Review Article

Migration of leukocytes through the vessel wall and beyond

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Summary

The migration of leukocytes from the vascular lumen to sites of infection and/or injury in the extravascular tissue involves a series of sequential and coordinated molecular and cellular events with the resultant primary response being that of reduced leukocyte velocity within the blood stream, followed by leukocyte firm adhesion to endothelial cells lining the vessel wall and eventually migration through the vessel wall. Despite the growing knowledge of the mechanisms that mediate initial interaction of leukocytes with the endothelium, very little is known about the mechanisms that mediate and regulate leukocyte migration through the venular wall, the endothelium and its associated perivascular basement membrane. This review, whilst giving a brief outline of the stepwise cascade of molecular interactions involved in this process and the methods employed to investigate leukocyte migration *in vivo*, focuses primarily on mechanisms of leukocyte transmigration, the final step in the process of leukocyte emigration. Furthermore, special emphasis is placed on discussing the process and the mechanisms involved in leukocyte migration through the basement membrane, a structure that presents significant impedance to transmigrating leukocytes but is seldom investigated in the context of leukocyte transmigration *in vivo*. The review also discusses the growing evidence supporting the concept that leukocyte transmigration is not only a response that describes the passage of leukocytes through the venular wall, but also acts as a means of regulating leukocyte responsiveness beyond the vessel wall, i.e. within the extravascular tissue.

Keywords

Leukocyte, transmigration, basement membrane

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Introduction

Migration of leukocytes to sites of injury or inflammation is a crucial component of both innate and adaptive immunity. To achieve this, finely co-ordinated mechanisms exist by which intravascular leukocytes are able to penetrate the vascular wall and migrate to sites of injury or infection without causing any perceptible damage to the vessels from which they emigrate. Within this scenario, leukocyte transmigration through vessel walls (predominantly post-capillary venules) is the final stage of a stepwise cascade of leukocyte responses mediated by a series of sequential molecular interactions that initially mediate the slowing down of leukocyte rolling velocity followed by leukocyte firm adhesion to the endothelium and eventually migration through the vessel wall (Fig. 1). As illustrated and discussed in this review, leukocyte transmigration not only acts as a means of directing the emigration of leukocytes from the vascular lumen to the extravascular tissue but may also play a critical role in regulating the phenotype of the emigrated cells such that leukocyte behaviour in the form of responsiveness to chemoattractants, directional migration and interactions with components of the extravascular tissue may be regulated. Such

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a mechanism could clearly contribute to the ability of the immune system to mount an optimum, appropriate and localised tissue response to a wide range of extravascular stimuli without causing any untoward damage to the vasculature. However, in conditions of uncontrolled leukocyte infiltration that may be excessive either in magnitude and/or duration, or inappropriate in its location, transmigration-induced change in phenotype of leukocytes may lead to induction or exacerbation of deleterious inflammatory responses in the host. Collectively, understanding the molecular interactions that drive and regulate the response of leukocyte transmigration could therefore be of value in the development of therapeutic strategies aimed at promoting or suppressing the host's inflammatory response, interventions that may be of potential benefit in both physiological and pathological scenarios.

The principal aims of this brief review will be to discuss the profile, mechanisms and consequences of leukocyte emigration through stimulated venular walls in vivo. The mechanisms that mediate the early phases of leukocyte/endothelial cell interactions within the vascular lumen will be discussed very briefly and more emphasis will be placed on the molecular interactions that mediate the migration of leukocytes through the vessel wall, in particular, migration through the perivascular basement membrane, a barrier that is seldom investigated in the context of leukocyte emigration in vivo.

Multi-step model of leukocyte transmigration

Under physiological conditions, the endothelial lining of the vasculature presents an anti-thrombogenic and anti-adhesive surface to flowing blood and blood components thereby maintaining homeostasis. However, at sites of tissue injury or infection, in response to inflammatory stimuli such as cytokines and endotoxin, the endothelium is rendered adhesive for circulating leukocytes, initiating a stepwise process of leukocyte emigration. The earliest steps in this "multi-step" model of leukocyte transmigration (Fig. 1) involve tethering to and rolling of leukocytes along the vascular endothelium, responses mediated by weak and reversible molecular interactions between the endothelial and leukocyte selectins (E-selectin, P-selectin and L-selectin) and their counter ligands (1, 2). In addition, certain integrins such as VLA-4, can also mediate leukocyte rolling under conditions of flow (3-5). The slowing down of leukocytes during rolling facilitates the ligation of specific and high affinity G-protein coupled receptors by lumenal endothelial cell associated activating factors such as chemokines and PAF. This interaction augments the ligand binding profile of integrins as achieved by increased affinity and/or avidity of the molecules (6, 7). Activation of integrins plays a key role in mediating the firm adhesive interaction of leukocytes to endothelial cells as well as the flattening of leukocytes over the endothelium, two critical steps in the process of leukocyte emigration. Principal integrins that are involved in this stage of leukocyte/endothelial cell interaction belong to the β_2 and β_1 family of integrins (e.g.

