and inflamed tissues, CFSE-labeled, congenic blood M$_{0}$ subsets were transferred into normal rats and rats with peritonitis. To examine migrating DCs, mesenteric LNs from young rats were removed and the afferent and efferent lymphatics were allowed to anastomose. Subsequent thoracic duct cannulation of these mesenteric-lymphadenectomized (MLNX) rats permits collection of intestinal lymph DCs (iL-DCs) that have just left the gut wall (13, 14). To investigate the relationship between M$_{0}$s and migrating DCs, we adoptively transferred labeled congenic M$_{0}$s into cannulated MLNX rats and analyzed the frequency and phenotype of donor-derived iL-DCs. The MLNX rats were not irradiated before the transfer to allow for examination of the M$_{0}$ contribution to iL-DCs under SS conditions.

Using this unique system, we show that CD43$^{\text{low}}$ and CD43$^{\text{high}}$ rat M$_{0}$s correspond to the CCR2$^{\text{high}}$CX$_{3}$CR1$^{\text{low}}$ and CCR2$^{\text{low}}$CX$_{3}$CR1$^{\text{high}}$ functionally distinct subsets identified in mice and humans, and importantly that CD43$^{\text{low}}$ M$_{0}$s are able to differentiate into CD43$^{\text{high}}$ M$_{0}$s in the blood stream without cell division. Finally, we show in vivo that CCR2$^{\text{low}}$CX$_{3}$CR1$^{\text{high}}$(CD43$^{\text{high}}$) blood M$_{0}$s can differentiate into a small proportion of iL-DCs in the absence of added phagocytic or inflammatory stimuli. These results define one origin and differentiation pathway of the CCR2$^{\text{low}}$CX$_{3}$CR1$^{\text{high}}$ subset of blood M$_{0}$s in vivo under SS conditions.
Materials and Methods

Rats and surgical procedures

PVG RT1<sup>e</sup> and congeneric RT1<sup>Rt7<sub>b</sub></sup> (RT7<sub>b</sub>) male rats were maintained under specific pathogen-free conditions and used at 12–24 wk of age in accordance with Home Office guidelines. Mesenteric lymphadenectomy, thoracic duct cannulation, and whole body perfusions were performed as previously described (12, 14).

Reagents

Twenty-five micrograms of R-848 (InvivoGen) or 50 μg of Salmonella typhimurium LPS (Sigma Aldrich) were injected i.v. in 0.5 ml of PBS. Peritonitis was induced by i.p. injection of 5 ml of sterile 4% thiglycolate broth and recruited cells were collected by peritoneal lavage. 7-aminocatinomycin D (7AAD) was purchased from Sigma Aldrich.

Antibodies

mAbs to rat Ags: CD4 (OX35), CD5 (OX19), CD6 (OX52), CD8a (OX8), CD11b/c (OX42), CD43 (W3/13), CD45RA (OX33), CD90 (OX7), CD103 (OX62), CD172a (OX41), CD200R (OX102), and RT<sub>b</sub> (His41) purified from cell culture supernatants were used for depletions or conjugated to biotin, FITC, or Alexa-488. Anti-CD32 (D34), later biotinylated, anti-PMN (RP-1-PE), anti-h-MHC-II (OX6-PerCP), and streptavidin-PE, and -allophycocyanin were all purchased from BD Pharmingen. Anti-CD11c (8A2), later biotinylated and anti-CD86 (24F-PE) was purified from Cedarlane Laboratories. Polyclonal rabbit anti-rat CX<sub>3</sub>CR1 was purchased from AMS Biotechnology, goat anti-rat CCR2 (sc-6226) and CCR7 (A-19) from Santa Cruz Biotechnology, and all three were later biotinylated in house.

