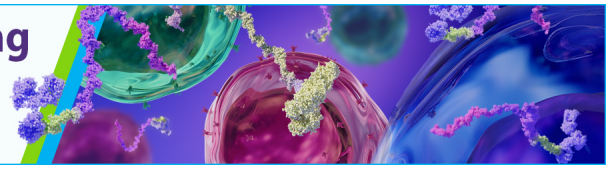

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BRIEF REVIEWS

The Physiology of Leukocyte Recruitment: An In Vivo Perspective

Björn Petri, Mia Phillipson,† and Paul Kubes[1]**

***The mechanisms of leukocyte recruitment have been studied extensively in vitro and have shed light on the basic molecular structure-function relationship of adhesion and signaling molecules involved in this essential immune response. This review will summarize how these in vitro observations extend to leukocyte behavior in inflamed blood vessels in the microcirculation. We highlight physiological results that might not have been predicted from in vitro systems. Special attention is placed on the physiology of rolling, adhesion, and intralumenal crawling in blood vessels. The importance of the glycocalyx, secondary tethers, shear, and the microenvironment are discussed. Docking structures forming rings of adhesion molecules together with a novel endothelial dome-like structure in vivo during transmigration are highlighted. Transcellular and paracellular emigration out of inflamed blood vessels is also discussed. The last section highlights leukocyte recruitment in some organs that do not always follow the accepted paradigm of leukocyte recruitment. The Journal of Immunology,* 2008, 180: 6439–6446.**

A necessary feature of any immune response is the movement of immune cells from one site to another to provide effector functions. Therefore, elucidating the molecular mechanisms underlying the migration of leukocytes from the blood to tissues is critical to our understanding of immune function. Over the last 20 years, leukocyte recruitment has been extensively studied and a paradigm formulated that can be found in most pathology and immunology texts (Fig. 1*A*). Much of this work has come from in vitro systems using leukocyte binding to endothelium under either static conditions or using flow chambers that allow the introduction of shear forces that appear to contribute significantly to the steps of the recruitment cascade. Despite the simplicity of these in vitro systems, it is quite remarkable how accurately many of the major steps of leukocyte recruitment have been predicted and confirmed in in vivo preparations such as the mesentery, skin, or cremaster muscle. In this regard, selectins mediate the initial tethering and rolling of leukocytes on endothelium and firm adhesion is mediated by a combination of chemokines and integrins, the former activating the latter. This then permits the leukocytes to emigrate out of the vasculature. There are emerging issues that suggest additional steps in this cascade, including intralumenal crawling, that bridge adhesion to emigration. Moreover, questions have been raised as to whether environmental conditions established in vitro actually truly reflect conditions in different organs such as liver and brain (Fig. 1, *B* and *C*), particularly under various inflammatory conditions.

The importance of leukocyte rolling: selectins

Selectins have been extremely well characterized and numerous excellent reviews regarding their molecular structure-function relationships exist (1, 2). Briefly, the selectins are a family of calcium-dependent, type I transmembrane glycoproteins (reviewed in Ref. 3) consisting of E-selectin and P-selectin on venular endothelium and L-selectin on many leukocyte subsets. P-selectin is constitutively expressed and stored in secretory granules known as α-granules (in platelets) or Weibel-Palade bodies (in endothelium). When stimulated by inflammatory mediators such as thrombin, cysteinyl leukotrienes, oxidants, or histamine, Weibel-Palade bodies within minutes fuse with the plasma membrane, increasing surface expression of P-selectin. Therefore, it is not surprising that P-selectin is often involved in early leukocyte recruitment during an inflammatory response. P-selectin transcription can be induced via selected cytokines including IL-4 and IL-13 (4, 5). It is important to note that whereas in human endothelium in vitro P-selectin does not appear to be synthesized by TNF and IL-1, in vivo in mouse systems these cytokines are potent activators of P-selectin synthesis. By contrast, in human endothelium in vitro TNF and IL-1 induce primarily E-selectin (6). Although it is tempting to conclude that this is a discrepancy between in vitro and in vivo systems, it has been demonstrated that murine but not human P-selectin promoter contains an NF-κB binding site (6). Therefore, it is quite likely that where P-selectin might dominate in certain inflammatory conditions in mouse, E-selectin may be more important in humans. Nevertheless in nonhuman primates in vivo, P-selectin expression could be induced through oncostatin M rather than TNF-α (7).

