Leukocyte Migration into Inflamed Tissues

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http://dx.doi.org/10.1016/j.immuni.2014.10.008

Leukocyte migration through activated venular walls is a fundamental immune response that is prerequisite to the entry of effector cells such as neutrophils, monocytes, and effector T cells to sites of infection, injury, and stress within the interstitium. Stimulation of leukocytes is instrumental in this process with enhanced temporally controlled leukocyte adhesiveness and shape-changes promoting leukocyte attachment to the inner wall of blood vessels under hydrodynamic forces. This initiates polarized motility of leukocytes within and through venular walls and transient barrier disruption facilitated sequentially by stimulated vascular cells, i.e., endothelial cells and their associated pericytes. Perivascular cells such as macrophages and mast cells that act as tissue inflammatory sentinels can also directly and indirectly regulate the exit of leukocytes from the vascular lumen. In this review, we discuss current knowledge and open questions regarding the mechanisms involved in the interactions of different effector leukocytes with peripheral vessels in extralymphoid organs.

INTRODUCTION

Circulating blood leukocytes are required to migrate to sites of tissue injury and infection with the principal aim of eliminating the primary inflammatory trigger and contributing to tissue repair. In innate immunity, this process is largely initiated by pathogen-associated molecular patterns (PAMPs), released by invading microorganisms, and damage-associated molecular patterns (DAMPs), derived from damaged and/or dead-cells, or in response to tissue and/or cellular stress (Medzhitov, 2008). In addition, antigens, largely through activation of resident memory T cells, can trigger recruitment of leukocytes via secretion of various primary inflammatory cytokines. Tissue sentinel cells, including mast cells, macrophages, and dendritic cells (DCs), play a key role in detection of such danger signals and can release a wide range of proinflammatory mediators to promote leukocyte recruitment.

The primary step in leukocyte migration is the establishment of weak and transient adhesive interactions between leukocytes and endothelial cells of postcapillary venular walls in close vicinity to inflamed tissues (Figure 1). This facilitates in situ stimulation of leukocytes by endothelial presented chemoattractants displayed on the luminal side of blood vessels, propagating firm leukocyte arrest, adhesion strengthening, crawling, and subsequently migration of cells out of the blood vasculature (reviewed by [Ley et al., 2007]). This series of sequential but overlapping steps termed the leukocyte-adhesion cascade, is primarily mediated by two major adhesion receptor families, selectins (expressed on leukocytes and endothelial cells) and integrins (leukocytes) (reviewed by [Ley et al., 2007]) (Figure 1). Activation of endothelial cells is a decisive step in this process and can occur in a rapid and protein-synthesis-independent manner (within minutes) resulting in cell-surface expression of preformed adhesion molecules involved in initiating rapid attachment of leukocytes to blood vessels (e.g., P-selectin). In addition, endothelial cell activation can occur more slowly (within hours) and involve transcriptional induction of numerous leukocyte-trafficking molecules (primarily endothelial cell selectins, integrin ligands, and de novo transcribed chemoattractants [reviewed by Pober and Sessa, 2007]). Rapid activation of endothelial cells can be induced by inflammatory stimuli such as histamine and PAF while slow activation can be driven by cytokines (e.g., interleukin-1β [IL-1β] and tumor necrosis factor [TNF]). These modes of endothelial cell stimulation have been termed type I and type II activation, respectively (Pober and Sessa, 2007). Shortly after arresting on their target blood vessel endothelial cells, leukocytes must integrate additional chemotactic cues—primarily chemokines or lipid chemoattractants (reviewed by [Alon and Shulman, 2011; Rot and von Andrian, 2004]). These cues govern the site and route of leukocyte migration along and through the endothelial cell barrier, determining a potential need for chemotactic crawling on the apical aspect of the endothelium to seek permissive sites and/or additional exit cues. The latter is supported by the ability of leukocytes to extend ventral protrusions through junctions between adjacent endothelial cells or into the endothelial cell body, facilitating a sensing mechanism for detection of chemotactic gradients associated with the endothelium or in the subendothelial cell space.

Beyond the endothelium, leukocytes are required to traverse through the pericyte layer embedded within the venular basement membrane, a phase of leukocyte trafficking that can also involve leukocyte sensitization by tissue-derived inflammatory signals (Figure 1). The collective breaching of the venular wall is a highly instructive process during which the transmigrating leukocytes and both cellular and matrix components of the vasculature undergo extensive alterations via spatially coordinated bidirectional signaling events details of which have begun to unfold (reviewed by [Nourshargh et al., 2010]). Most notably, transmigrated leukocytes exhibit altered phenotype, enhanced survival, and increased effector functions, such as greater ability

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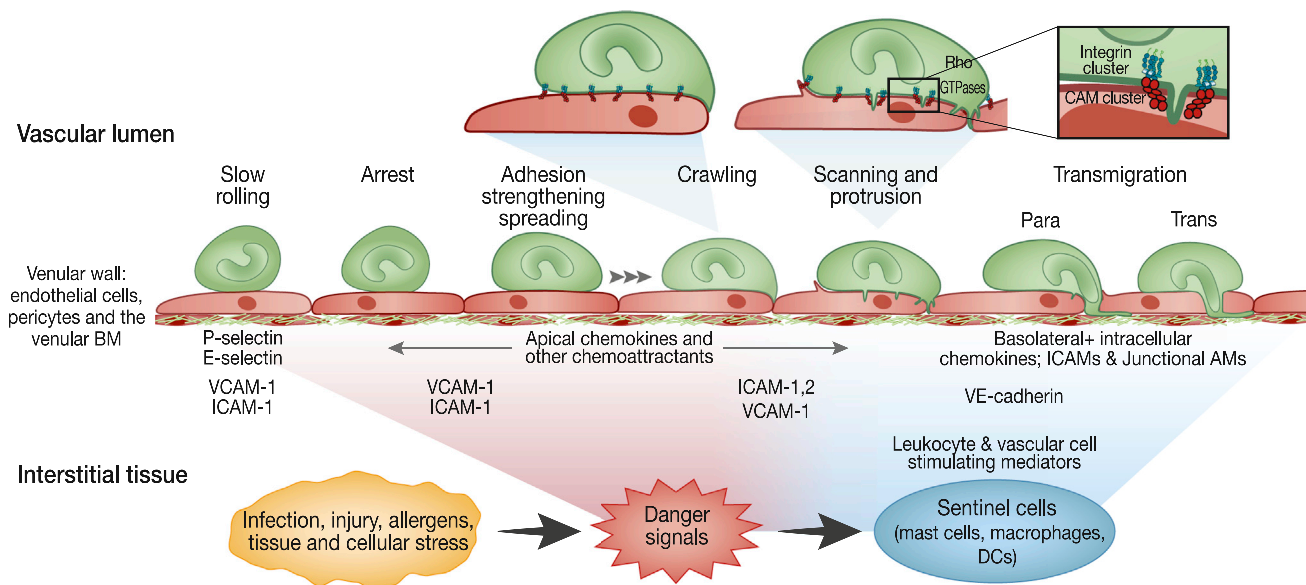

Figure 1. Leukocyte-Vessel Wall Interactions
In response to a diverse range of proinflammatory triggers, released danger signals and other proinflammatory mediators can stimulate leukocytes and vascular cells to initiate a cascade of leukocyte adhesion and motility responses on the luminal aspect of venular endothelial cells. This enables optimal scanning of the vascular lumen for exit signals. Leukocyte rolling, firm attachment, and intravascular crawling are sequentially mediated by the indicated endothelial cell adhesion molecules and leukocyte endothelial selectin and integrin ligands, responses that are prerequisites to leukocyte migration through venular walls. A delicate balance between integrin-ligand microclusters and actomyosin machineries (inset) allows arrested leukocytes to scan the endothelial lumen for chemotactic exit signals under hydrodynamic forces. This balance is spatially and temporally regulated by multiple GTPases activated primarily by chemoattractant signals. For simplicity, initial capturing and fast rolling steps are omitted. More details of the molecular interactions are provided in the text and in Box 1. BM, basement membrane.