Isolation of cells

Blood M<sub>ϕ</sub>s. The vascular perfusate was collected and spun on a Percoll gradient (Amersham Bioscience) as described (12). Mononuclear cells were depleted of B, T, and NK cells using anti-CD6, -CD8, and -CD45RA Abs, followed by goat anti-mouse Dynabeads (Dynal). This gave 96–98% pure CD172ahighCD103low MOs. In adoptive transfer experiments followed by cannulation, enriched MOs were depleted of CD43<sup>low</sup> BM<sub>ϕ</sub>s using anti-CD32, -CD200R, and -CD62L resulting in >93% pure CD43<sup>high</sup> M<sub>ϕ</sub>s (containing cells were exclusively CD43<sup>high</sup> M<sub>ϕ</sub>s).

Bone marrow (BM) M<sub>ϕ</sub>s. BM cells were flushed from femurs and tibias and RBC lysed with ACK lysis buffer. The cells were depleted of lymphocytes and precursors using anti-CD5, -CD6, -CD8, -CD45RA, and -CD90. In adoptive transfer experiments followed by cannulation, CD34<sup>high</sup> M<sub>ϕ</sub>s and polymorphonuclear leukocytes (PMNs) were depleted using anti-CD4 and -CD45 giving >93% pure CD172ahighCD34<sup>low</sup> M<sub>ϕ</sub>s. In cannulation experiments, involving R-848 injection the CD43<sup>low</sup> M<sub>ϕ</sub>s were further purified using anti-CD11b-biotin and anti-biotin MACs beads (Miltenyi Biotec) generating >80% pure CD43<sup>low</sup>CD11b<sup>+</sup> M<sub>ϕ</sub>s (contaminating cells were exclusively PMNs).

In experiments where blood and BM M<sub>ϕ</sub> subset recruitment to inflamed tissues and subset differentiation in blood was studied, enriched M<sub>ϕ</sub>s were sorted into CD43<sup>high</sup>, CD43<sup>low</sup>, and CD11b/c<sup>+</sup> subsets. Sorted M<sub>ϕ</sub>s were transferred into normal rats. After 18 h, some transferred CD43<sup>low</sup> blood M<sub>ϕ</sub>s had up-regulated CD43 to intermediate levels whereas the majority of transferred CD43<sup>low</sup> BM M<sub>ϕ</sub>s were still CD43<sup>low</sup> (Fig. 2B). Sixty hours after transfer, the same rats that had been bled at 18 h were perfused and recovered M<sub>ϕ</sub>s were analyzed for CD34 and CFSE. In addition, donor CD43<sup>low</sup> blood M<sub>ϕ</sub>s and CD43<sup>low</sup>CD11b<sup>+</sup> BM M<sub>ϕ</sub>s were now CD43<sup>high</sup> (Fig. 2C).

CD43<sup>low</sup> M<sub>ϕ</sub>s differentiate into CD43<sup>high</sup> M<sub>ϕ</sub>s in vivo

The developmental relationships between subsets of M<sub>ϕ</sub>s are uncertain, particularly in the SS. To examine M<sub>ϕ</sub> differentiation in vivo, CFSE-labeled M<sub>ϕ</sub>s subsets were transferred (Fig. 2A) and transferred i.v. into normal rats. After 18 h, some transferred CD43<sup>low</sup> blood M<sub>ϕ</sub>s had up-regulated CD43 to intermediate levels whereas the majority of transferred CD43<sup>low</sup> BM M<sub>ϕ</sub>s were still CD43<sup>low</sup> (Fig. 2B). Sixty hours after transfer, the same rats that had been bled at 18 h were perfused and recovered M<sub>ϕ</sub>s were analyzed for CD34 and CFSE. All donor CD43<sup>high</sup> and low blood M<sub>ϕ</sub>s and CD43<sup>low</sup>CD11b<sup>+</sup> BM M<sub>ϕ</sub>s were now CD43<sup>high</sup> (Fig. 2C). Importantly, their CFSE content showed that none of these M<sub>ϕ</sub>s had divided. In contrast, some donor CD43<sup>low</sup>CD11b<sup>+</sup> BM M<sub>ϕ</sub>s had divided and had also up-regulated CD43.

