Immunity, Vol. 19, 71–82, July, 2003, Copyright ©2003 by Cell Press

Blood Monocytes Consist of Two Principal Subsets with Distinct Migratory Properties

Frederic Geissmann,[1,3] Steffen Jung,[1,3,4] and Dan R. Littman[1,2,*]
[1]Molecular Pathogenesis Program and the
[2]Howard Hughes Medical Institute
Skirball Institute of Biomolecular Medicine
New York University School of Medicine
540 First Avenue
New York, New York 10016

Summary

Peripheral blood monocytes are a heterogeneous population of circulating leukocytes. Using a murine adoptive transfer system to probe monocyte homing and differentiation in vivo, we identified two functional subsets among murine blood monocytes: a short-lived $CX_3CR1^{lo}CCR2^{+}Gr1^{+}$ subset that is actively recruited to inflamed tissues and a $CX_3CR1^{hi}CCR2^{-}Gr1^{-}$ subset characterized by CX_3CR1-dependent recruitment to noninflamed tissues. Both subsets have the potential to differentiate into dendritic cells in vivo. The level of CX_3CR1 expression also defines the two major human monocyte subsets, the $CD14^{+}CD16^{-}$ and $CD14^{lo}CD16^{+}$ monocytes, which share phenotype and homing potential with the mouse subsets. These findings raise the potential for novel therapeutic strategies in inflammatory diseases.

Introduction

The mononuclear phagocyte system (MPS) (van Furth, 1988; Wiktor-Jedrzejczak and Gordon, 1996) is a specialized tissue distributed throughout the body and composed of strikingly diverse differentiated cell types. These include liver Kupffer cells, lung alveolar macrophages, serosal (peritoneal) macrophages, brain microglia, dendritic cells (DC), and osteoclasts, as well as granuloma-forming macrophages at sites of inflammation (Wiktor-Jedrzejczak and Gordon, 1996). The MPS is of hematopoietic origin and is thought to require continuous reconstitution. This could be achieved by self-renewal of differentiated cells (Lawson et al., 1992; Merad et al., 2002), by proliferation of bone marrow (BM)-derived precursors in peripheral tissues (Martinez del Hoyo et al., 2002; Takahashi et al., 1996; Yamamoto et al., 1996), or by the continuous extravasation and differentiation of circulating blood MPS precursors such as blood monocytes or preimmunocytes (Bruno et al., 2001; Kennedy and Abkowitz, 1998; Lagasse and Weissman, 1997). These alternatives are not mutually exclusive and might operate in parallel in the regeneration of the individual MPS subsets.

Monocytes are a population of mononuclear leukocytes that develop in the BM from dividing monoblasts, are released in the bloodstream as nondividing cells, and enter tissues, where their fate remains largely a topic for speculation (for review see Wiktor-Jedrzejczak and Gordon, 1996). With half of the circulating monocytes leaving the bloodstream under steady-state conditions each day, monocytes could constitute a considerable systemic reservoir of myeloid precursors. This notion is supported by studies with cytokine-driven culture systems that allow in vitro differentiation of monocytes into macrophages and their tissue-specific representatives as well as into DC (Akagawa et al., 1996; Chapuis et al., 1997; Sallusto and Lanzavecchia, 1994; Zhou and Tedder, 1996; Geissmann et al., 1998; Kiertscher and Roth, 1996; Randolph et al., 1998; Schreurs et al., 1999). However, experimental data on monocyte differentiation and function in vivo are scarce, and the physiological role of this versatile cell type has only been inferred from histological and immunohistochemical observations (Crofton et al., 1978; Randolph et al., 1999; Thomas et al., 1976; Wiktor-Jedrzejczak and Gordon, 1996).

Monocytes are defined as blood mononuclear cells with bean-shaped nuclei, expression of CD11b, CD11c, and CD14 in humans and CD11b and F4/80 in mice, and lack of B, T, NK, and DC markers. However, in the same way that cells originally described as lymphocytes based on their appearance in peripheral blood smears are now known to be comprised of many physiologically distinct subsets, monocytes are morphologically and phenotypically heterogeneous. The term monocyte may therefore describe cells with overall similar appearance but different roles (Muller, 2001).

Mononuclear phagocytes, as represented by macrophages and DC, are critical for both innate and adaptive immunity. Interaction of antigen-specific T lymphocytes with antigen-presenting dendritic cells triggers the induction of adaptative immune responses and is also likely to be critical for the control of tissue inflammation and the maintenance of immune tolerance. As putative precursors for microglia and osteoclasts, monocytes may also be involved in the physiology of the central nervous system and in bone remodeling. Understanding of the role and fate of functional subsets of circulating monocytes in vivo is therefore an important and long-standing issue.

To accurately study the fate of blood monocytes in vivo, the experiment of choice is the adoptive transfer of marked blood monocytes into congenic recipient mice. However, the difficulty of isolating mouse monocytes, due to their relative rarity (Mouse Phenome Database, http://aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home), their phenotypic heterogeneity, and their lack of proliferation have in the past largely hampered transfer experiments. Using adoptive transfer of GFP-labeled monocytes, we report here that monocytes can be divided into two main subsets: a short-lived "inflammatory subset" that homes to inflamed tissue, where it can trigger immune responses, and a "resident subset," with a longer half-life, that homes to noninflamed tissues.

*Correspondence: littman@saturn.med.nyu.edu
[3]These authors contributed equally to this work.
[4]Present address: The Weizmann Institute of Science, Department of Immunology, Rehovot 76100, Israel.

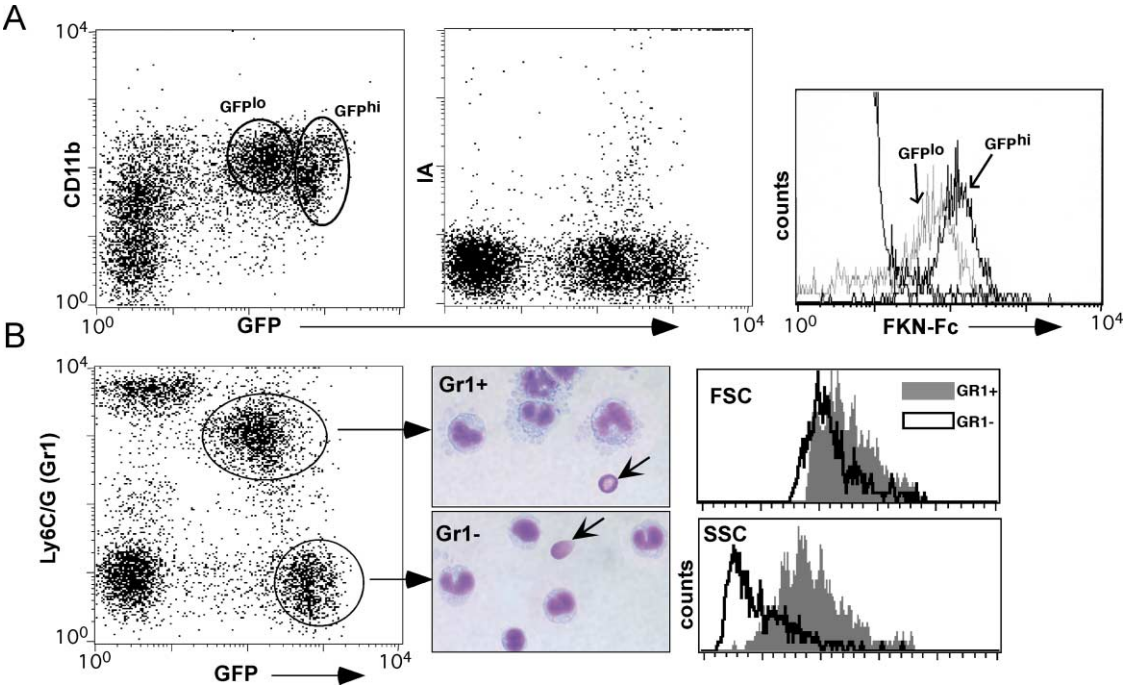

Figure 1. Definition of Mouse Monocyte Subsets by CX_3CR1 Expression

(A) Blood mononuclear cells from *$CX_3CR1^{gfp/+}RAG^{-/-}$* mice were stained for CD11b and I-A (left and middle panels) and FKN-Fc (right panel). Binding of FKN-Fc is shown for gated populations of GFP^{lo} cells (gray histogram) and GFP^{hi} cells (black histogram).

(B) Blood mononuclear cells from *$CX_3CR1^{gfp/+}RAG^{-/-}$* mice were stained for Ly6C/G (Gr1) and sorted, according to the indicated gates, for microscopic analysis (middle panels). The arrows indicate red blood cells of 7 μm diameter. The forward and side light scatter profiles of $GR1^-GFP^{hi}$ (open histograms) and $GR1^+GFP^{lo}$ (filled histograms) cells are shown in the panels at right.

Accumulation of the "resident monocytes" in diverse tissues is facilitated by the expression of the chemokine receptor CX_3CR1. The dichotomy in monocyte subsets has been conserved during evolution, as we have identified corresponding subsets in human blood.