to kill and clear invading pathogens and tumor cells. Consequently, breaching of venular walls not only provides a regulated process for facilitating leukocyte migration into inflamed tissues but also acts as a key process through which tissue-infiltrated leukocytes are primed for delivering an effective immune response (Nourshargh et al., 2010; Stark et al., 2013). Once in the interstitial tissue, leukocytes can exhibit multiple forms of leukocyte migration patterns where numerous cellular and molecular regulatory mechanisms have been proposed (Lämmermann and Germain, 2014; McDonald and Kubes, 2011; Weninger et al., 2014). This review will provide a brief outline of recent advances in our current knowledge of the bidirectional interactions of effector leukocytes with different vascular beds followed by a more in-depth discussion of the mechanisms that regulate leukocyte breaching of postcapillary venules in nonlymphoid tissues.

Luminal Leukocyte-Vessel Wall Interactions in Postcapillary Venules

Within the leukocyte-adhesion cascade, each step is conditional on the next (Figure 1) and multiple molecular choices at each step provide a large combinatorial diversity and high specificity required for selective leukocyte recruitment at the right tissue and within the correct context (reviewed by [Ley et al., 2007]). Integrins constitute a family of about 30 heterodimers that participate in a wide spectrum of cellular functions and whose ligand-binding activity is rapidly regulated by cytoskeletally controlled conformational changes and mechanical forces, as well as by redistribution from intracellular pools (Herter and Zarbock, 2013). With the exception of effector lymphocytes and certain monocyte subsets that express adhesive integrins (Carlin et al., 2013; Lek et al., 2013; Shulman et al., 2012), all circulating leukocytes maintain their integrins in largely inactive states. Leukocyte integrins must develop high affinity and avidity for their specific endothelial ligands in order to establish firm shear-resistant adhesions (Alon and Dustin, 2007; Carman and Springer, 2003; Ley et al., 2007). This transition requires freely flowing leukocytes to be reversibly captured on the endothelium, a step that is mediated by leukocyte glycoprotein (e.g., PSGL-1) interactions with members of the selectin family P- and E-selectin. Selectins can be induced on acutely or chronically stimulated postcapillary venules (e.g., P-selectin and P- and E-selectin, respectively), as well as on platelets or platelet microparticles deposited on injured blood vessels (reviewed by [Ley et al., 2007; Zarbock et al., 2011]). Free-flowing leukocytes can also interact with attached leukocytes through binding of leukocyte L-selectin to leukocyte PSGL-1 (Walcheck et al., 1996).

Selectin-mediated leukocyte rolling is often stabilized by leukocyte microvilli flattening that slows down the rolling leukocyte and further enhances the topographical availability of its chemokine receptors and integrins for interactions with their respective endothelial ligands (Chen and Springer, 1999). This response is further supported by elongation of rear tethers as well as by cell autonomous adhesive substrates termed slings (Sundd et al., 2012). These rolling interactions increase the efficiency of leukocyte encounters with endothelial cell-expressed chemoattractants (largely chemokines) and

Box 1. Diversity of Integrin Regulatory Machineries Critical for Luminal Leukocyte-Endothelial Cell Interactions

Initial attachment of leukocytes to the luminal aspect of venular walls is mediated by a complex and highly diverse array of signaling molecules that collectively translate apical chemokine and/or chemoattractant signals transmitted to leukocyte GPCRs into Gi-protein-mediated stimulation of multiple activating guanine exchange factors (GEFs). This can trigger different Rho family GTPase members and Rap-1 GTPases and their downstream effector molecules leading to conformational integrin activation followed by the microclustering of ligand-occupied integrins in numerous ventral focal points (reviewed by [Herter and Zarbock, 2013]). Stimulated GPCRs can also coactivate Rho and Rap-1 by triggering JAK PTKs independently of Gi-protein activation (Montresor et al., 2013). In some settings, the recruitment of the GPCR adaptor β-arrestin might be critical for optimal integrin activation (Molteni et al., 2009). Rho GTPases are thought to both directly (Bolomini-Vittori et al., 2009) and indirectly activate talin-1 by activating Rap-1 (Montresor et al., 2013). Importantly, different GEFs regulate the multiple Rho GTPase activities critical for leukocyte arrest and subsequent crawling and formation of protrusions and the mode of action of these GEFs and their GTPase targets varies with both the leukocyte type and the composition of endothelial displayed trafficking signals (García-Bernal et al., 2006; Nombela-Arrieta et al., 2004; Shulman et al., 2006). Some of these GEFs can be regulated by different PI3K- and DAG-dependent PKC isoforms. To facilitate the high adhesion turnover required for crawling and detachment of the leukocyte rear during breaching of the endothelium, integrin function must be spatially and negatively regulated by one or multiple mechanisms. This includes a rapid return of integrin activating GTPases to their inactive GDP-bound states, negative crosstalk between coexpressed integrins (Porter and Hogg, 1997), activation of Rho family GTPases that antagonize Rho and Rap integrin activating activities (Bolomini-Vittori et al., 2009), and myosin-II driven detachment forces of the uropod (Morin et al., 2008). These signals can be counterinhibited by inhibitory receptors such as neutrophil PILRα (Wang et al., 2013), the TGF-β superfamily myeloid cell member GDF-15 (Kempf et al., 2011), and GPCR agonists that suppress Gi signals (Chigaev et al., 2008).

subsequent chemoattractant- and integrin-dependent leukocyte arrest (Lefort et al., 2012). Neutrophil rolling on endothelial E-selectin engages the leukocyte PSGL-1 and assembles a multicomponent signaling machinery that triggers establishment of short lived and/or weak bonds between leukocyte integrins (e.g., LFA-1 and VLA-4) and their associated endothelial cell ligands (e.g., ICAM-1 and VCAM-1), leading to slowing down of selectin-mediated rolling [Block et al., 2012; Sriramarao and Broide, 1996; Stadtmann et al., 2011; Zarbock et al., 2008]). VAP-1, an ectoenzyme, induced at various sites of inflammation, might also contribute to stabilization of rolling via modifications of endothelial and leukocyte ligands (Jaakkola et al., 2000).

Leukocyte arrest on endothelial cells of stimulated venules requires activation of at least one of the major leukocyte integrins, LFA-1 (all effector leukocytes) or Mac-1 (neutrophils and monocytes), as well as VLA-4 and/or α4β7 (monocytes, eosinophils, and various effector T and B cells). This occurs by a strong inside-out stimulatory signal, usually transmitted by chemoattractant-mediated activation of G-protein-coupled receptors (GPCRs) on rolling leukocytes (reviewed in [Alon and Feigelson, 2009; Dixit and Simon, 2012]). Lymphocyte arrest involves rapid GPCR triggered activation of high-affinity integrin-ligand bonds within focal adhesive contacts, postulated to consist of microclusters of ligand-occupied integrins (Figure 1). These integrins are initially bidirectionally stimulated within a fraction of a second by coordinated cytoplasmic rearrangements of their subunit tails and the binding of their own extracellular ligands as mediated by primarily two cytoskeletal coactivators, the focal adhesion proteins talin-1 and kindlin-3 (Lefort et al., 2012; Moser et al., 2009; Ye et al., 2013). A subset of these focal contacts undergo further adhesion, strengthening via recruitment of diffusive integrins at distinct leukocyte compartments (Constantin et al., 2000; Smith et al., 2005).