To determine whether up-regulation of CD43 was accompanied by changes in other markers, blood M<sub>ϕ</sub>s were sorted by CD43 expression and BM M<sub>ϕ</sub>s were sorted into CD11b<sup>+</sup>CD43<sup>high</sup> to avoid the dividing cell population. Sorted cogenic M<sub>ϕ</sub>s subsets were transferred i.v. into naive recipients. After 40 h, recipients were perfused and donor M<sub>ϕ</sub> numbers and phenotypes were assessed. CD43<sup>high</sup> M<sub>ϕ</sub>s retained their original phenotype (Fig. 3A), whereas all donor CD43<sup>low</sup> M<sub>ϕ</sub>s, independent of origin (blood or BM), had become CD43<sup>high</sup>, expressed uniform high levels of CD4, and had up-regulated CX<sub>3</sub>CR1 (Fig. 3, B and C). In addition, the adoptively transferred CD43<sup>low</sup> M<sub>ϕ</sub>s had down-regulated CD62L, CCR7, and to a lesser extent CCR2.

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These experiments show that after adoptive transfer under SS conditions, CD43low MOs gradually acquire the phenotype of CD43high MOs without cell division. Some CD43high blood MOs differentiate into SS iL-DCs without cell division.

The fate of murine CCR2highCX3CR1low MOs in tissues has been examined recently (3, 9, 11) but the fate of CCR2lowCX3CR1high MOs is less understood. Immature CX3CR1+/H11001 DCs present in the lamina propria of the small intestine constitutively sample intestinal contents (16). Their origins and migratory fate(s) are however unclear. To determine whether both the subset MOs can become lymph-borne migratory intestinal DC, we transferred CFSE-labeled CD43high blood MOs or CD43low BM MOs, into thoracic duct-cannulated MLNX rats (Fig. 4A). Eighteen hours after transfer of 45 × 10⁶ MOs, donor MOs represented 3–4% of total blood monocytes, and at the time of cannulation they constituted 1–2% (data not shown). Analysis of iL-DCs showed that a small but clearly identifiable proportion derived from the transferred CD43high blood MOs (Fig. 4B, top row of panels). Furthermore, this differentiation had occurred without cell division. A small population of donor-derived iL-DCs, where the majority had undergone cell division, could also be observed in rats that had received CD43low BM MOs (Fig. 4B, top row of panels).

Donor-derived cells in lymph with a MO phenotype (SSClowCD172a+CD103−MHC-IIlow) were also detected and as noted with the monocyte-derived iL-DCs, only the lymph MOs originating from the CD43low BM MOs had divided (Fig. 4B, middle row of plots). No transferred CD43high blood MOs and very few, if any, CD43low BM MOs developed into PMNs in lymph (Fig. 4B, bottom row of plots).

These experiments show that MOs can give rise to DCs that migrate via intestinal lymph and that the MO-derived iL-DCs originating from CD43high blood MOs do this without cell division.

Some CD43high blood MOs differentiate into SS iL-DCs without cell division

The fate of murine CCR2highCX3CR1low MOs in tissues has been examined recently (3, 9, 11) but the fate of CCR2lowCX3CR1high MOs in tissues is less understood. Immature CX3CR1+ DCs present in the lamina propria of the small intestine constitutively sample intestinal contents (16). Their origins and migratory fate(s) are however unclear. To determine whether both the subset MOs can become lymph-borne migratory intestinal DC, we transferred CFSE-labeled CD43high blood MOs or CD43low BM MOs, into thoracic duct-cannulated MLNX rats (Fig. 4A). Eighteen hours after transfer of 45 × 10⁶ MOs, donor MOs represented 3–4% of total blood monocytes, and at the time of cannulation they constituted 1–2% (data not shown). Analysis of iL-DCs showed that a small but clearly identifiable proportion derived from the transferred CD43high blood MOs (Fig. 4B, top row of panels). Furthermore, this differentiation had occurred without cell division. A small population of donor-derived iL-DCs, where the majority had undergone cell division, could also be observed in rats that had received CD43low BM MOs (Fig. 4B, top row of panels).