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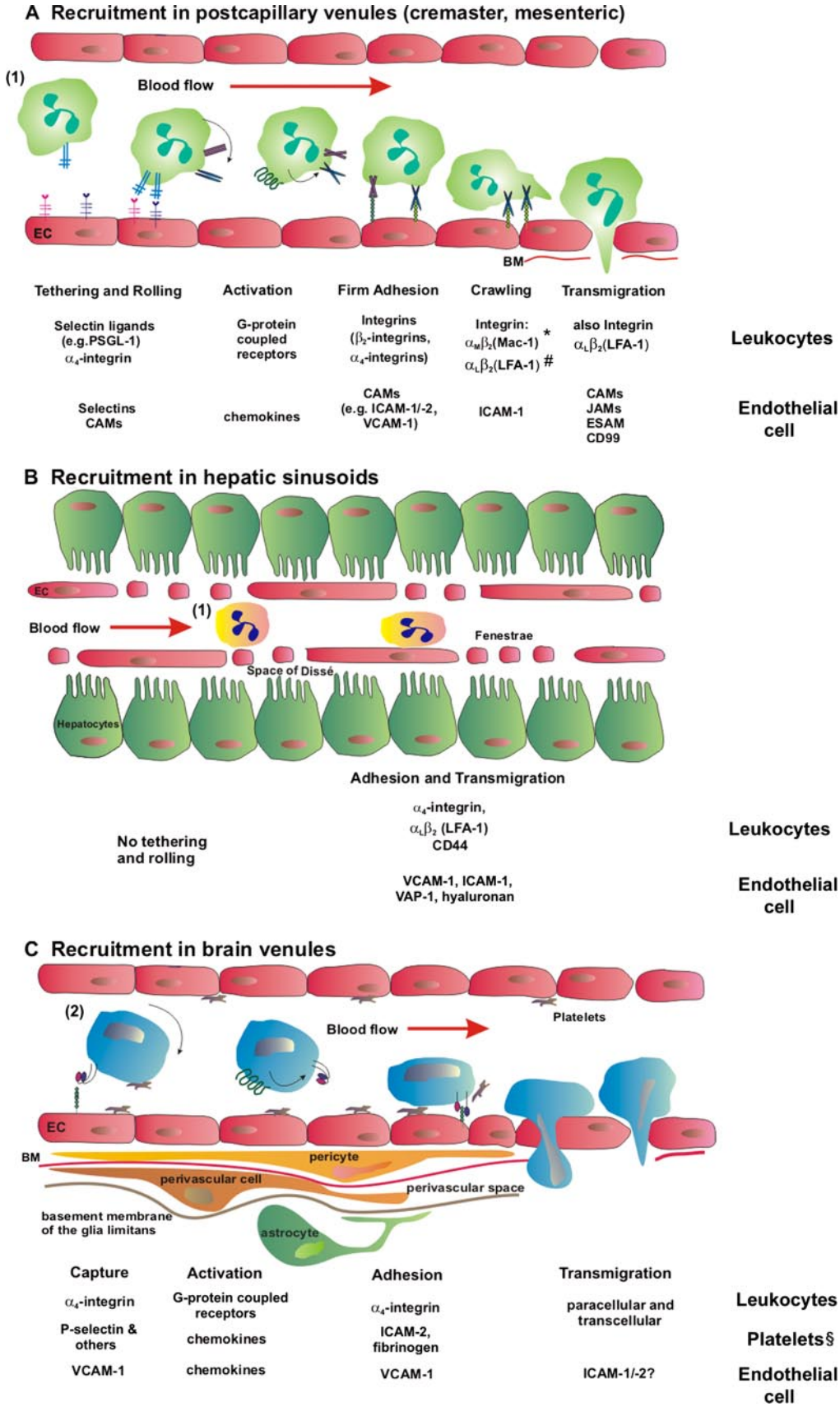

FIGURE 1. Organ-specific recruitment of leukocytes in inflamed vessels of three different organs: Mesenteric or cremasteric postcapillary venules (*A*), hepatic sinusoids (*B*), and brain postcapillary venules (*C*). Leukocyte recruitment in postsinusoidal venules of the liver is not shown, because the mechanisms in these venules are similar to the recruitment shown in cremaster (*A*). Currently known interactions between adhesion molecules and cellular components (platelets, endothelium, and leukocytes) involved in the process are listed beneath the figures. Mononuclear and polymorphonuclear cells (1) are shown in *A* and *B*, whereas lymphocytes (2) are depicted in *C*. §, Participation of platelets in the brain has been shown only for polymorphnuclear cells so far. BM, Basal membrane; CAMs, cellular adhesion molecules; EC, endothelial cell; JAMs, junctional adhesion molecules. *, As demonstrated for neutrophils; #, as demonstrated for monocytes.