Results

CX_3CR1 Expression Defines Two Subsets among Mouse Peripheral Blood Monocytes

In heterozygous *$CX_3CR1^{GFP/+}$* mice, one allele for the gene encoding CX_3CR1, the receptor for the membrane-tethered chemokine fractalkine (CX_3CL1 or Fkn), has been replaced with the gene encoding green fluorescent protein (GFP), which results in GFP labeling of all circulating $CD11b^+$ $F4/80^+$ cells (Jung et al., 2000; Palframan et al., 2001). GFP intensity and CX_3CR1 surface expression define two discrete subpopulations of monocytes that are roughly equally represented in the blood of *$CX_3CR1^{GFP/+}$* mice (Palframan et al., 2001; Figures 1A and 1B). CX_3CR1^{lo} monocytes are large granular mononuclear cells of 10–14 μm diameter, while CX_3CR1^{hi} cells are smaller mononuclear cells with a diameter of 8–12 μm (Figure 1B). Both subsets were noncycling in the blood ($Ki67^-$), expressed the hematopoietic antigens CD45R0 and CD44, and lacked expression of lymphoid lineage markers, such as NK1.1 and DX5 (NK cells), CD90 (Thy1) and CD3 (T cells), CD19 and CD45R/B220 (B cells), and MHC class II ($I\text{-}A^b$) and CD11c (dendritic cells) (Figure 1 and Table 1). The two monocyte subsets were also characterized by the differential expression of a number of surface markers (Table 1). CX_3CR1^{low} monocytes expressed CD62L (L-selectin), Ly6C/G (Gr1), α2 and α4 integrin (VLA2, VLA4), LFA1, and CCR2, while CX_3CR1^{high} cells expressed only LFA1 and VLA4.

CX_3CR1 Expression Also Defines Two Major Human Monocyte Subsets

To investigate whether differential CX_3CR1 expression among monocyte subsets is conserved between rodents and primates, we stained human peripheral blood cells with a Fkn-Fc fusion protein specific for CX_3CR1. Several discrete CX_3CR1^+ populations were identified among human leukocytes (Figure 2A). A population of $DR^-CX_3CR1^+$ cells expressed CD2, CD16, and CD56 and corresponds to NK cells, as previously described (Imai et al., 1997 and data not shown). A population of $DR^+CX_3CR1^{low}$ cells (population 1 in Figure 2A) was $CD14^{hi}$, $CD11b^{hi}$, $CD11c^{hi}$ but was negative for T cell (CD2), B cell (CD19, CD24), interferon-producing cell (IPC also called PDC or DC2) (BDC2A, CD123), and NK cell (CD2, CD56) markers (Figure 2A, Supplemental Figure S1 at http://www.immunity.com/cgi/content/full/19/1/71/DC1, and data not shown), and therefore corresponds to classical $CD14^+$ monocytes. These cells resemble murine $CX_3CR1^{low}Gr1^+$ monocytes in that they have high forward and sideward scatter profiles and express CD62L (Figure 2A). A population of DR^+ CX_3CR1^{high} cells (population 2 in Figure 2A) was charac-

Table 1. Phenotype of Mouse Monocyte Subsets

	Subset 1 (Inflammatory)	Subset 2 (Resident)
Monocyte marker		
CD11b	+	+
F4/80	+	+
Ly6C/G	+	−
Chemokine receptors		
CX3CR1	lo	hi
CCR2	+	−
Adhesion molecules		
CD31	++	+
LFA1	+	++
VLA1	−	−
VLA2	+	−
VLA4	+	+
L-selectin (CD62L)	+	−
Miscellaneous		
CD16/32	+	+
CD44	+	+
CD45	+	+
CD45 RA, RB, RC	−	−
KI67	−	−
T, B, NK, IPC, DC markers		
CD90	−	−
B220 (CD45R)	−	−
TCRβ	−	−
CD11c	−	−
IA	− (<5%)	−
NK1.1	−	−

terized as being $CD14^{low}CD16^{hi}$, $CD11b^{+}$, and $CD11c^{+}$ but was negative for T, B, IPC, and NK cell markers. These cells correspond to the previously defined $CD16^{+}$ monocytes (Passlick et al., 1989) and resemble murine $CX_3CR1^{hi}Gr1^{-}$ monocytes, as they are smaller and less granular than the $CX_3CR1^{low}CD14^{+}$ monocytes and do not express CD62L (Figure 2A). Although both subsets expressed CXCR4, expression of inflammatory chemokine receptors, including CCR1, CCR2, CXCR1, and CXCR2, was restricted to the CX_3CR1^{low} $CD14^{+}$ monocytes (Figure 2B). We conclude that human monocytes, although differing from mouse monocytes in terms of MHC class II and CD11c expression, can be divided into two subsets that share size and granularity, as well as adhesion molecule and chemokine receptor expression patterns, with the murine monocyte subsets.

$CX_3CR1^{lo}Gr1^{+}$ and $CX_3CR1^{hi}Gr1^{-}$ Monocytes Have Distinct Homing Properties

The differential expression of adhesion molecules and chemokine receptors by the two monocyte subsets in both mice and humans prompted us to establish an adoptive transfer system to compare their in vivo migration properties. Although monocytes represent less than 2% of peripheral blood mononuclear cells in wild-type C57BL/6 mice (Mouse Phenome Database, http://aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home), they constitute, due to the absence of B and T lymphocytes, 45%–50% of leukocytes in RAG-deficient mice. To facilitate isolation of monocytes, we therefore crossed the *CX_3CR1^{GFP}* allele onto a lymphocyte-deficient C57BL/6 *$RAG2^{-/-}$* background. The phenotype of monocytes, macrophages, and DC subsets is unaffected in RAG-deficient mice (data not shown). GFP-expressing monocytes were isolated from heparinized blood of *$CX_3CR1^{GFP/+}$ $RAG2^{-/-}$* C57BL/6 mice ($CD45.2^{+}$) by Ficoll density gradient separation and depletion of MHC class II ($I\text{-}A^{b}$)- and CD11c-positive cells to exclude interferon-producing cells (IPC) and circulating DC. $CX_3CR1^{lo}Gr1^{+}$ and $CX_3CR1^{high}Gr1^{-}$ monocytes were then fractionated by immunomagnetic sorting (Supplemental Figure S2), and 10^5 cells of each subset were separately injected intravenously (IV) into nonirradiated C57BL/6 $CD45.1^{+}$ recipient mice. The allotypic CD45 marker represents an additional tool to identify the $CD45.2^{+}$ graft in $CD45.1^{+}$ hosts. The number of monocytes injected (10^5) corresponded approximately to the total circulating in a C57BL/6 mouse. Recipients of the monocyte transfer were bled and sacrificed at various time points and perfused to remove remaining cells from blood vessels. Single-cell suspensions of various organs were prepared by collagenase digestion and analyzed by flow cytometry for the presence of $CD45.2^{+}/CD45.1^{-}$ $GFP^{low/hi}$ donor-derived cells.

Adoptively transferred $CD45.2^{+}/CD45.1^{-}GFP^{hi}Gr1^{-}$ cells were observed after transfer in the blood, spleen, lung, liver, and brain of recipient mice, but not in the lymph nodes, thymus, peritoneum, and bronchoalveolar fluid (Figure 3A). In contrast, $CD45.2^{+}/CD45.1^{-}GFP^{low}Gr1^{+}$ cells were almost undetectable in any tissue except spleen, as early as 1 day after transfer. Similar results were obtained when $Gr1^{+}$ and $Gr1^{-}$ monocyte subsets were injected in the same mice or in separate mice.

Monocytes are known to home to inflamed tissues (van Furth, 1988). We therefore compared the homing potential of the $CX_3CR1^{lo}Gr1^{+}$ and $CX_3CR1^{hi}Gr1^{-}$ monocyte subsets during inflammation, using the model of intraperitoneal (IP) thioglycollate injection (Lagasse and Weissman, 1996). Upon transfer into an inflamed host, the ephemeral $GFP^{low}Gr1^{+}$ monocytes invaded the peritoneum (Figures 3B and 3C). This finding is in accordance with their expression of CD62L and CCR2, the receptor for the proinflammatory chemokine MCP-1 (Ajuebor et al., 1998; Boring et al., 1997; Kurihara et al., 1997; Lu et al., 1998; Palframan et al., 2001). In contrast, the migration pattern of the $GFP^{hi}Gr1^{-}$ monocyte subset appeared to be only slightly affected by inflammation, as most of these cells were found in blood and noninflamed peripheral organs (Figures 3B and 3C). Similar results were obtained when $Gr1^{+}$ and $Gr1^{-}$ monocyte subsets were injected in the same mice (Figure 3C) or in separate mice (Figure 3B), indicating that $Gr1^{-}GFP^{hi}$ monocytes did not differentiate into $Gr1^{+}GFP^{low}$ monocytes. Two to five percent of both populations of grafted monocytes were recovered from recipients after these procedures.

$CX_3CR1^{lo}Gr1^{+}$ Monocytes Differentiate into Dendritic Cells in Inflamed Tissues and Trigger Naive T Cell Proliferation In Vivo

The adoptive monocyte transfer system allowed us to study the differentiation potential of donor monocytes. Before the adoptive transfer, both $CX_3CR1^{hi}Gr1^{-}$ and $CX_3CR1^{lo}Gr1^{+}$ monocyte subsets are characterized by the absence of the dendritic cell markers CD11c and MHC class II ($I\text{-}A^{b}$) (Supplemental Figure S2). Both mono-