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of observations made after administering TLR ligands to cannu-
lated rats (13, 14). In these studies, we have shown that after giving
i.v. LPS (13, 14) or R-848 (27), TLR4 and TLR7/8 ligands, re-
spectively, there is virtually a complete loss of intestinal lamina
propria DCs. This is accompanied by a TNF-α-dependent 15- to
30-fold increase in numbers of iL-DCs. Thus, following transfer of
labeled MOs, cannulated rats were given i.v. LPS or R-848 and
lymph collected for 18 h. LPS and R-848 both stimulated a large
increase in the frequency (see included histograms of CD103 ex-
pression in density plots, Fig. 5
C
) and total numbers of iL-DC.
Importantly, of the LPS and R-848-released iL-DCs, 0.15% and
0.21%, respectively, were donor-derived compared with 0.12% in
the SS (Fig. 5
C
C). As suggested by SS experiments (PBS), the fre-
quencies of the CD172a high and CD172alow subsets were almost
identical between donor- and host-derived iL-DCs (Fig. 5
C
).
MOs express TLR4 and 7/8 and we could detect up-regulation of
MHC-II expression on both donor derived and host MOs 18 h after
i.v. injection of LPS (Fig. 5
B
). In contrast, no change in MHC-II
expression was observed on MOs after R-848 administration (Fig.
5
B
). However, we could detect a dramatic increase in the fre-
quency of CD43 low blood MOs in R-848-treated rats. This was not
due to down-regulation of CD43 as transferred CD43 high blood
MOs remained CD43 high and most likely reflects increased release
of CD43 low MOs from the BM (Fig. 5
D
).
As 10–15% of blood MOs are CD43 low, we had thus transferred
around 15 × 10⁶ CD43 lowCD11b + MOs when total blood MOs
were transferred (Fig. 5
C
). We repeated the experiment transfer-
ring 18 × 10⁶ CD43 lowCD11b + BM MOs and 70 × 10⁶ CD43 high
blood MOs into separate rats and then administered R-848 i.v.
CD43 high CFSE + RT7 + MOs developed into iL-DCs that both
contain the dye and express the congenic marker (Fig. 5
E
, left
panel). This confirms that the CFSE + iL-DCs we recover (Figs. 4
B

and 5
C
) have not acquired the label through phagocytosis of trans-
ferred MOs. Moreover, no donor-derived CFSE + cells were de-
tected among iL-DCs in rats that had received CD43 lowCD11b +
BM MOs (Fig. 5
E
, middle panel).
These experiments show that a small number of blood MOs,
most likely the CCR2 lowCX3CR1 high(CD43 high) subset, can enter
the intestine and, within 3–4 days, differentiate into the two main
subsets of iL-DCs.

Discussion
MOs constitute a heterogeneous population of mononuclear leu-
kocytes that play a critical role in host innate immune responses as
they are rapidly recruited to inflamed tissues. MOs are also crucial
for the replenishment of tissue macrophages but their role in DC
production is controversial. Phenotypically distinct MO subsets
have been described in humans, mice, rats, and swine but most
studies have focused on humans and mice and as different markers
have been used between the species, there is a lack of clarity. We
have therefore carefully examined the phenotype of the two main
rat blood MO subsets, previously defined as CD43 high and CD43 low

