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Emerging Understanding of Roles for Arterioles in Inflammation

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Abstract

Arterioles, capillaries and venules all actively change their cellular functions and phenotypes during inflammation in ways that are essential for maintenance of homeostasis and self-defense, and are also associated with many inflammatory disorders. Endothelial cells (ECs), together with pericytes and extracellular matrix proteins, can regulate blood flow, the coagulation cascade, fluid and solute exchange, and leukocyte trafficking. While capillary and venular functions in inflammation are well characterized, the arteriolar contribution to inflammation has only recently come into focus. Arterioles differ from venules in structure, EC morphology, shear environment, expression and distribution of surface ligands, hence regulation and function of arteriolar wall cells during inflammation may also be distinct from venules. Recent work indicates that in response to pro-inflammatory stimuli, arterioles alter barrier function, and support leukocyte and platelet interactions through upregulation of adhesion molecules. This suggests that in addition to their role in blood flow regulation, arterioles may also participate in inflammatory responses. In this review we will discuss mechanisms that characterize arteriolar responses to proinflammatory stimuli. We will detail how distinct arteriolar features contribute to regulation of barrier function and leukocyte-EC interactions in inflammation, and further highlight the potential priming effects of arteriolar responses on venular function and progression of inflammatory responses.

Keywords

endothelial cells; morphology; permeability; leukocytes; neutrophils; adhesion molecules; shear stress; inflammatory responses

Endothelial cells form a semi-permeable barrier that is a primary player in regulating both the exchange of fluid and solutes and leukocyte trafficking in both healthy tissue and disease. This barrier is essential for proper tissue function and homeostasis. A very widely accepted paradigm is that small venules and capillaries are the main site for solute and fluid exchange, while leukocyte extravasation occurs mainly in post capillary venules. However, emerging evidence (34–36, 96, 98) suggests that arterioles, while playing a key role in regulation of blood pressure and flow, can also alter their barrier function in response to stress or pro-inflammatory stimuli, thus contributing to fluid and solute exchange. Furthermore, activated arterioles can support leukocyte interactions (8, 37, 50, 100), which may have important implications given recent findings suggesting that in microvessels, leukocyte engagement of surface endothelial receptors contributes significantly to the regulation of barrier function (5, 98). Not surprisingly, dysregulation of arteriolar EC function results in significant changes, including thickening of the vessel wall and impaired

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metabolic and inflammatory responses; these changes are critically associated with multiple disorders, including thrombosis, atherosclerosis, hypertension, heart disease and stroke.

Endothelial cells and barrier function

In an integrated microvascular network, small arterioles diverge into capillaries, which in turn converge into post capillary venules. Vascular ECs lining the lumen of these vessels serve as a semi-permeable barrier that separates blood contents from the interstitial compartments (64). Small molecular weight substances including gases, ions and small solutes can cross the endothelium by passive diffusion or via active carriers, driven ultimately by their concentration gradient. However, both the transport of larger solutes such as albumin, as well as diapedesis of immune cells that reside in the blood, are restricted, and regulation of these processes is substantially, although of course not exclusively, a function of the endothelial monolayer. Gradients of macromolecules such as albumin contribute to the net flux of water (73, 108), as well transport of nutrients, lipophilic fatty acids, hormones, and other metabolic by-products across the vessel wall (109). Thus maintaining proper barrier function, with tight regulation of solute and water exchange between the blood and the extravascular space, is essential for tissue homeostasis under both normal and pathological conditions.

Importantly, in addition to regulating solute and fluid exchange, ECs play an important role in regulating leukocyte trafficking, both for surveillance purposes in normal tissue (10) and leukocyte recruitment during inflammation (18, 62, 66). In response to inflammatory stimuli, the barrier becomes more permissive to water and macromolecules as well as to extravasating leukocytes. Unless the inflammation is resolved and the inflammatory cues subside, these alterations in the endothelial barrier will ultimately be manifest as tissue edema and/or inflammatory diseases including, for example, acute respiratory distress syndrome, rheumatoid arthritis, inflammatory bowel disease and atherosclerosis.

Both fluid and solute exchange and leukocyte TEM are generally assumed to occur exclusively in capillaries and postcapillary venules, thus the regulation of capillary and venular permeability in normal and inflamed tissue has been extensively studied (reviewed in (64, 65)). Similarly, leukocyte interactions with venular ECs have been well characterized (reviewed in (62, 67, 83)). Arterioles on the other hand, have been somewhat neglected, and their barrier function properties are still poorly understood.

Why should we care about arterioles?

The immediate answer is because arteriolar EC function changes in response to inflammatory cues. As arterioles do not exist in isolation, but are a part of an integrated vascular network, responses upstream in the arteriolar components of the network are likely to affect cellular functions downstream (in capillaries and the venular microcirculation), thus contributing to the global outcome.