Post arrest, effector leukocytes either rapidly protrude and translocate their body through the endothelial barrier, predominately at paracellular endothelial cell junctions, or use their integrins to translocate (crawl) on the apical aspects of blood vessels in search for exit cues (Phillipson et al., 2009) (Figure 1). In most inflammatory settings, leukocyte crawling is both chemokine-GPCR stimulated and integrin-dependent and is tightly regulated by canonical actomyosin machineries serially triggered by GPCR-activated small GTPases and integrin occupancy events (Shulman et al., 2009) (Box 1). In vivo, intravascular chemokine gradients have been postulated to provide a means through which leukocytes are directed through healthy tissues toward foci of sterile damage, thereby reducing potential collateral damage of infiltrating effector leukocytes (McDonald et al., 2010). As in other migratory processes, leukocytes reorganize their actin cytoskeleton to generate a protrusive leading edge and a contractile uropod (Hyun et al., 2012). Integrin recycling to the leading edge of the leukocyte (Katagiri et al., 2006), as well as polarized fusion of vesicles containing various signaling molecules, might also contribute to directional leukocyte crawling toward venular exit sites.

During crawling, leukocytes generate numerous millipede-like integrin-mediated contacts with the lumenal aspect of stimulated vessels (Shulman et al., 2009) (Figure 1). Consequently, physiological leukocyte crawling can persist also in opposite or perpendicular to the direction of blood flow, allowing optimal scanning capacity of the endothelial luminal surface (Carlin et al., 2013; Phillipson et al., 2006; Sumagin et al., 2010). In some settings, leukocytes bypass chemokine signals and use their own integrins for activating leukocytes via integrin outside-in signaling (Dixit et al., 2011; Jakus et al., 2009; Phillipson et al., 2009). Another modality of integrin activation, used by subsets of neutrophils and monocytes involves TNF receptor signaling on these leukocytes, as triggered by apically displayed TNF (Sumagin et al., 2010; Woodfin et al., 2009). Endothelial-displayed TNF can activate adherent neutrophils directly via amplifying integrin signaling or indirectly via promoting autocrine chemokine and/or chemoattractant activation of the crawling

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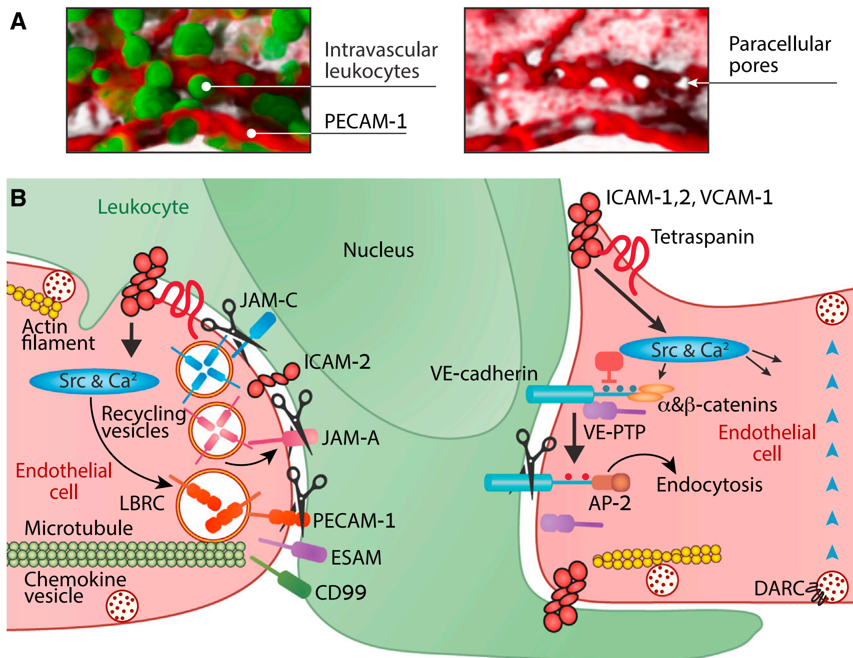

Figure 2. Paracellular Leukocyte TEM

(A) Paracellular leukocyte migration through endothelial cell junctions is the primary mode through which leukocytes breach the endothelial cell barrier in the peripheral vasculature (A). The images are video micrographs of a stimulated postcapillary venule of *Lys-EGFP-ki* mice (expressing green neutrophils & monocytes) in which endothelial cell borders have been stained red with an anti-PECAM mAb. The left image illustrates luminal leukocyte-venular wall interactions. The right image, acquired in the red channel only, shows intense PECAM-1 labeling of endothelial cell contact points and also nonjunctional PECAM-1-labeling that likely represents intracellular pools of PECAM-1 (e.g., within the LBRC). Importantly, the image shows micron-sized pores in PECAM-labeled regions between adjacent endothelial cells that represent sites of paracellular TEM.

(B) Paracellular TEM involves the coordinated disassembly of VE-cadherin assemblies and of other homophilic molecular interactions by serial leukocyte occupancy events. ICAM-1, ICAM-2, VCAM-1, and other CAM clustering events transduce multiple outside-in signals, which involve activation of endothelial Src and rise in cytosolic free Ca^{2+}. These events modulate numerous endothelial targets, including cytoskeletal remodeling machineries that facilitate junction opening and leukocyte crossing of endothelial cell junctions. VE-cadherin internalization and recycling are mediated by multiple tyrosine phosphorylation and dephosphorylation events temporally controlled by distinct endothelial sensors triggered by leukocyte occupancy. Other endothelial cell border molecules can also be subjected to local proteolytic cleavage and changes in cytoskeletal anchorage states that further facilitate their disassembly and transient internalization and recycling. Although the LBRC was originally defined as a depot for PECAM-1, it is likely that additional junctional adhesion molecules need to recycle between the endothelial surface and the LBRC, and other intracellular vesicles and VVOs. The cytoplasmic tails of these multiple adhesion molecules might target the structures within which they are stored to fuse with the plasma membrane at specific endothelial compartments engaged by emigrating leukocytes. Some of these machineries might also be used for the relatively rare transcellular TEM route. These various intracellular pools are distinct from vesicular stores of endothelial-produced chemokines that are tethered to submembranal actin filaments, as well as from DARC vesicles specialized to transcytose basolateral chemokines to the apical endothelial membrane. For simplicity, only representatives of key molecules expressed between adjacent endothelial cells are shown without their counterparts on neighboring endothelial cells, and leukocyte integrins and CAMs are omitted.

leukocytes (Smith et al., 2004). The usage of chemoattractant GPCR, integrin outside-in, and TNF machineries provides enormous diversity and ensures robustness of leukocyte migration on and through the endothelium (Box 1). These responses are also tightly regulated by the relative density of available integrin ligands, chemoattractants, and other endothelial displayed cytokines and adhesive ligands encountered by the arrested and crawling leukocytes (Shulman et al., 2012; Williams et al., 2011).