FIGURE 2. Transferred CD43 lowCD11b + MOs from blood and BM up-regulate CD43 expression in blood without cell divisions. CD172a +CD43 high or
low blood MOs and CD172a +CD43 lowCD11b + or − BM MOs were FACS-sorted into subsets as indicated by the arrows and (4) reanalyzed for purity. The
subsets were then CFSE-labeled and injected i.v. (4 × 10⁶) into naive RT1c rats. B, FACS analysis for CD43 expression by donor (CFSE +) MOs, recovered
by bleeding 18 h after transfer. C, FACS analysis of total MOs (CD172a +) recovered from the same rats that were previously bled in B but now perfused
60 h after transfer. The results are representative of three independent experiments.
Because of the amenability of rats to experimental manipulation not possible in the mouse, we also followed their fate in tissues under SS and inflammatory conditions. Our phenotypic analysis shows that CD43<sup>high</sup> MOs express CX<sub>3</sub>CR1 and CD11c but low levels of CD62L and CCR2 whereas in contrast the CD43<sup>low</sup> subset expresses low levels of CX<sub>3</sub>CR1 and CD11c but high levels of CD62L and CCR2. The subset-specific expression of these chemokine receptors has also been described in mice and humans and are associated with distinct mechanisms of recruitment into tissues in naive and immunologically challenged animals, resulting in the classification of “resident” CCR2<sup>low</sup>CX<sub>3</sub>CR1<sup>high</sup> and “inflammatory” CCR2<sup>high</sup>CX<sub>3</sub>CR1<sup>low</sup> subsets (3). Consistent with our data in rats, CD43 has recently also been shown to be expressed at higher levels by murine CCR2<sup>low</sup>CX<sub>3</sub>CR1<sup>high</sup>(Ly6C<sup>low</sup>) MOs compared with the Ly6C<sup>high</sup> subset (7). Moreover, we show that CD32 is expressed at higher levels by the CCR2<sup>high</sup>CX<sub>3</sub>CR1<sup>low</sup>(CD43<sup>low</sup>) which correlates with human peripheral blood MOs where CCR2<sup>low</sup>CX<sub>3</sub>CR1<sup>low</sup>(CD16<sup>+</sup>) MOs have been shown to express high levels of this FcR (4). In rats, the proportion of CCR2<sup>low</sup>CX<sub>3</sub>CR1<sup>mid</sup>(CD43<sup>low</sup>) MOs in blood is significantly higher than in mice and humans. Although this could be because we perfuse the animals and therefore obtain the marginating pool of MOs, this is most likely not the explanation as even after bleeding rats, the CCR2<sup>low</sup>CX<sub>3</sub>CR1<sup>high</sup>(CD43<sup>high</sup>) MOs constitute the major proportion of blood MOs.

It is clear from our study as well as from studies in mice (7) that the major subset of MOs in BM is the CCR2<sup>high</sup>CX<sub>3</sub>CR1<sup>low</sup>(CD43<sup>low</sup>) subset. This observation, together with finding that the CCR2<sup>high</sup>CX<sub>3</sub>CR1<sup>low</sup>(Ly6C<sup>high</sup>) subset appears before CCR2<sup>low</sup>CX<sub>3</sub>CR1<sup>high</sup>(Ly6C<sup>low</sup>) cells during repopulation of blood MOs after depletion by clodronate, has led to the suggestion that CCR2<sup>high</sup>CX<sub>3</sub>CR1<sup>low</sup> murine MOs may be precursors of CCR2<sup>low</sup>CX<sub>3</sub>CR1<sup>high</sup> MOs (7). Additionally, human CCR2<sup>high</sup>CX<sub>3</sub>CR1<sup>low</sup> (CD16<sup>+</sup>) MOs come to resemble CD16<sup>+</sup> blood MOs after in vitro culture with TGFβ (18). To directly address this point, we transferred sorted subsets of MOs and followed their differentiation in blood. Our results show for the first time that in vivo under SS conditions CD43<sup>low</sup> MOs from blood or BM mature into CD43<sup>high</sup> MOs without cell division. This differentiation is not just restricted to CD43 as expression of CD4, CD62L, CX<sub>3</sub>CR1, CCR7, as well as CCR2, albeit to a lesser degree, are also modulated on the transferred CD43<sup>low</sup> MOs. Importantly, the donor CD43<sup>high</sup> MOs recovered in rats receiving CD43<sup>low</sup> MOs cannot be preferentially surviving CD43<sup>high</sup> contaminants for two reasons. First, the numbers of transferred and recovered MO subsets are incompatible with this. Forty hours after transfer of sorted donor CD43<sup>high</sup> or low MOs, 1–2% or 0.5–1%, respectively, of the transferred cells were recovered by perfusion in three independent experiments. In these experiments, the contamination of sorted donor...
CD43low MOs with CD43high MOs was 0.5–2%. Given a similar recovery of contaminating CD43high MOs, these cells cannot account for >8% of the recovered CD43high MOs. Second, the increase in CD43 expression is gradual–18 h after transfer of CD43low MOs, the recovered MOs are CD43int but at 60 h they are CD43high in the same rats.

