Hypothesis

The local physicochemical environment conditions the proinflammatory response of endothelial cells and thus modulates leukocyte recruitment

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Abstract The locations at which vascular endothelial cells recruit leukocytes during physiological or pathological inflammatory responses are influenced by direct effects of local haemodynamics on leukocyte adhesion. However, the expression of genes by endothelial cells, and their ability to respond to inflammatory cytokines also depend on the flow forces to which they are exposed. In addition, cells of the underlying stroma can modify the phenotype and responsiveness of endothelial cells, and hence their ability to recruit leukocytes. Thus, endothelial cells are plastic in their responses, and we hypothesise that the pattern of recruitment of leukocytes to tissues is critically dependent on the variable modulation of the endothelium by the local physicochemical microenvironment.

Keywords: Endothelium; Shear stress; Stromal cell; Inflammation; Leukocyte recruitment

1. Introduction – endothelial recruitment of leukocytes in inflammation and regulation by the local environment

Endothelial cells (ECs) contribute to the regulation of vascular tone, haemostasis, angiogenesis, and passage of soluble compounds and cells of the immune system to and from tissue. Of particular importance during inflammatory responses is the ability of vascular EC to both initiate and control the recruitment of leukocytes [1,2]. They achieve this by responding to cytokines and other inflammatory agonists, to modify surface expression of adhesion receptors and chemokines required for the capture of leukocytes from the flowing blood and migration into tissue. However, the characteristics or phenotype of EC vary between different levels of the vascular tree and between different organs, with respect to patterns of gene expression, morphology, secretory potential and permeability [3,4]. This variation also applies to their ability to support adhesion and migration of leukocytes. Endothelium in venules of lymphatic organs is specialised for continuous capture and migration of recirculating lymphocytes, and post-capillary venules of other tissues are typically the dominant sites for phased recruitment of granulocytes and mononuclear cells following inflammatory insult.

There is much evidence that the phenotype of ECs in different vessels may be conditioned by the varying haemodynamic forces exerted on them from the vascular lumen, and by interactions with the underlying substrate and stromal cells (e.g. [3,5,6] and Table 1). Thus, it has been suggested that even within a single organ such as the lung, different haemodynamic and stromal conditioning of the ECs in alveolar vs. bronchial microcirculation, might explain the different adhesive mechanisms used for leukocyte recruitment in those regions [7]. Even after isolation from their normal environment, ECs from different sources can retain different adhesive characteristics, although generally their phenotype converges with time. It was shown recently that EC isolated from heart or lung of mice both supported adhesion of flowing T-cells but that the behaviour of the adherent T-cells and the chemokines utilised for their recruitment differed [8]. In humans, primary cultures of sinusoidal ECs from liver retain specific adhesion receptors for lymphocyte migration [9], not found for instance in more widely studied human umbilical vein ECs.

Fig. 1 illustrates the steps by which flowing leukocytes attach to and migrate through ECs which have been exposed to inflammatory cytokines, and the local environmental factors that may influence this process. While the propensity for ECs to recruit leukocytes is likely to be conditioned by fluid forces and stromal cells, direct evidence has only become available recently (e.g. [10–12]). Here, we advance the specific hypothesis that the sensitivity of ECs to inflammatory mediators and the ability to recruit leukocytes are modulated by local conditions.

2. Direct and indirect effects of haemodynamics on leukocyte adhesion and migration

During inflammation, leukocyte recruitment occurs typically in the microcirculation in post-capillary venules where the wall shear stress (i.e. the frictional force exerted by the flowing blood on the vessel wall) is relatively low, and not in adjacent arterioles with higher shear stresses [13]. Increasing shear forces generally reduces the efficiency with which cells can attach to an adhesive surface. However, the differences in recruitment between vessels cannot be explained by the direct physical effects of flow on adhesion alone. Acute artifical reduction in flow rate through arterioles in inflammed tissue did not induce adhesion

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Table 1
Examples of effects of co-culture with different stromal cells on functions of ECs