Breaching of the Endothelium

Multiple molecular and cellular events enable crawling leukocytes to initiate breaching of the endothelium and exhibit transendothelial cell migration (TEM). This includes detection of exit cues that provide chemotactic and haptotactic guidance out of the vascular lumen and beyond (see below), accurately timed adhesive interactions with the luminal aspect of the endothelium, and changes in leukocyte morphology that guide the large leukocyte nucleus through tight endothelial junctions and pores. Such responses generally coordinate the polarized movement of leukocytes through endothelial cells in a luminal to abluminal direction (Nourshargh et al., 2010). Leukocyte TEM can occur via migration of leukocytes through junctions between adjacent endothelial cells (paracellular TEM) or through the body of the endothelium (transcellular TEM). In vitro and in vivo studies have illustrated that TEM across stimulated endothelial cells of the peripheral circulation is largely via the paracellular route (~70%–90%) with transcellular TEM being a relatively low-frequency event (Ley et al., 2007; Muller, 2011; Woodfin et al., 2011) (Figure 2). Brain vascular endothelial cells seem to be an exception to this rule as they support a higher proportion of transcellular leukocyte TEM, a phenomenon that has been attributed to the specialized tight junctional structures expressed by brain endothelial cells that can restrict paracellular TEM (Engelhardt and Ransohoff, 2012).

Both paracellular and transcellular modes of TEM are supported by leukocyte-driven molecular changes in the endothelium. For example, integrin-mediated leukocyte adhesion can trigger clustering of endothelial ICAM-1, and concomitant recruitment of VCAM-1 into membranous structures that provide platforms for stable leukocyte firm arrest and TEM (Barreiro et al., 2008; Carman and Springer, 2004). Ligation of ICAM-1 and VCAM-1, and their tetraspannin or pentaspanin partners (e.g., CD9, CD151 or CD47, respectively) (Azcutia et al., 2012), by adherent and crawling leukocytes can also elicit multiple signaling events in endothelial cells postulated to reduce endothelial barrier properties (Figure 2). This includes increased intracellular Ca^{2+} (Huang et al., 1993; Pfau et al., 1995), reactive oxygen species (ROS) generation (Deem et al., 2007; Martinelli et al., 2009), and activation of p38 mitogen-activated protein kinase (MAPK) (Hu et al., 2000). In addition, ICAM-1 ligation can result in tyrosine phosphorylation of key endothelial cell junctional molecules through activation of endothelial proline-rich

tyrosine kinase 2 (Pyk2) and Src kinase (Allingham et al., 2007). These responses can couple to the triggering of RhoA (a small GTPase involved in regulation of actin cytoskeletal networks) and its downstream Rho-associated protein kinase (ROCK), as well as to endothelial myosin light-chain kinase (Saito et al., 2002). ICAM-1 occupancy by leukocytes can also lead to the translocation of clustered ICAM-1 to actin- and caveola-rich domains that recruits vesiculovacuolar organelles (VVOs) to form intracellular channels through which leukocytes can breach endothelial cells via a transcellular pore (Carman et al., 2007; Millán et al., 2006).

Once leukocytes have engaged with endothelial cell junctions, breaching of the endothelium is exquisitely regulated by adhesion molecules expressed selectively, or at high density, between adjacent endothelial cells (Figure 2). Two key junctional structures are adherens junctions that include VE-cadherin, and tight junctions that incorporate members of the junctional adhesion molecule (JAM) family (e.g., JAM-A, JAM-B, JAM-C, and endothelial cell-selective adhesion molecule [ESAM]) and claudins (Dejana, 2004). A number of other adhesion molecules are also enriched at borders of adjacent endothelial cells, such as PECAM-1, CD99, ICAM-2, and the polio virus receptor (PVR) (Muller, 2011; Nourshargh et al., 2010). The roles of these multiple molecules and their respective leukocyte ligands in leukocyte TEM are well established, but most probably vary for different leukocytes, different venules, and inflammatory models (reviewed by [Ley et al., 2007; Muller, 2011; Nourshargh et al., 2010; Vestweber, 2012; Voisin and Nourshargh, 2013]).

Intriguingly, expression of endothelial cell border adhesion molecules can be temporally and spatially regulated by inflammatory stimuli and TEM (Figure 2) (Muller, 2011; Voisin and Nourshargh, 2013). Such molecular reorganization of the endothelial cell contact points can play key roles in regulation of vascular permeability to macromolecules and leukocyte TEM (Vestweber, 2012). Leukocyte TEM in itself can affect the cell surface redistribution and internalization of key cell border structures, the recycling of intracellular pools of these molecules and their enzymatic cleavage (reviewed by [Ley et al., 2007; Muller, 2011; Vestweber, 2012; Voisin and Nourshargh, 2013]). Notably, multiple junctional endothelial cell adhesion molecules are thought to recycle in a variety of intracellular compartments and/or vesicles including the membranous lateral border recycling compartment (LBRC), endosomes, and possibly VVOs (reviewed by [Ley et al., 2007; Muller, 2011; Vestweber, 2012]). Although relatively little is known about these intracellular pools in terms of their potential crosstalk and regulation by leukocyte occupancy, they appear to contribute to maintaining the integrity of the endothelium and to provide new membranous pools that surround the leukocyte as it passes across endothelial cells (Mamdouh et al., 2003). For example, the LBRC acts as a depot for molecules such as PECAM-1, CD99, JAM-A, and PVR and supports leukocyte TEM through efficient recruitment of key molecules to sites of leukocyte diapedesis (Muller, 2011) (Figure 2). This structure although primarily implicated in paracellular leukocyte TEM has also been associated with transcellular leukocyte TEM (Mamdouh et al., 2009). The precise endothelial signals that stimulate LBRC and other vesicle trafficking and recycling are still unclear but might involve Src kinase activities (reviewed by [Muller, 2011]). Better understanding of the spatio-temporal distribution and function of individual endothelial cell border molecules might help identify previously unknown regulatory pathways involved in the onset and resolution of leukocyte trafficking.

Successful paracellular leukocyte TEM also depends on a transient loss of cell-surface VE-cadherin (Weber et al., 2007). VE-cadherin plays a critical role in maintaining the integrity of endothelial cell contacts via homotypic associations between neighboring endothelial cells, as well as maintaining the barrier function of the endothelium to macromolecules and emigrating leukocytes (Vestweber, 2012). These numerous functions of VE-cadherin are regulated by multiple cytoplasmic catenins that control the functional interaction between VE-cadherin and the cortical actin endothelial cytoskeleton (reviewed by [Weber et al., 2007]). VE-cadherin is also constitutively associated with a specialized phosphatase, VE-PTP, that needs to dissociate from its neighbor VE-cadherin complex in response to leukocyte occupancy (Vockel and Vestweber, 2013). This promotes tyrosine phosphorylation events of both VE-cadherin and its associated catenins (Broermann et al., 2011), while a distinct SHP-2-dependent tyrosine dephosphorylation event recruits the endocytic adaptor α-adaptin to drive a reversible endocytosis of VE-cadherin in close vicinity of adherent leukocytes (Wessel et al., 2014). Indeed, leukocyte diapedesis is strongly suppressed in vivo in several mice models genetically perturbed in VE-cadherin endocytosis and turnover (Broermann et al., 2011; Vestweber, 2012). VE-cadherin endocytosis is not triggered by ICAM-1- or VCAM-1-occupancy events and is therefore mechanistically distinct from the numerous ICAM-1 and VCAM-1 transduced signaling events discussed above. Of importance, leukocyte TEM and increased endothelial permeability to solutes are mediated by two distinct phosphorylation states of VE-cadherin (Wessel et al., 2014). Although a gatekeeper of paracellular endothelial junctions, VE-cadherin is dispensable for transcellular leukocyte TEM.