MOs enter tissues and differentiate into macrophages and possibly DCs but the replenishment of these cells is greatly influenced by inflammation or injury. The differentiation of human MOs into migratory DCs under different levels of inflammatory conditions has been extensively studied in vitro in a system of transendothelial migration (18, 19) but only recently have attempts been made to address this question in vivo in mice (9–11). In this study, we have shown that CCR2highCX3CR1low (CD43low) MOs are selectively recruited to the peritoneum during induced sterile peritonitis. Very few, if any, transferred CCR2lowCX3CR1high (CD43high) MOs were recruited to the site of inflammation. Some CD43low MOs also gave rise to CD11b+/c MHC-IIint cells in the draining PLNs. A similar subset-specific appearance of CD11c+ cells derived from transferred CCR2highCX3CR1low MOs in the PLNs of mice with peritonitis has been reported previously (3). In both species, the transferred CCR2highCX3CR1low MOs could have arrived via lymph as at least in rats this subset of MOs express CCR7. However, these cells could also have entered the LN from blood via high endothelial venules as in both species the MOs express CD62L. In addition, murine CCR2highCX3CR1low MOs are recruited from the blood to inflamed LNs via high endothelial venules in a CCL2-dependent manner (5).

MOs have been shown to give rise to cells with DC characteristics in the spleen (3, 20). In one of these studies, it was shown that within 2 days after transfer into naïve mice, both subsets of blood MOs could enter the spleen, where some CCR2lowCX3CR1high (Ly6Chigh) MOs up-regulated CD11c and MHC-II (3). In the skin, some murine CCR2highCX3CR1low (Ly6Chigh) MOs, recruited by intracutaneous bead injection, become bead-containing CD11c+ MHC-IIhigh cells, strongly suggesting their differentiation into migratory lymph DCs in vivo (10, 11). These DCs were suggested to preferentially arise from Ly6C+, CCR7/8+ MOs (9). These studies could not however determine the fate of CCR2lowCX3CR1high MOs in vivo after entering nonlymphoid tissues as such cells were not recruited. To determine directly whether MOs can enter tissues and differentiate into migratory DCs under SS conditions, we transferred labeled congenic subsets of MOs into cannulated MLNX rats. Using this model, we show for the first time that CCR2lowCX3CR1high MOs can differentiate into a small number of DCs that migrate from the gut toward the MLN in the absence of any inflammatory stimuli and that this occurs without cell division. We cannot however exclude the possibility that an unidentified subset of CD43high MOs gives rise to lymph DCs. iL-DCs that derive from CCR2highCX3CR1low (CD43low) MOs could also be identified, some of which had gone through several rounds of cell division. We do not know whether these transferred CD43high MOs had entered the intestine and then differentiated into intestinal DCs or if they had first differentiated into CD43high cells, which we could detect in blood, before entering the tissue. DCs that originate from CD43high BM MOs that had divided probably represent the CD11b− subset as in blood these cells divide and up-regulate CD11b. This suggests that

![FIGURE 4. MOs differentiate into iL-DCs in the SS.](image-url)
RT7b blood MOs were enriched by one round of depletion and analyzed for the expression of surface markers. BMOs differentiate into both main subsets of iL-DCs.

**FIGURE 5.** A total of 90 × 10⁶ blood MOs were enriched, CFSE-labeled and injected i.v. into individual RT1c MLNX rats. Sixty-five rats were cannulated for 24 h and the lymph samples were pooled. The hours later, a blood sample was taken and the thoracic duct of the recipient was collected for an additional 18 h, at which time point blood samples were taken from the individual rats when not stated otherwise and control rats received no MOs. Numbers show the percentage of cells in each gate or quadrant. The results are representative of three independent experiments.