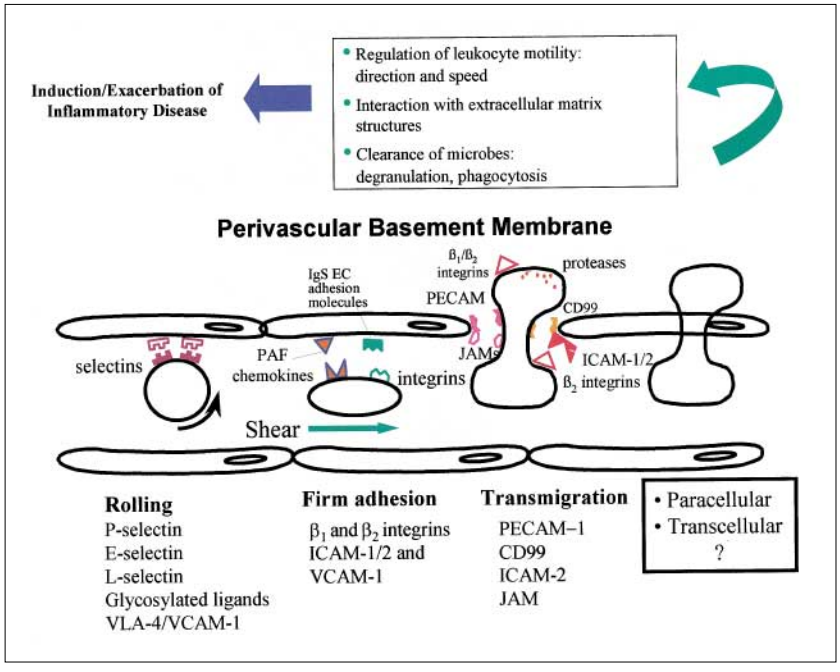

Figure 1: Schematic diagram showing the sequential steps involved in leukocyte transmigration through stimulated vessel walls, indicating the key molecules involved and some potential transmigration-induced changes in phenotype of the emigrating leukocytes (see text for details).

CD11b/CD18 and VLA-4), molecules that can interact with endothelial cell counter ligands such as ICAM-1 and VCAM-1, members of the Ig superfamily (8). For further details of the molecules involved in leukocyte/endothelial cell interaction, their structure, function and regulation of expression, the reader is referred to recent reviews (9, 10).

Once captured and activated, adherent leukocytes are stimulated to de-adhere (by mechanisms that are still unclear) and migrate through the vessel wall by traversing the endothelial cell layer and the basement membrane, two distinct but adjacent and interacting barriers. Relative to our enhanced knowledge of the mechanisms that mediate the early stages of leukocyte emigration, many unanswered questions remain regarding the molecular interactions governing the migration of leukocytes through the endothelium, basement membrane and the extravascular matrix. One factor that has contributed to the rather slow progress of this aspect of leukocyte biology relates to the difficulties, and indeed complexities, of studying leukocyte transmigration in vivo, an issue that will be addressed in the following sections. Before discussing the methods employed to study transmigration, it is appropriate to consider the composition of the barriers which leukocytes have to penetrate during transmigration *in vivo*.

Structure of venular walls

Venular walls are composed of endothelial cells and pericytes embedded within a tough, thin and distensible basement membrane, composed of several extracellular matrix proteins such as laminin, collagen type IV and entactin (11). Under normal circumstances vessel walls provide a formidable barrier to cellular and non-cellular components of flowing blood, the barrier being effectively breached, however, by emigrating leukocytes and plasma proteins at sites of inflammation.

Endothelial cells

Endothelial cells are polygonal in shape, ≈10-15μm wide by ≈25-40 μm long and ≈0.1-0.5μm thick, except in specialised post capillary venules found in the high endothelial venules of lymphoid organs, where they are high walled and cuboidal. They are arranged as a single layer of cells that line the lumen of vessel walls and are connected at intercellular junctions by complex molecular interactions involving structures such as tight junctions, adherens junctions and gap junctions. These molecules form a network of transmembrane proteins that regulate the permeability of vessels to macromolecules as well as providing a barrier to passage of leukocytes. Of relevance, the endothelial cells of arteries and arterioles show continuous and elaborate tight junctions in comparison to "leaky" post capillary venules that have loosely organised endothelial junctions. Details of the structure and interaction of endothelial cell junctional complexes are beyond the scope of this article but are covered in detail by other reviews in this series as well as recently published reviews (12).