E-selectin is not presynthesized in endothelial cells in vitro and in vivo, but an important exception to this rule is the observation that skin does indeed express E-selectin constitutively (together with P-selectin), allowing for the continuous rolling of leukocytes in this vascular bed (8). Expression can occur on the endothelial surface as quickly as 2 h after TNF-α stimulation in vitro and in vivo and decline within 24 h (1). Despite delayed expression, E-selectin overlaps with P-selectin, temporally helping to enhance leukocyte recruitment during inflammation. One distinguishing functional feature between E-selectin and P-selectin is a dramatic decrease in rolling velocity on E-selectin that is thought to enhance the probability of subsequent adhesion (9). The slow rolling on E-selectin is thereby stabilized via the contribution of β_2-integrins (10). However, a reduction in rolling velocity has also been seen for some (leukotriene C_4) and not other (histamine) P-selectin stimuli (11). Interestingly, a similar observation was made in vitro on human endothelium, but rather than quantitative differences in P-selectin expression, rolling velocity appeared to be more dependent on whether P-selectin clustered in clathrin-coated pits (12). In addition, leukotriene C_4 could also possibly induce the activation of leukocyte integrins, thereby contributing to slow rolling. The physiologic importance of these observations is that the slower rolling with leukotriene C_4 vs histamine in vivo provided leukocytes a greater propensity to firmly adhere at much lower concentrations of an activating molecule like platelet-activating factor (11).

The E- and P-selectin found on endothelium are long molecules 30–40 nm in length that reach out into the vessel and tether leukocytes. Reducing the length of an endothelial selectin by deleting half of the consensus repeat regions reduces the ability of these molecules to tether leukocytes (13). Expressing these shortened selectins in cells that lack a glycocalyx reconstitutes tethering and rolling. These data would support the view that the length of selectins is needed to reach past the glycocalyx to grab leukocytes. This remains a very interesting area of study as numerous investigators have suggested that unlike in vitro, in vivo the negatively charged glycocalyx may extend up to 500 nm, which would completely obscure selectins. Whether microvilli on leukocytes can reach into the glycocalyx to contact selectins, whether the glycocalyx is eliminated during inflammation, or whether the glycocalyx is much shorter in postcapillary venules where it would allow the selectins to reach past this barrier remains unclear. Because the depth of the glycocalyx in vivo is still controversial, new visualization techniques are needed to clarify this issue in different vascular beds (14, 15). It also remains unclear how the de novo expression of a selectin might push through the glycocalyx and into the lumen. It may be that as Weibel-Palade bodies devoid of glycocalyx fuse with endothelial membrane, patches of P-selectin would be exposed. Leukocytes also have a negatively charged surface in part due to long sialic acid-rich molecules like CD43 that have been shown to function as anti-adhesive molecules not dissimilar to the endothelial glycocalyx (16). These negatively charged moieties on leukocytes and endothelium may have important anti-adhesive functions in capillaries where leukocytes rarely interact with the vessel wall.

L-selectin has only two consensus repeats, but its localization to microvilli protruding from the leukocyte body allows for optimal leukocyte-endothelial cell interactions (17, 18). Although no one would dispute a role for L-selectin as an essential molecule for lymphocyte trafficking to lymph nodes, its role and mechanism of action in the periphery remains quite unclear, mainly because an endothelial ligand has been difficult to find. In fact, rather than modulating leukocyte-endothelial interactions, there is evidence in vitro that leukocytes in the mainstream of blood will tether to an already rolling leukocyte via L-selectin and P-selectin glycoprotein ligand-1 (PSGL-1).[2] These interactions permit the untethered leukocyte to be brought to the endothelial surface where it begins to roll. This phenomenon is called a secondary tether and has been beautifully demonstrated in vitro as lines or strings of rolling leukocytes that can be prevented with an L-selectin Ab (19). However, the addition of RBCs to a flow chamber along with leukocytes greatly reduced these secondary tethers and string formation (20). These data raised questions about the physiologic importance of secondary tethers. The strings of leukocytes seen in vitro are not obvious in vivo, and although groups of cells are often seen to roll in venules, secondary tethers were documented to be a very rare event in vivo (21). A recent study did suggest that secondary tethers occur in large vessels like the aorta during atherosclerosis (22). Finally, recent work has proposed that residual fragments from leukocytes may leave PSGL-1 on the endothelial surface that might now tether additional leukocytes, thereby explaining how L-selectin might mediate some rolling in the periphery independent of secondary tethers (23).