Arterioles are small resistance vessels and unlike venules are wrapped circumferentially by one or more layers of smooth muscle cells (71, 106). This morphology, together with their ability to locally vasodilate or vasoconstrict in response to a wide variety of chemical and electrical messages, defines arterioles as modifiers of resistance to flow and regulators of blood pressure. However, in addition to these fundamental homeostatic functions, (which in turn have the capacity to increase convective flux of material across the arteriolar wall), there is emerging evidence (presented in this review) that arterioles also contribute directly to solute exchange via changes in permeability, and furthermore, play a role in regulating leukocyte trafficking. Although the combined surface area of capillaries and venules is usually estimated as being at least five times that of arterioles, arterioles nevertheless offer a

considerable surface area that can support leukocyte-EC interactions and the exchange of solutes and fluid, thus their contribution to tissue homeostasis has the capacity to be significant. There is therefore good reason to seek to understand mechanisms regulating the permeability of arterioles to solutes and fluid on the one hand, and to leukocytes on the other, and to determine whether they differ from or are similar to these processes as observed in venules.

Arteriolar responses to inflammatory cues

It is well established that in response to inflammatory stimuli post capillary venular permeability to both water and solutes dramatically increases. It is also established that venules support leukocyte interactions, and during inflammation, through upregulation of a variety of surface adhesion molecules, these vessels permit rapid mobilization of leukocytes from the circulation into the interstitium. Importantly, similarly to venules, arteriolar responses to inflammatory stimuli are also characterized by increased expression of EC adhesion molecules, enhanced solute permeability, and initiation of leukocyte-EC interactions, as we discuss below.

Changes in adhesion molecule expression and distribution

EC adhesion molecules act as both adhesive ligands for leukocytes and as signaling receptors for ECs, thus expression and distribution of adhesion molecules is essential for leukocyte recruitment and EC function. In venules, members of the selectin family, *viz* P-, E- and L-selectin (54), as well members of the immunoglobulin superfamily, such as ICAM-1 (57, 115), VCAM-1 (15, 105), and PECAM-1 (68), are essential for leukocyte recruitment. Similarly, VE-Cadherin expressed at the interendothelial junctions is essential for the integrity of the vessel wall and, along with VE-cadherin-associated junctional accessory molecules, can play an important role in leukocyte TEM (6, 92, 99). We have shown that under normal (unstimulated) conditions arteriolar ECs, similarly to venular ECs, express low but detectable levels of P- and E-selectin, as well as ICAM-1 and VCAM-1 (100, 101). Importantly, numerous studies have shown that the expression of adhesion molecules in arterioles in various tissue beds is significantly increased during inflammation. For example, four hours activation with TNF α results in a dramatic increase in the expression of P- and E-selectin, as well as ICAM-1 and VCAM-1 in cremaster muscle arterioles (100). Similarly, treatment with exogenous Ang II, or treatment with L-NAME to block eNOS, (which produces elevated levels of endogenous Ang II) results in upregulation of these same four adhesion molecules in arterioles (69, 78). Increased ICAM-1 expression has also been observed in arterioles in transplanted rat lungs post reperfusion (20), and after PAF stimulation of cultured coronary microvascular ECs (33). Similarly, VCAM-1 expression is increased in kidney arterioles in renal disease (13).

Leukocyte interactions with ECs are dependent on, and are defined by, the expression of surface adhesion molecules, thus increased expression of adhesion molecules in arteriolar ECs is likely one of the main reasons for the induction of leukocyte-EC interactions in arterioles. Additionally, some adhesion molecules appear to serve as signaling receptors regulating EC solute permeability. Specifically, ICAM-1 mediated signaling has been directly implicated in increased albumin permeability in arterioles (96, 98).

Induction of leukocyte-EC interactions

While leukocyte rolling interactions are observed in control venules (43, 49, 100), and leukocyte adhesion and TEM are observed after activation (54), in arterioles leukocyte-EC interactions are induced only under certain inflammatory conditions. For example, leukocyte rolling is observed in cremaster muscle arterioles after treatment with the pro-inflammatory

cytokines IL-1 and TNF α (50, 96, 101, 104). Leukocyte adhesion in arterioles is stimulated by ischemia and reperfusion (40, 56), and in the presence of physiologically relevant doses of Ang II, whether through exogenous administration (8), or release of endogenous Ang II after blockade of NOS (69).