Apart from molecules whose principal function is to maintain vascular integrity and regulate permeability, endothelial cells express molecules at their cell borders that play a direct and critical role in leukocyte transmigration. These include PECAM-1 (CD31), CD99, ICAM-2 and members of the JAM family, molecules that will be discussed in detail in other reviews in this series and briefly below in relevant sections.

Perivascular basement membrane

In vivo, analysis of endothelial cell basement membranes by electron microscopy reveals a dense layer (20-100nm thick) consisting of pericytes embedded in a meshwork of fine fibrils (*lamina densa*). In addition, the basement membrane may have electron-lucent layers on one or both sides of the lamina densa called the *lamina rarae* or *lamina lucidae*. The electron-dense material of the basement membrane consists of a meshwork of collagen type IV that is exclusive to basement membranes.

Pericytes are associated abluminally with all post-capillary venules, and together with endothelial cells, may contribute to the formation of venular basement membranes (13). These cells have long processes that parallel the long axis of venules tapering to smaller processes that encircle venular walls as well as making direct contact with endothelial cells. The nature and physiological function of pericyte/endothelial cell interactions are at present unknown.

The term basement membrane derives from the fact that it was first recognised as the membrane beneath the basal cells of surface epithelia. This membrane is a highly specialised distensible matrix, distributed not only at the interface between epithelial and mesenchymal tissues but also around muscle and nerve fibres as well as blood vessels where the endothelial cells rest on a basement membrane. Key properties of the venular basement membrane include maintaining the venular architecture, providing a selective barrier to leukocytes and macromolecules, regulating endothelial cell adhesion and mitogenesis as well as acting as a depository for regulatory proteins such as growth factors, proteinases, proteinase inhibitors and thrombotic agents. All basement membranes contain laminin, entactin/nidogen-1, type IV collagen and proteoglycans although in recent years the complexity of the basement membrane has increased as new components are described (11). The current basement membrane model proposes two networks, one consisting of collagen IV and the second made up of laminin, interconnected by entactin-1. Although collagen IV endows the basement membrane with stability, laminin plays an essential role in basement membrane formation due to multiple interactions with itself and other components of the structure.

Laminins are large heterotrimeric glycoproteins (800KDa) consisting of genetically distinct α, β, and γ chains that can assemble into more than ten different isomeric forms (14). Of

interest there is evidence for tissue specific and developmentally regulated expression patterns of laminin isoforms highlighting the role of laminins in determining heterogeneity in basement membrane functions/composition in different tissues and during development and repair. Despite the tremendous interest in characterising the functional significance of individual isoforms, information in this area remains limited. To date, endothelial cell basement membranes have been found to express two laminin isoforms, laminin 8 (composed of laminin α_4, β_1 and γ_1 chains) and laminin 10 (composed of laminin α_5, β_1 and γ_1 chains), depending on their tissue of origin and state of growth or activation (15-17). Such selective expression profile of different laminin isoforms further indicates functional diversity within the laminin family and perhaps the specific receptors with which they interact. In this context, integrins are the major class of receptors for laminins and although several integrins can bind laminin eg $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$ and $\alpha_6\beta_4$, $\alpha_6\beta_1$ appears to be specific for members of the laminin family and is certainly the principal leukocyte receptor for laminins. Recent studies from our laboratory have indicated an important role for this molecule in leukocyte migration in vivo (18), findings that will be discussed in this review.