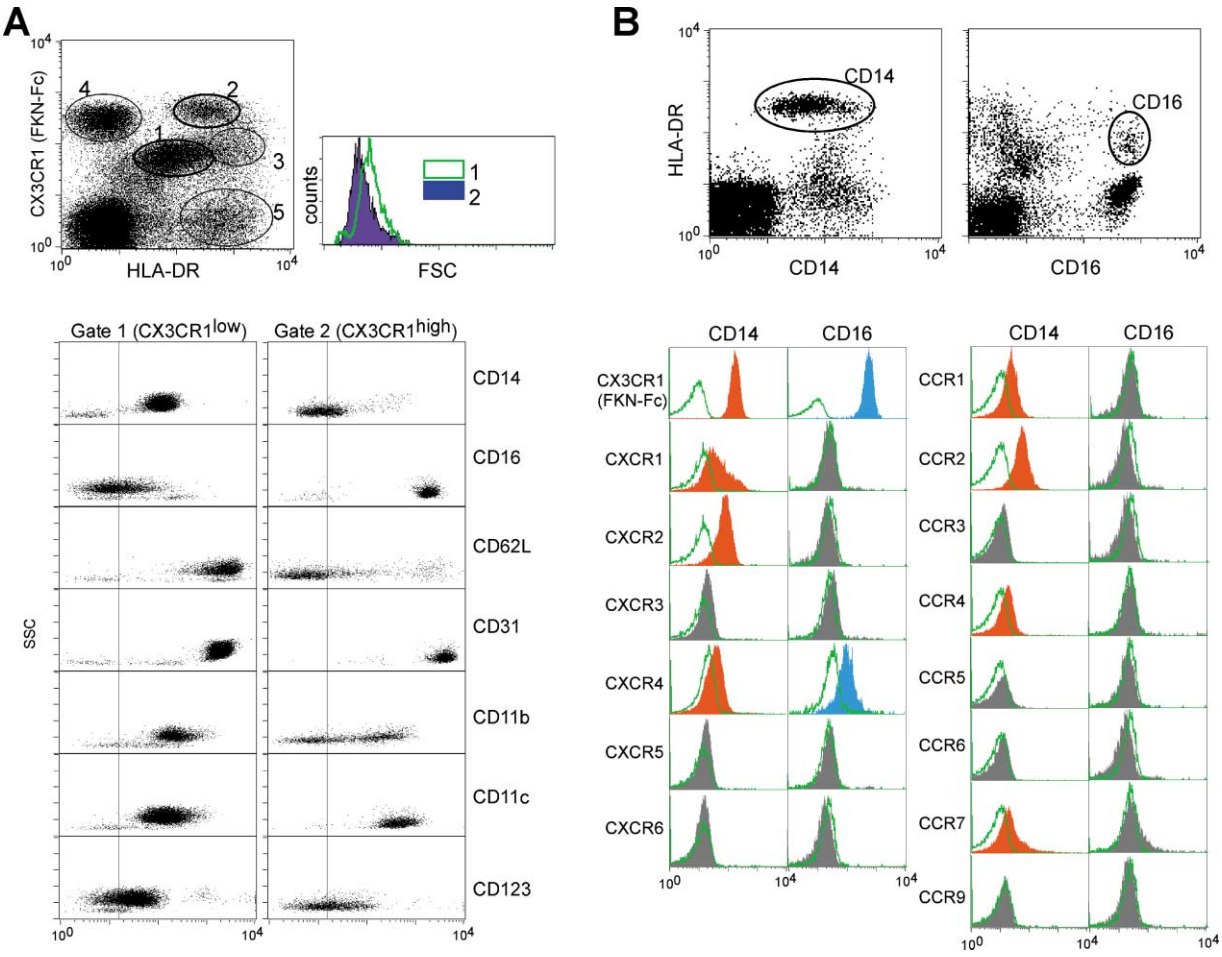

Figure 2. CX_3CR1 Expression on Two Distinct Human Monocyte Subsets

(A) Human peripheral blood mononuclear cells (PBMC) were stained with anti-HLA-DR, FKN-Fc, and one of the indicated antibodies. The forward scatter profiles of gated populations 1 and 2 and expression of surface markers on these populations are shown at right and below, respectively. Gate 3 corresponds to $HLA\text{-}DR^+CD14^-CD16^-CD19^-BDCA2^-$ cells (known as circulating DC or DC1), gate 4 corresponds to $CD16^+HLA\text{-}DR^-$ NK cells, and gate 5 contains $CD19^+$ B cells and IPC (see also Supplemental Figure S1 at http://www.immunity.com/cgi/cont/full/19/1/71/DC1).

(B) PBMC were stained with FITC-conjugated anti-CD16, PerCp-conjugated anti-HLA-DR, APC-conjugated anti-CD14 antibodies, and FKN-Fc followed by Cy3-conjugated goat anti-human IgG, or either one of PE-conjugated antibodies against CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR9, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, and isotype controls. Chemokine receptor expression levels on gated $CD14^+$ and $CD16^+$ monocytes are shown below. Open green histograms represent isotype control staining, and filled histograms represent specific staining. The red-filled histograms indicate significant expression above background, and blue-filled histograms indicate differences in relative levels of expression between the monocyte populations.

cyte subsets gave rise to $CD11c^+$ MHC class II^+ dendritic cells after in vitro culture with GM-CSF and IL-4 (data not shown). To investigate the potential of the transferred monocytes to differentiate into DC in vivo, we analyzed recipient mice for the presence of $CD45.1^+/CD45.2^+GFP^+CD11c^+$ MHC class II^+cells.

We first investigated the fate of CX_3CR1^{low} monocytes. Strikingly, 18 hr after IV transfer into a recipient with an inflamed peritoneum, a major fraction of donor-derived $CD45.2^+GFP^{low}Gr1^+$ monocytes that had entered the peritoneum had differentiated to express CD11c and I-A (Figures 3C and 3D). Some $CD45.2^+GFP^{low}$MHC class II $(I\text{-}A^b)^+$ cells were also observed in peripheral blood (Figures 3C and 3D). After 2 days, $CD45.2^+GFP^{low}CD11c^+$ cells were rare in the peritoneum and were absent from blood and inguinal lymph nodes, but were detected in parathymic lymph nodes that drain the peritoneal cavity (Supplemental Figure S3).

Blood monocytes do not stain for Ki67, a sensitive marker of cycling cells. The rapid acquisition of CD11c and I-A expression by the transferred monocytes is therefore likely to be due to differentiation rather than to expansion of a $CD11c^+I\text{-}A^+$ or $I\text{-}A^-$ DC precursor. The results suggest that CX_3CR1^{low} monocytes can differentiate into DC in vivo. However, DC are best defined by their ability to stimulate antigen-specific naive T cells (Jung et al., 2002; Mellman and Steinman, 2001). We therefore examined whether a monocyte graft can reconstitute priming of naive $CD8^+$ T cells in a MHC class I-deficient host. To this end, we injected MHC class I-deficient recipient mice ($\beta_2m^{-/-,\ CD45.1^+}$) intravenously with CFSE-labeled $CD8^+$ T cells from mice expressing

an ovalbumin-specific transgenic TCR (10^6 naive CD45.2$^+$ OT-I CD8 T cells [Hogquist et al., 1994]). The mice then received an IP injection of thioglycollate and were grafted intravenously with monocytes (10^5 cells, depleted of I-A$^+$/CD11c$^+$ cells). Six hours after monocyte transfer, recipient mice received an IP injection of OVA peptide (50 μg SIINFEKL). As expected, OVA peptide-induced CD8$^+$ T cell proliferation, which was readily observed in wild-type recipients, was impaired in the MHC class I-deficient host. However, the adoptive monocyte graft was able to partially restore antigen-specific proliferation of class I-restricted T cells in lymph nodes draining the peritoneum of the class I-deficient host (Figure 3E). These results strongly suggest that CX$_3$CR1^{low}Gr1$^+$ monocytes, which acquire DC markers in inflamed peritoneum, differentiate into functional DC, which have the ability to stimulate naive T cells. A fraction (25%) of CD45.2$^+$GFP$^+$CX$_3$CR1^{high} grafted cells also expressed CD11c and MHC class II (I-A^b) in the spleen in the absence of inflammation 2 days after transfer (see Figure 4A), suggesting that some CX$_3$CR1^{high} monocytes can acquire a DC phenotype in vivo. However, additional studies will be required to confirm that the Gr1$^-$ monocyte subset can differentiate into functional DC.

Entry of CX$_3$CR1^{hi}Gr1$^-$ Monocytes into Uninflamed Tissues Is Sensitive to Pertussis Toxin

It is well established that monocytes can be recruited to inflamed tissues by chemokines that bind to CCR2 and/or CXCR3 on their surface (Ajuebor et al., 1998; Boring et al., 1997; Janatpour et al., 2001; Kurihara et al., 1997; Lu et al., 1998; Palframan et al., 2001). CD45.2$^+$CX$_3$CR1^{high} donor monocytes, which do not express CCR2, home to spleen, lung, liver, and brain of recipient mice in the absence of inflammation (Figure 3A). Upon histological examination, GFP$^+$ cells were found outside blood vessels in the marginal zone of the spleen and beneath bronchial epithelium in the lungs of mice that had received CX$_3$CR1^{high} monocytes (Figure 4B).

Pretreatment of CX$_3$CR1^{high} monocytes with pertussis toxin, which inhibits $G_{\alpha i}$-mediated chemokine receptor signaling (Cyster and Goodnow, 1995), prevented accumulation of grafted monocytes within recipient spleens but did not affect their circulation in the blood of recipient mice (Figure 4C). This finding suggests that CX$_3$CR1^{high} monocyte entry into tissues involves an active mechanism, presumably engaging $G_{\alpha i}$-coupled chemokine receptor(s).

Engraftment of CX$_3$CR1^{hi}Gr1$^-$ Monocytes Is Dependent on CX$_3$CR1

Fractalkine (CX$_3$CL1), the ligand for CX$_3$CR1, is a transmembrane chemokine that is expressed on endothelial cells and can promote adhesion of monocytes (Bazan et al., 1997; Fong et al., 1998; Goda et al., 2000). To investigate a potential role for CX$_3$CR1 in the migration of monocytes, we performed transfer experiments with a 1:1 mixture of heterozygous *CX$_3$CR1$^{+/GFP}$* (*CD45.1/CD45.2*) and homozygous mutant *CX$_3$CR1$^{GFP/GFP}$* (*CD45.2/CD45.2*) monocytes into untreated *CD45.1/CD45.1* recipient mice (Figure 5A). After transfer of CX$_3$CR1^{high} cells, *CX$_3$CR1$^{GFP/GFP}$* (GFP$^+$CD45.1$^-$) cells were markedly reduced in recipient blood and tissues in comparison to *CX$_3$CR1$^{+/GFP}$* (GFP$^+$CD45.1$^+$) monocytes (Figure 5B and Table 2). The ratio of cotransferred *CX$_3$CR1$^{+/GFP}$* and *CX$_3$CR1$^{GFP/GFP}$* NK cells remained constant (1:1) and thus served as an internal control. While CX$_3$CR1 deficiency affected homing of CX$_3$CR1^{high}Gr1$^-$ monocytes, CX$_3$CR1^{low}Gr1$^+$ monocytes entered the inflamed peritoneum of recipients irrespective of their CX$_3$CR1 genotype (Figure 5C).

