# New observations on the trafficking and diapedesis of monocytes

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**Purpose of review**

Monocytes play multiple roles in immune system functions and inflammatory diseases such as atherosclerosis. These roles are coupled to diverse trafficking and cellular migration behaviors. Here, we review recent advances in our understanding of such behaviors with emphasis on broad scale trafficking patterns and the cellular and molecular mechanisms regulating diapedesis, a central aspect of trafficking.

**Recent findings**

Monocytes consist of 'inflammatory' and 'resident' subsets, which exhibit differential functions and trafficking properties. Notably, the spleen has recently been identified as a reservoir of inflammatory monocytes, which are readily recruited to injured myocardium and possibly other tissues. Resident monocytes have been shown to undergo long-range crawling within the lumen of the microvasculature, which facilitates immune surveillance and rapid response to infection. Monocyte diapedesis has been demonstrated to utilize both para and transcellular migration routes facilitated by endothelial 'transmigratory cups'. A significant number of new adhesion molecules and signaling pathways have recently been uncovered as functional mediators and modulators of these processes.

**Summary**

Our improving understanding of monocyte trafficking and migration mechanisms has begun to shed light on the functions of these often enigmatic cells. Continued progress in this area will be critical for elucidating roles of monocytes in disease and for developing therapeutics that target monocytes.

**Keywords**

adhesion, atherosclerosis, diapedesis, endothelium, migration, monocyte

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## Introduction

Effective immune system function requires dynamic orchestration of diverse innate and adaptive immune cell activities. Cells of the 'innate' immune system include phagocytes of the myeloid lineage (e.g. monocytes, macrophage, dendritic cells, neutrophils, basophils and mast cells) and natural killer (NK) cells of the lymphoid lineage. These are collectively able to internalize and digest bacteria or kill infected or abnormal host cells. Cells of the adaptive immune system (e.g. T and B lymphocytes) are responsible for developing immunological memory and require innate immune cells in this process.

Monocytes, which constitute between 4 and 10% of the circulating leukocytes, were once thought simply as macrophage and dendritic cell precursors [1–3]. Recently, however, these cells have been gaining widespread attention as true multitaskers of the immune system with critical roles in innate and adaptive immunity, immune surveillance, scavenging, host defense and both promotion and resolution of inflammation [1–3]. Monocytes are also recognized as critical mediators of inflammatory diseases such as atherosclerosis, multiple sclerosis and rheumatoid arthritis (RA) [1,4–7].

It is increasingly appreciated that monocytes have taken a 'divide and conquer' approach to fulfilling their many duties, with distinct monocyte subsets playing distinct functional roles [1,8,9] (Table 1 [10–15,16••,17]). In broad terms, the 'inflammatory monocyte' subset are through to play more prominent roles in promotion of inflammation, whereas 'resident monocytes' are more linked to steady-state surveillance of noninflamed tissues and resolution of inflammation/wound healing (Table 1) [1,8,9]. The detailed characterization of these subsets, their functional roles and their descendants has been extensively reviewed elsewhere [1,5–8,10,18] and will not be discussed here in detail. Rather, here, we will focus on the dynamic trafficking and migration behaviors of monocytes as critical determinants of their function.

## An overview of monocyte trafficking

As expected from differences between adhesion molecules and chemokine receptors, the major monocyte