Changes in macromolecular permeability

It is well recognized that both capillary and venular permeability are increased during inflammation. Interestingly, there is evidence that coronary arteriolar permeability is also regulated in a dynamic way, as coronary arterioles increase their permeability to proteins as an adaptation to exercise training (34), or, acutely, during exposure to adenosine (35). These findings provided some of the first direct evidence for regulated solute exchange in arterioles. Although capillaries provide the vast majority of surface area for exchange, the finding that arteriolar permeability is also regulated is of great significance as metabolically active tissue also, of course, lies in proximity to arterioles, which in cardiac tissue, appears, in focal areas, to be devoid of capillaries (35). Recently we extended these findings to demonstrate, in a skeletal muscle model, that arterioles not only support solute exchange, but that this exchange is tightly regulated in arterioles just as it is in venules under both normal non-stimulated conditions and during inflammation (96, 98). Using intravital confocal microscopy we showed that arteriolar permeability to albumin under non-inflamed conditions was controlled through PKC-mediated signaling. However upon activation with TNF α this was rapidly altered to become independent of PKC but dependent on Src activation (98). These data imply that different mechanisms are key for regulation of permeability in unstimulated vs activated conditions, an idea that has yet to be tested directly but which has important implications for how we view this regulation as part of homeostatic compared to inflammatory mechanisms. Increased arteriolar permeability has also been observed in diabetic or VEGF activated rat retinas (11). Furthermore in this work the authors found that the observed changes in vascular permeability were initiated in arterioles and later observed in post-capillary venules, suggesting that indeed, in intact vascular networks, upstream responses (in arterioles) can affect the function of downstream vessels (venules).

Distinct features of arterioles as compared to venules and consequent effects on permeability and leukocyte-EC interactions

Arterioles differ from venules in their structure, EC morphology, and shear environment, as well as in expression and distribution of surface ligands, suggesting that the regulation of endothelial barrier function in arterioles may also be distinct from venules. In the next paragraphs we discuss the possible implications of these distinct features of arterioles on regulation of solute permeability and leukocyte-EC interactions.

Structure

In venules, macromolecules and leukocytes crossing the vascular wall sequentially encounter the glycocalyx, endothelial cells, an extensive although discontinuous pericyte layer, and the extracellular matrix (ECM) (53). Arterioles are distinct from venules in that arteriolar ECs are also circumferentially wrapped by one or more layers of SMC on the abluminal side of the EC basal lamina. Thus, in contrast to the venular wall that is relatively thin, the arteriolar wall is a thick, multilayered structure, as illustrated in the electron micrographs (Fig 1). This wall structure supports rapid communication between ECs and SMCs (27, 55, 113), and is pivotal for regulation of vascular resistance and hence is essential for regulation of blood flow (110). Interestingly, whether this wall structure impedes or promotes solute exchange and recruitment of immune cells is still not clear. One possibility is that the additional SMC layer in arterioles might pose an obstacle for

macromolecular flux across arteriolar wall and potentially affect leukocyte TEM. Indeed, to date leukocyte TEM has not been observed in arterioles. A tight barrier is presumably beneficial for healthy tissue where efflux of luminal antigens, blood proteins and immune cells must be contained. On the other hand, bi-directional communication between ECs and SMCs might contribute to regulation of responses that facilitate solute exchange. Indeed, there is clear evidence that macromolecules can cross both the EC and SMC layers as they traverse the arterial wall. Early work using electron microscopic observations in normal blood vessels showed that large molecules such as horse radish peroxidase (~ 40 KD) can cross both EC and SMC layers within 15 min of intravenous injection in both rat aortas and mouse carotid arteries (23). Moreover, under pathological conditions such as formation of atherosclerotic plaques or thrombosis, the SMC layer undergoes drastic reorganization leading to a compromised vessel wall barrier, thus allowing enhanced leakage of luminal contents (41).

In capillaries and post-capillary venules, ECM components and the pericyte sheath appear to play an important role in maintaining blood vessel integrity and regulation of both vascular permeability and leukocyte extravasation. For example, expression of the ECM component hyaluronan has been directly linked to the endothelial barrier, with higher hyaluronan expression being associated with lower permeability (82). EC attachment to the ECM (through integrin receptors) can stabilize EC junctions, thus limiting vascular leakage (64). Not surprisingly, patients with collagen vascular diseases show increased capillary permeability (60). Furthermore, dysregulation of matrix metalloproteases impairs matrix integrity hence EC function and survival (85). Moreover ECM-EC interactions have the ability to induce signaling events in ECs, thus regulating EC barrier function. For example, ECM interactions with $\alpha_v\beta_3$ integrin result in activation of VEGFR-2 (94), ligation of which can increase endothelial permeability (76).

Arteriolar ECs are also known to assemble ECM, and to recruit pericytes (9). However, to date the contribution of the pericyte sheath and the ECM to regulation of barrier function and leukocyte-EC interactions in arterioles has not been explored.

Interestingly it has been noted that matrix protein composition is different between microvessel and large vessel endothelia (16). Since different ECM proteins interact with different endothelial receptors, these findings suggest that the distribution of ECM proteins and subsequent specific ECM-EC interactions could help determine the barrier properties of different types of microvessels. This also raises the possibility that matrix composition surrounding venules might be different from that surrounding arterioles; an idea that should be explored in future work.