Discussion

During the past several decades, lymphocytes have been progressively subdivided into a growing number of phenotypically discrete subsets bearing distinct functions. Careful examination of the functions of these subsets and the regulation of their differentiation has been instrumental toward our current understanding of adaptive immune responses and is contributing to the development of diagnostic and therapeutic tools for many diseases. In contrast, there has been relatively little effort until recently to characterize cells involved in innate immune responses, particularly cells of the myeloid lineages. In the last few years, there has been a growing appreciation that dendritic cells, the specialized antigen-presenting cells that link innate and adaptive immune responses, can be subdivided into at least two functionally distinct subsets, the CD11c$^+$ DC and the interferon-producing cells (IPC, also called plasmacytoid DC, or DC2 in human) (Cella et al., 1999; Siegal et al., 1999). Cells of the mononuclear phagocyte system, including DC and macrophages, can be derived from cytokine-stimulated monocytes in vitro. However, it has been difficult to demonstrate that these cells correspond to the tissue-specific macrophages and DC that are found in vivo, and it has not been formally determined whether such cells differentiate from circulating monocytes that extravasate into tissues. In addition, although peripheral blood monocytes of human and mice had been shown to be heterogeneous, functional subsets were not defined.

In this study, we describe an adoptive monocyte transfer system that has allowed us to characterize two major morphologically, phenotypically, and functionally distinct subsets of circulating monocytes in the mouse. Furthermore, we were able to identify putative human counterparts of these cells based on strikingly conserved interspecies phenotypic similarities. A short-lived CX$_3$CR1^{low}Gr1$^+$ murine subset, which we term "inflammatory monocytes," represents immediate circulating precursors for antigen-presenting DC and CD11c$^-$ myeloid cells in inflammatory conditions. We have identified a second CX$_3$CR1^{high}Gr1$^-$ monocyte subset that persists longer in tissues and serves as a precursor for resident myeloid cells, including CD11c$^+$I-A$^+$ DCs, in noninflamed tissues including liver, lung, brain, and spleen (Figure 6).

We show that CX$_3$CR1 expression also defines the two main subsets of monocytes in human. Previously described human CD16$^+$ monocytes (Passlick et al., 1989; Ziegler-Heitbrock, 2000), whose in vivo role remains enigmatic, share many features with mouse CX$_3$CR1^{high} monocytes. They are smaller in size and less

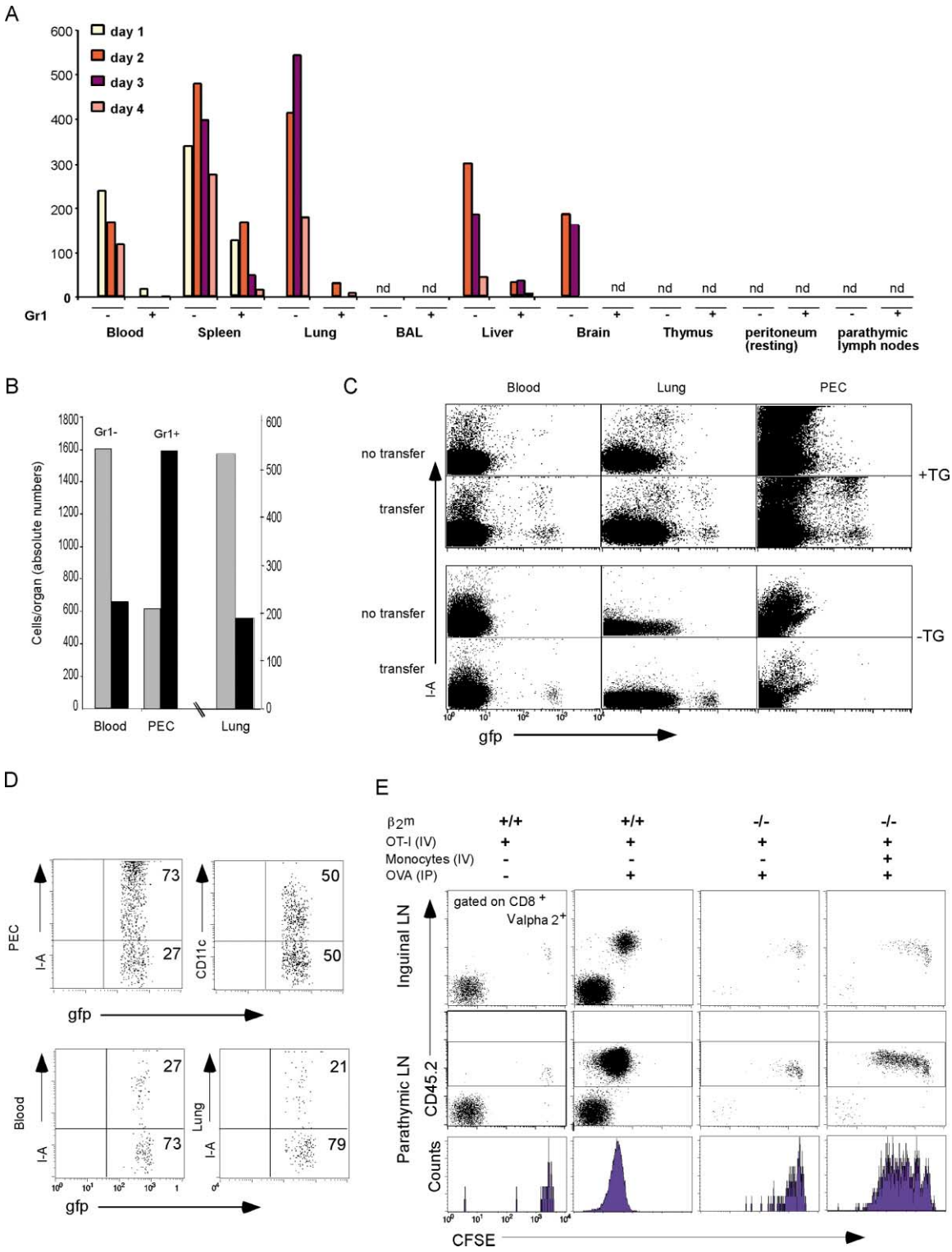

Figure 3. Migration and Differentiation Properties of Monocyte Subsets

(A) Recruitment of monocyte subsets to tissues in the absence of inflammation. 1×10^5 purified monocytes of each subset were injected intravenously (IV) into nonirradiated CD45.1$^+$ C57BL/6 recipient mice. At the indicated time points, recipient mice were analyzed for the presence of GFP$^+$CD45.2$^+$NK1.1$^-$ cells in each organ. Results are representative of six independent experiments. In each experiment, nongrafted CD45.1$^+$ C57BL/6 mice were used as staining controls (data not shown). Similar results were obtained when monocyte subsets were injected separately (as shown here) or coinjected. Coinjected cells were distinguished from each other by the intensity of the GFP signal. nd, not detected.

(B) Recruitment of monocyte subsets to inflamed tissue. Peritonitis was induced in recipient CD45.1$^+$ C57BL/6 mice by intraperitoneal injection of 1 ml thioglycollate. Six hours later, 1×10^5 CX$_3$CR1^{low}Gr1$^+$ and 1×10^5 CX$_3$CR1^{high}Gr1$^-$ monocytes were injected IV into separate recipients.

granular than the $CD14^+$ monocytes, express a high level of CX_3CR1, and are negative for CCR2 and L-selectin. Human $CD16^+$ monocytes also lack expression of CCR1, CXCR1, and CXCR2, receptors for inflammatory chemokines. This observation suggests that, like murine $CX_3CR1^{high}Gr1^-$ cells, the $CD16^+$ monocytes are excluded from inflamed tissues. Interestingly, $CD16^+$ monocytes have been shown to transmigrate through a layer of resting endothelial cells in vitro more efficiently than $CD14^+$ monocytes (Randolph et al., 2002). This is compatible with a shared potential of human and mouse CX_3CR1^{high} monocytes to give rise to resident tissue cells, including DC. Of note, human $CD14^+$ and $CD16^+$ monocyte subsets can both give rise to DC in vitro (Sallusto and Lanzavecchia, 1994; Sanchez-Torres et al., 2001). Accordingly, both murine monocyte subsets were able to give rise to $CD11c^+$MHC class II^+ dendritic cells in vitro after culture with GM-CSF and IL-4 (data not shown). The monocyte subsets described here are distinct from interferon-producing cells, which are CX_3CR1^- in human (see Table 1 and Supplemental Figure S1 at http://www.immunity.com/cgi/content/full/19/1/71/DC1), and have been described as $B220^+$, $CD11c^+$ in mice (Asselin-Paturel et al., 2001).