**Table 1 Properties of monocyte subsets in human and mouse**

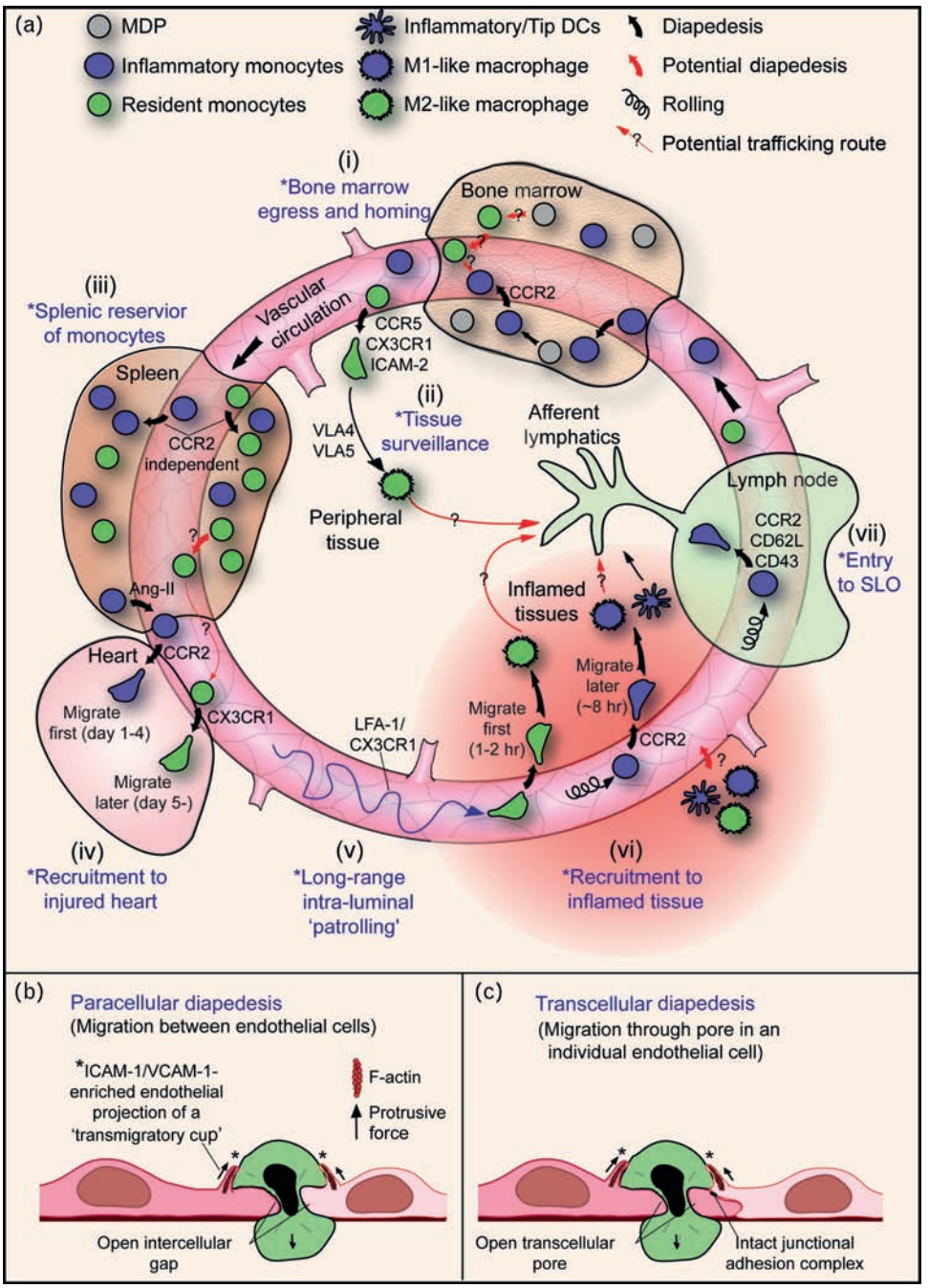
| Type [1,8,9] | Species | Antigen[a] [1,8,9] | General features[b] [1,4,8,10] | Trafficking features[c] |
|---|---|---|---|---|
| Inflammatory | Human | $CD14^{hi}$, $CD16^{-}$, $CCR2^{+}$, $CX3CR1^{low}$, $CD62L^{+}$, $CD115^{+}$ | Constitute 80–90% (human) or 50% (mice) blood monocytes, produce high levels of IL-10 (human), TNFα and IL-1 (mouse), Contribute to antimicrobial defense, replenish tissue Mϕ and DCs, increase number in response to hypercholesterolemia [11] | Released from the bone marrow in response to CCL2 [1,8,10,13,14], return to bone marrow [15], recruited to inflamed tissues and lymph nodes *in vivo* [1,8,10,14], pooled in the spleen [16••], recruited to the inflamed myocardium from spleen reservoir [16••], accumulate in atheromata |
| | Mouse | $Ly6C^{hi}$, $CCR2^{+}$, $CX3CR1^{low}$, $CD62L^{+}$, $CD115^{+}$, $F4/80^{+}$, MHC class $II^{-}$, $CD11c^{-}$ | | |
| Resident | Human | $CD14^{+}$, $CD16^{+}$, $CCR2^{-}$, $CX3CR1^{hi}$, $CD62L^{-}$, $CD115^{+}$ | Constitute 10–20% (human) or 50% of (mouse) of blood monocytes, produce TNFα at high (human) or modest (mouse) levels, replenish tissue Mϕ and DCs, recruit other leukocytes early in inflammation via TNFα [12], reduce inflammation and promote wound healing [9], involved in angiogenesis [9] | Recruited to all noninflamed tissues [8,10]; patrol the luminal surface of endothelial cells in noninflamed skin, mesentery and brain [12,17]; recruited very early into inflamed skin, mesentery and brain [12,17]; pooled in the spleen [16••]; accumulate in atheromata |
| | Mouse | $Ly6C^{low}$, $CCR2^{-}$, $CX3CR1^{hi}$, $CD62L^{-}$, $CD115^{+}$, $F4/80^{+}$, MHC-II | | |

CCL, C–C-chemokine ligand; CCR, chemokine (C–C motif) receptor; CX3CR, CX3C chemokine receptor; DC, dendritic cell; IL, interleukin; Mϕ, macrophage; MHC, major histocompatibility complex; TNF, tumor necrosis factor.
[a] In humans evidence exists for an additional $CD14^{dim}$, $CD16^{+}$ monocyte population that remains functionally uncharacterized [1].
[b] Largely derived from mouse data.
[c] Largely derived from mouse data.