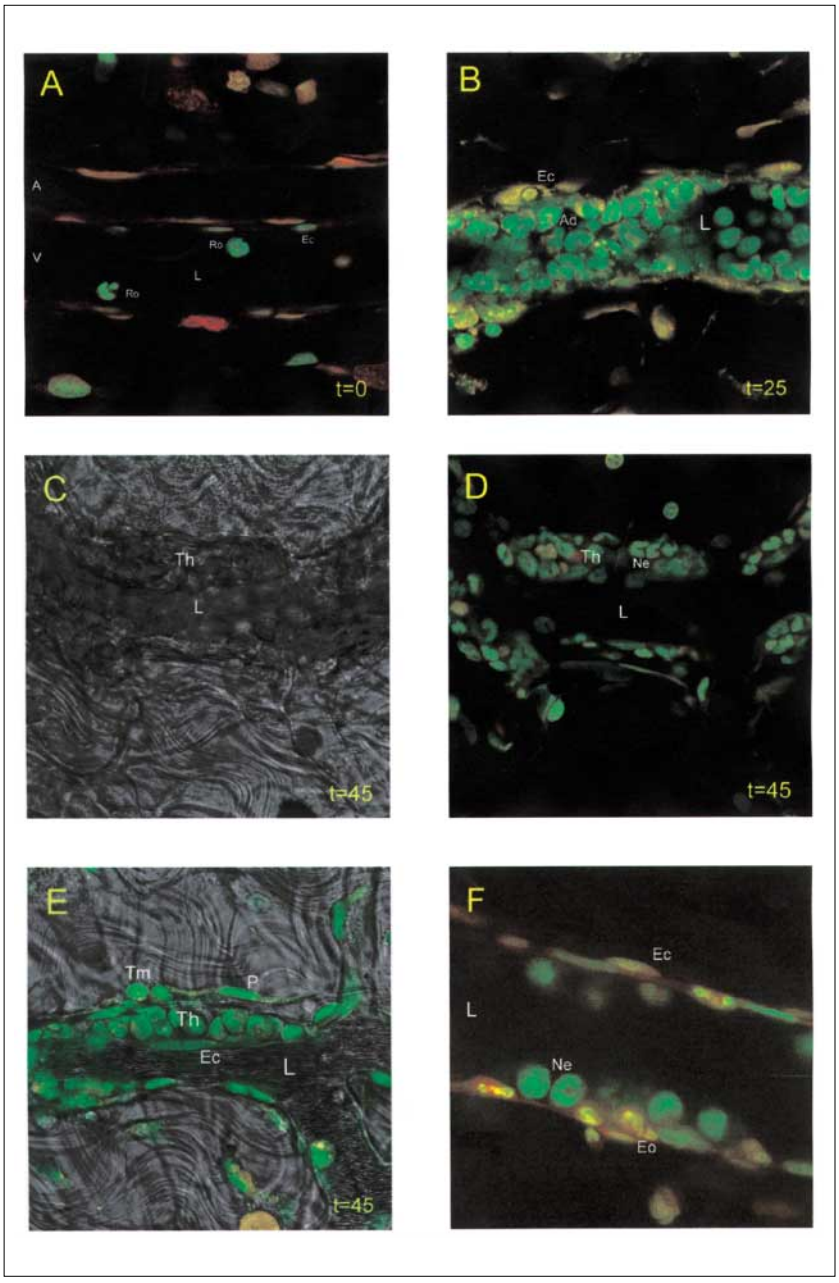

Figure 2: Leukocyte transmigration through rat mesenteric post capillary venules as visualised by brightfield and confocal intravital microscopy. Chemoattractant-stimulated venules (~20-40 μm) were stained with topical acridine orange and observed at different time-points post stimulation. Panel A shows an unstimulated post-capillary venule exhibiting rolling neutrophils only. Adherent neutrophils can be visualised within the lumen of the vessel 25 minutes following application of the chemoattractant (Panel B). Panels C and D show a post capillary venule at 45 minutes, as observed by brightfield and confocal microscopy, respectively. The images clearly show thickened venular walls as a result of tightly packed neutrophils between the endothelium and the perivascular basement membrane. Panel E is a merged brightfield and fluorescent image, further demonstrating the accumulation of neutrophils within venular walls during transmigration. Panel F demonstrates the ability of confocal intravital microscopy to distinguish neutrophils and eosinophils. The image shows a mouse cremasteric venule stimulated by the eosinophil specific chemokine eotaxin (CCL11) in which neutrophils are seen rolling but an eosinophil is seen transmigrating through the vessel wall. A=arteriole; V=venule; Ro=rolling neutrophils; Ec=endothelial cells; Ad=adherent neutrophils; L=lumen; Th=thickened vessel, Ne=neutrophils; P=pericyte; Tm=transmigrated neutrophils; Eo=eosinophils.