We also identified an additional human mononuclear cell subset, expressing intermediate levels of CX_3CR1 and representing about 1% of PBMC (gate 3 in Figure 2A). These cells are $CD14^-CD16^-CD11c^+DR^{high}$ (data not shown) and correspond to circulating DC. Interestingly, in C57BL/6 mice maintained under SPF conditions, the murine counterpart of this population is exceedingly rare ($I\text{-}A^+$ cells, see Figure 1A). However, $GFP^+CX_3CR1^{low}I\text{-}A^+$ cells do appear in the blood of mice that have received a CX_3CR1^{low} monocyte graft and an IP injection of thioglycollate (Figure 3D). This suggests that monocytes can develop into cells with the features of blood DC in animals that have ongoing tissue inflammation.

The chemokine receptor CX_3CR1 has been shown in vitro to have two distinct functions upon interacting with its only known ligand, CX_3CL1 (fractalkine), a type 1 transmembrane protein with a chemokine domain tethered on a extended mucin-like stalk (Bazan et al., 1997; Imai et al., 1997). It mediates adhesion to cells that express cell surface fractalkine and triggers chemotaxis in response to soluble fractalkine released by enzymatic cleavage. CX_3CR1 is expressed on blood monocytes and NK cells, as well as a small subset of T lymphocytes, while fractalkine is expressed on endothelial cells, epithelium, and neurons. We have previously failed to demonstrate a function for CX_3CR1 in models of toxoplasma-induced inflammation and microglial response to nerve damage (Jung et al., 2000). We have demonstrated here a function for this chemokine receptor, since we show that CX_3CR1 has a key role in the engraftment of $CX_3CR1^{high}Gr1^-$ monocytes. Membrane bound or soluble fractalkine encountered by $CX_3CR1^{high}Gr1^-$ monocytes within blood vessels may provide adhesive and/or chemotactic functions to enhance migration of monocytes into noninflamed tissues. This interpretation is supported by the recent report showing that the corresponding human $CD16^+$ monocytes are better suited than $CD14^+$ monocytes to migrate across endothelial monolayers (Randolph et al., 2002). Binding of fractalkine to its receptor may also deliver a survival signal to $CX_3CR1^{high}Gr1^-$ monocytes, as suggested by the observation that, in the absence of CX_3CR1, these cells fail to persist in the blood (Table 2). A role for CX_3CR1 signaling in preventing monocyte apoptosis is supported by previous reports that CX_3CL1 provides survival signals for CX_3CR1^+ microglia as well as human intestinal epithelial cells (Boehme et al., 2000; Brand et al., 2002). Moreover, other chemokine receptors, particularly CXCR4, have been shown to couple to the antiapoptotic Akt/PKB signaling pathway (Tilton et al., 2000). It will be interesting to investigate whether or not resident monocytes can be further divided into subsets that home to distinct sites, e.g., skin, bone, lung, or brain, as has been demonstrated for subsets of memory lymphocytes.

Information about monocyte subsets and their functions may impact our understanding of diseases and the design of therapeutic strategies. $CCR2^-CX_3CR1^{high}$ monocytes that home constitutively to tissues appear to belong to a different group than $CCR2^+CX_3CR1^{low}$ monocytes that home only when the tissue is inflamed. The two subsets of monocytes may exhibit different responses to pathogen products, and it will hence be interesting to determine whether they differ in expression of lectins and Toll-like receptors. Moreover, $CCR2^+CX_3CR1^{low}$ monocytes are likely to be involved in innate inflammatory responses, contributing to clearing *Listeria monocytogenes* from infected spleen (Serbina et al., 2003 [this issue of *Immunity*]), as well as in triggering of the adaptive response toward pathogens. In contrast, cells derived from resident $CCR2^-CX_3CR1^{high}$ monocytes may be involved in tissue homeostasis, as populations of resident macrophages include special-

Donor-derived monocytes in blood, peritoneal exudate (PEC), and lung were analyzed after 18 hr. Similar results were obtained when $CX_3CR1^{low}Gr1^+$ and $CX_3CR1^{high}Gr1^-$ cells were coinjected in the same recipients. Results are representative of three experiments.

(C) Recruitment of monocyte subsets in the absence or presence of inflammation. Analysis of control (no transfer) mice and recipient (transfer) mice 1 day after IV transfer of unfractionated monocytes. In the upper panels, peritoneal inflammation was induced with thioglycollate injection (+TG). The lower panels are controls without inflammation (−TG). Dot plots represent GFP and I-A levels on blood, lung, and peritoneal leukocytes.

(D) Expression of I-A and CD11c on $CD45.1^-NK1.1^-CX3CR1^{lo}$ monocytes recovered from peritoneal exudates, blood, and lung 18 hr after IV transfer.

(E) Flow cytometric analysis of transferred CFSE-labeled OT-I $CD8^+CD45.2^+$ T cells 60 hr after immunization of wild-type C57BL/6 (B6.SJL, $CD45.1^+$) and MHC class I-deficient mice ($\beta_2m^{-/-}$, $CD45.1^+$) with the antigenic SIINFEKL peptide. The monocyte graft consisted of 10^5 unfractionated blood monocytes, depleted of $I\text{-}A^+$ and $CD11c^+$ cells. Dot plots represent cells gated on $CD8^+$ and TCR $V\alpha5^+$ T cells. Note that the CFSE-negative population represents host $CD45.2^-V\alpha5^+CD8^+$ T cells. Histograms represent cells gated according to CD8, $V\alpha5$, and CD45.2 expression.

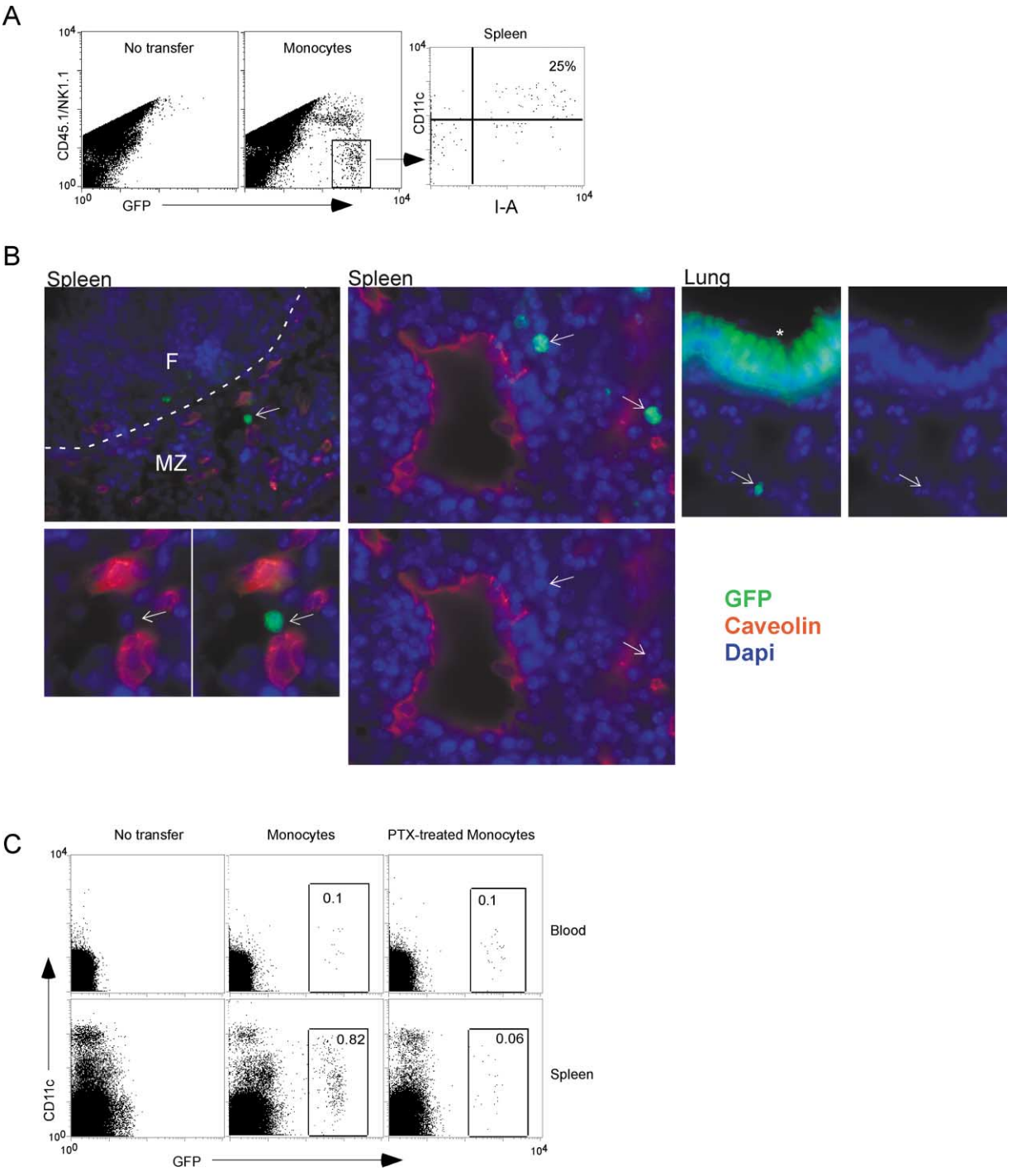

Figure 4. CX_3CR1^{hi} Monocyte Entry into Noninflamed Tissues Is Sensitive to Pertussis Toxin

(A) Analysis by flow cytometry of the expression of CD11c and I-A^b on CD45.1^-, NK1.1^- monocytes from a recipient spleen 2 days after transfer of blood Gr1^- (CX_3CR1^{high}) CD45.2^+ I-A^- CD11c^- monocytes from *$CX_3CR1^{gfp/+}$* mice. The analysis included the NK1.1 marker since the donor mice contain low numbers of GFP-expressing NK cells that were not depleted in this experiment.

