Monocyte trafficking across the vessel wall

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Abstract
Monocytes fundamentally contribute to immune surveillance and the inflammatory response in immunoinflammatory diseases like atherosclerosis. Recruitment of these cells to the site of injury requires their trafficking across the blood vessel wall. A series of events, including capture, rolling, slow rolling, arrest, adhesion strengthening, and lateral locomotion, precede monocyte transmigration. Recent investigations have revealed new aspects of this cascade. This article revisits some conventional paradigms and selectively highlights new findings, including novel insights into monocyte differentiation and recently identified functional mediators, signalling pathways, and new structural aspects of monocyte extravasation. The emerging roles of endothelial junctional molecules like vascular endothelial-cadherin and the junctional adhesion molecule family, adhesion molecules such as intercellular adhesion molecule-1, molecules localized to the lateral border recycling compartment like cluster of differentiation 99, platelet/endothelial cell adhesion molecule-1, and poliovirus receptor (CD155), as well as other cell surface molecules such as cluster of differentiation 146 and ephrins in transendothelial migration are discussed.

Keywords
Monocyte migration • Monocyte subsets • Extravasation

This article is part of the Spotlight Issue on Leucocyte Trafficking.

1. Migration of monocytes into tissue

Monocytes play a pivotal role in tissue homeostasis, protective immunity, and both promotion and resolution of inflammation. They and their offspring, macrophages and dendritic cells, are essential for the innate and adaptive immune responses to pathogens. Monocytes exert many of their functions outside the vascular compartment; thus, trafficking and migration are required. Recruitment of blood monocytes to the site of injury or infection and their diapedesis through the endothelium (also called extravasation) are crucial events in early inflammation, followed by monocyte differentiation and subsequent downstream events of the inflammatory response.

In atherosclerosis, monocytes show a profound inflammatory response that involves hypercholesterolemia-associated trafficking to inflamed arteries, mainly dependent on the chemokines CCL2 and CCL5, extravasation, subendothelial accumulation, and differentiation into macrophages. Eventually, these cells give rise to pathogenic lipid-laden foam cells.

A tightly regulated, multistep process, consisting of a series of interactions between the immune and the endothelial cells, precedes the actual transmigration step. For monocytes, this process is essentially believed to follow the ‘cascade’ paradigm originally established for neutrophils, but some molecules are of specific importance in monocyte recruitment and will be particularly highlighted. A summary of the most important molecules and events in monocyte extravasation is provided in Table 1 and Figure 1.

Initially, inflammatory cytokines such as tumour necrosis factor-α or IL-1β, originating from tissue macrophages, transiently activate the endothelium. They induce the rapid expression of adhesion molecules, most importantly the adhesion molecules E- and P-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), and the presentation of tethered chemokines on the luminal surface of the endothelium. Selectins interact with O-glycosylated carbohydrate ligands displayed on P-selectin glycoprotein ligand-1 (PSGL-1) and expressed on all monocytes. This interaction allows monocytes to roll on the endothelium and negotiate the high shear stress exerted by the blood flow in the vasculature. PSGL-1 is expressed at a significantly higher level by inflammatory Ly6CIlow monocytes than by resident Ly6Clow monocytes (see classification given below and Table 2), which likely facilitates their adhesion to atherosclerotic lesions.

Atherosclerosis is characterized by an up-regulation of endothelial adhesion molecules like E- and P-selectin and VCAM-1 in lesion-prone areas. Deficiency in P- and E-selectin as well as in ICAM-1 was shown to significantly reduce atherosclerotic lesion size in apoE−/− mice individually. In inflammatory conditions, monocyte rolling has also been shown to strongly depend on monocyte-expressed very late antigen-4 (VLA-4 αβ1 integrin) and CD44. VCAM-1 is an endothelial adhesion molecule that binds to VLA-4 and mediates slow rolling on cytokine-activated endothelium, thus facilitating the transition between rolling and firm arrest.

