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Review

# Tissue stroma as a regulator of leukocyte recruitment in inflammation

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RECEIVED SEPTEMBER 14, 2011; REVISED NOVEMBER 24, 2011; ACCEPTED NOVEMBER 27, 2011. DOI: 10.1189/jlb.0911458

## ABSTRACT

**The stromal milieu (cellular and matrix components) helps establish tissue "address-codes" that direct leukocyte behavior in inflamed tissue. Coordinated interactions among the stroma, leukocytes, and ECs dictate which leukocytes are recruited, whether they are retained within the inflamed site, and how long they survive. Herein, we discuss how the stromal milieu influences the leukocyte recruitment cascade. Moreover, we explore how corruption of the stromal phenotype in chronic inflammatory diseases contributes to undesired, continuous recruitment of leukocytes. Emerging complex, multicellular, multilayered (co-)culture models are now addressing the molecular circuitry involved in regulating stromal organization during inflammation. Understanding context-specific changes in pro- or anti-inflammatory agents derived from the stroma, such as IL-6 (and its cofactors), is important for the generation of therapeutic strategies that restore the balance between recruitment and clearance of the inflammatory infiltrate in chronic disease.** ***J. Leukoc. Biol.*** **91: 385–400; 2012.**

## Introduction

Inflammation is a physiological response, which defends against microbial infection and facilitates repair of damaged tissue. It depends on the local and controlled influx of circulating leukocytes (typically neutrophils initially, followed by mononuclear cells; e.g., ref. [1]), whereas resolution of the response requires their subsequent clearance (e.g., ref. [2]). The duration and intensity of this reaction must be tightly regulated, as persistent leukocyte infiltration can cause tissue damage that is associated with chronic inflammatory diseases such as RA and atherosclerosis [3, 4]. Vascular ECs act as the immediate regulators of leukocyte recruitment, responding to inflammatory agents by expressing adhesion molecules and activating agents, such as chemokines, for the recruitment of specific types of circulating leukocytes [5–7]. The combination of these receptors and agents is often referred to as the recruitment postcode, which can vary depending on inflammatory stimuli or site. However, the endothelial responses are themselves influenced by the local milieu, as physicochemical stimuli arising from local hemodynamics and from underlying stroma (matrix and cellular) modulate endothelial phenotype [8]. In addition, stromal cells may produce chemokines, which are transferred and presented to the leukocytes by the endothelium [9]. Thus, each inflammatory response is contextual, defined by the nature of the stromal response to inflammation within the tissue affected.

In this review, we consider the concept that the stroma is an active regulator of endothelial responses and of leukocyte recruitment in health and disease. Thus, the BM on which ECs grow and the underlying stromal cells (such as fibroblasts or SMCs) not only act as a support for the vasculature and a barrier to infiltrating leukocytes but also actively engage in controlling infiltration. Specifically, we concentrate on the process of inflammation and how the stromal milieu (cells and BM) can influence the recruitment of leukocytes by endothelium, provide migratory cues for captured cells, and influence their retention and subsequent fate in tissue (**Fig. 1**). The role of the interstitial tissue matrix in these processes has been reviewed recently in detail (see ref. [10]) and will not be considered here. We recently proposed "a stromal address code defined by fibroblasts"…"that directs leukocyte behavior within tissues" [11]. Here, we explore the importance of such stromal address codes in the generation of recruitment postcodes, examining the possibility that stromal address codes become corrupted in chronic inflammatory diseases and contribute to undesired, continuous recruitment of leukocytes. Recruitment to lymphoid tissues will not be discussed, although there, also, tissue-specific stromal cells may influence homing of lympho-

Abbreviations: BBB=blood brain barrier, BM=basement membrane, DARC=Duffy antigen receptor for chemokines, EC=endothelial cell, GAG=glycosaminoglycan, JAM=junctional adhesion molecule, $LTB_4$=leukotriene $B_4$, MS=multiple sclerosis, OA=osteoarthritis, RA=rheumatoid arthritis, SMC=smooth muscle cell, TLO=tertiary lymphoid organ

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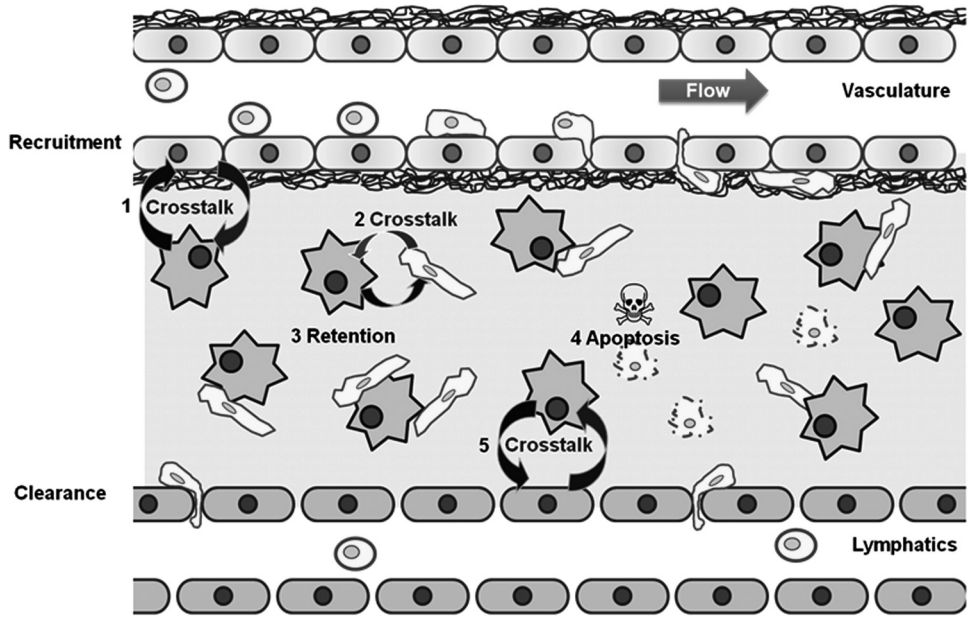