Contractile pericytes that are wrapped around capillaries in retina and cerebral circulation have been implicated in regulation of blood flow by controlling capillary diameter (79). Pericytes that are intimately associated with venular ECs within the region of the venular basement membrane have been implicated in regulation of leukocyte TEM, with migrating leukocytes appearing to traverse gaps in the pericyte sheath (81). Similarly, variations in the composition and density of the venular basement membrane are implicated in regulation of leukocyte TEM (107). Furthermore, leukocyte binding to ECM components during TEM can affect leukocyte function. For example, neutrophil β_2 -integrin interactions with the ECM protein fibrinogen regulate neutrophil apoptosis, hence contributing to neutrophil clearance during inflammation (114). Since the cellular morphology of the arteriolar wall with respect to the arrangement and density of SMCs, pericytes, and ECM, differs substantially from that in venules (71), it is not unreasonable to infer that regulatory processes for permeability of the arteriolar wall to macromolecules and to leukocytes will also likely differ from mechanisms defined in the venular microcirculation.

Intriguingly, we and others have shown that leukocyte TEM in venules is not random, but is localized to specific regions, which we have termed “portals”. The majority of these portals are tri-cellular EC junctions enriched in ICAM-1 expression (14, 99). Together with the findings outlined above, this suggests that there are preferred locations for leukocyte TEM across the intact venular wall. It is thus reasonable to speculate that the portals in the endothelial monolayer may be aligned with gaps in the loose network of pericytes, and with the low-density regions in ECM proteins. If this is indeed the case, then through as yet undefined signaling mechanisms, ECs may have the capacity to indicate to migrating leukocytes (possibly through clustering of ICAM-1) the location of regions with preferred local morphology and protein expression such as will support their egress into the tissue. Whether these phenomena contribute to differences in permeability responses between arterioles and venules is not yet clear, but certainly EC morphology (discussed next) differs between arterioles and venules in a way that suggests that this factor has the capacity to be a contributing factor.

EC morphology

Morphometric analysis in both isolated cell systems and in intact blood perfused microvessels has revealed that size and shape of individual ECs varies in different sized vessels, and that ECs are distinctly different between arterioles and venules (4, 99, 111). In arterioles, EC surface area increases as a function of vessel size, from $\sim 300 \mu\text{m}^2$ in small arterioles (10–30 μm in diameter) to greater than $1500 \mu\text{m}^2$ in larger arterioles ($> 100 \mu\text{m}$ in diameter). In contrast, the size of venular ECs does not change in different sized vessels (and remains $\sim 1200 \mu\text{m}^2$) (95). Importantly, ECs in arterioles are distinctly long and narrow (length/width ratio $\sim 8:1$) compared to venules, which are significantly more rounded (length/width ratio $\sim 2.5:1$) (95, 99). Intriguingly in arterioles with larger diameters, ECs become more elongated, while in larger venules ECs become significantly wider (95).

This distinct EC morphology in arterioles is beneficial for rapid EC communication with SMCs as each elongated EC can directly interact with three or more SMCs to induce vasoactive responses. EC morphology in arterioles is also likely to affect the way leukocytes interact with the vessel wall. For example by default, EC morphology and alignment dictates the number of tri-cellular junctions per unit vessel length. As tri-cellular junctions are preferred locations for leukocyte transmigration (14, 99), the number of available locations will affect the efficiency of leukocyte TEM. Indeed we have recently shown that while the total junctional length per surface area of vessel wall is not different between arterioles and venules, the number of tri-cellular junctions is significantly lower in arterioles compared to venules (99). Furthermore, enhanced leukocyte TEM occurs in venular convergences (97), which due to their EC morphology have a significantly higher number of tri-cellular junctions per unit vessel length compared to straight venular regions (99). In contrast, the number of tri-cellular junctions in arterioles is not increased in divergences, presumably because the long and narrow arteriolar ECs are able to bend appropriately to properly accommodate vessel geometry (Fig 2). The specific characteristic of tri-cellular junctions that enables them to function preferentially as portals has yet to be determined; understanding what confers this specificity on some EC junctions and not others in neighboring cells is an important question that, when answered, should contribute significantly to a more integrated understanding of regulation of vessel wall permeability and/or TEM.

Adhesion molecules

Expression levels and spatial distribution of adhesion molecules on the endothelial surface will determine the timing and location of leukocyte interactions with ECs, and hence will significantly impact inflammatory responses. P- and E-selectins mediate leukocyte rolling

interactions in venules (1, 39, 43, 49), and the roles of members of the CAM family in mediating leukocyte adhesion and transendothelial migration in postcapillary venules have also been well characterized (15, 99, 115). While the distribution of some these molecules axially along the venular tree has been described (21), local variations in adhesion molecule density on the EC surface, in relation to leukocyte interactions, have to our knowledge, only been functionally documented for P-selectin and ICAM-1 (43, 101). While this information is at present limited to these two adhesion molecules, it has facilitated an enhanced understanding of the roles of arterioles and venules in microvascular wall permeability to macromolecules and leukocytes, as outlined next.