Monocyte firm adhesion to the endothelium is coordinated
by C-C and C-X-C chemokines such as CCL2 and IL-8. Monocytes can also use VLA-4 for firm adhesion. Blockage of VLA-4 in atherosclerosis-prone mice was shown to inhibit monocyte adhesion in carotid arteries. A recent study has found that the hormone resistin facilitates monocyte–endothelial cell adhesion by up-regulation of ICAM-1 and VCAM-1 on endothelial cells. Monocyte arrest is followed by a directional chemotactic and mechanotactic step, in which monocytes spread, polarize, and subsequently locomote laterally to find preferred sites of extravasation. This intraluminal crawling depends on the leucocyte integrins lymphocyte function-associated antigen-1 (LFA-1) and macrophage-1-antigen (Mac-1) as well as the endothelial ligands ICAM-1 and ICAM-2, as blocking of these adhesion molecules was shown to disable crawling and the subsequent transmigration. This step involves probing of the apical surface with membrane protrusions called lamellipodia. Note that this crawling is different from patrolling, which is strictly intravascular and does not lead to extravasation in steady state, as outlined below. Finally, to exit the vessel, monocytes must negotiate the endothelium, the lamina basalis and the embedded pericytes (Figure 1).

### 2. Models and methods employed to elucidate monocyte transmigration

The recent past has seen major progress in determining the molecular pathways associated with monocyte transendothelial migration.

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**Table 1. Important molecules involved in monocyte trafficking across the vessel wall**

<table>
<thead>
<tr>
<th>Class</th>
<th>Common name</th>
<th>Immunological name</th>
<th>Gene name (mouse)</th>
<th>Function</th>
<th>Main ligands</th>
<th>Expressed on</th>
<th>Number of clinical trials</th>
<th>Of which open</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Integrins</strong></td>
<td>LFA-1</td>
<td>CD11a, CD18</td>
<td>Itgal, Itgb2</td>
<td>Patrolling, locomotion, arrest</td>
<td>ICAM-1, -2</td>
<td>Monocytes</td>
<td>39</td>
<td>8</td>
<td>8–10</td>
</tr>
<tr>
<td></td>
<td>Mac-1</td>
<td>CD11b, CD18</td>
<td>Itgam, Itgb2</td>
<td>Arrest, locomotion, transmigration, activation</td>
<td>Multiple</td>
<td>Monocytes</td>
<td>575</td>
<td>180</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>VLA-4</td>
<td>CD49d, CD29</td>
<td>Itgα4, Itgβ1</td>
<td>Adhesion, transmigration</td>
<td>VCAM-1</td>
<td>Monocytes</td>
<td>9</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td><strong>Immunoglobulin superfamily</strong></td>
<td>ICAM-1</td>
<td>CD54</td>
<td>Icam1</td>
<td>Adhesion, transmigration</td>
<td>LFA-1</td>
<td>EC</td>
<td>119</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>ICAM-2</td>
<td>CD102</td>
<td>Icam2</td>
<td>Adhesion, transmigration</td>
<td>LFA-1</td>
<td>EC</td>
<td>81</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MCAM</td>
<td>CD146</td>
<td>Mcam</td>
<td>Transmigration</td>
<td>ICAM-1, JAM-A, Mac-1 CAR</td>
<td>EC</td>
<td>12</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>JAM-A</td>
<td>CD322</td>
<td>Jam2</td>
<td>Transmigration</td>
<td>VLA-4, JAM-C Mac-1 CAR</td>
<td>EC</td>
<td>202</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>JAM-B</td>
<td>CD322</td>
<td>Jam2</td>
<td>Transmigration</td>
<td>VLA-4, JAM-C Mac-1 CAR</td>
<td>EC</td>
<td>164</td>
<td>20</td>
<td>12,13</td>
</tr>
<tr>
<td></td>
<td>JAML</td>
<td></td>
<td>Anica1</td>
<td>Reverse transmigration, Arrest, transmigration</td>
<td>VLA-4</td>
<td>Monocytes</td>
<td>116</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>VCAM-1</td>
<td>CD106C</td>
<td>Vcam1</td>
<td>Arrest, transmigration</td>
<td>PECAM-1</td>
<td>EC</td>
<td>93</td>
<td>20</td>
<td>10</td>
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<tr>