**Figure 1. Stromal microenvironment is an active regulator of inflammation, controlling leukocyte entry, transit through tissue, and exit.** Regulation of leukocyte recruitment, fate, and egress from tissue during inflammation involves a series of coordinated regulatory processes, including crosstalk among tissue stroma, leukocytes, and ECs. Dynamic conversations between stromal components (e.g., pericytes, fibroblasts, or ECM proteins) and ECs, which line the blood or lymphatic vasculature, influence the phenotype of the endothelium and consequently, their ability to support leukocyte interactions. In the first instance, this crosstalk allows vascular endothelium to act as the entrance gatekeepers, controlling leukocyte recruitment to and entry into the inflamed site (1). Once in, recruited cells are exposed to an array of signals from released, soluble agents or arising from physical contact with stromal cells (2). These conversations ultimately determine the fate of the recruited cell: for example, influencing migratory potential to retain leukocytes at the site of action (3), inducing clearance of unwanted cells by triggering apoptosis (4) or promoting migration through the tissue to the lymphatic barrier. Finally, lymphatic endothelial cells act as the exit gatekeepers, where stroma crosstalk may influence their ability to support leukocyte migration out of the tissue as part of the resolution process (5).

cytes and their destination in secondary lymphoid organs (e.g., reviewed in ref. [12]).

## MECHANISMS UNDERLYING THE DIFFERENT STAGES OF LEUKOCYTE RECRUITMENT—POTENTIAL INPUT FROM THE STROMA

In inflammation, leukocytes are recruited to the walls of postcapillary venules by ECs responding to locally produced agonists. Infectious agents and tissue damage cause tissue-resident macrophages to release cytokines (such as TNF-$\alpha$, IL-1$\beta$, and IFN-$\gamma$) or rapidly acting agents (such as histamine). ECs may also respond directly to bacterial components, activated complement fragments, or products of the hemostatic response, such as thrombin. In established inflammatory responses, T cells may also contribute to cytokines, such as IFN and IL-4. Over minutes or hours, these different agonists all have the ability to cause the endothelium to express specialized adhesion receptors that are able to capture flowing leukocytes (VCAM-1 and E- or P-selectin) [5, 7, 13, 14]. These receptors typically support a dynamic, rolling form of adhesion, which brings the leukocytes in contact with surface-presented chemokines or lipid-derived mediators (such as platelet-activating factor and $LTB_4$), which cause activation of leukocyte integrins. Activated $\alpha_4\beta_1$-integrin firmly binds VCAM-1, whereas activated $\alpha_L\beta_2$- and $\alpha_M\beta_2$-integrins bind ICAM-1 to stabilize adhesion and support onward migration.

The patterns of adhesion receptors and chemokines used are specific to the leukocyte being recruited and the stimulus driving the EC response [15–20]. For instance, in flow models in vitro, when ECs were stimulated with TNF or IL-1, neutrophils were captured by selectins, and CXC-chemokines stabilized attachment by inducing $\beta_2$-integrin binding to ICAM-1 (**Fig. 2**) [17, 21–23]. For PBLs, capture through $\alpha_4\beta_1$-integrin binding to VCAM-1 was stabilized by chemokines acting through CXCR3 for ECs treated with TNF and IFN [16, 24].

Additional signals may be needed for diapedesis. For example, we recently identified a signal from $PGD_2$ as a promoter of efficient transendothelial migration of neutrophils or T cells following their chemokine-induced activation, imparting a lasting, promigratory phenotype on T cells in vitro [25, 26]. The leukocytes can migrate between or directly through ECs (paracellular or transcellular routes, respectively). A series of receptors associated with cell–cell junctions has been proposed to regulate the paracellular route, including CD31, CD99, and JAM-A, -B, and -C (see reviews; refs. [27–30]).

Neutrophils migrate across stimulated endothelial monolayers in minutes, and within tens of minutes thereafter move into tissue in vivo [31], across filters in vitro [32, 33], or into collagen gels [34]. The kinetics of T cell migration across endothelium, during inflammation in vivo has not been reported, but experimentally, they can migrate into secondary lymphoid tissue within tens of minutes of their infusion [35, 36]. In vitro, lymphocytes migrate across cytokine-treated ECs in minutes [15, 16, 18], although many may then migrate continuously back and forth across the monolayers in a "frustrated" manner [34]. They show relatively little penetration of underlying substrates (such as collagen gels or filters) even after hours [34, 37, 38], and it may be that additional migratory signal(s) from stroma are required to mediate efficient onward migration [33, 34]. The migration process itself may also modify characteristics of leukocytes. This has been best