Surface expression of P-selectin and ICAM-1 in unstimulated arterioles is significantly lower than in unstimulated venules (100), however after stimulation with inflammatory cytokines, expression of these molecules is increased not only in venules, as has been well recognized (12, 63, 101), but also in arterioles (69, 101). Interestingly, although perhaps not surprisingly, leukocyte-EC interactions closely correlate with the expression levels of adhesion molecules in these two microvessel types. Thus, leukocyte rolling interactions are readily observed in unstimulated venules, however unstimulated arterioles, where adhesion molecule expression on the EC surface is close to undetectable, do not support leukocyte interactions with the vessel wall. Activated arterioles show increased expression of P-selectin and ICAM-1 to levels that are typically observed in unstimulated venules (100). Intriguingly, under these conditions, leukocyte rolling interactions are now supported in arterioles, but firm adhesion is rarely observed. In activated venules, expression of both P-selectin and ICAM-1 is further increased over the levels seen in unactivated venules or activated arterioles, resulting in leukocyte adhesion and TEM. Thus in general, relatively low expression of these adhesion molecules in both arterioles and venules is sufficient to sustain leukocyte rolling, however, a significantly higher expression is needed to support firm adhesion and TEM. The inability of activated arteriolar ECs to raise adhesion molecule expression to levels typical of activated venules presumably reflects one aspect of the larger question of what are the mechanisms underlying the differentiation of arteriolar versus venular ECs into cells of differing phenotypes. Importantly, while significantly increased over unstimulated conditions, the low expression of adhesion molecules in activated arterioles relative to activated venules is likely to be one of explanations for the absence of leukocyte emigration from activated arterioles. However, while leukocyte interactions in arterioles do not ultimately lead to TEM, we have recently shown that leukocyte interactions with ICAM-1 (which is also upregulated in inflamed arterioles) serve a different purpose, which is contribution to the regulation of arteriolar barrier function. Specifically, following TNF α treatment, engagement of ICAM-1 by rolling leukocytes in arterioles as well as venules results in changes in permeability to albumin (96, 98). Depletion of circulating neutrophils prevents this TNF α mediated increase in arteriolar albumin permeability (96). Importantly, antibody crosslinking of ICAM-1 in unstimulated, neutrophil-depleted arterioles increases permeability to a level similar to that seen following TNF α treatment (96, 98). Together, these findings argue that there is a direct contribution of leukocyte-EC interactions to regulation of arteriolar permeability specifically through engagement of ICAM-1.

In addition to differences in the expression levels, the distribution of adhesion molecules also varies between arterioles and venules, both with respect to specific regions of the microcirculatory network, and among individual ECs in equivalent microvessels. Immunostaining of intact blood perfused microvessels revealed high heterogeneity in the surface expression of both P-selectin (43) and ICAM-1 (101). Expression of these adhesion molecules in some regions along the vessel wall was significantly higher than in other regions (illustrated in Fig 3A). Not surprisingly these regions support increased leukocyte interactions.

Similarly, the distribution patterns of adhesion molecules differ from one molecule to another. For example P-selectin is expressed as punctuate clusters over the EC surface (Fig 3B, and (29, 43) and thus mediates stochastic leukocyte rolling interactions (43, 46). Moreover, while overall expression levels increase, the expression patterns of P-selectin do not change with inflammatory stimulus (100). This is consistent with the role of P-selectin in mediating initial interactions between leukocytes and ECs. ICAM-1 expression on the other hand is more diffuse over the EC surface (Fig 3B, bottom panel), and exhibits great variability in expression density both between adjacent ECs, as well as across the surface of individual cells (101). Interestingly, in inflamed vessels ICAM-1 expression is clustered at the regions of migrating leukocytes (99) and around actively migrating leukocytes (15).

While clustering of ICAM-1 can be observed in individual venular ECs, the expression of ICAM-1 is increased in all venular ECs upon activation (Fig 4A, (101)). In contrast, upon activation arteriolar ECs show redistribution of ICAM-1 among cells, where some cells increase and some decrease their ICAM-1 surface expression, magnifying the overall variability of ICAM-1 in the arteriole (Fig 4B, (101)). We speculate from this finding that some arteriolar ECs play a more important role in the inflammatory response than do others, but this idea, along with elaboration of the mechanisms that enable some of them to upregulate ICAM-1 surface expression while others appear to downregulate it, remains to be tested. One possibility is that ECs that downregulate surface ICAM-1 have upregulated expression of another key molecule in the overall inflammatory response, arguing possible specialization of specific regions of the arteriolar wall to mediate distinct functions, but again this idea will require testing. This general concept, ie that different ECs are specialized to express different components of the inflammatory response is a compelling speculation that could be generalized to all regions of the microcirculation.