**A**

- CD172a
- CD103
- MHC-II

**B**

- Pre
- Post LPS
- Post R-848

**C**

- PBS
- LPS
- R-848

**D**

- All MOs
- Transferred MOs

**E**

- CD43<sup>high</sup>
- CD43<sup>low</sup>
- Control

CD11b<sup>-</sup>CD43<sup>low</sup> cells are at an earlier state of maturation than the CD11b<sup+</sup>CD43<sup>low</sup> MOs.

After transfer of approximately the number of CD43<sup>high</sup> MOs obtained by perfusing one rat into a MLNX rat, the frequency of M<sub>0</sub>-derived DCs was 0.02–0.04%. However, 18 h after transfer, the frequency of donor cells among total recipient blood MOs was 3–4% and at the time of cannulation they represented 2%. Several explanations for the low frequency of donor-derived DCs need to be considered. The low frequency may relate to the effects of in vitro handling on M<sub>0</sub>s as when this was minimized higher frequencies of both donor-derived M<sub>0</sub>s present in blood at the time of cannulation and M<sub>0</sub>-derived iL-DCs were detected. Additionally, it may reflect the normal proportion of CD43<sup>low</sup> M<sub>0</sub>s that gives rise to DCs rather than macrophages. Moreover, we are sampling the number of M<sub>0</sub>-derived DC exiting only the gut and for a limited amount of time. Most likely, M<sub>0</sub>s also enter a number of other tissues. As we in this study wanted to address DC differentiation under SS conditions, the rats were not irradiated. The transferred DC precursors will therefore have to compete with host precursors, which will presumably occupy most of the available niches and that have not been handled in vitro. Finally, it is possible that a large proportion SS iL-DCs do not derive from M<sub>0</sub>s but from an as yet unidentified blood-borne precursor. What this study does however demonstrate is that iL-DCs can derive from M<sub>0</sub>s under SS.

After transfer of both subsets of M<sub>0</sub>s, we could detect cells with a M<sub>1</sub> phenotype in lymph. These cells were not iL-DCs or PMNs as they were CD103<sup+</sup> MHC-II<sup+</sup>and CD11b<sup+</sup>CD43<sup>low</sup> cells. This is an interesting population of M<sub>0</sub>s as their existence in pseudoafferent lymph implies that at least some M<sub>0</sub>s migrate via afferent lymph under SS conditions. Importantly, we did not detect any donor-derived PMNs in lymph and the M<sub>0</sub>s we identified were not contaminating cells from blood as they were found in lymph free of RBC. In addition, these lymph-M<sub>0</sub>s were in contrast to blood M<sub>0</sub>s all CD43<sup>high</sup> (U. Yrlid and G. G. MacPherson, unpublished observation). We are currently further investigating the migration pattern of lymph M<sub>0</sub>s.