Leukocyte recruitment independent of rolling by selectins

In vitro when endothelium was stimulated with TNF or other cytokines, blocking selectins reduced adhesion (1, 2). However, in vivo when animals were pretreated with anti-selectin inhibitors, leukocyte recruitment and inflammation often persisted. There is a generally held view that a linear relationship exists between rolling and adhesion such that a 50% inhibition in rolling inhibits 50% adhesion and 50% inflammation. However, this theoretical view seems not to be true in vivo. To observe any effect on adhesion in vivo, the rolling of leukocytes has to be inhibited at least by 90–95% (24). This suggests that many more cells are rolling compared with the cells that are needed for efficient adhesion and transmigration. In addition, shear in inflamed blood vessels is quite heterogeneous. Anti-selectin therapy inhibited most of the rolling in all of the blood vessels, but leukocyte adhesion was inhibited only in those blood vessels with higher shear (24), suggesting that lower shear allowed neutrophils to adhere independently of selectins. Indeed, much of the tethering and adhesion was entirely dependent on the β_2-integrin. It has been reported that reducing shear in an inflamed vessel could induce brief leukocyte rolling and subsequent adhesion via the β_2-integrins in vivo (25). In vitro, extension of the β_2-integrin in an intermediate affinity state showed a mixed phenotype converting rolling to adhesion. Locking the β_2-integrin in an extended but still low affinity state supported rolling on ICAM-1 (26), suggesting some overlap of function between selectins and β_2-integrins. A recent study demonstrated that this intermediate conformational change of β_2-integrin occurred via PSGL-1-induced Syk activation and was important in vivo in permitting the transition from rolling to adhesion (27). Overlapping functions between selectins and integrins

[2] Abbreviations used in this paper: PSGL-1, P-selectin glycoprotein ligand-1; ESAM, endothelial cell-selective adhesion molecule; VAP-1, vascular adhesion protein-1.

have also been investigated in vivo. In LFA-1$^{-/-}$ mice the leukocyte rolling velocity was significantly increased and could be further enhanced with Abs against $\alpha_4\beta_1$ integrin. This indicates that LFA-1 as well as the $\alpha_4\beta_1$-integrin participate in rolling along vessels after an inflammatory stimulus (28).

It is also worth noting that some organs are completely resistant to anti-selectin therapy. Indeed, Doerschuk and coworkers clearly demonstrated that using anti-selectin therapy had absolutely no impact upon leukocyte recruitment into the inflamed lung under certain conditions (29). The recruitment of leukocytes in lungs occurs in both venules and capillaries, an observation also made in liver (30). Intravital microscopy of liver revealed that leukocytes rolled in venules but not in sinusoids. Rather, leukocytes appeared to adhere in sinusoids without any rolling (Fig. 1*B*). Blocking the selectins prevented leukocyte rolling and adhesion in liver venules but not in sinusoids (30). Although physical trapping was evoked as a possible mechanism for the recruitment of leukocytes in the capillaries of liver as well as lung, new evidence is emerging that at least in liver sinusoids the recruitment of leukocytes relies on adhesion molecules (discussed later).

Ten years ago, in vitro studies clearly demonstrated that α_4-integrin induced the rolling of leukocytes (31, 32). Similar data were reported for rolling in vivo in numerous inflammatory models (33). The importance of this observation was apparent in that the selectins could be bypassed entirely by integrins on leukocytes. It is becoming clear that there are numerous molecules that can replace the selectins in addition to α_4-integrin-like CD44 (34) and vascular adhesion protein-1 (VAP-1) (35). The endothelial cell surface-expressed oxidase VAP-1 thereby is a unique molecule that can also act as an ectoenzyme catalyzing chemical modifications. Induction of E- and P-selectin could be demonstrated in a VAP-1 enzyme activity-dependent manner in vivo (36). Thus, via an as yet unknown ligand, VAP-1 can prime the endothelium for enhanced leukocyte recruitment due to its enzymatic activity. However, VAP-1 can also mediate adhesion independently of its enzymatic activity (37) and can substitute in some situations for selectins.

Adhesion and crawling: molecularly distinct events

The integrins were discovered many years ago, at least in part due to patients that lacked CD18 and therefore presented with various recurring infections. It became clear that the neutrophils from these patients were able to roll, but all steps downstream of this phenomenon, including adhesion and emigration, were significantly impaired. Moreover, crawling on substrata was also impaired in neutrophils from these patients (38). The β_2-integrins are made up of a common β_2 subunit and one of four different α subunits (39). Neutrophil adhesion is mediated largely by the high affinity state β_2-integrin LFA-1. This is especially true under shear flow, when LFA-1-dependent binding to its ligand (ICAM-1) is absolutely necessary. Shear flow also seems to be a crucial factor to initiate adhesion, as it mediates the activation of β_2-integrins via E-selectin signaling (40). Even with low-level β_2-integrin ligand activity, stimulatory shear stress in combination with chemoattractant signals is able to promote more robust adhesion and transmigration of neutrophils (41).