<td></td>
<td>PECAM-1</td>
<td>D31</td>
<td>Pecam1</td>
<td>Transmigration</td>
<td>EC, monocytes</td>
<td>7</td>
<td>2</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poliovirus receptor</td>
<td>CD155C</td>
<td>Pvr</td>
<td>Transmigration</td>
<td>CD96, DYNAM-1, TIGIT PILR</td>
<td>EC, monocytes</td>
<td>11</td>
<td>5</td>
<td>16</td>
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<tr>
<td></td>
<td>MIC2</td>
<td>D99</td>
<td>Cd99</td>
<td>Transmigration</td>
<td>EC, monocytes</td>
<td>115</td>
<td>49</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ICD99L2</td>
<td></td>
<td>Cd99</td>
<td>Transmigration</td>
<td>EC, monocytes</td>
<td>7</td>
<td>0</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selectins</td>
<td>E- selectin</td>
<td>CD62E</td>
<td>Sele</td>
<td>Rolling</td>
<td>ESL-1, PSLG-1</td>
<td>EC</td>
<td>78</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CD44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P- selectin</td>
<td>CD62P</td>
<td>Selp</td>
<td>Rolling, arrest, signalling</td>
<td>PSLG-1</td>
<td>EC</td>
<td>72</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>L-selectin</td>
<td>CD62L</td>
<td>Sell</td>
<td>Rolling, arrest, signalling</td>
<td>PSLG-1, CD34</td>
<td>Monocytes</td>
<td>44</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>CCR1</td>
<td>CD191</td>
<td>Ccr1</td>
<td>Arrest</td>
<td>CCL5</td>
<td>Monocytes</td>
<td>249</td>
<td>71</td>
<td>22</td>
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<tr>
<td></td>
<td>CCR5</td>
<td>CD195</td>
<td>Ccr5</td>
<td>Spreading, arrest, transmigration</td>
<td>CCL5</td>
<td>Monocytes</td>
<td>226</td>
<td>83</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>CCR2</td>
<td>CD192</td>
<td>Ccr2</td>
<td>Adhesion, transmigration</td>
<td>CCL2, -7, -12</td>
<td>Monocytes</td>
<td>277</td>
<td>97</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>CD144</td>
<td>Cdh5</td>
<td>Transmigration</td>
<td>EC</td>
<td>7</td>
<td>3</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VLA-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eph A1, -2</td>
<td></td>
<td>Epha1</td>
<td>Transmigration</td>
<td>Ephrin-A ligands</td>
<td>Monocytes</td>
<td>8</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Eph B1–4</td>
<td></td>
<td>Ephb1</td>
<td>Transmigration</td>
<td>Ephrin-B ligands</td>
<td>Monocytes</td>
<td>17</td>
<td>5</td>
<td>26</td>
</tr>
</tbody>
</table>
Blocking antibodies that arrest monocytes at different stages of diapedesis have generated valuable insights into the spatial and temporal regulation of this complex process. Additionally, function-blocking antibodies directed against signalling molecules and receptors have been used to identify different players involved in migration to the site of inflammation and extravasation. Among others, blocking antibodies against platelet/endothelial cell adhesion molecule-1 (PECAM-1) and cluster of differentiation 99 (CD99) were used in vitro and subsequently validated in vivo, as discussed below. Inhibiting antibodies also constitute a potent therapeutic approach to treating inflammatory conditions. In addition to impeding transmigration with antibodies, tissue-specific conditional knockout and inducible and cell type specific gene knockout and knockin animals have proved to be powerful tools for uncovering the intricacies of transmigration in vivo. More recently, microscopy-based approaches for live cell visualization have led to an expanded definition of the leucocyte adhesion cascade and have greatly enhanced the understanding of dynamic changes during transmigration. Among others, intravital real-time imaging, multiphoton and confocal laser scanning microscopy, time-lapse video microscopy, and electron microscopy were employed to meticulously study the extravasation process in vitro and in vivo.