subsets have been progressively demonstrated to exploit distinct trafficking patterns that are coupled with their discrete functional roles [1,8,9] (Table 1). Monocytes, macrophages and conventional dendritic cells all derive directly from the common macrophage dendritic cell precursor (MDP) in the bone marrow [1,8,9]. It remains unclear whether resident monocytes derive from the inflammatory subset or directly from MDPs [1,8,9]. Egress of monocytes from the bone marrow requires migration across the monolayer of endothelial cells that line the vascular circulatory system (i.e. diapedesis) in order to enter the circulation (i.e. intravasation). For inflammatory monocytes, this process relies on the chemokine (C–C motif) receptor *2* (CCR2)-mediated signals in response to its ligands C–C-chemokine ligand (CCL) 7 and CCL*2* [1,11,13,14] (Fig. 1 a.i) [19–31]. Thus, inflammation (which is associated with increased CCL*2* in circulation) strongly enhances inflammatory monocyte emigration. Upon the resolution of inflammation, inflammatory monocytes rapidly return to the bone marrow by migrating from the circulation into the bone marrow parenchyma (i.e. extravasate) [15]. In the presence of a local inflammatory stimulus, circulating inflammatory monocytes quickly extravasate/traffic into affected non-lymphoid tissues in a CCR*2*–CCL*2*-dependent manner, where they differentiate into certain macrophage and dendritic cell (i.e., inflammatory dendritic cells including Tip dendritic cells) subsets [1,8,10] (Fig. 1 a.iv and vi). These dendritic cells then migrate through the interstitium, in a manner dependent on the integrins very late antigen (VLA)-4 and VLA-5 [10], and enter secondary lymphoid organs (SLOs) via the afferent lymphatics (Fig. 1 a.vii). In response to chemokines CCL*2* and CXC chemokine ligand 9, inflammatory monocytes can also enter inflamed SLO directly by migration across the high endothelial venules through use of the adhesion molecules L-selectin, CD43 and $\beta_2$ integrins [8,10,20] (Fig. 1 a.vii).

**Figure 1** (*Continued*)
'patrolling' on the luminal surface of the microvasculature. Upon recognition of diverse inflammatory signals, these cells serve as a 'first responder' population of immune cells that enter the tissue and signal recruitment of other leukocytes (e.g. neutrophils and inflammatory monocytes) through secretion of TNFα and IL-1β. Resolution of inflammation requires recruited monocytes to eventually be cleared from the peripheral tissues. In addition to trafficking to draining lymphatics, it is also believed that monocytes may undergo 'reverse migration' in which they intravasate directly into the vasculature [19]. (vii) Circulating inflammatory monocytes directly enter the SLOs in response to tissue-specific inflammatory recruitment signals via the so-called 'remote control' mechanism [20]. Additionally, tissue monocytes and their descendants enter the lymph nodes via intravasation across afferent lymphatics. (b and c) Transmigratory cups for para and transcellular diapedesis. (b) Monocyte is depicted in the process of disrupting endothelial adherens junctions to form a paracellular gap for diapedesis. (c) Monocyte has opened a transcellular pore in an individual endothelial cell leaving the adherens junctions intact. This process has recently been demonstrated to be dependent on dynamic probing and progressive extension of leukocyte ILPs [21,22]. In both (b) and (c), ICAM-1, VCAM-1, ERM and actin-enriched protrusive structures proactively formed by the endothelium are shown 'embracing' the migrating monocytes. These seem to function in providing adhesion scaffolds that help guide leukocyte migration across endothelial barriers [23–31]. CCL, C–C-chemokine ligand; CCR, chemokine (C–C motif) receptor; CX3CL, CX3C chemokine ligand; DCs, dendritic cells; ERM, ezrin/radixin/moesin; ICAM, intercellular adhesion molecule; IL, interleukin; ILPs, invadosome-like protrusions; MDP, macrophage dendritic cell precursor; MI, myocardial infarction; SLO, secondary lymphoid organ; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule.

**Figure 1 Monocyte trafficking and diapedesis**




(a) Basic monocyte trafficking routes in mice. This schematic shows some key aspects of monocyte trafficking largely defined from murine systems. Importantly, tissue and stimulus variations are not comprehensively illustrated. Trafficking events that remain unexplored or controversial are denoted by red arrows and question marks. (i) Adult bone marrow resident and inflammatory monocytes derive from MDPs. Studies [13,15] demonstrate that inflammatory monocytes intravasate from the bone marrow in a CCL2-dependent manner and also home back to bone marrow after removal of inflammatory stimuli [15]. It remains unclear, precisely where resident monocytes develop (i.e. in bone marrow or circulation) and, therefore, if and how resident monocytes intravasate from the bone marrow. (ii) A fraction of circulating resident monocytes extravasate into noninflamed peripheral tissues, where they replenish certain subsets of macophages and DCs. This occurs in a largely CX3CL1, CCL2 and ICAM-2-dependent manner. (iii and iv) A large fraction of total inflammatory and resident monocytes has recently been recognized to be pooled in the spleen [16••]. Although the migratory cues driving spleen homing are not well established, they appear to be CCR2-indepenent [16••]. In response to MI splenic inflammatory monocytes are mobilized to the circulation in an angiotensin-II-dependent manner and then subsequently recruited into damaged heart in a CCR2/CCL2-dependent fashion [16••]. Inflammatory monocytes arrive in injured heart early (days 1–4) and are followed by arrival of resident monocytes (beginning at day ~5) in a CX3CR/CX3CL1-dependent manner [9]. Where these resident monocytes are recruited from, in particular, whether they are mobilized from the splenic reservoir, and what the mobilization stimuli are, remain undetermined. (v and vi) In noninflamed tissues, resident monocytes undergo long-range