In vitro, in static assays there appeared to be good evidence that redundancy between LFA-1 and another β_2-integrin, namely Mac-1, existed (3). The fact that in vitro LFA-1 and Mac-1 are both able to bind ICAM-1 strengthened the argument for redundancy. However LFA-1 and Mac-1 bind to distinct domains of ICAM-1 (42, 43). In addition to ICAM-1, Mac-1 is able to bind numerous other ligands including fibrinogen (44), and fibrinogen binds ICAM-1 and can bridge Mac-1 to endothelial ICAM-1. One alternative explanation to redundancy is that each β_2-integrin plays a distinct and sequential role in the recruitment cascade. Indeed, an in vitro study predicted sequential usage of LFA-1 and Mac-1: an early LFA-1–dependent adhesion to ICAM-1-transfected cells and then a sustained adhesion dependent on Mac-1 (30). Henderson and colleagues, in a systematic assessment of the role of Mac-1 and LFA-1 using intravital microscopy, revealed that whereas LFA-1 mediated adhesion, Mac-1 appeared to be more important in the emigration process. This was based on the fact that Mac-1 blockade did not affect adhesion but fewer neutrophils emigrated (28). Clearly, Mac-1 either played an important role in emigration or else an as yet unidentified step existed between adhesion and emigration.

Wojciechowski and Sarelius, using time-lapse intravital microscopy, reported that after adhesion most leukocytes begin to crawl in venules in the absence of an inflammatory stimulus (45). Schenkel and colleagues (46) reported monocyte crawling in a static in vitro assay on activated endothelium and suggested that the monocytes used crawling to find endothelial junctions, a step essential for subsequent emigration. In this process the interaction of both β_2-integrins, LFA-1 (CD11a-CD18) and Mac-1 (CD11b-CD18), with their ligands ICAM-1 and ICAM-2 were important for proper locomotion of monocytes to junctional extravasation sites. This latter study did not examine crawling under flow conditions, which was a concern because under flow conditions in vivo endothelial cells are more elongated and narrower, increasing the propensity that leukocytes would adhere on junctions. In fact, Burns and colleagues reported that, in vitro, leukocytes rolled along junctions and arrested on or very near junctions (47). This raised the possibility that crawling was not a necessary recruitment step in flow systems.

Using time-lapse video microscopy to visualize leukocyte behavior in vivo, it became clear that the crawling step also occurred in inflamed venules (48). Together with a PECAM-1 Ab stain to identify junctions, this imaging approach revealed that, within inflamed postcapillary venules, immediately upon adhesion there is significant intraluminal crawling of all neutrophils to distant emigration sites. In dramatic contrast, Mac-1$^{-/-}$ neutrophils adhered extremely well but failed to crawl (48). Although only a few LFA-1$^{-/-}$ neutrophils adhered in inflamed venules, those that did crawled efficiently (48). ICAM-1 but not ICAM-2 mediated the Mac-1-dependent crawling whereas neither Ab against ICAM-1 nor ICAM-2 alone could inhibit adhesion. These studies together support the view that adhesion and crawling are separate events and at least in neutrophils depend upon LFA-1 and Mac-1, respectively (48). Leukocytes can also use α_4-integrin to adhere. Whether this molecule will mediate adhesion and/or crawling in some cell types remains unknown. It is noteworthy that cross-linking β_2-integrin in vitro does mobilize β_1-integrins on neutrophils, perhaps suggesting a downstream role for the β_1-integrins (49). In vivo, increased β_1-expression has been reported on emigrated neutrophils (50) and contributes to crawling outside the vasculature; but

whether this molecule contributes to intralumenal crawling remains unknown.

Neutrophil adhesion and crawling ultimately contribute to efficient and timely emigration out of the vasculature. Indeed, Schenkel and colleagues (46) reported that when monocyte crawling under static conditions was inhibited, emigration was also prevented. The importance of crawling to emigration was less obvious in inflamed blood vessels in vivo. Mac-$1^{-/-}$ neutrophils could not crawl but eventually emigrated in similar numbers to wild-type neutrophils; however, the process took longer (5 min in wild-type vs 30 min in $Mac^{-/-}$ mice) (48). It remains unclear why the in vitro and in vivo studies do not agree. One possibility could be that the in vitro assays were not sufficiently long to detect the delayed emigration. It is also possible that the difference is related to the macrovascular endothelium used in vitro vs the microvascular endothelium in vivo. Alternatively, shear was necessary to allow for emigration as was shown previously for lymphocytes and eosinophils (51, 52).