3. Monocyte heterogeneity and homeostasis

The understanding of monocyte ontogeny and differentiation has changed significantly during the past few years. Monocytes and their descendants constitute a highly complex and dynamic cellular system. Blood monocytes account for 4 and 10% of the circulating leucocytes in mice and humans, respectively. This review will highlight some key aspects of the recent findings, but does not claim to be comprehensive. References [2,3] and [45] offer deeper insights into functions and the development of monocytes.

Monocytes are grouped into subsets on the basis of their location, phenotype and function, as well as by characteristic chemokine

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**Table 2** Nomenclature and features of blood monocytes

<table>
<thead>
<tr>
<th>% monocytes</th>
<th>Subset</th>
<th>Surface markers</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>50</td>
<td>LY6C&lt;sup&gt;hi&lt;/sup&gt;, inflammatory</td>
<td>LY6C&lt;sup&gt;hi&lt;/sup&gt; CCR2&lt;sup&gt;+&lt;/sup&gt; Gr1&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Pro-inflammatory, pathogen defence, phagocytosis</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>LY6C&lt;sup&gt;low&lt;/sup&gt;, patrolling</td>
<td>Ly6C&lt;sup&gt;low&lt;/sup&gt; CX&lt;sub&gt;3&lt;/sub&gt;CR1&lt;sup&gt;+&lt;/sup&gt; Gr1&lt;sup&gt;+&lt;/sup&gt; LFA1&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>Patrolling, promotion of tissue repair</td>
</tr>
<tr>
<td>Human</td>
<td>80–90</td>
<td>Classical</td>
<td>CD14&lt;sup&gt;+&lt;/sup&gt; CD16&lt;sup&gt;-&lt;/sup&gt; CD62L&lt;sup&gt;+&lt;/sup&gt; CCR2&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Phagocytosis</td>
</tr>
<tr>
<td></td>
<td>2–11</td>
<td>Intermediate</td>
<td>CD14&lt;sup&gt;+&lt;/sup&gt; CD16&lt;sup&gt;-&lt;/sup&gt; CCR2&lt;sup&gt;+&lt;/sup&gt; CX3CR1&lt;sup&gt;+&lt;/sup&gt; CCR5&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Pro-inflammatory</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>Non-classical patrolling</td>
<td>CD14&lt;sup&gt;+&lt;/sup&gt;(CD14&lt;sup&gt;dim&lt;/sup&gt;) CD16&lt;sup&gt;-&lt;/sup&gt; CX3CR1&lt;sup&gt;+&lt;/sup&gt; CCR5&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Local surveillance of tissue, antiviral</td>
</tr>
</tbody>
</table>

+ = approximately 10-fold increase in surface expression compared with isotype control; ++ = approximately 100-fold increase in surface expression compared with isotype control.
receptor expression and the presence of specific surface molecules \(^1,6,66\) (Table 2). They arise from haematopoietic stem cells in the bone marrow via a lineage-negative (Lin\(^-\)) CD117\(^+,\) CD135\(^+\) clonotypic monocyte and dendritic cell precursor (MDP) founder cell, in a macrophage colony-stimulating factor receptor (MCSF-R, CD115)-dependent manner. 5 Emerging data suggest the existence of a developmentally committed clonogenic, monocyte-, and macrophage-specific progenitor downstream from MDP. 66 The largest human monocyte subpopulation expresses high levels of the LPS co-receptor CD14 (CD14\(^++\)). These monocytes are called classical monocytes, express CCR2, and produce IL-10 in response to LPS. CD14\(^+\), CD16 (low-affinity IgG receptor) \(^++\) non-classical monocytes lack CCR2, but express high levels of CX3CR1. A small intermediate group with high CD14 and moderate CD16 expression \(^67\) (CD14\(^++\)CD16\(^-\) intermediate subset in Table 2) has recently been reported to not merely be a transitory state as previously assumed, but also to possess unique features and differ from traditional subsets inter alia with respect to inflammatory cytokine secretion. 68

The exact correspondence between mouse and human monocyte subsets is subject to debate. There is evidence that suggests that Ly6C\(^\text{low}\) monocytes may be equated to the CD14\(^+\)CD16\(^-\) non-classical human monocytes, based on gene expression profiles. 69,70 Similarly, the Ly6C\(^\text{high}\) subset seems to correspond to the human CD14\(^++\)CD16\(^-\) classical subgroup. Although data comparisons and lineage tracing studies between the potentially matching subsets show overall correspondence, significant differences, both, in subset-specific gene expression and functional behaviour remain, thus forbidding direct translation between murine and human subpopulations. Reference 69 offers a comprehensive review on the correlation of monocyte subsets within a spectrum of species. Some have proposed that Ly6C\(^\text{high}\) cells can give rise to a Ly6C\(^\text{low}\)CX3CR1\(^+\) subset, but this is highly controversial. 4,45,71,72

Ly6C\(^\text{low}\) mouse monocytes and CD14\(^\text{dim}\) human monocytes constitutively crawl on the luminal side of the non-inflamed endothelium (‘patrolling monocytes’). They are thought to scavenge microparticles and debris from the endothelial surface in a Toll-like receptor 7 (TLR7)-dependent manner. 32,33 The differentiation and survival of such patrolling monocytes critically depends on the nuclear receptor Nur77 (Nrf4a1). 73 Interaction of the integrin LFA1 with its endothelial ligands ICAM-1 and -2 mediates patrolling of Ly6C\(^\text{low}\) monocytes on the endothelium. 33 In resting tissue, \(<1\%\) of all patrolling monocytes cross the endothelium. 35 Interestingly, in the event of tissue damage, Ly6C\(^\text{low}\) monocytes rapidly extravasate and transiently produce inflammatory cytokines, thus initiating a very early innate response. 33,32 The role of monocyte subsets in vascular remodelling is unknown. As little is known about molecules, pathways, and signals specifically involved in transmigration of patrolling monocytes, the emphasis of this review lies on extravasation of Ly6C\(^\text{hi}\) monocytes in an inflammatory setting.