On the contrary, resident monocytes constitutively migrate into noninflamed tissues in a manner largely dependent on the chemokines CCL3 and CX3C chemokine ligand 1 (CX3CL1) and the endothelial intercellular adhesion molecule (ICAM)-2 [8,15] (Fig. 1 a.ii). This contributes to the homeostasis of subsets of peripheral resident macrophages and dendritic cells. As described below, these basic trafficking patterns have recently undergone significant expansion.

## A mobilizable splenic reservoir of monocytes

Recent studies have demonstrated in mouse that the spleen contains an unexpectedly large fraction (i.e. exceeding that in circulation) of the bodies of both inflammatory and resident monocytes [16••] (Fig. 1 a.iii). Critically, these studies also show that this pool, as is the case for bone marrow monocytes, serves as a mobilizable reservoir [16••]. In response to heart injury by myocardial infarction (MI), large amounts of splenic inflammatory monocytes intravasate and subsequently migrate into the damaged myocardium [16••] (Fig. 1 a.iii and iv). Importantly, this mobilization was independent of CCR2 and was instead mediated by angiotensin II (Fig. 1 a.iii). Previous work [9] by this group established that inflammatory monocytes enter the injured heart, in a CCR2/CCL2-depenendent manner, early (days 1–4), where they function to phagocytize damaged cell material (Fig. 1 a.iv). This is then followed by the CX3CR/CX3CL1-dependent entry of resident monocytes (beginning at day ~5), which help to initiate tissue repair and angiogenesis [9]. Whether these resident monocytes are also recruited from the splenic reservoir and what stimulates their mobilization are important open questions. Moreover, whether the splenic reservoir of inflammatory and resident monocytes can be mobilized in other settings of tissue inflammation or damage also awaits characterization.

## Resident monocytes 'patrol' the intravascular space

New model systems have revealed that resident monocytes undergo long-range lateral migration within noninflamed peripheral microvasculature of the skin, mesentery and central nervous system [12,17] (Fig. 1 a.v). Initial studies by Auffray *et al.* [1,12] demonstrated that resident monocytes continuously migrate over the luminal surface of dermal and mesenteric microvascular endothelium, apparently patrolling for signs of infection or tissue damage, in a manner dependent on the integrin lymphocyte function-associated antigen 1 (LFA-1) and CX3CR1. Wide ranging inflammatory stimuli, including chemical irritants, aseptic wounding and peritoneal infection with *Listeria monocytogenes*, caused rapid extravasation of patrolling monocytes, preceding neutrophil accumulation by at least 1 h and inflammatory monocytes by several hours (Fig. 1 a.vi). During this early phase, the newly extravasted resident monocytes were the main secretors of tumor necrosis factor alpha (TNFα) and interleukin (IL)-1 and thereby served as sentinels initiating the inflammatory response. Similar patrolling behavior of resident monocytes was also observed within the microvasculature of the brain [17]. In this setting, patrolling was dependent on angiopoietin-2. During endotoxemia, the number of patrolling monocytes increased in an angiopoietin-2, TNFα and IL1β-mediated fashion, and within several hours a fraction of these migrated into the perivascular space.

The above studies are suggestive of a potentially broader role for luminal leukocyte–endothelial patrolling during immune surveillance [32]. Indeed, similar observations have been made for NK T cells patrolling the liver sinusoidal endothelium [33,34]. Vascular endothelial cells also express major histocompatibility complex class I and II along with costimulatory molecules [35,36], suggesting potential for antigen-specific stimulation of $CD4^+$ and $CD8^+$ lymphocytes. It is interesting to consider whether memory and effector lymphocytes may also exhibit luminal patrolling behaviors similar to those defined above.

## Monocyte reverse migration

Several studies have begun to document the so-called 'reverse migration', whereby inflammatory 'mononuclear cells' leave the peripheral tissues during inflammation by reversing their migratory path and undergoing intravasation to re-enter the vascular circulation directly [19]. For monocytes, specifically, large-scale emigration to the draining lymphatics has been implicated as one mechanism for inflammation resolution [37–43]. However, emerging evidence support a possible role for reverse migration of monocytes from inflamed tissues [44,45•] including atherosclerotic plaques [7]. Further elucidation of the mechanisms of this process could be of great translational value.