Flow environment

It is well established that ECs from different regions of the circulation are variously influenced by flow-dependent mechanical deformations, resulting in characteristically different gene expression and signaling in ECs residing in arterial versus venous vessels (reviewed by Chien (17)). In the microvasculature, shear rates in resistance arterioles are typically higher than in venules and range in general from 200 to 4000 sec^{-1} (89, 102). Interestingly, unlike in venules, where the shear environment can be predicted by vessel diameter (97), in arterioles, due to their dynamic nature and vasoactivity, shear rates cannot not be correlated to vessel size (Fig 5). These differences in shear environment may well contribute directly or indirectly to the differences in leukocyte-EC interactions and regulation of barrier function in arterioles and venules. Despite evidence that leukocyte interactions with venular ECs appear to be largely uninfluenced by the hemodynamic environment (52), particularly at higher shear rates (80), fluid shear forces can indeed affect leukocyte-EC interactions in venules (86), although the observed effects appear to vary with the experimental approach. For example, in isolated cell systems leukocyte delivery, hence leukocyte rolling and adhesion, is significantly decreased under high hydrodynamic flow (51). However we found that this is not the case in intact blood perfused venules where increased adhesion is observed in smaller venules ($<40\mu\text{m}$) that typically display an elevated shear environment (97). In these vessels, basal expression of ICAM-1 is significantly higher compared to larger venules (97), further supporting the finding that *in vivo*, adhesion molecules play a more prominent role in regulating leukocyte-EC interactions. Arterioles, as discussed above, have typically higher shear rates compared to venules, and expression of adhesion molecules in control and inflamed arterioles is significantly lower than in venules under similar conditions. Indeed leukocyte rolling is induced in inflamed but not unstimulated arterioles. To initiate contact with endothelial cells, leukocytes must exit the center line of the flow stream and marginate towards the vessel wall. Vessel bifurcations,

characterized by stagnation regions (low blood flow, (97)) are the primary locations where initial contact between leukocytes and ECs occurs. We argue therefore that only in activated arterioles are the levels of surface adhesion molecules sufficient that leukocytes can remain in continuous contact with ECs. Further, we argue that in arterioles, the shear environment would be expected to have a more potent effect on leukocyte-EC interactions than in venules. Indeed under unactivated conditions, leukocyte adhesion in arterioles is rarely observed. It is however possible to induce adhesion in these vessels by locally reducing blood flow via partial occlusion of the inflow vessel (80, 100)), indicating that the shear environment does indeed contribute to the observed lack of leukocyte adhesion in these vessels. There is good evidence suggesting that variations in shear rate can affect expression of EC adhesion molecules. For example, expression of ICAM-1 is significantly increased when ECs in flow chambers are subjected to high shear environments (70). Interestingly however, we note that in small arterioles that typically support higher shear flow than their downstream venules, the expression of adhesion molecules is significantly lower than in venules, suggesting that in intact networks other factors must contribute to the regulation of adhesion molecules expression. In addition to lower expression of adhesion molecules, the elevated shear rate in arterioles is thus another cause for the lack of adhesion and hence leukocyte migration in arterioles.

Hemodynamic factors can also affect endothelial barrier function. In arterioles, where flow patterns are more variable, in-vivo permeability to albumin is directly correlated to spatial variations in shear stress (32) and is sensitive to flow reversal (2). The effects of fluid dynamics on endothelial barrier function must be due, at least in part, to the specific effects of fluid shear on endothelial morphology, intracellular signaling and cytoskeletal and junctional organization (59, 75).

Physiological and pathological consequences of arteriolar responses to inflammatory stimuli

As has been discussed above, wall structure, EC morphology, shear environment and expression of adhesion molecules are different in arterioles compared to venules. However, inflammatory activation of arterioles results in increased expression of EC adhesion molecules, induction of leukocyte-EC interactions, and changes in barrier function (macromolecular permeability). While leukocytes do not appear to extravasate from arterioles to any significant extent, induction of leukocyte rolling in arterioles clearly has alternative physiologically relevant consequences, including increased arteriolar permeability and the potential priming of both leukocytes and the downstream venules (discussed below).

Physiology

As discussed earlier in this review, increased expression of ICAM-1 on the luminal surface of arteriolar ECs during inflammation and its engagement by interacting leukocytes results in increased endothelial permeability to macromolecules such as albumin (18, 96, 98). Given the surface area of resistance arterioles, their contribution to total solute exchange is potentially significant. We have not explored whether other molecules implicated in leukocyte-EC adhesive interactions may also directly activate ECs and hence affect arteriolar permeability, as has been shown for the products released by neutrophils following interactions between neutrophils and venular ECs (25, 53), but there is no reason to suppose that there are not other mediators of permeability changes in arterioles yet to be described. Certainly, as discussed earlier, coronary arterioles show both acute and adaptational changes in permeability in response to adenosine, further attesting to the potential contribution of these microvessels to regulation of fluid homeostasis.

An important consequence for leukocyte-EC interactions in arterioles is the potential for these interactions to “prime” both the leukocytes and the downstream capillaries and venules for subsequent inflammatory responses. For example, it is established that activation/conformational changes of leukocyte β_2 -integrins are initiated by leukocyte rolling (87, 88). As activation of β_2 -integrin is essential for binding of endothelial adhesion ligands, including ICAM-1 and VCAM-1 (22), and thus transitioning the leukocyte to firm adhesion, it is a reasonable speculation that rolling interactions in arterioles might initiate β_2 -integrin activation at this earlier stage in the leukocyte’s passage through the microvasculature, and thus support more efficient adhesion and TEM when these cells reach venules. Indeed, in parallel plate flow chamber assays, early leukocyte rolling on E-selectin is essential for β_2 -integrin activation and transitioning to firm adhesion, through interactions with ICAM-1 (93). As E-selectin is expressed in activated arterioles, and ICAM-1 is found in abundance in activated venules, the findings in flow chambers may thus be simulating consecutive events in an integrated *in vivo* vascular network.