In pathogen-challenged mice, numbers of circulating LY6Chi monocytes do not substantially contribute to the tissue macrophages at steady state. 2,77,78 Compelling lineage analyses have shown the existence of macrophages that derive from embryonic pre-haematopoietic precursors, persist to adulthood, and maintain themselves by self-renewal, 78–80 thoroughly independent of monocyte replenishment. In fact, recent studies suggest that recruited endogenous monocytes do extravasate, but may not imperatively differentiate into macrophages or dendritic cells. Instead, these monocytes serve as surveillance and effector cells and migrate to lymph nodes. 80 Blood monocyte contribution to the tissue macrophage compartment seems to be largely limited to inflammatory settings. However, there is strong variation between tissues: while there is evidence that the pool of resident macrophages of the CNS, the microglia, originates from primitive myeloid precursors, 78,81 and the pool of intestinal macrophages crucially requires CCR2-dependent influx of Ly6C\(^\text{hi}\) monocytes. 82 Other tissues show a mixture of both early seeding with self-renewal and monocyte-derived recruitment. An interesting recent study proposes a third model of macrophage provenance, providing evidence that lung alveolar macrophages perinatally derive from fetal monocytes, followed by self-maintenance throughout life. 83

In macrophages, two main activation states represent the extremes of a spectrum of possible functional polarizations. In these two well-described conditions, macrophages exert opposing functions. M1 macrophages have been described to play a pro-inflammatory role, whereas a wound healing role has been attributed to M2 macrophages. 85,86 Emerging evidence suggests that the macrophage activation status depends on several variables, such as location, cytokines, and other microenvironmental cues. 87–89 For example, immediately after cardiac ischaemia, the damaged myocardium recruits Ly6C\(^\text{low}\) macrophages that give rise to an abundance of inflammatory M1 macrophages. 90 References [34,91] and [92] suggest that in the following reparative phase, the predominant macrophage phenotype in the ischaemic tissue changes towards an alternatively activated state, clearly expressing M2-macrophage-associated genes.

### 4. Two modes of monocyte transmigration across the vascular endothelium

The thin monolayer of endothelial cells (cell thickness 0.1 in the periphery to 1 \(\mu\)m over the nucleus) constitutes the primary physical barrier between blood and tissue. Endothelial cells have no true tight junctions. Adherens junctions between the cells and integrin and cadherin anchors to the basement membrane (BM) form a network that tightly regulates vascular homeostasis and restrains leucocyte transendothelial migration. 93 In addition to the predominant paracellular route that leads monocytes through the junctions between endothelial cells and requires junctional remodelling, a different route directly through fusing vesicles in the endothelial cell cytoplasm constitutes an established mode of monocyte transmigration \(^62,94–96\) (Figure 1). Under most circumstances, monocytes choose transcellular migration in only 10–30% of events. 96,97 Strong activating stimuli are thought to increase overall occurrence of transcellular transendothelial migration in monocytes. 96,97 Live cell visualization in vitro \(^62\) and live imaging through confocal intravital microscopy \(^95\) have greatly advanced our understanding in this field. However, it remains difficult to accurately quantify paracellular vs. transcellular migration, because light
microscopy cannot clearly determine whether a cell is migrating through a junction or merely very close to it.98