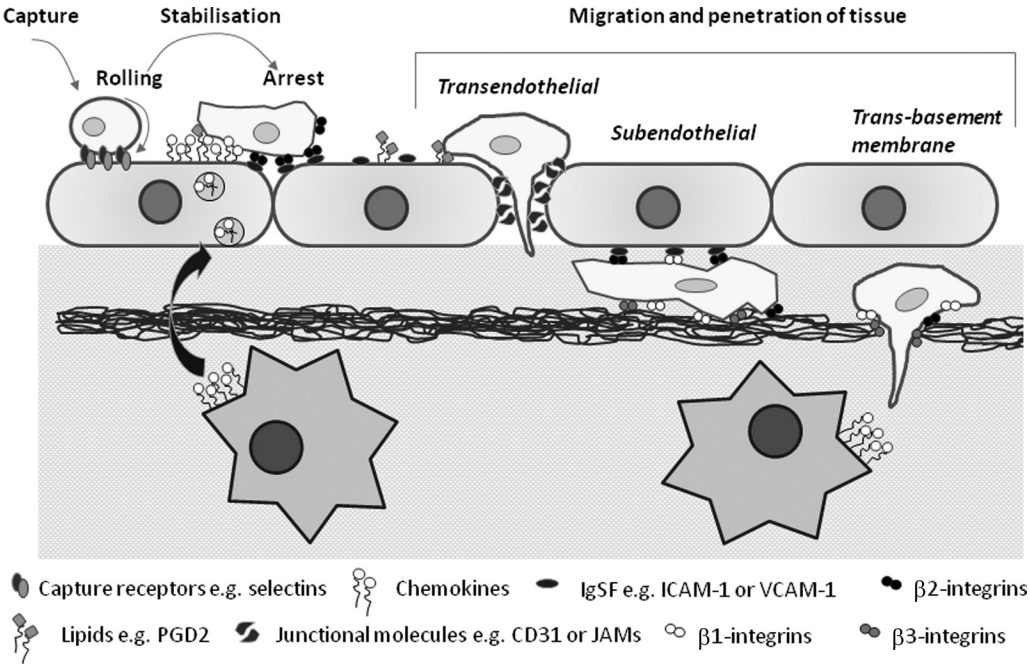


**Figure 2. Neutrophil recruitment cascade—interactions with tissue stroma.** Neutrophil recruitment and fate during inflammation are tightly regulated by a series of signals. Crosstalk within inflamed or diseased tissue triggers the activation of vascular ECs, such that they are capable of supporting interactions with circulating neutrophils. Initial tethering and rolling are mediated by E- and P-selectin. Endothelial chemokines or transcellular trafficking of fibroblast-derived chemokines, such as CXCL5, to the endothelial surface by DARC can rapidly stabilize adhesion. $PGD_2$ signaling through it's receptor DP-1 is required for subsequent efficient migration. Crawling over and migration through the endothelial layer are dependent on $\beta_2$-integrin, in addition to a series of cell–cell junction-associated receptors (CD31, CD99, and JAM-A, -B, and -C). Movement within the subendothelial space is supported by a combination of $\beta_1$- and $\beta_2$-integrins, whereas transit across the BM also requires $\beta_3$-integrin. Migration within the tissue itself may be promoted through stroma-derived chemotactic gradients directing neutrophils to the site of injury. IgSF, Ig superfamily.

demonstrated for neutrophils, which undergo changes in integrin expression and use after migrating through endothelium (e.g., refs. [21, 39–42]). In addition, migration of T cells across blood vascular endothelium promotes their subsequent migration across lymphatic ECs by modifying the function (but not expression) of CCR7, the major chemokine receptor regulating transit of the lymphatic barrier [26].

The processes outlined above can largely be supported by ECs cultured in isolation in vitro, but this is clearly not the situation in vivo, and elements of the stroma have the potential to influence various steps, as illustrated in Fig. 1. It is widely agreed that stromal-derived chemokines play a role in the successful navigation of circulating leukocytes into the inflamed site (e.g., reviewed in refs. [43–45]) and indeed, might be essential for efficient lymphocyte migration away from the subendothelial space [33, 34]. However, it is also possible that stromal cells act as a source of cytokines that directly activate or modulate activation of the endothelium. The BM presents a barrier to leukocyte migration, and as the substrate on which ECs grow, might also influence the responses of those cells. After transendothelial migration, contact with stromal-derived signals may influence leukocyte motility [26, 33, 34] and fate (changes in phenotype or survival within tissue; e.g., ref. [46]). Another interestingly possibility is that the stroma influences exit of leukocytes from tissue, controlling their transit across the lymphatic endothelial barrier, an area yet to be investigated.

The effects of different components of stroma on the different stages during leukocyte recruitment are considered below. In general, whereas many structural components or cells can be shown to modify leukocyte behavior, mechanisms underlying these effects are hard to define. Moreover, difficulties in constructing models of tissue, where each step in recruitment occurs in order and can be dissected, make it hard to interpret how findings in simpler models relate to the situation in vivo. We start by considering some of the experimental aspects.

## EXPERIMENTAL MODELS FOR STUDYING THE EFFECTS OF STROMA ON LEUKOCYTE RECRUITMENT

For the purposes of this review, the stroma can be divided into the subendothelial BM and the underlying connective tissue containing stromal cells (including pericytes, SMCs, fibroblasts, and more organ-specific cells, such as hepatocytes or podocytes). ECM proteins (such as collagens, laminins, and fibronectin) and GAGs (such as heparan sulfate) and the proteoglycans with which they are associated are condensed in the BM (see reviews; refs. [47, 48]) and also dispersed on and between the underlying stromal cells (reviewed in ref. [10]). It is possible to observe the migration of leukocytes through these structures using intravital microscopy (e.g., ref. [49]). However, relatively few studies have directly observed recruitment from the luminal surface into tissue in inflammation (e.g., refs. [50–52]). Experimentally, it is difficult to isolate the effects of specific stromal components in vivo and indeed, to modify these components in a controlled manner.