There is accumulating evidence that leukocyte engagement of EC ligands results in release from leukocytes of a variety of signal molecules (of which CAP37 is a classic example (25)), that are potent mediators of enhanced solute permeability in venules. Similarly, the neutrophil products cathepsin G and elastase can both affect endothelial permeability (30), as do interleukins 1, 6, and 8 as well as TNF α (7, 74), among others. Clearly, leukocyte interactions with arteriolar ECs raise the possibility that released substances can be convectively carried in the blood stream to induce permeability changes in distal microvessels and thus contribute to inflammatory responses in venules.

A common geometry in many microvascular beds is the parallel alignment of descending arterioles and ascending collecting venules (91), or arteriole-venule pairs crossing each other, illustrated in Fig 6. This geometry is reinforced by a complex network of capillaries that fills the spaces between parallel arterioles and venules. The proximity of these different vessel types creates a high likelihood that paracrine cross-talk may occur between them. Indeed, the dilatory effects of vasoactive substances that diffuse from venous blood to the adjacent arterioles have been demonstrated (28, 31). In some cases, such as in mesenteric arterioles of high cholesterol mice, it has been shown that arteriolar tone is dependent on leukocyte-EC interactions in nearby venules, and can be modulated by inhibition of leukocyte β_2 -integrins (45). Given that arterioles can change their barrier function in inflammation, thus contributing to solute and fluid exchange, it is clearly possible that inflammatory mediators released in activated arterioles could also diffuse towards neighboring capillaries and venules, thus affecting their function. Indeed it is well established that signaling molecules, including inflammatory mediators, released as a consequence of leukocyte-EC interactions in venules can affect arteriolar function (44, 45) hence the proposed reversal in signaling direction (from arterioles to venules to prime the inflammatory response) appears entirely reasonable, but will require further exploration.

Pathology

Increased vascular permeability and accumulation of immune cells is a hallmark of inflammation-based disorders, including chronic obstructive pulmonary disease, cystic fibrosis, inflammatory bowel diseases, arthritis, atherosclerosis and thrombosis. The microvasculature, and specifically arterioles, are affected by these disorders, and play a pivotal role in facilitating pathological symptoms. For example, thrombi can form in mesenteric and cremaster arterioles, leading to downstream occlusion and tissue ischemia (61, 72). Furthermore, thrombus formation has been observed in cerebral arterioles (26), increasing the risk for stroke. Intriguingly, both increased expression of EC adhesion molecules and leukocyte-EC interactions, as observed in inflamed arterioles are identified as

risk factors for thrombosis (3). Under normal physiological conditions arterioles that express low levels of adhesion molecules do not support leukocyte interactions, and thus are protected from thrombus formation. However in inflammation, leukocyte interactions with ECs often result in de-endothelization and exposure of sub-endothelial matrix, thus promoting thrombus formation. One of the key players promoting pathological thrombus formation is circulating platelets (48). In inflamed arterioles, activated ECs upregulate the levels of P-selectin and release VWF (42, 72, 100). Intriguingly, P-selectin has been shown to mediate both platelet adhesion, as well as leukocyte recruitment towards the arteriolar wall during inflammation (24, 112). Similarly, VWF released by ECs can bind to the exposed sub-endothelial matrix, and serve as an adhesive ligand for both circulating platelets (90) and inflammatory monocytes (38). Moreover, platelets can independently promote leukocyte recruitment and accumulation at the vessel wall by directly mediating leukocyte attachment (77), and indirectly through secretion of leukocyte chemoattractants (116). Inhibition of both leukocyte-EC interactions and absence of either P-selectin or VWF can delay thrombus formation (72). These examples link inflammation and thrombosis, arguing that these are interdependent processes that activate and perpetuate each other in arterioles. While the underlying mechanisms are the subject of ongoing research, it is clear that upregulation of adhesion molecule and chemokine expression on the arteriolar wall during inflammation plays an important role in promoting this vascular pathology.

Another example of where arterioles play a critical role in manifestation of a disease occurs in diabetes. Diabetic patients in most cases also suffer from hypertension (103), both well-defined risk factors for atherosclerosis. While atherosclerosis is characterized by increased stiffness of large arteries, in both diabetic and hypertensive patients the microcirculation (specifically small arterioles) is also remodeled. Increased cross-sectional area (thickening of the arteriolar wall), and increased collagen deposition are characteristic of diabetic arterioles (84). These changes likely result from impaired myogenic responses (58), increased wall stresses and alterations in the vascular ECM leading to endothelial dysfunction (47). Components of the immune system including circulating blood cells, as well as expression levels of cytokines and chemokines are also altered in diabetic arterioles (19). Thus here too, increased expression of surface adhesion molecules hence increased leukocyte-EC interactions, are likely contributors to vascular dysfunction and disease.