Breaching Venular Walls Post Transendothelial Cell Migration

To fully exit venular walls, leukocytes that have penetrated the endothelial cell barrier are subsequently required to cross the pericyte sheath and the venular basement membrane (BM) (reviewed by [Nourshargh et al., 2010]). Pericytes are mural cells that form the second cellular component of all venules and are typically found in a discontinuous manner wrapped around endothelial cells and embedded within the venular BM (Armulik et al., 2005; Nourshargh et al., 2010). While pericytes have long been considered as important players in vascular development and function (Armulik et al., 2005), there is now a growing body of evidence highlighting the significance of these cells as regulators of immune responses and inflammation and interpreters of danger signals (reviewed by [Nourshargh et al., 2010; Pober and Tellides, 2012; Voisin and Nourshargh, 2013], Figures 1 and 3). Specifically, pericytes can inducibly express key adhesion molecules (e.g., ICAM-1, VCAM-1), chemokines (e.g., human and murine CXCL1, CXCL8, MIF), and receptors for proinflammatory molecules (TNFRI, TNFRII, IL-1R, TLRs, Nod-like receptors) (Pober and Tellides, 2012; Stark et al., 2013; Voisin and Nourshargh, 2013). In vitro, pericyte-expressed adhesion molecules and chemokines support leukocyte attachment,

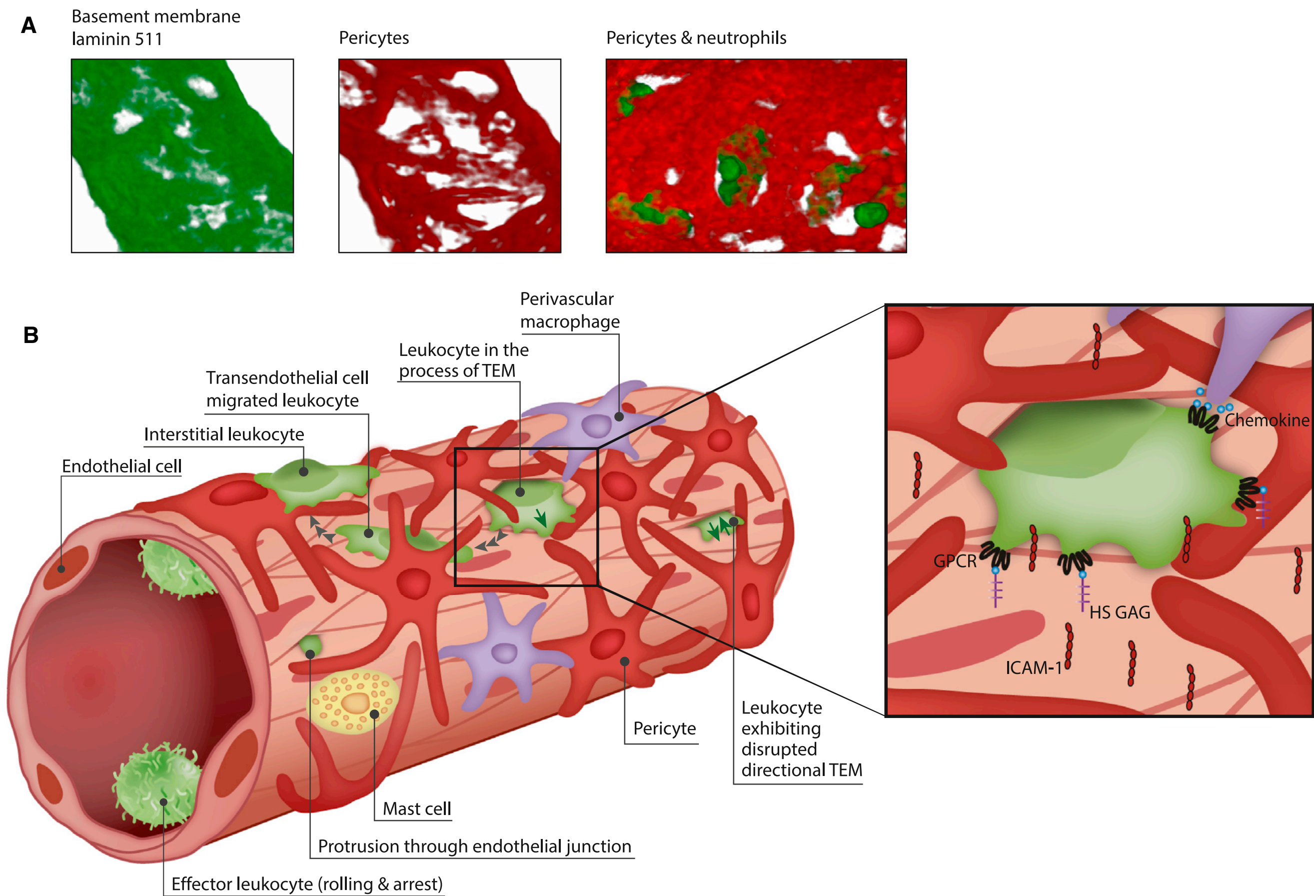

Figure 3. Establishment of Exit Cues for Leukocytes Breaching Venular Walls
Multiple mechanisms support the efficient migration of leukocytes through endothelial cells, the venular BM, and the pericyte layer. This includes preferential sites of migration and the establishment of chemotactic and haptotactic gradients that translate initial leukocyte protrusions into a stable leading edge. The confocal images shown in (A), acquired from fluorescently-labeled stimulated mouse cremaster venules, demonstrate the existence of low protein deposition regions within the laminin-511 network of the venular BM (left panel). These regions (termed LERs) are aligned with gaps in the pericyte sheath (middle panel) and are used by emigrating leukocytes preferentially to breach the venular wall (right panel). In addition, components of the vasculature, perivascular cells, and possibly migrating leukocytes themselves can generate series of chemotactic signals to support a directional luminal-to-abluminal leukocyte transmigration response (B). This includes endothelial-cell-derived chemotactic molecules as well as proinflammatory and promigratory signals released by pericytes, perivascular leukocytes (macrophages and mast cells), and recently emigrated leukocytes. Soluble and GAG-immobilized chemokines recognized by leukocyte GPCRs (B, inset) can cooperatively guide emigrating leukocytes out of the vasculature. Loss of net attractant cues might result in disrupted directional motility of leukocytes. Pericyte chemokines and cytokines (not shown) can also guide and instruct emigrating leukocytes with costimulatory and possibly prosurvival signals. Emigrating leukocytes can also use their β2 integrins (not shown) to migrate on both the abluminal endothelial aspects and on pericytes expressing ICAM-1 (inset) and get instruction signals of costimulation and survival. Lymphocyte β2 integrins might be alternatively triggered by local cognate antigen-driven signals presented by perivascular macrophages (not shown). In some settings, leukocyte β1 integrins can recognize specific ECM ligands within the basement membrane (not shown). Further details are described in the text.

costimulation, and differentiation (Ayres-Sander et al., 2013; Pober and Tellides, 2012; Stark et al., 2013; Voisin and Nourshargh, 2013). A large variation in the profile of pericyte-leukocyte communications has been reported, very likely due to the heterogeneity of pericytic cells from different blood vessels, tissues, species, isolation procedures, and inflammatory settings. The relevance of such interactions to leukocyte trafficking in vivo is only just beginning to emerge (Voisin and Nourshargh, 2013). The application of confocal intravital microscopy to analysis of neutrophil-pericyte interactions within cytokine-stimulated mouse cremaster muscles identified a key role for pericytes in subendothelial cell neutrophil motility (Proebstl et al., 2012). Specifically, pericytes were observed to provide an adhesive substrate for neutrophils crawling within the venular wall and seeking portals to the extravascular tissue (Proebstl et al., 2012) (Figure 3). This response was mediated through the interaction of pericyte-expressed ICAM-1 with neutrophil Mac-1 and LFA-1. Of relevance, the use of an endothelial cell pericyte in vitro coculture model indicated that the TEM process itself can prime neutrophils for enhanced interactions with pericytes (Ayres-Sander et al., 2013). Adhesion of fully extravasated myeloid cells with the abluminal aspect of pericytes has also been proposed as a mechanism through which various leukocyte effector functions are enhanced by specific instructing signals presented by subsets of inflamed pericytes (Stark et al., 2013). Collectively, pericytes appear to fine-tune leukocyte trafficking and instruct emigrated leukocytes for optimized navigation and effector responses at sites of inflammation. The impact