On the other hand, in vitro studies of interactions between leukocytes and stroma have often used reductive models incorporating individual stromal cells or proteins. These may mimic regulatory processes to a degree, but as noted above, migrat-

ing leukocytes are conditioned by their passage through endothelium, and it is desirable to include this conditioning in models if possible. Moreover, the structure of the BM and matrix is imperfectly replicated by use of isolated proteins and GAGs. ECs can generate a BM in vitro, but this requires prolonged culture [53, 54]. It is also possible to construct models, e.g., where ECs are cultured for prolonged periods on porous filters or on collagen gels with stromal cells in close proximity, in which leukocyte recruitment can be followed (e.g., refs. [33, 42, 53]). Such constructs may be more representative of the in vivo situation, where the proximity of the stroma to the endothelium allows bidirectional cell–cell messages. Nevertheless, to date, in vitro studies have largely investigated effects of BM, matrix structures, or stromal cells separately, and lack of unified models or consistency in methods makes it hard to be sure what are the key regulatory phenomena.

## EFFECTS OF BM AND ECM PROTEINS ON LEUKOCYTE RECRUITMENT

### BM function in inflammation

The BM is generally perceived as an inert structural support, which physically separates the vascular endothelium from the underlying tissue [55]. In principle, the BM may condition the responses of endothelium, which grow upon it, with the capacity to present necessary adhesive substrates and act as a reservoir for bioactive molecules [56]. It is widely assumed, as a result of structural similarities, that the chemokine binding properties of heparan sulfate proteoglycans (perlecan) in the BM will be comparable with those associated with ECs (syndecan), which itself has been examined extensively [57–59]. Thus, in principle, the BM could present chemotactic agents (endothelial and stromal in origin) to migrating leukocytes and play an active role in recruitment.

Interestingly, the structure of the BM varies between organs and may change during disease, but the consequences for leukocyte recruitment are hard to predict from existing knowledge. Recently, confocal imaging revealed heterogeneity in the density of some BM components coinciding with gaps in the pericyte coverage of venules in various vascular beds in mice [50–52]. Migration of leukocytes occurred preferentially in these low-density regions, and the density was reduced further by migrating neutrophils, which actively remodeled the BM upon transit [50]. Other histological studies have revealed that the BM undergoes structural changes in a number of chronic inflammatory pathologies, such as RA, asthma, and diabetes [60–62]. Whether changes in BM are a trigger or a result of inflammatory conditions remains uncertain. However, heterogeneity, in its composition and coverage, lends itself to the idea that the BM can actively influence leukocyte recruitment, as well as representing a mechanical barrier.

### BM and modulation of EC phenotype

Several studies suggest that the BM may be able to modify the behavior of ECs growing upon it. A number of in vitro studies have demonstrated that EC proliferation and migration can be modified by the composition of the adhesive substrate. Although most of these studies used purified proteins, rather than a "complete" BM, they illustrate the principle. Human dermal microvascular ECs cultured on laminin had a greater proliferation rate than those grown on fibronectin or collagen type IV [63]. Another study showed that expansion of subconfluent monolayers of HUVEC was significantly quicker on collagen type IV, compared with an uncoated control surface, and quicker still on laminin or fibronectin [64]. Human saphenous vein ECs migrated more quickly on laminin 511 or collagen type IV compared with fibronectin, albumin, or laminin 411 [65]. Other EC properties can be also be modified by manipulation of the culture surface; for example, when bovine aortic ECs were seeded on matrices from different bovine organs (lung, liver, or kidney), there were modifications in EC-surface glycosylation [66].

However, there are very few studies examining how BM could modify ECs to impact on the process of leukocyte recruitment in inflammation. One study showed that HUVEC cultured on collagen type IV expressed VCAM-1 at a lower level on unstimulated and IL-1$\beta$-stimulated monolayers than HUVEC cultured on collagen type I [67], although ICAM-1 was not affected. Our own work demonstrated that when HUVEC were cultured for a prolonged period and deposited a discrete BM, they were sensitized to TNF so that neutrophil adhesion and transmigration occurred at lower concentrations of cytokine compared with endothelium grown for shorter periods [54]. This priming effect was partially reproduced when fresh endothelium was seeded onto BM deposited over 20 days [54], indicating that the interaction with the deposited matrix modified endothelial behavior.

### Leukocyte migration on BM and matrix proteins

Studies of neutrophil migration through the walls of inflamed venules in mice have recently shown that transit through the BM is a separately regulated stage in recruitment. The $\alpha_6\beta_1$-integrin was required for penetration of the BM in cremaster muscle stimulated with IL-1$\beta$, and ligation of CD31 during transendothelial migration acted to up-regulate expression of this integrin [40, 51, 68]. It was also observed that neutrophils preferentially migrated through regions that had low density of BM components, concomitant with gaps between pericytes, and tended to follow each other through these routes [52]. Interestingly, there was further reduction in density during migration at these sites, which was reversed afterward [50]. As neutrophils also carried laminin fragments with them [50], the results suggested that active digestion of structures occurred during migration. Elastase has been demonstrated to contribute to digestion of matrix proteins by migrating neutrophils in vitro at least (e.g., refs. [69, 70]), although there is not marked inhibition of neutrophil recruitment in elastase-deficient mice [71]. There is also evidence indicating that neutrophil proteases, including proteinase 3, can facilitate the onward migration of neutrophils across epithelial barriers [72]. However, there remains uncertainty regarding the role of proteolytic enzymes in migration of leukocytes through the subendothelial space.