Methods of investigating leukocyte transmigration

The field of leukocyte transendothelial migration has benefited immensely from in vitro techniques that allow in depth analysis of molecular and signalling mechanisms involved in the process, under defined experimental conditions. However, observations from such in vitro studies have to be extrapolated to the in vivo situation with much caution, as even the most elaborate in vitro models do not completely mimic the in vivo vessel wall structure. Some clear discrepancies between the in vivo and in vitro scenario that need to be considered include: (i) commonly used cultured endothelial cell monolayers do not form physiologically relevant levels of junctional complexes except under special culture conditions (19). (ii) The process of cell culture may by itself alter the true phenotype/responsiveness of the endothelial cells, (iii) pericytes, an integral part of the vessel wall, and rarely incorporated into in vitro models, are known to express key adhesion molecules on their cell surface (20) and may play an active role in regulation of transmigration. (iv) Commonly used pseudo-basement membrane structures, such as matrigel, do not closely mimic the true vascular endothelium basement membrane in composition and may therefore provide a non-physiological adhesive substrate (and by extension, signals) for the endothelium and leukocytes. The differences in profiles of leukocyte transmigration as observed using *in vitro* and *in vivo* models are clearly illustrated by the noted differences in their time-courses. For example, neutrophil migration through venular endothelial cells in vivo occurs within 15-45 minutes (Fig. 2) as compared to transendothelial cell migration detected in vitro which reportedly occurs within 2 minutes (21).

Leukocyte transmigration is studied *in vivo* using a number of models. The most common approach is to quantify the migration of leukocytes into a body cavity such as the pleural or peritoneal cavity following the local instillation of a non-specific pro-inflammatory stimulus such as thioglycollate or glycogen, or as commonly used by our group, a defined inflammatory mediator such as IL-1β or IL-4 (18, 22, 23). Of course, leukocyte infiltration can be induced in any organ (e.g. skin, lung, heart) and tissues analysed *ex-vivo* by standard histological and/or electron microscopic techniques. Whilst providing valuable information, the above approaches are not ideal as quantification of leukocyte migration into a cavity does not distinguish between the different stages of leukocyte emigration and *ex-vivo* analysis of tissue sections clearly fails to provide data in real-time. One experimental approach that has been invaluable in dissecting the molecular interactions involved at different stages of leukocyte emigration in real-time is the technique of intravital microscopy (IVM).

Intravital microscopy allows the direct observation of leukocyte/endothelial cell interactions within the microcirculation of living animals and usually requires surgical exteriorisation and exposure of the tissue of interest in anaesthetised animals, though chronically implanted chambers, such as the rabbit ear chamber, allow observations of leukocyte/endothelial cell interactions in conscious animals. A number of tissues have been studied using this experimental approach, the most commonly used being the cremaster muscle, mesentery and the hamster cheek pouch and dorsal skinfold chamber (24). The transparency of the aforementioned organs allows the microcirculation to be visualised with ease by trans-illumination, whereas solid organs such as brain, liver and spleen require epi-illumination and fluorescent labelling of leukocytes to elucidate leukocyte/endothelial cell interactions within their microcirculation. Despite the increasing application of IVM to investigations of leukocyte/endothelial cell interactions in different organs, a large percentage of studies employing this technique are still limited to a small selection of tissues (e.g. cremaster muscle and mesentery), an issue that may ultimately affect the progress of our understanding of tissue-specific inflammatory events (e.g. 25).

The great strength of IVM is that it enables direct observation, and hence investigation, of the different stages of leukocyte migration, i.e. rolling, firm adhesion and transmigration, within a microvascular bed in real-time. However, IVM, as is commonly performed using standard bright field microscopy also has its limitations. For example, different leukocyte sub-types cannot be accurately distinguished during the experimental procedure and hence the nature of the emigrated leukocytes is often determined ex-vivo following dissection and staining of tissues. Furthermore, and of particular relevance to our interest in the mechanism of leukocyte migration through vessel walls, bright field IVM does not allow observations of leukocyte behaviour within the venular wall, ie between the endothelium and the perivascular basement membrane. We have recently overcome these limitations by extending our standard IVM procedure with bright field microscopes to a confocal microscope, as illustrated by the images in Figure 2. Careful use of the laser light as a source of illumination provides images of such high resolution that it allows distinctions to be made between different leukocyte sub-types, enabling the experimenter to quantify temporal and differential leukocyte migration profiles in real time. Furthermore, the ability of a confocal microscope to provide optical sectioning means that the use of this experimental approach has enabled us to directly visualise leukocytes at various stages of their migration through vessel walls, ie pre-, during, and post-migration through endothelial cells in 3D in time-lapse (Fig. 2).