Box 2. Regulatory Roles of Perivascular Mast Cells in Leukocyte Diapedesis

Along with tissue-resident macrophages, mast cells are the prototypical tissue-resident immune sentinels that reside in most peripheral tissues, often in close vicinity of arterioles, venules and postcapillary venules (Duffy et al., 2012; Kunder et al., 2011). These granule rich cells store a multitude of inflammatory (e.g., inflammatory cytokines, myeloid-attracting chemokines) and vasoactive mediators (e.g., histamine, prostaglandins, leukotrienes, and thromboxanes), critical for triggering the onset of acute and chronic inflammatory reactions (Abraham and St John, 2010). Mast cell secretion is tightly regulated by a variety of inflammatory and stress signals, including tissue damage, microbial infections and the binding of allergen-coated crosslinked immunoglobulin E to their FcRe receptors (Cheng et al., 2013). During acute allergic responses, infection, and injury, mast cell degranulation results in immediate (type I) endothelial upregulation of endothelial stored P-selectin and rapid PAF synthesis critical for early neutrophil recruitment and extravasation (Lorant et al., 1991; Ostrovsky et al., 1998). Of interest, mast cells have been shown to act cooperatively with tissue macrophages to recruit neutrophils in response to LPS, with TLR stimulation providing rapid neutrophil recruitment via release of preformed CXC chemokines nearby vessels followed by slower macrophage-mediated recruitment of the leukocytes deeper into the extravascular tissue (De Filippo et al., 2013). Despite such studies and the clear potential role of mast cells in inflammation, further investigations are required to fully elucidate the functions of these versatile sentinel cells in leukocyte trafficking.

of specific leukocyte-pericyte interactions on the phenotype, morphology, and immune responses of both cell types requires further investigations.

Like their endothelial cell counterparts, pericytes contribute to the generation and barrier properties of the venular BM. The venular BM is composed of a complex network of laminins (primarily isoforms −411 and −511) and collagen IV, interconnected by molecular bridges involving numerous other glycosaminoglycan (GAG)-decorated glycoproteins such as nidogens and perlecan (Sorokin, 2010). This structure provides a formidable barrier to emigrating cells, but the mechanism through which leukocytes breach the venular BM is not fully understood (Rowe and Weiss, 2008). Proteolytic cleavage of BM constituents by emigrating leukocytes has long been considered as a possible mechanism, because it has been implicated in cancer cell invasiveness and intravasation (Sabeh et al., 2009). However, the physiological implications of degrading a structure that is key to maintaining vascular integrity renders this option unlikely and indeed contentious (Rowe and Weiss, 2008). A more plausible mechanism is the potential existence of leukocyte-permissive regions within the venular BM. In this context, immunofluorescent studies have demonstrated the existence of regions within the venular BM that exhibit low deposition of certain matrix proteins (laminin-511 and type IV collagen) (Voisin and Nourshargh, 2013; Voisin et al., 2010; Wang et al., 2006) (see Figure 3). These sites, which have been termed low-expression regions (LERs), constitutively exist in all vascular beds analyzed and are commonly directly aligned with gaps between adjacent pericytes, indicating the importance of pericytes in generation of BM constituents. As predicted by their permissive nature, LERs act as "gates" for transmigrating neutrophils and monocytes in vivo (Voisin and Nourshargh, 2013; Voisin et al., 2010; Voisin et al., 2009; Wang et al., 2006). Interestingly, LERs are transiently enlarged during neutrophil transmigration through subtle disassembly and physical carriage of neutrophil-bound BM components, most notably laminins (Voisin et al., 2010; Voisin et al., 2009; Wang et al., 2006). Such molecular remodeling might be facilitated by leukocyte adhesion to the venular BM as numerous studies have indicated roles for $\beta1$ integrin laminin receptors $\alpha6\beta1$ and $\alpha3\beta1$ in neutrophil migration through the venular BM (Dangerfield et al., 2002; Hyun et al., 2012). Other mechanisms that could contribute to leukocyte migration include possible "thinning" of the BM as caused by tractional forces exerted on the matrix networks by pericyte shape-change (Finsterbusch et al., 2014; Proebstl et al., 2012) or other physical and enzymatic processes (Rowe and Weiss, 2008). In addition, subunits of laminins with lower capacity for crosslinking with collagen type IV molecules (e.g., the short $\alpha4$ chain of laminin-411) might provide more penetrable LERs due to easier dissociation of the laminin and collagen IV networks during cell migration.

Exit Cues for Leukocyte Transmigration

The contribution of chemokines and other chemoattractants for distinct steps of leukocyte TEM has been extensively studied both in vitro (reviewed by [Shulman and Alon, 2012]) and in vivo (reviewed by [Blanchet et al., 2012; Ingersoll et al., 2011]). While endothelial cells are accepted as a major source of chemotactic molecules for effector leukocytes, pericytes (Proebstl et al., 2012), fragments of the venular BM, perivascular macrophages (Abtin et al., 2014), certain subsets of mast cells (see Box 2), antigenic signals (Box 3), and recently extravasated leukocytes are postulated to also send short-range guidance cues for transmigrating leukocytes.

Type II protein synthesis-dependent activation of endothelial cells can result in the expression of a variety of chemoattractants for myeloid leukocytes and effector T cells (Pober and Sessa, 2007). Chemokines constitute a key component of the repertoire of endothelial cell-derived exit cues and the expression of these molecules on the endothelium is regulated by numerous modes, most notably via binding to heparin sulfate (HS) glycosaminoglycans (GAGs) (Proudfoot, 2006). These large GAGs decorate a variety of membranous and extracellular matrix proteins and can serve as chemokine scaffolds in multiple types of interstitial spaces (Sarris et al., 2012), as well as different types of blood vessels and lymphatics (Bao et al., 2010; Wang et al., 2005; Weber et al., 2013). A recent study suggests that blood vessels both in lymphoid and nonlymphoid tissues pattern steep gradients of HS scaffolds between their luminal and basolateral endothelial aspects (Stoler-Barak et al., 2014). The enriched HS GAGs might be necessary to maintain functional chemokine gradients toward the basolateral aspects of blood vessels. Indeed, excess deposition of the HS binding chemokine CXCL1 on apical aspects of inflamed blood vessels results in reduced neutrophil diapedesis (Yao et al., 2013). Of note, HS or other GAGs do not appear to be essential for all types of inflammatory reactions

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Box 3. A Role for Antigenic Signals in T Cell Diapedesis and Retention in Tissues