Studies of the equivalent processes in vitro have been made difficult by the lack of adequate experimental models. A num-

ber of studies have investigated leukocyte adhesion and migration on isolated matrix proteins or substrates deposited by short-term endothelial cultures, although many of the results are conflicting. For instance, resting neutrophils adhered more efficiently to laminin than type IV collagen [73], but the opposite was found for neutrophils stimulated with PMA [74]. Neutrophils activated with PMA adhered to collagen type IV and fibronectin using $\beta_2$-integrins [74, 75]. However, in a different study, migration of neutrophils through collagen gels after stimulation with fMLP was mainly dependent on $\beta_1$-integrins [39], and the importance of $\beta_2$-integrins was dependent on gel concentration in another report [76]. We compared neutrophil adhesion and migration on purified BM proteins and on substrates deposited by short-term or prolonged cultures of ECs [77]. Whereas levels of adhesion of stimulated neutrophils did not vary greatly between these surfaces, it was apparent that neutrophil migration and integrin use were modified on the complete BM compared with purified proteins or substrate deposited after short-term culture. Neutrophil $\beta_1$-integrins had a greater role in attachment and migration on the BM, whereas the neutrophils actually migrated more slowly on it [77].

Leukocyte migration underneath ECs and through BM has not been studied widely in vitro. Huber and Weiss [53] demonstrated that over 21 days, cultured HUVEC laid down a BM with content, morphology, and permeability comparable with that seen in vivo. They showed that neutrophils could migrate through this BM into collagen gels, but there appeared to be a delay after crossing the endothelial layer. We compared migration through 4- and 20-day cultures of HUVEC on porous filters or collagen gels and found the latter deposited a distinct BM that held up neutrophil migration in static or flow-based models [42]. The isolated BM itself was also shown to be a barrier to chemotaxis. Moreover, there was a specific role for $\beta_3$-integrins in neutrophil migration on and through fully formed BM, not detectable with short-term cultures [42]. Interestingly, we and others [78, 79] reported that neutrophils migrated underneath ECs faster than they migrated on the luminal surface. However, in the recent studies, the cells migrating underneath long-term cultures were slower than those underneath short-term cultures [42].

Taken together, these studies show that the BM can modify responses of ECs supporting migration and that migration through endothelial monolayers and contact with BM modify the migratory behavior of neutrophils. Indeed, efficient transit through the BM may require changes in integrin use. Whereas $\beta_2$-integrins support migration over and through endothelial monolayers, $\beta_1$- and $\beta_3$-integrins take on greater roles in migration through the subendothelium (Fig. 2). These integrins can also bind various proteins found in the interstitial space, although recent studies have questioned whether they are actually required for migration in that compartment [80]. Whether all of the above applies to monocyte and lymphocyte recruitment is less clear. Recent reports indicate that monocytes may use different mechanism to cross the BM than neutrophils in mice [81] and that in vitro, BM delays migration of monocytes and lymphocytes less dramatically than neutrophils [42].

## Modification of BM structure in disease

Change in BM and ECM histology occur in a number of diseases, ranging from circulatory pathologies to fetal abnormalities. For example, in atherosclerosis, collagen and proteoglycan constituents of the subendothelial matrix are modified in lesions [82, 83]; indeed, in advanced plaques, deposition of multiple BM appears to occur [84, 85]. Asthma is associated with vascular remodeling, including excess collagen deposition in the BM [61], an altered BM proteoglycan profile [86], and an associated increase in inflammatory infiltrate [87]. Thickening of the microvascular BM is a common feature of diabetes [62]. In inflammatory liver fibrosis, BM components collagen type IV and laminin are deposited around vessels and in portal tracts [88]. As well as a pathological thickening of the BM, there are numerous disease states where the BM is thinned or absent, e.g., in chronic rhinosinusitis [89], chronic pancreatitis [90], and idiopathic pulmonary fibrosis [91] where inflammatory cell infiltrates are found. In such disorders, the BM may be altered through the proteolytic activity associated with inflammation [92].

There are few in vitro studies to link BM modification in disease to functional changes in inflammation. When microvascular ECs isolated from abdominal skin of diabetic or nondiabetic adults were maintained in culture by serial passage, the BM deposited was indistinguishable in terms of protein composition and arrangement [93]. This suggests that whereas the local environment might have caused ECs to change BM deposition in vivo, a change in EC phenotype was not imprinted or maintained in a uniform culture system. Indeed, when ECs were cultured in high glucose, to model diabetes, the adhesion of monocytes to the BM was modified [94], an effect attributable to modification of the proteoglycans. This study supports the concept that endothelial conditioning can modulate the BM constitution, leading to functional changes, which impact on the process of leukocyte recruitment.

The studies reviewed above show that contact with BM influences endothelial responses and modulates behavior of leukocytes. Thus, BM has direct and indirect effects (via ECs) on leukocyte adhesion and migration. Moreover, BM acts as a specific barrier to leukocyte migration, and efficient penetration requires signals from earlier steps in recruitment. It is hard, however, to allocate effects to specific constituents, as when used in isolation, the various proteins do not reproduce structures or functions. Nor is it possible to state unequivocally how the changes in BM seen in disease contribute to inflammatory leukocyte recruitment. It is possible, for instance, that changes could increase endothelial sensitivity and leukocyte recruitment, creating a "vicious cycle". Further studies of how modification of BM structure modifies recruitment are needed in physiological and pathological contexts. In vitro, these might include overexpression or ablation of specific constituents made by ECs while maintaining the other components structure. Comparable manipulations in mice might be allied to intravital microscopy. However, there remains the caveat for in vitro studies that stromal cells such as pericytes may contribute to "true" BM as well as the endothelium.