Crawling in blood vessels may be a very important mechanism that extends beyond inflammation to normal surveillance. A recent study by Auffray et al. (53) described the existence of a resident monocyte subpopulation with patrolling behavior. These cells crawl continuously on resting endothelium in dermal and mesenteric blood vessels and rapidly invade tissue after an infectious stimulus. This group reported the need for LFA-1 and the chemokine receptor CX_3CR1 for the crawling of this population of monocytes in vivo, because the addition of an LFA-1 Ab detached these crawling cells. Moreover, a population of NKT cells has been reported to exist in the liver sinusoids where they patrol for foreign and perhaps endogenous lipid stimuli (54). The adhesion mechanism for this crawling remains unknown. Interestingly, the liver also has Kupffer cells, a population of mononuclear cells that reside in the vasculature; however these cells appear to be immobilized (55).

Emigration across endothelium: para or transcellular?

It is safe to say that the emigration process is likely the least well understood step in the leukocyte recruitment cascade, perhaps in part because different leukocyte subsets behave differently on different vascular endothelia under different inflammatory conditions. The molecular mechanisms of transmigration are summarized elsewhere (3). In this review we summarize some of the conflicting data regarding the route of emigration. No one would dispute the existence of leukocyte emigration out of the vasculature at junctional sites. Indeed most investigators that have demonstrated emigration through endothelium (transcellular route) reported $<$50% of this type of migration. Nevertheless, both in vitro and in vivo studies have previously shown that all leukocyte subtypes are able to migrate in a transcellular and paracellular fashion and that both pathways are likely to coexist. The prevailing view is that neutrophils preferentially migrate at tricellular corners of endothelium (56) whereas monocytes seem to favor tight junctions between two endothelial cells (57). Evidence is accumulating that lymphocytes may migrate transcellularly. Indeed one in vitro study showed that human T lymphocytes preferentially used the transcellular pathway whereas human neutrophils favored the paracellular route across a TNF-α stimulated human umbilical vein endothelium (58). Although recent work by Carman and colleagues (59) suggested that lymphocytes use the transcellular route more often across microvascular endothelium (30% of the time) rather than across HUVECs (10% of the time), this still suggested that in 70% of cases of diapedesis the cells emigrated paracellularly.

In vivo, Feng et al., reported the striking finding that as many as 90% of all neutrophils migrated transcellularly out of skin vasculature in response to a bacterial peptide (60). This study stands out as an example of transcellular migration being dominant. Intriguingly, the study was also unique in that it used extensive serial sectioning and electron microscopy to detect transcellular migration, the most accepted method of assessing these dynamic events using electron microscopy. Using confocal fluorescence microscopy in vivo, Phillipson et al. (48) reported only $\sim$10% of neutrophils emigrated transcellularly in response to a chemokine. In that study, 50% of neutrophils adhered at junctions while the remaining neutrophils adhered at nonjunctional sites but then crawled to junctions. However, inhibition of neutrophil crawling via Mac-1 Ab caused the neutrophils to emigrate where they adhered. As such, 50% or more of neutrophils emigrated transcellularly, suggesting that neutrophils can migrate transcellularly but normally use a paracellular route. Whether a bacterial product used by Feng et al. (60) signals the neutrophils differently than a chemokine used by Phillipson et al. (48) remains to be seen, but certainly it is known that these two chemoattractants have very different intracellular signaling pathways for chemotaxis (61).

A recent study demonstrated for the first time that neutrophils and lymphocytes may use different molecular mechanisms to emigrate out of vessels, potentially explaining the different modes of diapedesis (62). In vivo, the lack of the tight junction protein endothelial cell-selective adhesion molecule (ESAM) impaired neutrophil but not lymphocyte recruitment into inflamed peritoneum (62). These observations lend support to the view that neutrophils that migrate through junctions require the junctional protein ESAM whereas lymphocytes do not require this junctional protein. This suggests that lymphocytes migrate independently of ESAM and as such may indeed use a different route for diapedesis.

It should also be noted that a cell type can switch transmigration preference as the microenvironment changes. Neutrophil transmigration can switch from a paracellular to a transcellular route, depending on the environmental conditions. For example, Yang et al. demonstrated recently in vitro that human neutrophils prefer the paracellular pathway through human endothelium activated for 4 h with TNF-α (63). Stimulation for 24 h with TNF-α, however, resulted in a shift toward the transcellular pathway (63). TNF-α upregulated ICAM-1 a lot more at 24 h than at 4 h, and ICAM-1 appeared to play a key role in promoting the usage of the transcellular pathway by neutrophils. The cytoplasmic part of ICAM-1 was required for neutrophil transcellular migration as demonstrated by mutants lacking the entire cytoplasmic tail (63), suggesting that signaling into endothelium via ICAM-1 somehow facilitated transcellular migration. It is interesting that in this same study T cells did not migrate transcellularly in the same in vitro assays. The transmigration preference is not only leukocyte specific but may also be dictated via the type of stimulus the leukocyte is encountering, as shown in several in vivo studies (64–66).