5. VE-cadherin as a gatekeeper of transendothelial migration

The control of endothelial junctions constitutes a central event in determining the time and location of leucocyte extravasation. For paracellular transmigration, signals generated by adherent monocytes dissociate the adherens junctions that create cell contact integrity at endothelial cell–cell borders.25,99 One essential stabilizing component of adherens junctions is the membrane glycoprotein vascular endothelial-cadherin (VE-cadherin), linked to the actin cytoskeleton through α-catenin. Drugs disrupting this interaction drastically increase vascular permeability.100 Recently, the Vestweber group elegantly showed the VE-cadherin complex to be of dominant importance to transendothelial migration by replacing VE-cadherin with a VE-cadherin–α-catenin fusion protein, thus strongly inhibiting leucocyte extravasation.101 The endothelial-specific vascular endothelial tyrosine phosphatase associates with VE-cadherin and regulates its function by dephosphorylation. Monocyte adhesion to activated endothelial cells triggers the disruption of this association and has been shown to be critical for paracellular monocyte diapedesis.102 Non-receptor protein tyrosine kinases Src- and Pyk2-dependent phosphorylation of VE-cadherin dissociates its binding to α-catenin, thus removing it from the junction.103 Alterations in endothelial junctional structure during transmigration of monocytes were visualized in real-time in vitro, and ICAM-1 signalling was shown to affect VE-cadherin rearrangement to accommodate monocyte transmigration.104 Monocyte transendothelial migration may augment subsequent transmigratory activity by decreasing VE-cadherin expression.105

6. JAM family

The junctional adhesion molecule (JAM) family consists of six immunoglobulin-like proteins, JAM-A, JAM-B, JAM-C, JAM-4, ESAM, and CAR.72 They are expressed on endothelial cells and can be expressed on leucocytes. JAMs and JAM multimers mainly interact with each other and with integrin counter-receptors, for instance JAM-A binds LFA-119 and JAM-C interacts with Mac-1.107 JAM-C is specifically required to prevent reverse transmigration of monocytes back into the vascular lumen and provides regulated and polarized monocyte transmigration.13 In this study, anti-JAM-C antibodies significantly reduced monocytes in inflamed tissue, but increased the number of not transmigrated monocytes with a reverse-transmigratory phenotype. These monocytes show abluminal to luminal migration after primary extravasation, due to impaired JAM-C activity.13 Recently, a study revealed JAM-A to be of significant importance for arterial monocyte recruitment in the context of diet-induced atherosclerosis. Here, it was shown that the same number of monocytes adhered to JAM-A-deficient endothelial monolayers as to JAM-A-bearing endothelial cells, but the number of monocytes that transmigrated beneath the monolayer was markedly reduced in the JAM-A-deficient model.108 JAM-like protein (JAM-L) is closely related to the JAM family and is restricted to leucocytes, mainly monocytes and granulocytes. Under inflammatory conditions, monotypic JAM-L expression is up-regulated, and JAM-L and its endothelial counter-receptor CAR play a critical role in mediating monocyte migration across the endothelium in vitro. In this process, VLA-4 facilitates JAM-L-receptor binding.14,109

7. Other junctional molecules

The type 1 membrane glycoprotein PECAM-1 has long been known to be crucial for the diapedesis of monocytes, as monoclonal antibodies to PECAM-1 block monocyte transmigration by 70–90% in some models.110 Such antibodies have been demonstrated to attenuate inflammation in several in vivo models.118–121 PECAM-1 deficiency in ApoE double-KO mice (ApoE−/−/PECAM-1−/−) was found to reduce atherosclerotic plaque burden in some areas of the aorta.114–116

Recently, poliovirus receptor (PVR, CD155), located among others at the endothelial borders and on monocytes,117 was identified as a regulator of monocyte extravasation that acts downstream of PECAM-1. Antibody blockage of certain transmigration steps is reversible by thorough washing. The group showed that resumed extravasation of isolated adherent monocytes released from anti-PECAM-1 blockage could still be arrested by anti-PVR or antibodies against its monocyte ligand and DNAx accessory molecule-1 (DNAM-1) in vitro. The reverse (arresting diapedesis by anti-PECAM-1 after previous blockage with anti-PVR) was not possible, suggesting that monocytes have already passed the PECAM-1-dependent step when secondly arrested by anti-PVR.118

Antibodies directed against two other type 1 membrane proteins, CD99 and CD99L2,118 impair monocyte influx into the site of inflammation to an equal, if not greater degree in vitro14 as well as in vivo,17 compared with anti-PECAM-1. However, while anti-PECAM-1 and anti-CD155 antibodies arrest adherent monocytes on the apical surface of the endothelial cells, anti-CD99 and anti-CD99L2 arrest monocytes partway through the endothelial junction.14 This indicates that CD99 and CD99L2 both act downstream of CD155 and PECAM-1. PECAM-1, CD155, CD99, and CD99L2 all participate in the extravasation process but not in capture or adhesion to the blood vessel wall, as revealed by intravital microscopy. PECAM-1, CD99, and CD99L2 are enriched at endothelial cell junctions and expressed diffusely on the monocyte surface, and facilitate transmigration by homophilic binding between the molecules on the two cell types, respectively.14,118