The images in Figure 2 demonstrate the time-course and different stages of leukocyte transmigration. Following topical application of a chemoattractant such as the chemokine IL-8, an initial increase in leukocyte rolling flux and reduced rolling velocity is followed by a rapid leukocyte firm adhesion

response, often within 2-10 minutes of application of the chemokine. The number of adherent cells increases in a time-dependent manner such that maximal adhesion occurs at around 20-30 minutes after application of the mediator (Fig. 1B). As the adherent leukocytes begin to migrate through venular walls, a progressive thickening of the vessel wall is noted (30-45 minutes) (Fig. 1C). Transmigrated leukocytes in the extravascular tissue are first seen at ~45 minutes post application of the chemoattractant, with the response continuing whilst the tissue is exposed to the stimulus. Of interest, although the adhesion response appears to be generalised throughout the length of the post capillary venule, transmigration appears to occur preferentially at specific points along the vessel wall, with leukocytes very often budding off preferentially from one or two points along the vessel. The reason for this localised *in vivo* transmigration response is unclear but maybe related to the in vitro findings of Burns and colleagues, indicating preferred leukocyte transmigration through endothelial cell tricellular corners (19). It is also possible that once the endothelium and the basement membrane are breached by one leukocyte, sufficient "signals" are generated to promote/facilitate the migration of other leukocytes via the same route. These signals maybe in the form of released chemoattractants from the emigrating neutrophils or endothelial cells or maybe cleavage products of basement membrane constituents such as fragments of laminin reported to be chemotactic for neutrophils. Additionally, the signals maybe intracellular such as increased levels of Ca^{2+} in endothelial cells (26) signals that may promote a change in phenotype of endothelial cells leading to facilitated migration of leukocytes. Such a change in endothelial cell phenotype maybe associated with altered expression of adhesion molecules involved in leukocyte transmigration. In this regard, Muller and colleagues have recently shown that leukocyte transmigration is associated with increased availability of endothelial cell surface PECAM-1 (27), a mechanism by which leukocyte emigration through endothelial cell junctions may be supported and propagated.

Leukocyte migration through endothelial cells

Mechanisms and factors mediating the migration of leukocytes through the endothelial layer are described briefly in the following paragraphs, as they will be comprehensively covered in other reviews in this series.

Although the tight and adherens junctions provide an effective barrier to emigrating leukocytes, it is generally considered that the dominant route by which leukocytes penetrate the endothelial cell barrier is via a paracellular route i.e. through endothelial cell junctions. In this context, as already discussed, leukocytes may preferentially migrate through sites where the junctional complexes are less tight, i.e. at tricellular corners as has been demonstrated by Burns et al. (19) and/or may simply cause transient disruption of the VE-cadherin-catenin complex as they penetrate the endothelial cell junctions (28). There is however at present a renewed interest in the potential ability of leukocytes to migrate through endothelial cells via a transcellular route, i.e. migration through the body of the endothelium, a debate that has been rekindled by the work of Feng et al. In this study, through detailed electron microscopic analysis of FMLP-injected guinea-pig skin sites, the authors provide convincing results indicating leukocyte migration via a transcellular route (29). The significance of these observations to leukocyte migration in different tissues and in response to different stimuli is at present unclear.

Despite the above debate regarding the route of leukocyte emigration, there is no doubt that endothelial cell junctions do express key molecules that can support leukocyte transendothelial cell migration. These include PECAM-1 (CD31), CD99, ICAM-2, and members of the JAM family, molecules for which there is now convincing evidence with respect to a functional role in leukocyte transmigration from either in vitro and/or in vivo studies. Details of the precise involvement of such molecules is however currently unknown and is likely to be complex as the available data implies differential roles of endothelial cell junctional molecules in mediating different stages of leukocyte transmigration and/or transmigration of different leukocyte subtypes as well as perhaps mediating leukocytes transmigration in different vascular beds. For details, the reader is referred to recent reviews (30) or other articles in the present series.

Other key factors that are now known to play a significant role in regulating leukocyte migration through endothelial cells include the nature and localisation of chemokines, ie lumenal or ablumenal, that may determine the ability of leukocytes to migrate on and through the endothelium. In addition, there is now clear in vitro evidence for a requirement for physiological shear stress in promoting/accelerating transendothelial migration of leukocytes across stimulated cultured endothelial cells (31, 32).