(B) Analysis by fluorescence microscopy of recipient spleen and lung 2 days after transfer of purified monocytes depleted of I-A^+, CD11c^+, GR1^+, and NK1.1^+ cells. Sections are stained with DAPI (blue nuclear staining) and anti-caveolin-1 (red, endothelial cell staining). The arrows indicate GFP$^+$ cells. * indicates epithelial autofluorescence. In the spleen panel, the lower left figures are a magnification of the top left figure.

(C) Analysis by flow cytometry of the presence of CD45.1^-, GFP$^+$ donor-derived cells 2 days after transfer of pertussis toxin (PTX)- or control PBS-treated monocytes.

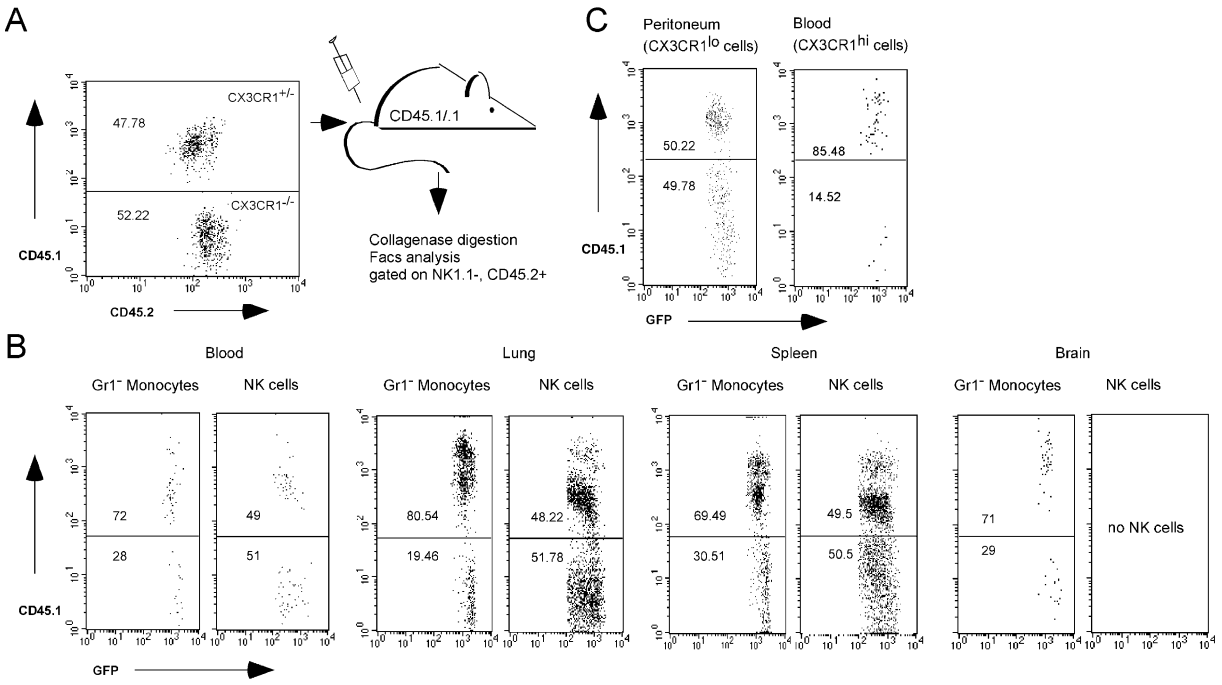

Figure 5. Engraftment of $CX_3CR1^{hi}Gr1^-$ Monocytes Is Dependent on CX_3CR1

(A) I-A$^-$CD11c$^-$ blood monocytes and NK cells were isolated from *RAG$^{-/-}$CD45.2/CD45.2 CX$_3$CR1$^{-/-}$* and *RAG$^{-/-}$ CD45.1/CD45.2 CX$_3$CR1$^{+/-}$* mice. Monocyte counts were assessed by flow cytometry, and equal numbers of Gr1$^+$ and/or Gr1$^-$ cells from *CX$_3$CR1$^{-/-}$* and *CX$_3$CR1$^{+/-}$* mice were transferred into *CD45.1/CD45.1* recipients. For analysis of donor-derived cells in recipient mice, blood was obtained by cardiac puncture, and the animals were then perfused with cold PBS and sacrificed. The indicated organs were removed and cells were prepared as described in the Experimental Procedures. Cell suspensions were then stained with CD45.1-PE, CD45.2-PerCp, and NK1.1-APC antibodies for four-color FACS analysis.

(B) CD45.1$^+$ recipients received monocytes depleted of GR1$^+$ cells IV and were analyzed 2 days after transfer.

(C) CD45.1$^+$ recipients were injected IP with thioglycollate, received unfractionated monocytes IV, and were analyzed 18 hr after transfer.

ized subsets, such as osteoclasts, Kupffer cells, and microglia. In light of their apparently different physiological functions, defects in one or the other subset may therefore result in different types of diseases. By targeting molecules restricted to $CCR2^+CX_3CR1^{low}$ monocytes, such as chemokine receptors, it may be possible to regulate their function in inflammatory diseases, without affecting the potential homeostatic role of $CCR2^-CX_3CR1^{high}$ monocytes in the brain or in the bone.

In summary, we have shown, through the use of an adoptive transfer system, the existence of two major subsets of circulating monocytes in mice and their conservation between rodents and primates, and have also performed an initial characterization of their migratory properties and immune functions. This report also demonstrates, in an in vivo system, that monocytes differentiate into DC that can stimulate naive T cells.

Table 2. Ratio of $CX_3CR1^{-/-}/CX_3CR1^{+/-}$ Cells after Transfer of Gr1$^-$ Monocytes

Time[a]	Blood	Lung	Spleen	Liver	Brain
18 hr	.2				
36 hr	.4	.24	.4	.5	.3
60 hr	.14	.3	.6	.6	.4

[a] Input at time 0 was at a ratio of 1.0.

Experimental Procedures

Animals

CX3CR1$^{gfp/+}$ mice were generated in the laboratory (Jung et al., 2000) and backcrossed for ten generations to C57BL/6 mice. *RAG2$^{-/-}$* and B6.SJL-*Ptprca Pep3b*/BoyJ (CD45.1) mice were obtained from Taconic (Germantown, NY), and OT-1 transgenic mice were obtained from Jackson Laboratories. All mice were bred and maintained in our specific pathogen-free animal facility according to institutional guidelines, and experiments were done at 6–12 weeks of age.

Adoptive Transfer of Monocytes

Blood monocytes from RAG2$^{-/-}$ $CX_3CR1^{gfp/+}$ CD45.2$^+$ C57BL/6 mice were separated from PMN on a Ficoll gradient and depleted of I-A+ and CD11c+ cells by immunomagnetic cell depletion using MACS technology (Miltenyi Biotec, Bergisch Gladbach, Germany). Ly6C/G$^+$ (Gr1$^+$) and Ly6C/G$^-$ (Gr1$^-$) monocytes were then separated by MACS sorting. NK1.1$^+$ cells were depleted by MACS before injection in some experiments (e.g., for histological analysis). Congenic CD45.1$^+$ C57BL/6 recipient mice were injected IV with 10^5 Gr1$^-$ or Gr1$^+$ monocytes or with 2×10^5 cells consisting of a 1:1 mix of Gr1$^-$ and Gr1$^+$ monocytes. Inflammation was induced by injecting 1 ml of a PBS solution containing 3% thioglycollate medium (Difco, Detroit, MI) into the peritoneal cavity of the mice 6 hr before transferring the monocytes. Peritoneal exudate cells (PEC) were harvested by lavage with PBS containing 5 mM EDTA. At various time points, mice were anesthetized using a cocktail of Ketamine (50 mg/kg), Xylazine (10 mg/kg), and Acepromazine (1.7 mg/kg) IV, and peritoneal and broncho-alveolar lavage (BAL) was performed, and peripheral blood was obtained. To perform BAL, the trachea was surgically exposed and cannulated. The airways were lavaged three times

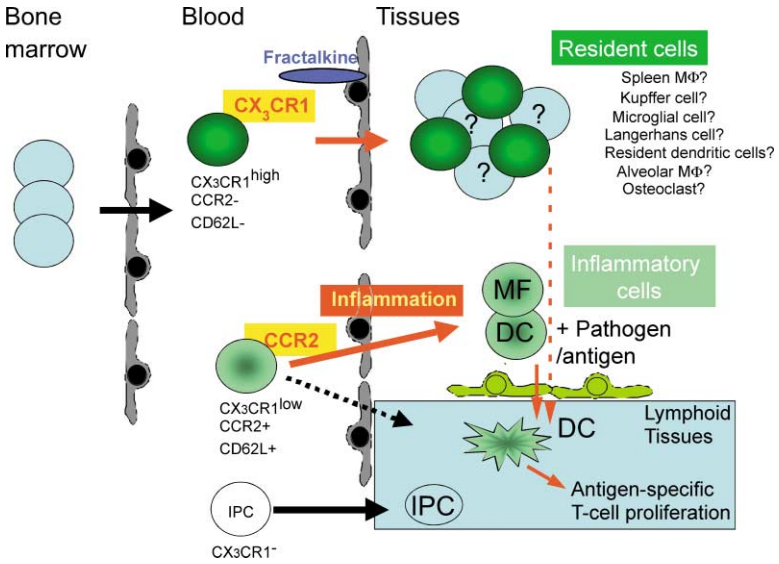

Figure 6. Model for Functional Dichotomy of Monocyte Subsets

Resident monocytes (CD16$^+$ in human) express high levels of CX_3CR1 which, upon interaction with fractalkine, facilitates extravasation into tissues, where these cells give rise to specialized cell types. Inflammatory monocytes (CD14$^+$ in human) express lower levels of CX_3CR1 but have high levels of other receptors that respond to inflammatory chemokines, resulting in migration of the cells to sites of inflammation, where they subsequently differentiate into dendritic cells.

with 1 ml of Ca^{2+}- and Mg^{2+}-free PBS (Gibco), containing 0.05 mM sodium EDTA, the BAL fluid was centrifuged, and the cells were resuspended in ice-cold HBSS. The peritoneum was lavaged with 10 ml of Ca^{2+}- and Mg^{2+}-free PBS with 0.05 mM sodium EDTA using a 10 ml syringe and an 18 gauge needle. Blood (500 μl) was obtained by cardiac puncture and collected in heparin-containing tubes (150 UI). Mice were then perfused with 20 ml of cold PBS and sacrificed. For FACS analysis, the indicated organs were then dissected and removed, washed in PBS, sliced, and incubated with collagenase D (1 mg/ml in PBS) for 45 min at 37°C, and cells were recovered on a Percoll gradient.