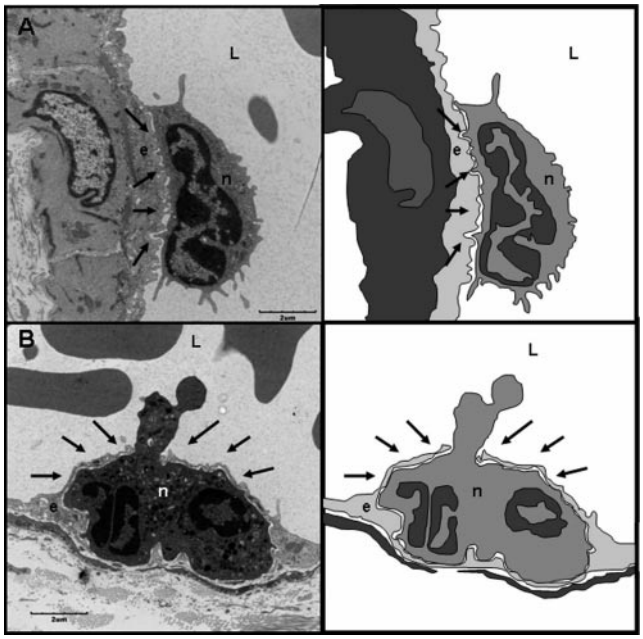

FIGURE 2. *A*, Crawling neutrophil with podosome-like protrusions (arrows) invaginating the endothelium. *B*, Novel "dome"-like structures (arrows) in vivo may be formed from docking structures or transmigratory cups. Abbreviations: e, endothelial cell; n, neutrophil; L, lumen.

Endothelium and leukocytes: active participants in emigration

Migration across the endothelium is a very dynamic process for both the leukocyte and the endothelial cell, and the latter contributes in a major way. Regardless of the route the leukocyte will use to cross the endothelial barrier, the endothelium contributes docking structures known as transmigratory cups. These structures allow very close contact between the leukocyte and the endothelium due to the microvillus-like protrusions that are formed by the endothelium. It has been shown that the expression of the integrins LFA-1 and VLA-4 ($\alpha_4\beta_1$) and their ligands on the endothelium (ICAM-1 and VCAM-1) are enriched in these transmigratory structures (57, 67). ICAM-1 and VCAM-1, clustered on the endothelial surface, were originally described as rings (67) and are connected through the adaptor proteins moesin and ezrin to the actin-cytoskeleton (68). Through the anchorage of the adaptor proteins with α-actinin and vinculin, the clustering of ICAM and VCAM leads to the reorganization of the cytoskeleton (69). It has been shown that vimentin as a member of the intermedia filaments is also located in the microvilli of the docking structures and that vimentin-deficient mice show a decreased homing behavior of T lymphocytes to secondary lymphoid organs, suggesting that vimentin may also play a role in lymphocyte transmigration (58).

A striking observation is the "dome" structure recently identified in vivo (70) and shown in Fig. 2*B*. The endothelium in vivo not only extends along the sides of the leukocytes but often forms a complete "dome" surrounding the leukocyte. Noteworthy is the intact endothelium; luminally and basolaterally, a part of the transmigrating neutrophil is still in the lumen before it is entirely enclosed by the endothelium (Fig. 2*B* and Ref. 70). It is tempting to suggest that the docking structures actually become domes that allow the basolateral membrane to be disrupted without any breach in the endothelial barrier function as recently shown by Phillipson and colleagues (70). It is intriguing that in electron micrographs observed in vitro this dome structure has not been reported (57–59, 67, 69), whereas in vivo these structures have been noted (60, 71, 72). It is worth mentioning that Mamdouh and colleagues reported membrane compartments near junctions that were mobilized during emigration (73). This perhaps could be a source of additional membrane for the formation of docking structures and domes.