Cell type	Endothelial response modified
Smooth muscle cells	Inhibited EC growth [48]; increased expression of adhesion molecules and leukocyte recruitment [11,38,40]
Alveolar epithelial cells	Increased adhesion of leukocytes [39]
Pericytes	EC growth and angiogenesis [48,49]
Fibroblasts (skin)	Formation of endothelial tube structures [50]
Astrocytes	Permeability of monolayers [51]
Glomerular epithelial cells (podocytes)	Augmentation of 'angiogenic' response [52]

at levels comparable to venules, indicating that the responses of the ECs were themselves different [14]. Comparative studies of arterioles and venules of the inflammed heart [15] or lungs [16] have supported the concept that endothelial as well as haemodynamic factors influence leukocyte adhesion in these microvessels. Leukocytes do not normally adhere to the endothelium of large arteries, possibly because local wall shear stresses are too high. However, blood-derived monocyte/macrophages and T-lymphocytes are found within atheromatous plaques and are important promoters of the development of these lesions [17]. Plaques form preferentially at bifurcations and regions of vessel curvature where the laminar pattern of blood flow is disturbed [18]. In these regions vortices occur, with regions of backflow (recirculation) and low wall shear stress (e.g. [19]). Such disturbances may directly facilitate leukocyte adhesion, but the local conditions may also modulate the state of ECs so that they are more responsive to inflammatory mediators. Indeed, ECs covering plaques express capture receptors (selectins and vascular cell adhesion molecule-1, VCAM-1), which are absent from healthy arteries [20,21].

The indirect (cell biological) effects of shear stress on adhesion are most probably mediated through the regulation of endothelial gene expression. Much attention has been paid in the last decade to the ability of ECs to respond to haemodynamic forces, chiefly shear stress, but also hydrostatic pressure which induces circumferential strain in the vessel wall and cellular compression [6]. It has become increasingly recognised that ECs can respond to changes in shear force, rapidly by releasing vasoactive substances such as nitric oxide, or more slowly through modification of gene expression. Numerous studies have defined changes in expression of genes, including those encoding receptors and chemokines relevant to leukocyte recruitment, when endothelial cels are cultured at different levels of shear stress (e.g. [10,22–27]). Some such changes are transient and may not be strictly relevant to the adhesive status of ECs which are continually exposed to flow. For instance, endothelial integrin ligand ICAM-1 and the chemokine MCP-1 were upregulated upon exposure to shear stress, but returned to basal level by 24 h [26,28]. Moreover, changes in gene expression have rarely been quantified when cytokine stimulation (which typically regulates adhesion molecule expression in inflammation) and shear stress are combined, while use of direct functional readouts such as leukocyte migration has also been uncommon.

In fact, signalling responses and activation of gene transcription factors induced by TNF are modified in ECs that have been cultured under flow even for quite short periods [29]. Perhaps more directly relevant to modulation of endothelial inflammatory responses are recent studies where prolonged periods of shear and cytokine treatment have been combined, and subsequent ability of endothelial cells to recruit leukocyte has been studied. Thus, we showed that exposing cultured ECs to relatively low shear stress (0.3 Pa; approximating levels in post-capillary venules) for increasing periods upto 24 h, steadily decreased the response of the EC to TNF, as judged by their ability to induce migration of adherent neutrophils [12]. When higher shear stresses were used (up to arterial levels of 2.0 Pa), the TNF treated ECs were no longer able to efficiently capture flowing neutrophils. The differential responses at low