Leukocyte migration through the perivascular basement membrane

The perivascular basement membrane (BM) provides a distinct barrier to emigrating leukocytes and as demonstrated in Figure 2, once leukocytes reach this structure, their continued emigration is delayed until the BM is penetrated. Details of the mechanisms used by leukocytes to cross the perivascular basement membrane are unknown though the lateral organisation of the BM protein networks (such as collagen IV and laminin) together with the known resistance of the BM to mechanical damage strongly suggests that this barrier cannot be easily penetrated. This argues against the possibility that leukocytes physically push their way through it. In this context, leukocyte proteases

are frequently considered as key players in the disruption of BM constituents and indeed a number of in vitro studies have implicated certain leukocyte proteases in this response (33, 34). The involvement of leukocyte proteases in the breaching of the BM is however a contentious field. In an elegant study employing a highly detailed in vitro model of the vessel wall, Huber and Weiss demonstrated that neutrophil migration through the subendothelial cell basement membrane was associated with a significant disruption of the retentive properties of the basement membrane though this was not dependent on neutrophil elastase or cathepsin G and was resistant to a broad range of leukocyte protease inhibitors (35). In support of these in vitro observations there is at present no *in vivo* evidence for the involvement of leukocyte proteases in migration of leukocytes through the perivascular basement membrane. Indeed as leukocyte migration through the perivascular basement membrane does not lead to any gross and/or irreversible disruption of the BM structure, it is more likely that if involved, leukocyte proteases play a subtle and regulated role in aiding this stage of leukocyte emigration. For example, it is conceptually possible that leukocyte proteases may be required for exposing key binding sites within BM constituents with which leukocyte receptors can then interact. In this context we are currently investigating the potential co-operative interaction of leukocyte proteases with leukocyte adhesion molecules, e.g. β_1 integrins, in mediating leukocyte migration through the perivascular basement membrane. Interestingly, although Huber and Weiss could not identify the mechanisms by which neutrophils disrupted the BM, the study did note that the transient loss in the integrity of the BM was repaired by the overlying endothelium via a mechanism that required active protein and RNA synthesis, a mechanism that maybe related to the generation of key components of the BM by the endothelium.

Apart from proteases, a number of adhesion molecules have also been implicated in the process of leukocyte migration through the perivascular basement membrane. One such molecule is PECAM-1, a molecule that appears to be unique in that there is now strong evidence from in vitro and in vivo studies for its involvement in leukocyte migration through both endothelial cells and its associated BM (30, 36-40). Initial indications for the involvement of PECAM-1 in leukocyte migration through the basement membrane came from in vitro studies of Muller and colleagues in which it was demonstrated that antibodies to Ig domain 6 of this molecule had no effect on monocyte transendothelial cell migration but blocked their subsequent migration into the underlying collagen gel (36). About the same time, our group was involved in a study where the effect of an anti-PECAM-1 polyclonal antibody, recognising rat PECAM-1, on neutrophil migration through IL-1 β-stimulated rat mesenteric venules was being investigated as observed by intravital microscopy. The study led to the surprising observation that the anti-PECAM-1 antibody blocked neutrophil migration at the level of the perivascular basement membrane, as analysed in detail by electron microscopy (40). More recently, defects in leukocyte migration through the basement membrane have been detected in genetically modified mice lacking PECAM-1 (37, 39). Whilst the localisation of PECAM-1 at endothelial cell junctions and its ligand binding profile supports a functional role for this molecule in leukocyte transendothelial cell migration, the mechanism by which PECAM-1 could regulate leukocyte migration through the BM appeared less clear and confusing. In this context, as there is no evidence for a direct interaction between PECAM-1 and components of the BM, the ability of PECAM-1 to regulate expression/function of leukocyte integrins (41-44) as a potential mechanism of PECAM-1-dependent leukocyte migration through the BM was considered. Hence, as laminin is a major constituent of mammalian basement membranes, and in light of the evidence for the involvement of the principal leukocyte laminin receptor $\alpha_6\beta_1$ in leukocyte migration through this barrier (32, 45-47), we have recently investigated the role of PECAM-1 in the functional role and regulation of expression of $\alpha_6\beta_1$ during neutrophil transmigration *in vivo*. In initial studies we demonstrated that an anti-α_6 integrin mAb blocked neutrophil migration through IL-1β-stimulated mouse cremasteric venules at the level of the perivascular basement membrane, as observed by intravital and electron microscopy (18). As this inhibition was only observed in wild-type mice and not in PECAM-1 deficient mice, we next investigated the possible association of PECAM-1 with $\alpha_6\beta_1$ expression. For this purpose we examined the expression of $\alpha_6\beta_1$ on transmigrated neutrophils in both wild-type and PECAM-1 deficient mice, studies that indicated a critical role for PECAM-1 in transmigration-induced increased expression of $\alpha_6\beta_1$. Furthermore, using chimeric mice that lacked either leukocyte or endothelial cell PECAM-1, as obtained by bone-marrow transfer, the study provided in vivo evidence for a role for both leukocyte and endothelial PECAM-1 in this response. Hence, homophilic PECAM-1 interaction mediates both neutrophil transmigration and increased expression of $\alpha_6\beta_1$ on transmigrated neutrophils in vivo. In more recent studies we have found evidence for further regulatory roles of PECAM-1 indicating additional mechanisms involving PECAM-1 that may be of relevance in the context of leukocyte transmigration through the BM. Specifically, we have found that MMP-9, the matrix metalloproteinase, is up-regulated on transmigrating eosinophils in vivo, a response that is absent in PECAM-1 deficient mice (48).