Adoptive Transfer of Antigen-Specific T Cells

Ovalbumin-specific CTL precursors, expressing a transgenic TCR specific for the SIINFEKL peptide presented in the context of MHC class I K^b, were isolated from OT-1 mice (Hogquist et al., 1994). T cells were isolated from spleens and LN and enriched by magnetic depletion of I-A^{b+}B220$^+$ non-T cells and positive enrichment of CD8$^+$ T cells (Miltenyi Biotech). Cells were labeled with the intracellular fluorescent dye carboxyfluorescein diacetate succimidyl ester (CFSE; Molecular Probes, C-1157) by incubating them in the absence of serum for 8 min at RT at 10^7 cells/ml in 5 μM CFSE. CFSE loading was stopped by addition of an equal volume of cold FCS. Cells were washed twice in complete RPMI medium. 10^6 clonotype-positive CD8$^+$ cells were injected in 200 μl of PBS into the tail veins of $\beta 2m^{-/-}$ or wild-type CD45.1$^+$ C57BL/6 recipient mice. Recipient mice received thioglycollate IP, followed by a monocyte graft (10^5 cells IV, depleted of I-A$^+$/CD11c$^+$ cells), and were immunized IP with ovalbumin (SIINFEKL) peptide. After 60 hr, the lymph nodes draining the peritoneum and control inguinal lymph nodes were removed, and donor-derived T cells were analyzed for CFSE intensity by four-color flow cytometry after staining with CD45.2, CD8, and TCR-Vα2 antibodies.

Pertussis-Toxin Treatment of Monocytes

Blood monocytes from *RAG*$^{-/-}$*CX$_3$CR1*$^{gfp/+}$ CD45.2$^+$ C57BL/6 mice were depleted of I-A$^+$ and CD11c$^+$ cells by immunomagnetic cell depletion. Monocytes were washed and resuspended in RPMI with 10% fetal calf serum, 2 mM glutamine, 100 mg/ml streptomycin, and 100 U/ml penicillin, and incubated with pertussis toxin (Sigma, P 7208) at 100 ng/ml or solvent as a control for 1 hr at 37°C in 5% CO_2. Washed cells were injected into CD45.1$^+$ C57BL/6 recipient mice.

Flow Cytometry

Human PBMC were incubated with goat or human IgG for 10 min at 4°C in Ca^{2+}/Mg^{2+}-free PBS with 0.5% BSA and 0.05% NaN3 (FACS medium), and stained with FITC-conjugated (anti-CD16 or anti-HLA-DR) and PE-conjugated antibodies (anti-CD1a, -CD2. -CD3, -CD11b, -CD11c, -CD14, -CD16, -CD19, -CD24, -CD56, -CD123) obtained from Pharmingen (San Diego, CA), with anti-BDCA-2 (Miltenyi Biotech), with anti-CCR1, -CCR2, -CCR3, -CCR4, -CCR5, -CCR6, -CCR7, -CCR9, -CXCR1, -CXCR2, -CXCR3, -CXCR4, -CXCR5, -CXCR6 antibodies (a generous gift from R&D), with PerCp-conjugated anti-HLA-DR and APC-conjugated anti-CD14 (from BD Pharmingen), and with FKN-Fc (a kind gift of Millenium Pharmaceuticals) followed by Cy5-conjugated goat anti-human IgG or a Cy3-conjugated goat anti-human IgG (Jackson ImmunoResearch).

Mouse cell suspensions were incubated with anti-mouse FcγRII/III (2.4G2) for 10 min at 4°C in FACS medium and then stained with the following anti-mouse antibodies from BD PharMingen: PE-coupled antibodies specific for Ly6C/G (Gr1), Ly6C, VLA-1, VLA-2, VLA-4, CD31, NK1.1, DX.5, LFA-1, CD90, CD11b, CD11c, I-A^b, CD44, CD45.1, CD45.2, CD45RA, CD45 RB, CD45RC, and V_α5; biotinylated anti-F4/80 (Caltag), CD11b, CD11c, and V_β8.1/2; and APC-coupled antibodies against TCRβ, B220, Ly6C/G (Gr1), CD11b, and CD11c. Cells were analyzed on a FACSCalibur cytometer or LSR cytometer (Becton Dickinson, Mountain View, CA) using CellQuest software (Becton Dickinson).

Cytological Analysis

Cells were stained with antibodies against Ly6C/G (Gr1) and sorted for expression of GFP and Gr1. GFP^{high}GR1$^-$ and GFP^{low}GR1$^-$ cells were then centrifuged onto glass slides by using a Cytospin (Shandon, Pittsburgh, PA), dried for 1 hr at room temperature, and stained with May-Grunwald-Giemsa, or fixed in acetone for 10 min and stained with an avidin-biotin-peroxidase method revealed by 3–39 diaminobenzidine as chromogen (Vectasin ABC Kit, Vector, CA), using antibody against Ki67 (BD Pharmingen).

Histological Analysis

Organs were washed in PBS, sliced, and fixed for 45 min at 4°C in 4% paraformaldehyde. Organs were then washed with PBS and incubated overnight at 4°C in 30% sucrose, washed again in PBS, embedded in OCT, and frozen. Fifty micron thick sections were analyzed by fluorescence microscopy after staining with a rabbit polyclonal serum against Caveolin-1 (Transduction Laboratories) followed by Cy-3-conjugated goat anti-rabbit Ig (Jackson ImmunoResearch) to visualize endothelial cells (Liu et al., 1999) and DAPI to visualize the nuclei. Visual data were acquired with an Axioplan 2 fluorescent microscope (Carl Zeiss, Jena, Germany) equipped with a Cooke Corporation SensiCam CCD camera using SlideBook software (Intelligent Imaging Corporation).

Acknowledgments

We are grateful to Giorgio Inghirami for discussions, John Hirst for cell sorting, and Mary-Jean Sunshine for her advice and help with generation of the mice. F.G. acknowledges the support of Brigitte Senechal and Anna Geissmann. This work was supported by grants to D.R.L. from the National Institutes of Health. F.G. was supported by a fellowship from the Human Frontier Science Program, and S.J. was supported by a Special Fellowship of the Leukemia & Lymphoma Society. D.R.L. is an investigator of the Howard Hughes Medical Institute.

Received: February 5, 2003
Revised: March 27, 2003
Accepted: April 28, 2003
Published: July 15, 2003

References

Ajuebor, M.N., Flower, R.J., Hannon, R., Christie, M., Bowers, K., Verity, A., and Perretti, M. (1998). Endogenous monocyte chemoattractant protein-1 recruits monocytes in the zymosan peritonitis model. J. Leukoc. Biol. *63*, 108–116.

Akagawa, K.S., Takasuka, N., Nozaki, Y., Komuro, I., Azuma, M., Ueda, M., Naito, M., and Takahashi, K. (1996). Generation of CD1+RelB+ dendritic cells and tartrate-resistant acid phosphatase-positive osteoclast-like multinucleated giant cells from human monocytes. Blood *88*, 4029–4039.

Asselin-Paturel, C., Boonstra, A., Dalod, M., Durand, I., Yessaad, N., Dezutter-Dambuyant, C., Vicari, A., O'Garra, A., Biron, C., Briere, F., and Trinchieri, G. (2001). Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. Nat. Immunol. *12*, 1144–1150.

Bazan, J.F., Bacon, K.B., Hardiman, G., Wang, W., Soo, K., Rossi, D., Greaves, D.R., Zlotnik, A., and Schall, T.J. (1997). A new class of membrane-bound chemokine with a CX3C motif. Nature *385*, 640–644.

Boehme, S.A., Lio, F.M., Maciejewski-Lenoir, D., Bacon, K.B., and Conlon, P.J. (2000). The chemokine fractalkine inhibits Fas-mediated cell death of brain microglia. J. Immunol. *165*, 397–403.

Boring, L., Gosling, J., Chensue, S.W., Kunkel, S.L., Farese, R.V., Jr., Broxmeyer, H.E., and Charo, I.F. (1997). Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C-C chemokine receptor 2 knockout mice. J. Clin. Invest. *100*, 2552–2561.

Brand, S., Sakaguchi, T., Gu, X., Colgan, S.P., and Reinecker, H.C. (2002). Fractalkine-mediated signals regulate cell-survival and immune-modulatory responses in intestinal epithelial cells. Gastroenterology *122*, 166–177.

Bruno, L., Seidl, T., and Lanzavecchia, A. (2001). Mouse pre-immunocytes as non-proliferating multipotent precursors of macrophages, interferon-producing cells, CD8alpha(+) and CD8alpha(−) dendritic cells. Eur. J. Immunol. *31*, 3403–3412.

Cella, M., Jarrossay, D., Facchetti, F., Alebardi, O., Nakajima, H., Lanzavecchia, A., and Colonna, M. (1999). Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Nat. Med. *5*, 919–923.

Chapuis, F., Rosenzwajg, M., Yagello, M., Ekman, M., Biberfeld, P., and Gluckman, J.C. (1997). Differentiation of human dendritic cells from monocytes in vitro. Eur. J. Immunol. *27*, 431–441.