8. The lateral border recycling compartment

Mamdouh and Muller recently discovered119 in vitro studies that approximately one-third of PECAM-1 and considerable amounts of CD99, CD155,118 JAM-A, and other molecules involved in transendothelial migration, but not in VE-cadherin, reside in a subjunctional, intracellular endothelial membrane reticulum, forming a ‘transmigration complex’ that is actively transported to the site of diapedesis by kinesin-microtubule motors and surrounds the transmigrating cell (Figure 2). This membrane reticulum has been named the lateral border recycling compartment (LBRC).121 The 50 nm vesicle-like structures are connected with each other and the endothelial cell border. In resting endothelial cells, there is an incessant flux of membrane between the junction and the LBRC. Homophilic interaction between PECAM on endothelial cells and PECAM on monocytes, however, triggers extensive and rapid targeted recycling of PECAM-bearing membrane to the site of transmigration.119 Thus, the migrating cell is supplied with functional molecules required for it to be able to transmigrate.
Inhibition of targeted recycling, for instance by depolymerization of microtubules or disruption of PECAM–PECAM interaction, blocks monocyte extravasation by over 85%, arresting adherent monocytes above the cell junctions, unable to initiate transmigration. These findings suggest a critical role for the LBRC and targeted recycling in trans-endothelial migration. The accumulation of adhesion molecules around the migrating cell is observed not only in paracellular, but the LBRC is also recruited to mediate transcellular migration and seems to fulfil nearly identical rules in the two crossing routes. However, suitable markers to study the LBRC in vivo remain to be identified; thus, the role of the LBRC in vivo is unknown.

**9. Transmigratory cups and endothelial calcium signalling**

To initiate cytoskeletal reorganization and facilitate transmigration, VCAM-1, ICAM-1, and E-selectin form clusters, employing their leucocyte integrin counter-receptors. The clusters, also called docking structures, initiate activation of Src, Rac-1, and RhoA and cause an increase of cytosolic-free calcium in endothelial cells. The resulting activation of Ca^{2+}/calmodulin-dependent myosin light chain kinase (MLCK) and phosphorylation of VE-cadherin are crucial to release the associated catenins, loosen the junctions, and allow diapedesis. Studies in monocytes, lymphocytes, and neutrophils revealed these clusters to frequently be located on upright endothelial membrane processes and organized to so-called transmigratory cups by tetraspanins. These structures seem to be generated as a response to endothelial cell binding to LFA-1 and VLA-4 and to partially envelop the leucocytes during extravasation. Here, VCAM-1 and ICAM-1 also interact directly with the ezrin/radixin/moesin complex, which serves as a cytoskeletal linker and a signal transducer in cytoskeletal remodelling. The transmigratory cups play a guiding role in paracellular, as well as in transcellular, extravasation. Under certain in vitro conditions, 96% of transmigrating monocytes were observed to be associated with such ICAM-1-enriched projections.
10. Metalloproteinases

Broad spectrum inhibition of metalloproteinase activity almost doubles the duration of the diapedesis step in monocytes. This delayed transmigration is accompanied by significant elevation of monocyte surface integrin Mac-1.63,126

11. Cluster of differentiation 146 (MCAM)

Cluster of differentiation 146 (MCAM, CD146) is a member of the immunoglobulin superfamily and is, among others, strongly expressed on blood vessel endothelium. The inflammatory cytokine TNF increases CD146 expression and triggers metalloproteinase-dependent release of soluble CD146. By the use of blocking antibodies and small-interfering RNA (siRNA), both soluble and tethered CD146 were reported to be crucial in monocyte trafficking across the endothelium. However, the mechanism by which this important role is exerted and a potential monocyte counter-receptor for CD146 remain to be discovered.11

12. LIM domain binding 2

A recent genetic study provided compelling evidence for the involvement of the transcriptional regulator LIM domain binding 2 (LDB2) in the extravasation of monocytes in atherosclerosis.127 The authors found that this co-transcription factor, which is present in mouse and human atherosclerotic lesions, regulates many genes associated with monocyte transendothelial migration in atherosclerosis. LDB2 deficiency leads to increased monocyte migration in vitro, responsible for significantly larger lesion size in ldbr−/-/ldbr−/-apoB100/100 mice. In younger, atherosclerosis-free ldbr−/- mice, 40 genes were differentially expressed, as assessed by transcriptional profiling.127 Among them, a considerable up-regulation of the adhesion molecule VCAM-1 became apparent in ldbr−/- monocytes as well as the arterial wall.