## EFFECTS OF STROMAL CELLS ON LEUKOCYTE RECRUITMENT

### Potential roles of stromal cells in inflammation

In addition to their structural role, stromal cells actively shape their local milieu through the secretion of ECM components, cytokines, chemotactic agents, and growth factors and through communication with neighboring cells (tissue-resident cells and the overlying endothelium; Fig. 1). The cellular composition of stroma varies greatly between different tissues, providing the potential for organ-specific modulation of leukocyte recruitment through several routes. Stromal cells might interact with ECs to condition their responses to inflammatory agonists; they may generate chemoattractants that are presented by ECs or deposited in matrix to encourage leukocyte migration; and they might interact directly with leukocytes to influence their fate and retention in tissue. In addition, stromal cells associated with sites of chronic inflammation may be "transformed" into active proinflammatory components of the afflicted tissue. For example, contractile SMCs convert to a secretory phenotype during the formation of an atherosclerotic plaque, resulting in enhanced proliferative and migratory capabilities [95], along with increased chemokine [CXCL8 (CXCL8), CCL2 (MCP-1)] and cytokine (TNF and IL-1) production [96–98]. Similar behaviors are associated with synovial fibroblasts in RA [99–101] and hepatic fibroblasts isolated from chronically diseased livers [102].

It could be proposed therefore that "normal" stromal cells play a role in regulating protective inflammatory cell infiltrates, whereas transformed cells/phenotypes may induce or perpetuate inappropriate and damaging leukocyte recruitment. Here, we examine these possibilities by considering how different types of normal or modified stromal cells affect leukocyte behavior in experimental models. These models broadly fall into two classes: leukocytes and stromal cells are brought together directly, or in more complex models, ECs or a transendothelial migration stage are included. By analogy to the studies on migration underneath ECs described above, it seems highly likely that the outcome of interactions between stromal cells and leukocytes will depend on the prior conditioning of the leukocytes by the earlier stages of recruitment.

## FIBROBLASTS

We have proposed that fibroblasts from different tissues are imprinted with a tissue-specific phenotype, which contributes to site-specific leukocyte recruitment and experimentally, is maintained ex vivo [11]. For instance, microarray technology revealed that fibroblasts isolated from the skin, synovium, or bone marrow displayed discrete transcriptional repertoires [103, 104]. When fibroblasts were cultured with ECs, both cell types showed changes in gene transcription, and the changes in the ECs were dependent on the anatomical source of the fibroblast [24, 105]. Comparable changes in gene expression may effectively alter leukocyte recruitment postcodes in disease, influencing establishment and/or the resolution of an inflammatory response by altering the endothelial phenotype or modulating behavior of migrating leukocytes.

### Fibroblasts from normal tissue

When dermal microvascular ECs were allowed to bind to cultures of dermal fibroblasts in a three-dimensional construct, they migrated to form tube-like structures with endothelial migration efficiency, depending on the subtype of fibroblast present [106]. However, endothelial recruitment of leukocytes has not been examined in such models. We have cocultured fibroblasts with ECs, in close contact on opposite sides of micropore filters, or on separate filters [24, 33, 105, 107]. Fibroblasts from noninflamed skin or synovium did not induce ECs to support adhesion of flowing neutrophils or lymphocytes [24, 107]. Indeed, when the cocultures were exposed to inflammatory cytokines (TNF+IFN), the fibroblasts caused a reduction in lymphocyte (but not neutrophil) attachment from flow [34]. This immunomodulatory effect was mediated by a combination of IL-6 and TGF-$\beta$, produced during the coculture period [24]. IL-6 itself was able to directly down-regulate the level of recruitment by ECs in monoculture. Consequently, we postulated that "healthy" fibroblasts were imprinted with a phenotype, which communicated to the overlying vascular endothelium, maintaining it in a basal/resting state and regulating its cytokine sensitivity to prevent uncontrolled leukocyte influx (see **Fig. 3A**).

The bone marrow, on the other hand, is a specialized organ in which immature leukocytes are retained until sufficiently mature to migrate through the sinusoidal endothelium into the circulation. In addition, aged neutrophils and pools of memory T cells are found to traffic back to the bone marrow, migrating from the blood into the tissue (e.g., ref. [108]). In the context of recruitment into the bone marrow, we recently observed that bone marrow fibroblasts induced a proadhesive state in cocultured endothelium, supporting lymphocyte recruitment in the absence of exogenous cytokines (unpublished results). The underlying mechanisms have yet to be determined, but the above results suggest that different stroma can contribute to induction or suppression of leukocyte recruitment by ECs, as appropriate to the tissue function.