## An overview of monocyte diapedesis

As suggested throughout the preceding discussion, a central aspect of leukocyte trafficking is the continuous transitions from the tissue into the blood circulation and *vice versa*. The vascular endothelium represents the interface between these two tissue compartments, serving as both a barrier to leukocyte trafficking and a sentinel to instruct leukocyte adhesion and transmigration. Thus, the crossing of the endothelium (i.e. diapedesis) represents a critical determinant of leukocyte trafficking behaviors. Although equally important, details of the intravasation process remain poorly studied and the vast majority of our understanding of diapedesis, and indeed trafficking in general, relates to extravasation.

## The 'five-step' cascade for extravasation

Extravasation begins with the accumulation of circulating leukocytes on the luminal surface of the endothelium through a classic three-step adhesion and activation cascade [46–48]. First, leukocytes undergo transient rolling interactions mediated by selectins (step 1), which facilitate sensing of, and responses to, chemokines presented on the endothelial surface (step 2). This in turn triggers high-affinity interaction of leukocyte integrin receptors (e.g. LFA-1, Mac-1 and VLA-4) with their endothelial ligands [e.g. ICAM-1, ICAM-2 and vascular cell adhesion molecule 1 (VCAM-1)] resulting in firm leukocyte arrest (step 3) [49,50]. Subsequently, leukocytes undergo actin-dependent spreading, polarization and integrin-dependent lateral migration on the luminal surface of the endothelium (step 4). This activity seems to allow leukocytes to search out sites permissive for endothelial barrier penetration [51,52]. Finally, the leukocyte must formally breach and transmigrate across the endothelium (step 5), a process referred to specifically as 'diapedesis'. A variety of new aspects of this process for monocytes, and leukocytes in general, have begun to emerge, as discussed below.

## Transmigratory cups

Endothelial cells contribute proactively to diapedesis, for example, by facilitating opening of intercellular junctions [10]. Recently, additional mechanisms have become evident. Imaging studies of monocyte adhesion to TNFα-activated endothelial cells *in vitro* demonstrated the formation of spike-shaped 'clusters' of E-selectin, VCAM-1 and ICAM-1 formed around the periphery of adherent monocytes [23]. These clusters required intact actin and RhoA signaling and were enriched in ezrin/radixin/moesin (ERM) proteins [23], cytoskeletal adaptor proteins important for microvilli formation [53]. Subsequent studies examining monocytes, neutrophils and lymphocytes demonstrated that these 'clusters', in fact, represented three-dimension 'cup-like' adhesion interfaces apparently assembled from extended microvilli, each enriched in actin, ICAM-1, VCAM-1 and ERM proteins. These surrounded and partially embraced the adherent leukocytes *in vitro* [24–26] and *in vivo* [54–59] (Fig. 1 b and c). These novel structures termed 'transmigratory cups' or 'docking structures' seem to function both in adhesion strengthening [23,24,27,29,30] and in facilitating/guiding diapedesis [25,26,31] (Fig. 1 b and c).

## Two routes for crossing the endothelial barrier

Until recently, only one basic pathway for diapedesis was widely recognized, the 'paracellular' route, in which leukocytes and endothelium cooperate to locally disassemble the interendothelial junctions to open a paracellular gap for leukocyte transmigration [60–64] (Fig. 1 b). In fact, however, a large number of studies demonstrate the coexistence of the paracellular route along with a quantitatively important second pathway termed the 'transcellular route' both *in vitro* and *in vivo* [21,65]. For transcellular diapedesis, leukocytes pass directly through individual endothelial cells via the formation of a transcellular pore (Fig. 1 c). Recently dynamic probing and progressive extension of novel, actin-, Wiskott–Aldrich syndrome protein- and src-dependent protrusive organelles similar to podosomes and invadopodia [66–68] (i.e. invadosome-like protrusions [21]) were shown to be critical for transcellular pore formation by monocytes and lymphocytes [22,69]. In-vivo settings most relevant for monocyte utilization of each of these migration pathways remain an important open question.

## Platelet endothelial cellular adhesion molecule-1 recycling dynamics during monocyte diapedesis

Platelet endothelial cellular adhesion molecule-1 (PECAM-1)-mediated homophilic interaction between leukocytes and endothelium supports efficient diapedesis [10,26,65,70]. This is associated with targeted recycling of membrane from the 'lateral border recycling compartment' to the site of diapedesis [70], a process that seems to be a functionally important role in monocyte trafficking [71•] (Table 2) [72–74,75••,76–81,82••,83,84,85•,86,87,88•,89–93]. It was recently established that this process is mediated by kinesin family molecular motors and microtubule-based transport in a manner dependent on PECAM-1 tyrosine residue Y663 [71•,94].

## Negotiating the basement membrane

In addition to endothelium, leukocytes need to breach vascular basement membrane to complete diapedesis [64,95]. Neutrophils have been shown to preferentially extravasate at preexisting regions of relatively low matrix protein deposition [64,95,96]. A recent study [97] demonstrates that monocytes and neutrophils penetrate these low resistant areas via different modes. Whereas neutrophils enlarge these sites within extracellular matrix for transmigration, monocytes drastically change their own shape and invade the interstitium in the absence of basement membrane remodeling. Implicit in these studies is the idea that monocytes are morphologically more plastic than other leukocyte types and, therefore, may have greater facility in negotiating tissue barriers in general. Such migratory 'freedom' would seem an ideal trait for a cell type charged with the responsibility of conducting constitutive and virtually ubiquitous tissue surveillance.