In summary, recent findings suggest that arteriolar barrier properties are tightly regulated under normal physiological conditions, as well as during inflammation. Arterioles have been identified as potential contributors to the maintenance of proper tissue homeostasis via regulation of solute and fluid exchange. Moreover changes in expression of surface adhesion molecules and induction of leukocyte-EC interactions in arterioles, while directly linked to pathological phenotypes, can potentially prime and enhance the responsiveness of downstream capillaries and venules during inflammation, thus providing further support for the integrated nature of signaling and responses in the intact microcirculation.

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List of abbreviations

EC	endothelial cell
ICAM-1	intercellular adhesion molecule 1
VCAM-1	vascular cell adhesion molecule 1

PECAM-1	platelet endothelial cell molecule 1
TEM	transendothelial migration
AngII	angiotensin II
L-NAME	N ω -nitro-L-arginine methyl ester
NOS (eNOS)	nitric oxide synthase (endothelial NOS)
PAF	platelet activating factor
SFK	src family kinases
VEGF	vascular endothelial growth factor
VEGFR-2	VEGF receptor 2
SMC	smooth muscle cell
ECM	extracellular matrix
VWF	Von Willibrand factor
BSA	bovine serum albumin

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Figure 1. Morphology of microvessel wall barrier differs in arterioles and venules

Scanning electron micrographs show a cross sectional view of a mouse cremaster muscle arteriole (left panel) and venule (right panel). Red blood cells can be seen in the arteriole, and leukocytes interacting with the vessel wall can be seen in the venule. Arrows indicate ECM surrounding each vessel; asterisks indicate skeletal muscle myocytes. Scale bar: 10 μm .

Figure 2. EC junctional morphology in arterioles and venules differs in a manner that will affect leukocyte interactions

Intact blood perfused cremaster muscle venules (left panel) and arterioles (right panel) were immunofluorescently labeled for PECAM-1 and ICAM-1 respectively using local introduction and perfusion of the appropriate primary and fluorescently tagged secondary antibodies in sequence. As illustrated by the representative images, PECAM-1 is primarily localized to the EC junctions, while ICAM-1 is excluded from EC junctions. Staining for both molecules allows cell borders of individual ECs to be identified. Different morphology and alignment of ECs in arterioles and venules by default dictates the arrangement and the number of tri-cellular junctions per unit vessel length; this in turn affects leukocyte interactions. Moreover, while in venules, relatively round ECs simply “fill in” the space in the converging region, in arterioles, long narrow ECs appear to appropriately traverse the bifurcation to properly accommodate vessel geometry.

Figure 3. Microvessels exhibit regional variations in expression levels and distribution of surface adhesion molecules

Intact blood perfused cremaster muscle arterioles were immunofluorescently labeled for ICAM-1 or P-selectin using local introduction and perfusion of the appropriate primary and fluorescently tagged secondary antibodies in sequence. **(A)** A representative image and a corresponding fluorescence intensity plot showing variability of ICAM-1 expression in an arteriole imaged at the central vessel plane (schematic at bottom of panel) using confocal microscopy. **(B)** An example of the expression patterns of P-selectin (upper panel) and ICAM-1 (lower panel) in TNF α stimulated cremaster arterioles, imaged at the upper plane of the vessel cross section (schematic at bottom of panel).

Figure 4. TNF α treatment enhances the heterogeneity in ICAM-1 expression patterns in arterioles but not venules

Representative immunofluorescence images, and the corresponding surface plots, of intact blood perfused cremaster muscle microvessels, showing that in response to TNF α , ICAM-1 expression in venules (Panel A) is increased in all ECs. Arterioles on the other hand (Panel B), initially express much less ICAM-1, undergo redistribution of ICAM-1 among ECs where some cells increase and some cells decrease their ICAM-1 expression, thus magnifying the variability of ICAM-1.

Figure 5. Shear rate in arterioles cannot be predicted by vessel size

Shear rate in TNF α stimulated (500ng, 4h, via intrascrotal injection) intact blood perfused cremaster muscle arterioles of various diameters was assessed by tracking the displacement of fluorescently labeled beads (0.5μm) which were injected into the blood stream via a catheter inserted into the jugular vein. No significant ($p<0.05$) correlation was found between vessel size and the prevailing shear rate in these vessels.

Figure 6. Alignment of arterioles and venules in close proximity of each other may elicit paracrine talk between them

To visualize the geometry of the microvascular bed in cremaster muscle of anesthetized mice, a feed arteriole to a region of the network was microinjected with fluorescently labeled BSA to perfuse the downstream section of the intact microvasculature. A representative field of view shows parallel alignment of descending arterioles and ascending collecting venules, as well as arterioles and venules pairs crossing each other (highlighted in white squares), suggesting that paracrine cross-talk is possible between these vessels.