13. Ephrins and netrin-1

The receptor protein tyrosine kinase family of eph receptors and their ligands ephrins was recently implicated in monocyte activation and migration into inflamed tissue and the development of atherosclerosis. Monocytes among others express EphA1 and 2 and EphB1–4 receptors.26 EphrinB1 and its cognate receptor, Eph receptor B2,128 as well as EphrinA1 and EphA4,129 are expressed on arterial and some venous endothelial cells and in the atherosclerotic plaque. It was demonstrated that ephrin-A1-induced EphA4 forward signalling in endothelial cells increases monocyte adhesion129 and ephrinA1-dependent activation of the EphA2 receptor influences the expression of VCAM-1.130 Monocytes were activated by interaction with EphrinB2.131 Through EphB4 forward signalling and ephrin B2 reverse signalling, Ephrin B2–Eph B interaction was shown to influence monocyte adhesion and transendothelial migration.132 Netrin-1 is crucial in retaining monocytes and macrophages, preventing them from leaving atherosclerotic lesions.133

14. Transcriptional changes in monocytes driven by transmigration

It has been established that the gene expression profile of trafficking monocytes is altered dramatically between constitutive and inflammatory conditions.134 Adhesion of monocytes to endothelial cells triggers regulation of extravasation-specific genes and initiates changes towards a more differentiated phenotype.135 Following monocyte diapedesis across activated endothelial cells, 489 genes have been shown to be up- and 203 to be down-regulated in monocytes. Among the highly up-regulated genes were MCP-1 (CCL2) and -3 (CCL7), both chemokines that attract monocytes, suggesting that monocytes may recruit other monocytes to the site of diapedesis.135 Other up-regulated genes identified are mainly involved in the immune response and inhibition of apoptosis.136 Taken together, these findings indicate that transmigration promotes further recruitment of monocytes and inhibits apoptosis, but additional stimuli are necessary for differentiation into macrophage or dendritic cell phenotypes.

15. Migration beyond the endothelium

After successfully overcoming the endothelium, monocytes are confronted with the vascular BM and encounter the pericytes that continuously wrap around the microvasculature. The non-cellular matrix of the BM constitutes a tight mesh-like network, formed mainly by collagen IV and laminins, interconnected by glycoproteins. In 2009, the Nourshargh group provided evidence that monocytes preferentially negotiate the BM at permissive regions of low matrix protein density by changing their shape and squeezing through small pre-existing gaps.137 reviewed in Nourshargh et al.138 Pericytes and the BM may have an active role in the innate immune response. Neuron-glial-2 negative (NG2+) pericytes line postcapillary venules (Figure 2), whereas NG2− pericytes reside in arterioles and capillaries. Monocytes first encounter NG2− pericytes and crawl along them. In response to inflammatory stimuli, NG2+ pericytes secrete chemoattractants and express ICAM-1, thus attracting the transmigrated cells.139 Discoidin domain receptor 1a (DDR1a), a surface receptor that has only been detected on leucocytes once they reach the extravascular space, seems to be involved in monocyte migration beyond the endothelium. Studies show that DDR1a interaction with collagen leads to shape changes that enable monocytes to travel through the tight collagen network of the BM.140

16. Concluding remarks

The abundance of molecules and pathways implicated in the migration of monocytes out of the blood stream (summarized in Table 1) is of clear translational potential. Thousands of clinical trials have been conducted on the molecules listed (see Table 1, right two columns). A humanized mAb against the alpha4 subunit of VLA-4, natalizumab, is FDA-approved for the treatment of multiple sclerosis and Crohn’s disease.141 In 2014, the integrin α4β7 mAb vedolizumab was introduced to the market as an inhibitor of inflammatory cell influx selectively into gut mucosal tissue in the setting of ulcerative colitis and Crohn’s disease.142,143 However, none of these treatments specifically inhibits the transmigration of monocytes; for example, α4β1 is also expressed on activated lymphocytes, endothelial cells, and other cells, and α4β7 is also expressed by activated lymphocytes. In addition to drugs, imaging agents and diagnostic biomarkers are also under development.

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References

Monocyte extravasation