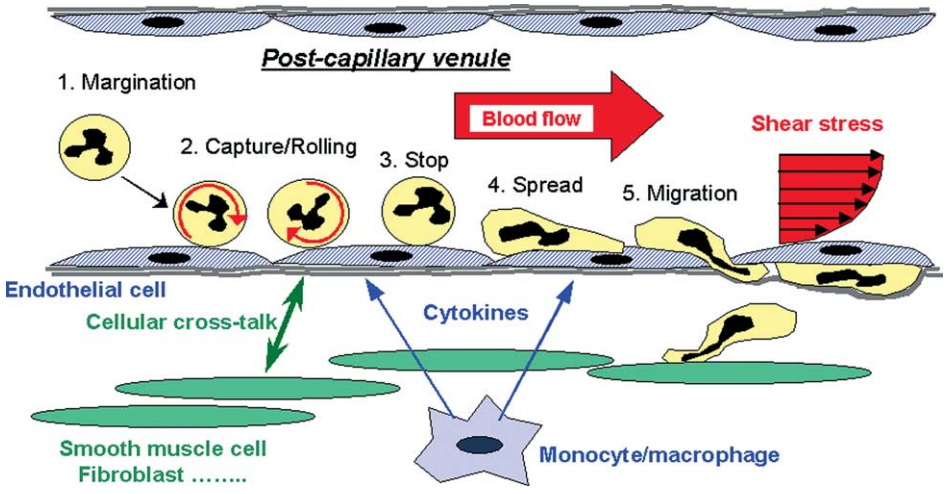

Fig. 1. Schematic representation of the steps by which leukocytes, such as neutrophils, are recruited from blood into inflammed tissue, and the local physicochemical environmental factors which condition the contribution of ECs to this process. Leukocytes become marginated by the central blood stream (1) and are captured and form unstable rolling attachments (2) using selectin receptors expressed on the surface of the ECs. Activating message(s) from agents such as chemokines presented on the ECs cause the leukocytes to become immobilised (3) through their activated integrin receptors, and then to spread on the surface (4) and migrate first over and then through the endothelial monolayer (5) via co-ordinated regulation of integrin adhesion and cytoskeletal rearrangements. Stromal cells such as tissue-resident monocyte/macrophages release inflammatory mediators and cytokines which induce the endothelial presentation of selectins and chemokines. Other cells such as smooth muscle cells or fibroblasts may add to the cytokine milieu and condition the responses of ECs. Shear stress applied to ECs by the flowing blood can modify their expression of genes and their responses to pro-inflammatory cytokines.

and high shear stress were attributable to reduced ability to upregulate chemokine and selectin expression, respectively. In addition, others demonstrated recently that ECs lining the aorta of rabbits showed reduced upregulation of VCAM-1 in response to TNF treatment when the aorta was perfused ex vivo at high compared to low shear stress [30].

These studies imply that ECs are conditioned by their shear environment and can take on different responsiveness to cytokines, and hence ability to recruit leukocytes and mount an inflammatory response. This 'chronic' conditioning might apply to different regions of the circulation where shear levels are continuously different. However, given that EC can quickly respond to changes in shear stress, acute shear modulation might be important in conditions such as ischaemia and reperfusion, or artifical cessation of flow during surgery, where sudden and relatively short-lived changes may occur. Indeed, it has been shown that if EC are conditioned to shear flow and it is then ceased, they quickly respond by generating and oxidative burst and then mobilise gene transcription factors [31]. It will be interesting to test whether responses to inflammatory agents rapidly increase under these circumstances, or whether hours are again necessary. In any case, the possibility arises that the pro-inflammatory responses of ECs are not only conditioned by their stable local flow environment, but can also fluctuate depending on changes in that environment.

3. Effects of stromal factors on ability of ECs to support leukocyte adhesion and migration

Endothelial cells exist in close contact with smooth muscle cells and pericytes in the vessel wall, are juxtaposed to specialised cells in specific organs such as the brain (astrocytes), liver (hepatocytes), kidneys (glomerular podocytes) and lungs (alveoler epithelial cells), and are surrounded by less obviously specialised fibroblasts in all tissues. All of these cells are believed to be able to condition the responses of ECs and may provide tissue-specificity (see Table 1 for examples). Macrophages and mast cells are resident in all tissue and can instigate inflammatory responses by releasing fast-acting agents such as histamine or cytokines tumour necrosis factor-α (TNF) and interleukin-1β (IL-1) [32]. These agents are capable of inducing ECs to support some or all of the stages of leukocyte recruitment outlined in Fig. 1. Smooth muscle cells found in the wall of most vessels above the size of capillaries have an essentially structural function. However, in disease states such as atherosclerosis they can transform to a secretory phenotype, releasing a battery of compounds including chemokines (IL-8 and MCP-1) and in some contexts, IL-1 and TNF [17,33]. A similar capability is found in tissue fibroblasts, which again classically have functions in structural support, but may also release a variety of cytokines and chemokines capable of modifying endothelial and leukocyte behaviour [34]. However, while stromal cells are widely regarded as capable of modifying inflammatory responses, their effects on the ability of endothelial cells to recruit leukocytes remain poorly defined.

The matrix proteins to which ECs are attached might also affect phenotype [3]. For instance, the growth characteristics or surface glycosylation of ECs are modified when cultured on specific components of extracellular matrix or matrices derived from different organs [35,36]. Recent studies in our laboratory indicate that in prolonged cultures, ECs progressively deposit matrix proteins and change their sensitivity to cytokine stimulation. However, a direct link between the two responses is unproven so far. Interestingly, responses of ECs to shear stress appear to originate from or are at least modulated by signals arising from integrins engaged with matrix proteins [37]. Thus, while the effects of interactions with specific substrate proteins on endothelial adhesive phenotype are uncertain, there appears to be a strong likelihood that the response to the physical environment, at least, will be modulated at this level.