Despite the growing evidence and the mechanistic observations implicating PECAM-1 in the process of leukocyte transmigration through the perivascular BM, there is evidence for PECAM-1-independent mechanisms. For example, the observed defect in leukocyte migration through the BM in PECAM-1 deficient mice is both transient and stimulus specific (37). In addition, our findings demonstrated a lack of inhibi-

tory effect of the anti-α_6 integrin mAb in PECAM-1 KO mice, indicating the existence of PECAM-1- as well as $\alpha_6\beta_1$-independent mechanisms of leukocyte transmigration (18). Future studies in our laboratory are aimed at identifying the PECAM-1-independent mechanisms of leukocyte migration through the perivascular basement membrane with the view of obtaining a better understanding of the molecular interactions that mediate this critical but poorly understood stage of leukocyte migration.

Consequences of leukocyte transmigration

In addition to aiding the passage of leukocytes through endothelial cells and the perivascular basement membrane, the process of transmigration appears to prepare the leukocytes for subsequent responses such as migration in the extravascular tissue, increased degranulation and phagocytosis, critical leukocyte effector responses in host-defence (Fig. 1). Full details of this change in leukocyte phenotype, that will without doubt vary depending on the vascular bed through which leukocytes emigrate, the leukocyte sub-types and inflammatory stimulus driving the transmigration response, are beyond the scope of this mini-review but some specific examples related to neutrophils are given below.

Although until recently neutrophils were considered to express very low levels of β_1 integrins, it is now very clear that transmigrated neutrophils express significant and functionally relevant levels of β_1 integrins. Specifically, the molecules $\alpha_6\beta_1$, $\alpha_4\beta_1$ and $\alpha_2\beta_1$ have all been shown to be up-regulated on transmigrated neutrophils, responses that can aid the migration of leukocytes through the basement membrane and the extravascular tissue (18, 49-51). Similarly, in vivo, transmigrated neutrophils express cell surface neutrophil elastase, a response that unlike regulation of expression of $\alpha_6\beta_1$ (see previous sections) (18) appears to be PECAM-1-independent (Wang et al., manuscript in preparation). The mobilisation of neutrophil elastase to the cell surface does however appear to be regulated by molecular events involved in the process of transendothelial cell migration as illustrated by the in vitro findings of Cepinskas et al. (52). As discussed in previous sections the functional role of neutrophil elastase is at present unclear but maybe related to regulation of leukocyte migration through venular walls and extravascular tissue as well as regulation of mediator generation/cleavage and/or leukocyte phagocytic responses at sites of inflammation (53).

It is well accepted that transmigrated neutrophils express increased levels of β_2 integrins and there is evidence to suggest that neutrophils adherent to extracellular matrix proteins undergo β_2 integrin mediated degranulation and release of reactive oxygen species when stimulated with chemoattractants (54). Furthermore, the integrin $\alpha_M\beta_2$ has been shown to be activated following ligation of PECAM-1 (42), an interaction that may enhance the ability of leukocytes to mediate particle engulfment and phagocytosis as well as FcγRIII mediated release of reactive oxygen species. Collectively, transmigration should be considered not only as a mechanism by which leukocytes traverse the vessel wall, but a cellular event through which leukocyte responsiveness beyond the endothelium is regulated.

Conclusion

Although leukocyte-endothelial interactions leading to leukocyte rolling, firm adhesion and transendothelial cell migration are common to all leukocytes and microvascular beds, a remarkable degree of diversity is built into these interactions. For example, the profile of leukocyte migration is highly dependent on the phenotype of the vascular endothelium that could in turn determine the profile of adhesion molecules and the nature of chemokines expressed by the vascular lumen. In addition, the distribution of chemokine receptors and integrins on leukocytes and their activation by different inflammatory stimuli, will govern the behaviour of leukocytes within stimulated vascular beds. Such factors may contribute to tissue-specific mechanisms of leukocyte transmigration leading to distinct patterns of leukocyte migration in different organs in response to the same stimulus (22, 55). A better understanding of tissue-specific mechanisms of leukocyte emigration could contribute to the development of more specific anti-inflammatory therapeutic strategies for the treatment of tissue-specific disorders such as asthma, rheumatoid arthritis and ARDS.

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