**Table 2 Molecules involved in monocyte trafficking**

| Class[a] | Molecule | Mono/EC[b] | Type | General function in monocyte trafficking[c] | Reference |
|---|---|---|---|---|---|
| Adhesion | ALCAM | EC | IgSF | Diapedesis across BBB *in vivo*, enriched in TC | [72] |
| | CD13 | Both | Ectoenzyme | Adhesion *in vivo* | [73] |
| | CD43 | Mono | GP | Entry to lymph nodes through high endothelial venules | [10] |
| | CD44 | Mono | GP | Rolling and transmigration *in vivo* | [45•] |
| | CD81 | EC | Tetraspanin | TC formation, adhesion | [30] |
| | CD146 | EC | IgSF | Diapedesis | [74] |
| | Del-1 | EC | GP | Reduced adhesion | [75••] |
| | EphB4 | Mono | RTK | Adhesion, diapedesis | [76] |
| | EphrinB2 | EC | EphB ligand | Adhesion, diapedesis | [76,77] |
| | E-selectin | EC | Selectin | Rolling, enriched in TC | [23] |
| | ICAM-1 | EC | IgSF | Adhesion, diapedesis, TC formation | [25,26,30] |
| | ICAM-2 | EC | IgSF | Adhesion, diapedesis, enriched in TC | [26] |
| | JAM-A | EC | IgSF | Diapedesis | [10] |
| | JAM-B | EC | IgSF | Diapedesis | [10] |
| | JAM-C | EC | IgSF | Diapedesis | [44] |
| | JAML | Mono | IgSF | Adhesion, diapedesis | [78,79] |
| | LFA-1 | Mono | Integrin | Adhesion, diapedesis, patrolling microvasculature | [12] |
| | CD62L | Mono | Selectin | Rolling | [45•,80] |
| | PECAM-1 | EC/mono | IgSF | Diapedesis, enriched in TC | [10,26] |
| | Mac-1 | Mono | Integrin | Adhesion, diapedesis, lateral migration | [51] |
| | PSGL-1 | Mono | GP | Tethering and rolling | [45•,80,81] |
| | SIRPα | Mono | GP | Reduced adhesion and diapedesis | [82••] |
| | VCAM-1 | EC | IgSF | Adhesion, TC formation | [11,23,25] |
| | VLA4 | Mono | Integrin | Ahesion, diapedesis, interstitial migration | [10] |
| | VLA5 | Mono | Integrin | Interstitial migration | [10] |
| Signaling | Akt1 | EC | Kinase | Diapedesis | [83] |
| | A20 | EC | Zn finger | Reduced rolling and adhesion | [84] |
| | Calcium | EC | Ion | Paracellular gap formation, TC formation | [10,25,30] |
| | CD39 | Mono | Apyrase | Reduced adhesion to BBB *in vivo* | [85•] |
| | $cPLA_2\beta$ | Mono | Phospholipase | Speed and directionality of chemotaxis, migration | [86] |
| | $iPLA_2\beta$ | Mono | Phospholipase | Speed of chemotaxis, migration *in vivo* | [86] |
| | Nitric oxide | Mono | Nitric oxide | Reduced diapedesis *in vivo* | [87] |
| | RhoA | EC | GTPase | Paracellular gap formation, TC formation | [10,23] |
| | ROS | EC | Reactive oxy | TPA-dep occludin degradation, diapedesis across BBB | [88•] |
| | STAT1 | Mono | TF | Reduced diapedesis | [89] |
| Chemo/cyto | Ang-II | Mono | Hormone | Resident mono patrolling of brain MV endothelial cells *in vivo* | [17] |
| | AngPT-II | Mono | GF | Mobilization of splenic inflammatory monocytes | [16••] |
| | CCL2 | Mono | Chemokine | Exit from BM, entry to inflamed tissue, cholesterol-induced increases in no. *in vivo* | [11,13,14] |
| | CCL20 | Mono | Chemokine | Entry to inflamed skin | [90] |
| | CX3CL1 | Mono | Chemokine | Entry to noninflamed tissue | [10] |
| | CXCL9 | Mono | Chemokine | Entry to SLO via HEV | [10] |
| | CXCL12 | Mono | Chemokine | Reduced adhesion, increase diapedesis | [91] |
| | IL-17 | Mono | Cytokine | Migration | [92] |
| | Oncostatin | EC | Cytokine | Reduced migration *in vivo* | [93] |
| Cytoskelal | Actin | EC | Microfilaments | Paracellular gap opening, TC formation | [23,25,26] |
| | Tubulin | EC | Microtubules | TC formation, LBRC regulation for diapedesis | [25,26,71•] |
| Adaptor | Ezrin | EC | ERM | TC formation | [23] |
| | Kinesin | EC | Motor | LBRC regulation for diapedesis | [71•] |
| | Moesin | EC | ERM | TC formation | [23] |