Considering direct contact between leukocytes and fibroblasts, several papers have demonstrated the ability of T cells to adhere to fibroblasts isolated from healthy skin [109, 110] or the rheumatoid joint [111]. Adhesion to synovial fibroblasts was reduced markedly when fibroblast ICAM-1 or its ligand $\beta_2$-integrin on T cells was blocked [112]. In contrast, $\alpha_4\beta_1$-integrin on Jurkat cells appeared to mediate binding to resting or cytokine-treated fibroblasts from healthy juvenile skin [113]. Studies of chemotaxis revealed that neutrophils and monocytes migrated through monolayers of fibroblasts from various tissues (healthy lung and skin, RA synovium) on filters within 1 h toward the complement fragment C5a using a combination of $\beta_2$- and $\beta_1$-integrins [114–116]. In these experiments, the leukocytes had not migrated previously through the endothelium, and it is evident that under such conditions, the fibroblasts were unable to induce movement through the filter in the absence of exogenous chemotactic agents. The studies compared the ability of healthy and diseased fibroblasts to support leukocyte migration by using fibroblasts obtained from normal lung tissue after lobectomy for neoplasm, fibroblasts

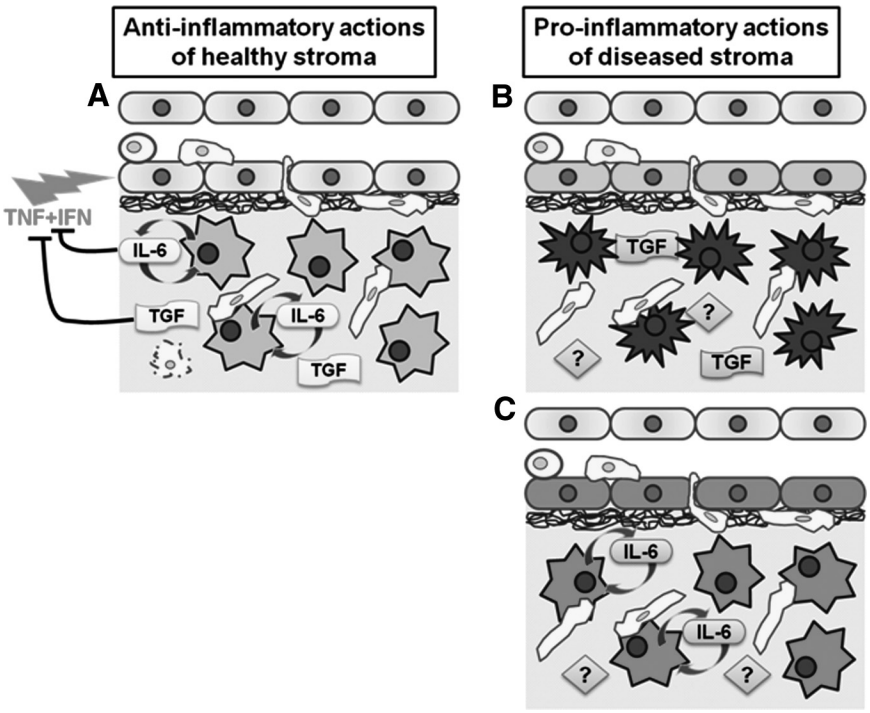


**Figure 3. Phenotype of stroma cells critically influences endothelial recruitment of leukocytes.** Certain cytokines are known to have dual roles with the ability to act in a pro- or anti-inflammatory manner. The outcomes arise from differences in levels and/or presence of other secreted cofactors generated by particular endothelial-stromal cell combinations (i.e., they depend on the stromal context). (A) In healthy fibroblasts, the presence of IL-6 in conjunction with TGF-$\beta$1 acts to suppress lymphocyte recruitment induced by TNF + IFN [24]. (B) In contrast, cofactors generated during cocultures with diseased SMCs allow TGF-$\beta$1 to act in a proinflammatory manner, priming leukocyte recruitment and platelet binding [25, 124, 129]. (C) Diseased fibroblasts generate a different profile of secretory factors transforming the actions of IL-6 into a proinflammatory agent. In this context, IL-6 indirectly induces recruitment of neutrophils and lymphocytes [24, 107].

from skin from children undergoing minor plastic surgery, and fibroblasts from the rheumatoid joint [114, 116]. However, no studies have directly compared healthy and diseased fibroblasts from the same tissue. In earlier studies, fibroblasts have been cultured for widely varying numbers of passages prior to use. However, it is now recognized that the phenotype of stromal cells drifts during in vitro culture [117], and so, consistent use of similar low passages is desirable for comparative studies.

## Fibroblasts from diseased tissue

OA and RA are common joint disorders characterized by tissue remodeling, including the proliferation of fibroblasts in the synovial membrane, in conjunction with persistent inflammation. In contrast to the fibroblasts from healthy tissue, rheumatoid synovial fibroblasts activated cocultured ECs such that they supported the recruitment of neutrophils and lymphocytes from flow in vitro (in the absence of additional stimuli) [24, 33, 105, 107]. Capture was mediated through P-selectin or VCAM-1 for neutrophils or lymphocytes, respectively [24, 107]. Adhesion was stabilized rapidly by the presentation of fibroblast-derived chemokines on the endothelial surface [105], resulting in neutrophil activation through CXCR2-binding CXCL5 (epithelial neutrophil-activating protein 78) and lymphocyte activation through CXCR4 engagement of CXCL12 (stromal cell-derived factor 1$\alpha$) [24, 105, 107]. Subsequently, the transcellular trafficking of fibroblast-derived CXCL5 to the endothelial surface by DARC was shown to underlie neutrophil activation [105]. For lymphocytes and neutrophils, recruitment was dependent on IL-6 generated during the coculture period, although treatment of EC with IL-6 alone did not cause adhesion [24, 105, 107].