Recent work by Carman et al. demonstrated that the lymphocyte itself also actively contributes to the transcellular migration. The lymphocyte forms an invasive podosome that palpates the endothelial surface. This forms podoprints on the endothelial surface at the docking structure leading to the formation of transcellular pores (59). The podosome formation was dependent on Src kinase and the actin regulatory protein WASP (Wiskott-Aldrich syndrome protein). The use of human lymphocytes bearing WASP mutations or the interference of podosome formation by pretreating human lymphocytes with Src inhibitors led to the blockade of transcellular pores and the inhibition of transcellular but not paracellular migration (59). Interestingly, the pores only formed at sites lateral to the nucleus where the endothelium was the thinnest and not directly above the nucleus. These intriguing observations were made using activated lymphocytes, and this begs the question of whether other leukocytes and naive lymphocytes in high endothelial venules behave similarly. Although the imaging remains less than optimal for visualizing the formation of these pores and podosomes in vivo, electron micrographs reveal similar structures for neutrophils in vivo (Fig. 2*A* and Ref. 70). Recently, Wang et al. (74) reported areas of low extracellular matrix protein localization and gaps between pericytes in the basement membrane of blood vessels, suggesting that neutrophils identify not just sites on endothelium but also outside the vessel where they then emigrate.

Organ-specific recruitment

Despite the fact that most current texts have accepted the leukocyte recruitment in mesentery or cremaster as a universal concept, it is becoming clear that each tissue holds some distinguishing features and some organs do not follow the proposed paradigm. Although we describe a few of these caveats, we draw the reader's attention to more detailed reviews (55, 75, 76). Likely the first observation of selectin- and β_2-integrin-independent neutrophil recruitment in vivo was provided by Doerschuk in some lung inflammatory models (reviewed in Ref. 77). Much of the adhesion was shown to occur in pulmonary capillaries. Similarly, adhesion of neutrophils was seen in liver sinusoids (Fig. 1*B*) in the presence and absence of the β_2-integrin ligand ICAM-1 (reviewed in Ref. 78). By contrast, $CD8^+$ T cells have been shown to use β_2-integrin as a major adhesive pathway in sinusoids (79, 80). The liver is a site where activated $CD8^+$ T cells are recruited and eliminated, terminating the immune response. Naive T cells can also bind in the liver sinusoids of uninflamed liver via β_2-integrins (81). Activated $CD4^+$ T cells by contrast adhere via either α_4-integrin or VAP-1 in sinusoids in Con A-induced hepatitis (82). CD4 T cells producing IFN-γ (Th1) bound sinusoidal VCAM-1 via α_4-integrin. T cells producing IL-4 (Th2) had no affinity for VCAM-1 in Con A-induced hepatitis but bound avidly to VAP-1 (82). In fact, VAP-1, a 170-kDa homodimeric glycoprotein, is expressed at

high levels in the human liver and promotes lymphocyte adhesion and transmigration across hepatic sinusoidal endothelial cells in vitro (83).

Another organ that fails to follow the standard leukocyte recruitment paradigm is the brain. The inflamed brain has an increased propensity for platelet recruitment in this vasculature during inflammation. Using intravital microscopy, numerous groups have reported an important role for platelets in the leukocyte recruitment into inflamed brain microvessels (84, 85). Platelets adhere to the brain endothelium, and depletion of platelets reduces leukocyte recruitment. It is conceivable that platelets could function as a bridge to tether and adhere leukocytes, although the molecular adhesive mechanism has not been elucidated. In vitro, platelets bind collagen and express very large amounts of P-selectin that can avidly recruit leukocytes (86). However platelets binding to activated brain endothelium expressed P-selectin in some (85) but not other models (84), suggesting different modes of platelet activation. Furthermore, in vitro studies under flow have shown that platelets express the LFA-1 ligand ICAM-2 and the chemokine RANTES (CCL5) on their surfaces (87–89), which led to platelet-dependent PSGL-1, CCR1/CCR5, and LFA-1-dependent recruitment on the brain endothelium, which appears to lack these signals. A dominant role for P-selectin in leukocyte recruitment has been reported in a number of models of brain inflammation including traumatic brain injury (90), permanent middle cerebral artery occlusion (91), and other inflammatory conditions (84), but not in some models of multiple sclerosis, where α_4-integrin was more important for mononuclear cell recruitment (92).

Summary

In summary, it is clear that the basic principles of the proposed mechanism of leukocyte recruitment, initially discovered in in vitro assays and confirmed in easily accessible vessels like the cremasteric and mesenteric venules (Fig. 1*A*), have served as an important basis for our understanding of leukocyte recruitment. However, it is also now clear that the paradigm that selectins mediate rolling and integrins mediate adhesion is far too simple a model for most vascular beds and for all leukocytes under physiologic and pathophysiologic conditions. Understanding these critical nuances of each organ and each condition will increase our ability to immunomodulate specifically certain inflammatory diseases.

Disclosures

The authors have no financial conflict of interest.

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