Ang-II, angiotensin II; AngPT-II, angiopoietin II; BBB, blood–brain barrier; BM, bone marrow; CCL, C–C-chemokine ligand; Chemo/cyto, chemoattractants including chemokines, cytokines, growth factors and hormones; $cPLA_2\beta$, cytosolic phospholipase $A_2\beta$; CX3CL, CX3C chemokine ligand; EC, endothelial cell; FN, fibronectin; GF, growth factor; GP, glycoprotein; HEV, high endothelial venule; ICAM, intercellular adhesion molecule; IgSF, immunoglobulin superfamily member; IL, interleukin; JAM, junctional adhesion molecule; JAML, junctional adhesion molecule like; LBRC, lateral border recycling compartment; LFA, lymphocyte function-associated antigen; MV, microvascular; PECAM, platelet endothelial cellular adhesion molecule; PSGL, P-selectin glycoprotein ligand; SIRP, signal regulatory protein; ROS, reactive oxygen species; RTK, tyrosine kinase; SLOs, secondary lymphoid organs; TC, transmigratory cup; TF, transcription factor; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

[a] Functional classification.

[b] Functional role on monocyte or endothelium.

[c] General properties/functions in monocyte migration and diapedesis.

## New adhesion molecules in diapedesis

A variety of adhesion molecules have been newly shown to function in monocyte trafficking (Table 2). The junctional adhesion molecule (JAM)-like (JAML) protein, a JAM family member, contributes to monocyte adhesion and particularly transmigration *in vitro* [78,79]. Contrasting to other JAMs, which are expressed on endothelial cells as well as leukocytes, JAML is selectively expressed on leukocytes (i.e. monocytes, neutrophils and T lymphocytes [78]) and is upregulated on human monocytes by CCL2 [79]. Monocyte JAML mediates adhesion to endothelial cells in a novel VLA-4-dependent manner; VLA-4 activation facilitates JAML dimerization, which in turn

facilitates binding of JAML to the coxsackie and adenovirus receptor and potentially other endothelial by ligands [78,79].

Eph receptors and ephrin ligands molecules were originally identified for their roles in neuronal pathfinding, and later in vasculogenesis [76,77,98]. The Eph receptors are composed of a family of receptor tyrosine kinases that bind to transmembrane ligand ephrins and modulate cell–cell contacts and bidirectional cell signaling. Whereas human and murine monocytes express EphB receptors, EphB2 and EphB4, arterial and some venous endothelial cells display luminal ephrinB2 expression, which partially associates with PECAM-1 [98]. EphrinB2–EphB interaction was shown to contribute to CCL2-stimulated monocyte adhesion and diapedesis across arterial endothelium in a manner dependent on both EphB4 forward signaling and ephrinB2 reverse signaling [76,77].

Tetraspanins are proteins that possess four membrane-spanning segments that are largely implicated in forming/stabilizing lateral protein–protein associations within the plane of the plasma membrane [99]. Studies with lymphocytes have demonstrated the tetraspanin CD9 and CD151 facilitate formation of transmigratory cups (apparently by promoting ICAM-1 and VCAM-1 clustering), and, in this way, enhance lymphocyte adhesion [27,28]. Recently, through genetic screens, the tetraspannin CD81 was demonstrated to be significantly upregulated in endothelial cells of atherosclerotic plaques in humans [30]. In-vitro studies demonstrated that CD81 overexpression enhanced the recruitment of ICAM-1 and VCAM-1 into transmigratory cups and facilitated adhesion of monocytes. These studies suggest that CD81 may contribute to atherosclerosis by enhancing monocyte adhesiveness in a transmigratory cup-dependent manner [30].

P-selectin glycoprotein ligand-1 (PSGL-1, also known as CD162) is well established to initiate tethering of leukocytes on activated endothelial cells under flow [64]. The role of PSGL-1 in monocyte trafficking, however, has been unclear. Recent studies demonstrate that inflammatory, but not resident, monocytes express significant amounts of PSGL-1 on their surface in humans and mice, which promotes early steps (i.e. tethering and rolling) in their adhesion to atherosclerotic lesions [80]. Additional studies have shown that inflammatory monocytes transmigrate across infected dermal venules *in vivo*, in a PSGL-1/L-selectin-dependent manner [81].

Activated leukocyte cell adhesion molecule 1 (ALCAM-1) is an endothelially expressed immunoglobulin superfamily (IgSF) protein that binds to the costimulatory molecule CD6 extracellularly and ERM proteins cytoplasmically. Transmigration of both lymphocytes and monocytes across the blood–brain barrier (BBB) endothelium *in vitro* and *in vivo* was recently shown to be dependent on ALCAM-1, which, interestingly, was found greatly enriched in transmigratory cups [72]. CD146 is another IgSF member that is constitutively expressed in human endothelial cells and further upregulated by TNFα. The function of CD146 in general is poorly understood, but was also recently shown to support monocyte adhesion and trans-endothelial migration through an as yet unknown monocyte receptor [74].