In the context of the adaptive immune response, the migratory patterns acquired by antigen-experienced effector T lymphocytes that egress lymphoid organs and migrate to affected tissues are still not fully elucidated (Agace, 2006). In addition to upregulating selectin ligands and integrins, as well as GPCRs for multiple inflammatory chemokines (Reinhardt et al., 2003; Weninger et al., 2001), these T cells likely undergo tissue-restricted imprinting of specific trafficking molecules depending on the organ they were generated in (Woodland and Kohlmeier, 2009). A long-standing question is whether and when antigenic signals presented to these effector T cells and their memory counterparts by the vasculature at the site of infection or inflammation also contribute to the recruitment of these lymphocyte subsets (Marelli-Berg and Jarmin, 2004; Pober et al., 2001). Apical presentation of cognate antigenic peptides by endothelial MHC-I and perivascular DCs might potentially augment integrin adhesiveness and diapedesis of CD8 subsets, especially within vascular beds deficient in adhesive and chemotactic activities (Savinov et al., 2003; Walch et al., 2013). Antigen-specific CD4 effectors can use cognate antigen-driven signals presented by MHC-II for entry into pancreatic islets in early stages of mouse autoimmune diabetes (Calderon et al., 2011) and extravasating CD4 T cells can readily interact with antigenic moieties presented by perivascular macrophages at various chronic sites of inflammation (Bartholomäus et al., 2009). It is likely, however, that such vascular and perivascular antigenic signals recognized by cognate effector T cells contribute to their emigration only when the expression of vascular and perivascular trafficking signals become limited.

because neutrophil chemoattractants like PAF, LTB_4, formylated peptides, and C5a lack affinity for GAGs (Goodarzi et al., 2003). As well as generating chemokines, endothelial cells can actively import chemokines produced by perivascular cells by various transcytosis machineries (Middleton et al., 1997). The atypical GPCR like molecule DARC (Duffy antigen) displays promiscuous affinity for most CXC chemokines and inflammatory CC chemokines (reviewed by [Mantovani et al., 2006]) and plays a major but probably not an exclusive role in both the transcytosis of perivascular chemokines and their correct positioning on the apical surface of the endothelium (Middleton et al., 1997) (Figure 2). Interestingly, DARC-mediated transcytosis of chemokines to the apical aspect of endothelial cells is also HS dependent (Middleton et al., 1997; Wang et al., 2005). The key role of DARC in chemokine transcytosis is supported by its expression profile, recently found to be restricted to postcapillary venules (U. von Andrian, personal communication).

Other vascular-derived sources of chemotactic cues are pericytes and the vascular BM (Figure 3). As discussed above, pericytes can generate chemokines and immobilize them on GAGs (Stramm et al., 1987). Conceptually, the generation of pericyte-derived chemokines and/or chemokine depots is likely to play a key role in facilitating continued migration of leukocytes through venular walls after the breaching of the endothelium. The constitutive existence of the venular BM LERs might also provide a mechanism through which guidance molecules generated in the extravascular tissue, or released from perivascular macrophages or mast cells, penetrate the venular wall and hence create a chemotactic gradient toward these preferential vascular exit sites. Thus, multiple mechanisms enable vascular and perivascular components to cooperatively guide the passage of leukocytes from the vascular lumen to the extravascular tissue. This includes the release of proinflammatory mediators and chemotactic molecules from extravasated or tissue-resident leukocytes. Specifically, because blood leukocytes are a rich source of preformed granular chemoattractants, such as cathepsin G, LL-37, and azurocidin (Borregaard et al., 2007; Soehnlein et al., 2008), and can also release lipid and protein chemotactic molecules (e.g., LTB_4, PAF, and various chemokines), recently extravasated myeloid leukocytes might further stimulate leukocyte recruitment via a relay phenomenon, typically observed in interstitial spaces (Afonso et al., 2012; Lämmermann et al., 2013). With respect to tissue-resident leukocytes (Figure 3; Box 2), in infectious models, neutrophils extravasate from inflamed dermal venules in close proximity to perivascular macrophages and mast cells that produce large amounts of both neutrophil and monocyte attracting chemoattractants (e.g., CXCL1, CXCL2, and CCL2) (Abtin et al., 2014; De Filippo et al., 2013). The repertoire of perivascular macrophages and mast cells is highly versatile with different subsets of these cells generating different profiles of inflammatory mediators in response to distinct danger signals (Figure 1) (Galli et al., 2011). Notably, later on during the inflammatory process, extravasating neutrophils phagocytosed by perivascular macrophages can negatively regulate further leukocyte extravasation by secreting a variety of resolution mediators such as resolvins, lipoxins (Wolf et al., 2008), TGF-β, and IL-10. These mediators can directly reduce leukocyte adhesion and responsiveness to chemoattractants, as well as attenuate endothelial expression of trafficking molecules (reviewed by [Soehnlein et al., 2009]). MMPs secreted from perivascular macrophages might also contribute to this resolution by cleaving chemokines (Wolf et al., 2008).

Aberrant Modes of Leukocyte Extravasation

A number of aberrant modes of leukocyte extravasation, at odds with the well-described sequence of molecular and cellular responses described by the classical leukocyte-adhesion cascade, have also been reported. As discussed, leukocyte crawling on the luminal aspect of the endothelium represents a key phase of the leukocyte-adhesion cascade that enables leukocytes to seek exit cues and preferential sites of leukocyte migration within the venular wall. Nevertheless, in certain noninflammatory settings, leukocyte crawling is not followed by emigration into tissues because it is mainly used for patrolling for intravascular signs of injury or infection (Auffray et al., 2007; Carlin et al., 2013). Specifically, $Ly6C^{lo}$ monocytes comprise a noninflammatory subset of monocytes that crawl at steady state, exhibiting long-range luminal lateral migration responses, within blood vessels of noninflamed tissues (Auffray et al., 2007; Carlin et al., 2013). This response is supported by basally expressed ICAM-1 and ICAM-2 and constitutively adhesive LFA-1 expressed by the crawling subset of monocytes (Carlin et al.,

2013). Of interest, this LFA-1-dependent crawling does not depend on a prior stimulation by a P-selectin-mediated rolling phase and does not require chemokine signals (Carlin et al., 2013). Furthermore, while patrolling small blood vessels in various organs (e.g., skin, kidney), and reportedly scavenging luminal endothelial microparticles as part of their tissue surveillance functions, these monocytes appear to recruit neutrophils upon encounter of stimuli, but rarely breach venular walls themselves (Carlin et al., 2013). Abortive emigration of leukocytes to blood vessels is observed in other settings including natural killer (NK) and NKT cell crawling inside liver sinusoids (Geissmann et al., 2005) and retention of immature B cells in the bone-marrow vasculature (Pereira et al., 2009). These and other forms of leukocyte-vessel wall interactions that are futile in terms of breaching the vessel wall could comprise an important range of essential physiological homeostatic leukocyte surveillance responses, important in leukocyte survival, differentiation, or reprogramming by organ-specific intraluminal vascular signals. Leukocyte-vessel wall interactions without the need for extravasation might also regulate vascular permeability through luminal release of leukocyte-derived vasoactive factors such as TNF (Finsterbusch et al., 2014) or via signaling to endothelial cells after ligation of key adhesion molecules such as ICAM-1 (Sumagin et al., 2011).

Unsuccessful breaching of vessel walls could also constitute pathological forms of leukocyte-vessel wall interactions as a result of excessive intraluminal leukocyte activation. Such responses can typically cause vascular injury through release of damaging neutrophil-derived factors (e.g., ROS, proteases), as exemplified by FcγRIIIB-triggered β2 integrin-dependent neutrophil arrest following immune-complex deposition on resting blood vessels (Mayadas et al., 2009). Of interest, multiphoton intravital microscopy has additionally illustrated that in noninflamed murine kidneys, neutrophils and monocytes are commonly retained in glomerular capillaries (static or crawling) and that the duration of this phenomenon is enhanced after immune-complex-elicited glomerular injury (Devi et al., 2013). Intravascular activation can also occur following severe sepsis, a condition during which live neutrophils can release neutrophil extracellular traps (NETs) within the vascular lumen while maintaining some irregular crawling responses (McDonald et al., 2012).