Crofton, R.W., Diesselhoff-den Dulk, M.M., and van Furth, R. (1978). The origin, kinetics, and characteristics of the Kupffer cells in the normal steady state. J. Exp. Med. *148*, 1–17.

Cyster, J.G., and Goodnow, C.C. (1995). Pertussis toxin inhibits migration of B and T lymphocytes into splenic white pulp cords. J. Exp. Med. *182*, 581–586.

Fong, A.M., Robinson, L.A., Steeber, D.A., Tedder, T.F., Yoshie, O., Imai, T., and Patel, D.D. (1998). Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. J. Exp. Med. *188*, 1413–1419.

Geissmann, F., Prost, C., Monnet, J.P., Dy, M., Brousse, N., and Hermine, O. (1998). Transforming growth factor beta1, in the presence of granulocyte/macrophage colony-stimulating factor and interleukin *4*, induces differentiation of human peripheral blood monocytes into dendritic Langerhans cells. J. Exp. Med. *187*, 961–966.

Goda, S., Imai, T., Yoshie, O., Yoneda, O., Inoue, H., Nagano, Y., Okazaki, T., Imai, H., Bloom, E.T., Domae, N., and Umehara, H. (2000). CX3C-chemokine, fractalkine-enhanced adhesion of THP-1 cells to endothelial cells through integrin-dependent and -independent mechanisms. J. Immunol. *164*, 4313–4320.

Hogquist, K.A., Jameson, S.C., Heath, W.R., Howard, J.L., Bevan, M.J., and Carbone, F.R. (1994). T cell receptor antagonist peptides induce positive selection. Cell *76*, 17–27.

Imai, T., Hieshima, K., Haskell, C., Baba, M., Nagira, M., Nishimura, M., Kakizaki, M., Takagi, S., Nomiyama, H., Schall, T.J., and Yoshie, O. (1997). Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. Cell *91*, 521–530.

Janatpour, M.J., Hudak, S., Sathe, M., Sedgwick, J.D., and McEvoy, L.M. (2001). Tumor necrosis factor-dependent segmental control of MIG expression by high endothelial venules in inflamed lymph nodes regulates monocyte recruitment. J. Exp. Med. *194*, 1375–1384.

Jung, S., Aliberti, J., Graemmel, P., Sunshine, M.J., Kreutzberg, G.W., Sher, A., and Littman, D.R. (2000). Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. Mol. Cell. Biol. *20*, 4106–4114.

Jung, S., Unutmaz, D., Wong, P., Sano, G., De los Santos, K., Sparwasser, T., Wu, S., Vuthoori, S., Ko, K., Zavala, F., et al. (2002). In vivo depletion of $CD11c^+$ dendritic cells abrogates priming of $CD8^+$ T cells by exogenous cell-associated antigens. Immunity *17*, 211–220.

Kennedy, D.W., and Abkowitz, J.L. (1998). Mature monocytic cells enter tissues and engraft. Proc. Natl. Acad. Sci. USA *95*, 14944–14949.

Kiertscher, S.M., and Roth, M.D. (1996). Human CD14+ leukocytes acquire the phenotype and function of antigen-presenting dendritic cells when cultured in GM-CSF and IL-4. J. Leukoc. Biol. *59*, 208–218.

Kurihara, T., Warr, G., Loy, J., and Bravo, R. (1997). Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. J. Exp. Med. *186*, 1757–1762.

Lagasse, E., and Weissman, I.L. (1996). Flow cytometric identification of murine neutrophils and monocytes. J. Immunol. Methods *197*, 139–150.

Lagasse, E., and Weissman, I.L. (1997). Enforced expression of Bcl-2 in monocytes rescues macrophages and partially reverses osteopetrosis in op/op mice. Cell *89*, 1021–1031.

Lawson, L.J., Perry, V.H., and Gordon, S. (1992). Turnover of resident microglia in the normal adult mouse brain. Neuroscience *48*, 405–415.

Liu, J., Razani, B., Tang, S., Terman, B.I., Ware, J.A., and Lisanti, M.P. (1999). Angiogenesis activators and inhibitors differentially regulate caveolin-1 expression and caveolae formation in vascular endothelial cells. Angiogenesis inhibitors block vascular endothelial growth factor-induced down-regulation of caveolin-1. J. Biol. Chem. *274*, 15781–15785.

Lu, B., Rutledge, B.J., Gu, L., Fiorillo, J., Lukacs, N.W., Kunkel, S.L., North, R., Gerard, C., and Rollins, B.J. (1998). Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. J. Exp. Med. *187*, 601–608.

Martinez del Hoyo, G., Martin, P., Arias, C.F., Marin, A.R., and Ardavin, C. (2002). CD8alpha+ dendritic cells originate from the CD8alpha-dendritic cell subset by a maturation process involving CD8alpha, DEC-205, and CD24 up-regulation. Blood *99*, 999–1004.

Mellman, I., and Steinman, R.M. (2001). Dendritic cells: specialized and regulated antigen processing machines. Cell *106*, 255–258.

Merad, M., Manz, M.G., Karsunky, H., Wagers, A., Peters, W., Charo, I., Weissman, I.L., Cyster, J.G., and Engleman, E.G. (2002). Langerhans cells renew in the skin throughout life under steady-state conditions. Nat. Immunol. *3*, 1135–1141.

Muller, W.A. (2001). New mechanisms and pathways for monocyte recruitment. J. Exp. Med. *194*, F47–F51.

Palframan, R.T., Jung, S., Cheng, G., Weninger, W., Luo, Y., Dorf, M., Littman, D.R., Rollins, B.J., Zweerink, H., Rot, A., and von Andrian, U.H. (2001). Inflammatory chemokine transport and presentation in HEV: a remote control mechanism for monocyte recruitment to lymph nodes in inflamed tissues. J. Exp. Med. *194*, 1361–1373.

Passlick, B., Flieger, D., and Ziegler-Heitbrock, H.W. (1989). Identification and characterization of a novel monocyte subpopulation in human peripheral blood. Blood *74*, 2527–2534.

Randolph, G.J., Beaulieu, S., Lebecque, S., Steinman, R.M., and Muller, W.A. (1998). Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. Science *282*, 480–483.

Randolph, G.J., Inaba, K., Robbiani, D.F., Steinman, R.M., and Muller, W.A. (1999). Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. Immunity *11*, 753–761.

Randolph, G.J., Sanchez-Schmitz, G., Liebman, R.M., and Schakel, K. (2002). The CD16(+) (FcgammaRIII(+)) subset of human monocytes preferentially becomes migratory dendritic cells in a model tissue setting. J. Exp. Med. *196*, 517–527.

Sallusto, F., and Lanzavecchia, A. (1994). Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J. Exp. Med. *179*, 1109–1118.

Sanchez-Torres, C., Garcia-Romo, G.S., Cornejo-Cortes, M.A., Rivas-Carvalho, A., and Sanchez-Schmitz, G. (2001). CD16+ and CD16- human blood monocyte subsets differentiate in vitro to dendritic cells with different abilities to stimulate CD4+ T cells. Int. Immunol. *13*, 1571–1581.

Schreurs, M.W., Eggert, A.A., de Boer, A.J., Figdor, C.G., and Adema, G.J. (1999). Generation and functional characterization of mouse monocyte-derived dendritic cells. Eur. J. Immunol. *29*, 2835–2841.

Serbina, N.V., Salazar-Mather, T.P., Biron, C.A., Kuziel, W.A., and Pamer, E.G. (2003). TNF/iNOS producing dendritic cells mediate innate immune defense against bacterial infection. Immunity *19*, this issue, 59–70.

Siegal, F.P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P.A., Shah, K., Ho, S., Antonenko, S., and Liu, Y.J. (1999). The nature of the principal type 1 interferon-producing cells in human blood. Science *284*, 1835–1837.

Takahashi, K., Naito, M., and Takeya, M. (1996). Development and heterogeneity of macrophages and their related cells through their differentiation pathways. Pathol. Int. *46*, 473–485.

Thomas, E.D.T., Ramberg, R.E., Sale, G.E., Sparkes, R.S., and Golde, D.W. (1976). Direct evidence for a bone marrow origin of the alveolar macrophage in man. Science *192*, 1016–1018.

Tilton, B., Ho, L., Oberlin, E., Loetscher, P., Baleux, F., Clark-Lewis, I., and Thelen, M. (2000). Signal transduction by CXC chemokine receptor 4. Stromal cell-derived factor 1 stimulates prolonged protein kinase B and extracellular signal-regulated kinase 2 activation in T lymphocytes. J. Exp. Med. *192*, 313–324.

van Furth, R. (1988). Phagocytic cells: development and distribution of mononuclear phagocytes in normal steady state and inflammation. In Inflammation: Basic Principles and Clinical Correlates, J.I. Gallin, I.M. Goldstein, and R. Snyderman, eds. (New York: Raven Press), pp. 281–295.

Wiktor-Jedrzejczak, W., and Gordon, S. (1996). Cytokine regulation of the macrophage (M phi) system studied using the colony stimulating factor-1-deficient op/op mouse. Physiol. Rev. *76*, 927–947.

Yamamoto, T., Naito, M., Moriyama, H., Umezu, H., Matsuo, H., Kiwada, H., and Arakawa, M. (1996). Repopulation of murine Kupffer cells after intravenous administration of liposome-encapsulated dichloromethylene diphosphonate. Am. J. Pathol. *149*, 1271–1286.

Zhou, L.J., and Tedder, T.F. (1996). CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells. Proc. Natl. Acad. Sci. USA *93*, 2588–2592.

Ziegler-Heitbrock, H.W. (2000). Definition of human blood monocytes. J. Leukoc. Biol. *67*, 603–606.