It is difficult to unequivocally demonstrate that in vivo variations in endothelial inflammatory responses arise from their stromal environment. However, in vitro co-culture of ECs with stromal cells is known to modify a range of responses of ECs (Table 1). Of particular relevance here, are the findings that ECs cultured with smooth muscle cells had augmented response to oxidised low density lipoprotein judged by ability to bind monocytic cells [38]. Moreover, ECs co-cultured with alveolar epithelial cells showed increased recruitment of mononuclear leukocytes upon cytokine stimulation [39]. We showed recently that smooth muscle cells in their secretory state (i.e., as found in atheromatous arteries) did not themselves cause ECs to become adhesive, but did greatly increase the response of ECs to TNF [11]. This was manifest as more efficient binding of flowing lymphocytes, monocytes and neutrophils, and ability to initiate binding at much lower levels of TNF. Others have found that co-culture alone caused upregulation of mRNA for adhesion receptors ICAM-1, E-selectin and VCAM-1, although adhesive functional effects were not reported [40]. It is possible that the apparent discrepancy might arise from different conditions of co-culture (growth factors, substrate, etc.). Interestingly, in a rare combination of stromal and physical environmental modulation, the 'intrinsic' receptor upregulation in co-cultures was modified when the co-cultured ECs were exposed to arterial levels of fluid shear stress [40].

Skin fibroblasts do not appear to have the same stimulatory capacity as smooth muscle cells [41], but this may not be the case in inflammed tissue. For instance, fibroblasts from arthritic synovium release elevated levels of cytokines such as IL-6 and MCP-1, and have been shown to modulate retention of lymphocytes in tissue, and to alter their migrational behaviour [34,42]. We are currently investigating whether in co-culture they can directly induce or modify endothelial recruitment of leukocytes. In fact it is likely that an evolving stromal environment plays a key role in the development of chronic inflammatory pathology, in part at least by modifying the responses of ECs. Recruited leukocytes such as T-cells and monocytes may also provide positive feedback [43,44]. Monocytes are able to directly induce ECs to upregulate adhesion receptors and support capture of flowing leukocytes [45]. This pro-inflammatory capability may decline during normal maturation into macrophages [46]. However, in abnormal tissues such as atheroma or the pannus of the rheumatoid joint, the monocyte-derived cells may themselves take on phenotypes (foam cells or type B cells respectively) that promote a pro-inflammatory state via interaction with endothelium.

4. Pathophysiological significance of endothelial conditioning in inflammation

The foregoing has implications for the regulation of 'physiological' protective inflammatory responses, and for

the institution and progression of pathologies linked to aberrant recruitment of leukocytes. Limitation of the sensitivity of ECs to cytokines at specific sites may allow controlled, local recruitment. Moreover, specific niches in organs may induce receptors peculiar to that tissue and adapted for its function (e.g. liver, lymph nodes, lungs). On the other hand, the local flow conditions, or changes in stroma may lead to predilection to disease. Atheroma typically forms in regions of arteries with complex patterns of low shear flow [18], which are believed to alter EC phenotype as well as directly affecting leukocyte recruitment to the wall [19,37]. Moreover, transformation of SMC to secretory phenotype may make the local EC more sensitive to circulating inflammatory cytokines and accelerate disease progression [11]. Acute changes in local conditions might also influence disease progression. During ischaemia, ECs might be conditioned so that they respond strongly upon return of flow (reperfusion) [31]. This might influence development of tissue infarcts, as well as outcome of surgical procedures or organ transplantation. It is also interesting to speculate that the typical increase in blood flow arising from arteriolar dilation at onset of inflammation could have a feedback effect on responsiveness of venular endothelium downstream. Moreover, progression to chronic disorders and failure to resolve inflammation could arise from changes in local stroma, which might be thought of as a 'rheostat' that is reset in persistent inflammation [47]. Thus in RA joint, there is proliferation and transformation of fibroblasts and macrophages in the developing panus. Local vessels develop with endothelial architecture reminiscent of lymphatics rather than post-capillary venules, and there is prolonged recruitment of all types of leukocytes with specific T-cells subsets retained in the tissue. While these features cannot all be attributed to changes in the endothelial phenotype, it may play a significant role.

5. Summary

In summary, we hypothesise that ECs are pliable cells whose pro-inflammatory phenotype is variable and not necessarily set for life. Their ability to upregulate expression of adhesion receptors and cytokines, and thus to support an inflammatory responses by recruiting leukocytes, are determined by their local physicochemical environment. Not only are the haemodynamic and stromal environments peculiar to specific types of vessel or organ, but they may be modified in disease states. By analogy to the adhesion receptors and chemokine 'post-code' for leukocyte recruitment borne by endothelium, there might effectively be a tissue post-code that conditions the EC responses. This tissue postcode may not only be important in determining sites or severity of initial inflammatory responses, but will also influence resolution of inflammation or its transformation to a chronic state.

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