Heterogeneity among afferent lymph DCs has been described in a number of species (17, 21–23) but whether they have common origins is not clear. We have previously shown that rat iL-DCs can be divided individual rats then received 50 µg of LPS or 25 µg of R-848 i.v. and lymph was collected for an additional 18 h, at which time point blood samples were taken from the individual rats. B, FACS analysis of the frequency of CFSE<sup+</sup> M<sub>0</sub>s among total CD172a<sup+</sup> mononuclear cells in blood samples taken at the time of cannulation (Pre) and at the end of the cannulation 18 h after LPS or R-848 (Post LPS and Post R-848). C, FACS analysis of iL-DC subsets in intestinal lymph collected from SS rats (PBS) or 0–18 h after LPS or R-848 injection. The histogram in the top left corner shows the expression of CD103 by collected 7AAD<sup+</sup> TDLs (after partial depletion of B and T cells). Indicated is also the gate used to allow for analysis of host (CFSE<sup+</sup>) and donor M<sub>0</sub>-derived (CFSE<sup+</sup>) iL-DCs shown in the large density plot. The two right histograms shows the expression of CD172a by the CFSE<sup+</sup> and iL-DCs gated out as indicated by the gates and arrows (note that the CFSE labeling of M<sub>0</sub>s already in blood in this experiment was lower but homogeneous and does not reflect any cell divisions). D. A total of 10 × 10⁶ CD43<sup>low</sup> sorted congenic blood MOs were injected i.v. into RT1<sup+</sup> rats. Forty-two hours later, recipients were bled (Pre R-848), then given 25 µg of R-848 i.v. 1 h later perfused (Post R-848). CD172a<sup+</sup> mononuclear cells were gated out and CD43 expression by either (all M<sub>0</sub>s) or (transferred M<sub>0</sub>s) was assessed by FACS. E, RT7<sup+</sup>CD43<sup>high</sup> blood MOs (70 × 10⁶) and RT7<sup+</sup>CD11b<sup+</sup>CD43<sup>low</sup> BM M<sub>0</sub>s (18 × 10⁶) were enriched, CFSE-labeled and injected i.v. into individual RT1<sup+</sup> MLNX rats. The MLNX rats were then given R-848 i.v., cannulated as in C, and CD103<sup+</sup> MHC-II<sup+</sup> TDLs were analyzed by FACS. Plots represent individual rats when not stated otherwise and control rats received no M<sub>0</sub>s. Numbers show the percentage of cells in each gate or quadrant. The results are representative of three independent experiments.
into two phenotypically and functionally distinct subsets distinguished by the expression levels of CD172a (17, 24). CD172aLow IL-DCs selectively carry intestinal apoptotic intestinal material to the T cell areas of the MLN while CD172aHigh DCs are largely excluded from this part of the MLN (24). This subset-specific function and localization in lymphoid tissue is shared with murine DC subsets from lymphoid tissues where CD8α−, but not CD8α+, DCs engulf apoptotic material and are primarily detected in T cell areas of spleen and LN (25). In this study, we show that both the major subsets of iL-DCs can be derived from Mψs in the absence of cell division and that the frequencies of the two subsets are very similar to those of total iL-DCs. This suggests that there is no preference for Mψs to differentiate into either of the subsets under SS conditions. To be able to analyze iL-DC subset frequencies more accurately, we administered TLR4 or TLR7/8 ligands which stimulate virtually total emptying of DC from the lamina propria of the gut. This procedure had only a minor effect on the overall frequency of Mψ-derived iL-DCs, we recovered but the significant increase in iL-DCs recovered enabled us to confidently confirm that subset frequencies among donor- and host-derived iL-DCs were essentially identical. Even though the Mψs transferred were not enriched for either subset, it is very unlikely that CD43Low Mψs would contribute significantly to either subset of iL-DCs, as when equivalent numbers of purified CD11b+CD43Low Mψs were transferred no donor-derived iL-DCs could be detected. This also suggests that those iL-DCs detected after transfer of CD11b−/−CD43− BM Mψs most likely derive from the dividing, CD11b− subset of CD43Low Mψs which could contain cells equivalent to the novel CD11b− progenitor of mononuclear phagocytes described in mice (26). CCR2highCXCRIIL1Y6Chigh) Mψs enriched from BM have also been shown to give rise to both the major subsets of murine splenic DCs (20). This study however used irradiated recipients and CD8α− and CD8α+ donor-derived CD11c+MHC-II+ splenic DCs were recovered 2 wk after transfer, whereas in our study blood Mψs were transferred to naive rats and Mψ-derived DCs were analyzed after 3–4 days.

In conclusion, we show that CD43 expression divides rat Mψs into two distinct subsets. CD43High Mψs, the major blood population, do not express CCR2, CCR7, or CD62L but express CXCR1 and CD11c. In contrast to CD43Low Mψs, CD43High Mψs do not migrate to inflamed peritoneum. Furthermore, we demonstrate unambiguously that in blood, in the absence of any phagocytic or inflammatory stimuli, CD43Low Mψs mature into CD43High Mψs without division. Finally, we show that a small number of blood Mψs can enter the intestine and differentiate into the two main subsets of iL-DCs. This study therefore provides evidence for one of the possible origins of CCR2highCXCR1high blood Mψs and demonstrates their ability to differentiate into migratory intestinal DCs in vivo in the absence of inflammatory stimuli.

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Disclosures

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References