Transmembrane ectoenzymes have been implicated in leukocyte trafficking events, largely through proteolysis of vasoactive peptides and chemokines. Interestingly, CD13 (aminopeptidase N) expressed on both monocytes and endothelium was recently shown to participate in direct homophilic adhesion and in this way enhance monocyte adhesion and diapedesis across endothelium *in vitro* and in an in-vivo model of peritonitis [73].

## New signaling molecules/pathways in monocyte diapedesis

Many aspects of the signaling molecules/pathways important for monocyte trafficking have been well characterized (see reviews [10,64,100]), yet many other are still emerging. Chemokine CCL2 is central for recruitment of monocytes into many inflamed tissues [100] including cholesterol-induced atherosclerotic plaques [11]. Mishra *et al.* [86] demonstrated for the first time that $Ca^{2+}$-independent phospholipase and cytosolic phospholipase differentially regulated monocyte migration speed and directionality in response to CCL2 via differential subscellular localization patterns.

Several new chemokines/cytokines have been implicated in monocyte trafficking. CCL20 (ligand for CCR6) promotes monocyte recruitment to inflamed skin [90]. CXCL12 (SDF-1) was shown to enhance monocyte migration and diapedesis across BBB endothelium *in vitro* [91]. Interestingly, SDF-1-enhanced migration apparently occurred through down modulation of LFA-1-mediated adhesion in a Lyn kinase-dependent manner [91]. However, as these experiments were in the absence of physiologic shear flow (in which LFA-1 is critical for initial monocyte arrest on endothelium), it remains unclear whether SDF-1 will have a net positive or negative effect of monocyte recruitment *in vivo*. Finally, IL-17 [a cytokine produced by the recently discovered proinflammatory T helper cell 17 (Th17) $CD4^+$ lymphocyte subset] has been shown to promote monocyte chemotaxis through p38 mitogen-activated protein kinases signaling, suggesting a mechanism for recruitment of monocytes during Th17-mediated diseases such as RA [92].

Recent studies have suggested a new signaling mechanism for breaching the endothelial barrier. Upon interaction

with monocytes, brain endothelial cells were shown to release extracellular protease tissue-type plasminogen activator (tPA). tPA, in turn, mediated breakdown of the tight junctional protein occludin, in an extracellular signal-related kinase 1/2-dependent manner, thereby promoting monocyte diapedesis [88•]. Other studies have revealed for the first time that phosphoinositide 3-kinase (PI3K)/Akt1 signaling in endothelium are key components of endothelial barrier disruption associated with acute inflammation and, apparently as a result, monocyte and neutrophil recruitment *in vivo* [83].

## Modulatory molecules in monocyte migration

Contrasting those that promote/mediate efficient monocyte diapedesis, endogenous molecules that negatively modulate this process have remained largely unknown. Expression of developmental endothelial locus-1 (Del-1, a matrix protein) by endothelium has been shown to be inversely related to monocyte and neutrophil adhesion *in vitro* and *in vivo* [75••]. Similarly, signal regulatory protein α (SIRPα), an IgSF member and ligand for CD47 (a transmembrane protein expressed on endothelium), has been reported to negatively regulate monocyte adhesion and diapedesis [82••]. Interestingly, both Del-1 and SIRPα act by inhibiting functions of key monocyte integrins, LFA-1 (for Del-1) and Mac-1 (for SIRPα) [82••]. Additionally, CD39 (expressed on endothelial cells and monocytes) functions to metabolize proinflammatory purinergic agonists. In this way, CD39 prevented recruitment of monocytes into ischemic cerebral tissues through repression of purinergic receptor P2X7-dependent upregulation of Mac-1 *in vitro* and *in vivo* [84]. In addition, the interferon-γ/Jak-STAT1 [89], oncostatin M/oncostatin M receptor-β/nuclear factor kappa B [93], CXCL12 (SDF-1α)/CXCR4/Src kinase/Lyn (discussed above) [91], A20/IκB [84] and thrombin/nitric oxide/protease-activated receptor 1/PLCβ/PI3K [87] signaling pathways have each separately been recognized as novel suppressors for monocyte trafficking (Table 2). Further characterization of such antagonistic mechanisms for monocyte recruitment will be of clear translational potential.

## Conclusion

Monocytes have become increasingly recognized as multifunctional contributors to immune system function and inflammatory disease. To accomplish their diverse roles, monocytes exhibit particularly diverse and dynamic trafficking properties. Recent advances in understanding of monocyte subsets, functions and trafficking have significantly expanded our understanding of these cells, but at the same time have raised many new questions. For example, it remains to be determined how splenic reservoirs of monocytes may contribute to responses in settings other than heart injury and how this pool of cells may contribute to inflammatory disease. Similar questions can be asked about the patrolling resident monocyte population. Moreover, the basis for monocyte interstitial migration in three-dimensional matrices remains largely unexplored, and the process of monocyte exit from tissues during resolution of inflammation (an area with particular therapeutic potential) needs much further elucidation. Finally, despite a growing list of molecules implicated in monocyte trafficking, much remains to be determined about how their functions are coordinated and, particularly, how these become dysregulated during development of inflammatory diseases such as atherosclerosis.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 69).

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