While leukocyte migration through endothelial cells commonly occurs in a luminal-to-abluminal direction, there is now unequivocal evidence for the occurrence of TEM in the basal to apical direction of the endothelium. This leukocyte reverse TEM (rTEM) response has been reported for human monocyte, neutrophil, and T cell migration through cultured endothelial cells (Buckley et al., 2006; Lee et al., 2009; Randolph and Furie, 1996) and for neutrophils in zebra fish embryos (Mathias et al., 2006). Neutrophil rTEM, and a TEM mode that involves multiple oscillatory movements within endothelial cell junctions (termed "hesitant" TEM), have also been observed in stimulated mouse cremasteric venules in vivo (Woodfin et al., 2011). Of importance, these events were totally localized to the endothelium, and leukocytes that had breached the pericyte and venular BM layer were never seen to exhibit reverse motility back into the vascular lumen. Neutrophil and monocyte rTEM is significantly enhanced under conditions of reduced expression and/or functionality of endothelial cell JAM-C (Bradfield et al., 2007; Woodfin et al., 2011), suggesting that JAM-C plays a key role in regulating the directional movement of leukocytes from the apical to basal aspect of the endothelium. The underlying mechanism of leukocyte rTEM is at present unclear but might involve disruption of local organization of exit cues, existence of competing gradients of chemoattractants and repellents, and/or desensitization of leukocyte GPCRs following high-receptor occupancy (Huttenlocher and Poznansky, 2008). Leukocyte migration into the vascular lumen can be physiologically important, such as encountered during trafficking of leukocytes from the bone marrow to the vasculature as part of leucocytosis and during lymphocyte migration from lymphoid tissues to the vascular compartment as part of immune surveillance (Huttenlocher and Poznansky, 2008). However, the functional role of this response within inflammatory scenarios remains unclear and requires further investigations. Interestingly, neutrophil rTEM following ischemia-reperfusion injury has been associated with dissemination of systemic inflammation (Woodfin et al., 2011), and rTEM could be involved in dissemination of pathogens from a primary site of infection to distant organs (Duffy et al., 2012). Furthermore, based on findings in zebra fish embryos where neutrophils have been seen to migrate away from the site of injury, leukocyte reverse motility has also been proposed as a means of resolving an inflammatory response (Deng and Huttenlocher, 2012).

Finally, important examples of aberrant modes of leukocyte trafficking relate to tissue-specific responses, in line with the specialized vascular morphology and/or rheological properties of certain organs such as the lungs, liver, and the brain (Engelhardt and Ransohoff, 2012; Lee and Kubes, 2008). Most notably, neutrophil migration into the lung parenchyma is selectin- and β2-integrin-independent where adhesion and TEM occurs in the absence of rolling in pulmonary capillaries (reviewed by [Doerschuk, 2001]). Furthermore, in the liver, blood drains through sinusoids, which are capillary-like vessels that express a unique type of endothelium that is discontinuous and fenestrated, lacking basal lamina and tight junctions (Jenne and Kubes, 2013). Liver sinusoidal endothelial cells also lack the capacity to express E- and P-selectins, and in line with this, leukocyte adhesion within sinusoids appears to occur independently of rolling. In addition, sinusoidal endothelial cells express low inducible expression of VCAM-1 but high constitutive expression of ICAM-1 and, most notably, of the adhesion molecule VAP-1 (Salmi and Jalkanen, 2001) and the CD44 ligand, hyaluronan (McDonald et al., 2008). Collectively, there is ample evidence to support the concept that organ-specific anatomical, cellular, molecular, and hemodynamic features are critical for mediating specific leukocyte trafficking responses in line with the specialized immune-surveillance requirements and functions of different organs.

Concluding Remarks and Future Perspectives

While the identity of key molecular and cellular players involved in orchestrating an effective leukocyte trafficking reaction is well established, there remains a need for better understanding of the intricacies, dynamics, cellular interplays, and diversities of this critical immune response. In this context, advancements in versatile imaging modalities have dramatically enhanced our

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ability to dissect these standing questions in different leukocyte and vessel types and in an organ-specific manner. In addition to building on these fundamental issues, a number of specific avenues not discussed in the present review also require further explorations. These include a better understanding of the impact and regulatory effects of the neuronal and the endocrine system on both vascular components and leukocytes. For example, almost all blood vessels are intimately associated with peripheral nerves, and at present it is unclear whether and how neurotransmitters affect leukocyte extravasation to peripheral and lymphoid tissues in homeostasis and inflammation. Furthermore, although there is much evidence for different susceptibilities of men and women to infections and inflammatory diseases (reviewed by [Libert et al., 2010]), the underlying molecular basis of this gender-difference on leukocyte trafficking and leukocyte-vessel-wall crosstalk remains unclear. Similarly, despite much interest in the impact of circadian rhythm on inflammatory responses (reviewed by [Scheiermann et al., 2013]), it remains unclear whether and how different endothelial cells, and their associated pericytes, are subjected to circadian modulation of transcription and how this impacts trafficking and recruitment of different leukocyte subsets during different times of the day. In recent years, the impact of aging on vascular morphology has received much attention, but there remains a paucity of understanding of whether and how vascular senescence impacts leukocyte-vessel-wall interactions. Other open questions include the variation of blood vessel glycocalyx and basement membrane composition between different organs and inflammatory conditions. In this regard, the pulmonary vasculature has been shown to contain a particularly thick HS stabilized glycocalyx believed to mask endothelial-expressed adhesion molecules including integrin ligands from marginating leukocytes before it is degraded by inflammatory signals (Schmidt et al., 2012). Nevertheless, similar masking functions have not been attributed to the glycocalyx of venules. Likewise, differing composition and barrier properties of distinct basement membranes might result in different subsets of leukocytes using different venular breaching strategies to extravasate these distinct perivascular barriers.

Experimentally, in spite of the increasing use of genetically modified mouse models in leukocyte trafficking studies, it is not yet possible to use similar models for silencing key endothelial and pericyte machineries involved in leukocyte trafficking, due to the high toxicity of such systemic genetic approaches. As a result, there is a growing need to not only temporally silence genes of interest in blood-vessel-wall cells, using newly developed conditional and cell-type recombinase-based targeting approaches, but also restrict the temporal gene silencing to a confined subset of vessels.

In summary, leukocytes are provided with a diverse range of highly versatile and selective options in responding to different molecular cues in breaching blood vessel walls at different sites of inflammation. The studies reviewed here highlight considerable heterogeneity in vessel-wall composition, resulting in extremely large combinatorial range of trafficking signals and endothelial barrier regulatory molecules that shape leukocyte recruitment and activation in distinct tissues. The assortment of vascular trafficking molecules discussed, and the large combinatorial diversity they provide, introduce new challenges to the future design of composite antimigration therapies and their implementations for personalized anti-inflammatory medicine.

ACKNOWLEDGMENTS

We wish to thank H. Vega for help with figures, S. Feigelson for helpful suggestions, and A. Woodfin and M.-B. Voisin for providing the confocal images. S.N.'s research is generously funded by a Wellcome Trust Senior Investigator Award (Ref. 098291/Z/12/Z). R.A. is an incumbent of the Linda Jacobs Chair in Immune and Stem Cell Research, and his research is supported by the Israel Science Foundation, the US Israel Binational Science Foundation, and the Flight Attendant Medical Research Institute Foundation.

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