As IL-6 was also required for the immunomodulatory effects of dermal fibroblasts noted above, it is likely its actions (pro- or anti-inflammatory) depend on other products secreted by particular EC–stromal cell combinations. For the RA fibroblasts, IL-6 secretion and in turn, neutrophil recruitment were suppressed by increasing doses of the glucocorticoid hydrocortisone [105], mimicking clinical effects of steroids and supporting the use of anti-IL-6 therapy in this disease. Synovial fibroblasts from patients with OA also promoted lymphocyte adhesion to otherwise "resting" endothelium, although the mechanism(s) were not demonstrated [24]. These results suggest that fibroblast residents within diseased synovium are imprinted with proinflammatory characteristics that can be translated to the overlying endothelium as modifications in the expression of adhesion receptors and chemokines, potentially creating and/or sustaining the chronic inflammatory milieu (see Fig. 3B).

In addition to modulating endothelial responses, stromal cells may drive onward migration of recruited leukocytes. In contrast to cells from healthy controls, dermal fibroblasts isolated from patients with scleroderma, who suffer from perivascular inflammatory infiltrates, promoted migration of mononuclear leukocytes through ECs cultured on an 8-$\mu$m pore filter [118]. This affect was apparent when the fibroblasts were cultured on the opposite side of the filter to the EC or in the well underneath, implying that soluble mediators released into the culture medium supported this phenomenon. Indeed, this enhanced migration was attributable to the presence of fibroblast-derived chemokines such as CCL2, rather than changes in the expression of endothelial adhesion receptors [118]. In an analogous system, where we cultured ECs above fibroblasts on separate filters, fibroblasts from healthy skin and RA syno-

vium promoted migration of T cells through the endothelial layer [33]. However, when T cells were brought into direct contact with the same fibroblasts, $CD4^+$ and $CD8^+$ cells migrated through the synovial fibroblasts much more efficiently than through the dermal fibroblasts [100]. This difference arose from greater secretion of CXCL12 by the former. Thus, fibroblasts from healthy and diseased tissue may modulate the migration and possibly retention of T cells in tissue, although the inflamed phenotype may generate additional attractants.

Surprising results, perhaps relevant to damaged tissue, occurred when dermal or synovial fibroblasts were cultured in close contact with ECs (on either side of 3 $\mu$m-pore filters). Lymphocyte capture from flow was ablated, and in a static assay, migration through the endothelium was reduced [33]. In contrast, the close contact had little effect on cytokine-driven capture or transendothelial migration of neutrophils but tended to increase the efficiency of neutrophil migration away from the endothelium [33]. The close endothelial-fibroblast contact may have cleaved structures required for the presentation of chemokines to lymphocytes but not to neutrophils [33].

Additionally, in some patients, elements of the rheumatoid synovium acquire lymphoid-like functions, creating regions within the inflamed tissue that architecturally resemble LNs (e.g., [119, 120]). TLO neogenesis is believed to be triggered by the reactivation of physiological pathways involved in embryonic development of secondary lymphoid organs (reviewed in detail in refs. [121, 122]). In RA, follicular DCs within TLO are thought to be derived by differentiation of local synovial fibroblasts or their precursors [11, 123]. Thus, synovial fibroblasts are subjected to at least two different reprogramming pathways (proinflammatory and then lymphoid neogenesis) during persistence of RA. In addition to the structural architecture created by the fibroblasts, recruited leukocytes may act as nonconventional lymphoid inducer cells within the joint, fueling TLO development (reviewed in refs. [121, 122]). Why cells recruited into acutely (rather than chronically) inflamed tissues do not take on the same functions is largely attributed to the absence of continual antigen/stimulus associated with chronically inflamed sites (reviewed in refs. [121, 122]). Whereas many questions remain to be addressed, it is clear that recruited leukocytes and stromal components acquire lymphoid inducer and organizer functions, resulting in the formation of TLO. Thus, an early component of the process may be the ability of transformed fibroblasts to induce continual leukocyte recruitment.

In summary, fibroblasts from healthy or diseased tissue can modulate the ability of vascular ECs to recruit flowing leukocytes and may also direct their onward migration. Fibroblasts imprinted with an inflammatory phenotype appear able to directly induce adhesion and migration, whereas those from healthy tissue may have immunomodulatory effects in the context of inflammatory cytokines from other sources. Based on the ubiquity of fibroblasts in all tissue, one might propose that they are fundamental to the control of inflammation. Important questions remain. Even normal fibroblasts from different tissues have different effects (e.g., bone marrow versus skin), and this raises the question of whether they contribute to or even institute tissue-specific recruitment of different types of T cells. In the context of chronic inflammation, it is uncertain at what stage stromal cell phenotype changes and thus, whether such changes are the cause or a consequence of disease or contribute specifically to maintenance of a disease state. In the foregoing, IL-6 had pro- or anti-inflammatory effects depending on the stromal context, and more information is required as to which cofactors operate to achieve these critical differences (see Fig. 3). Finally, it remains to be clarified how direct interactions noted between leukocytes and fibroblasts translate into effects on functional behavior of the leukocytes or their ability to move through or clear the tissue. Improved methods of tracking cells, as they move through endothelial and stromal layers and possibly into draining lymphatics, will be required to answer this question.

## SMCs

Studies of proinflammatory potential of SMCs have centered on cells in the secretory state that have been implicated in the development and persistence of atherosclerotic plaques. Indeed, SMCs cultured in vitro proliferate in a state resembling the disease-associate cells, although they can be reverted to a contractile phenotype by serum and growth factor deprivation [124]. The primary invading leukocytes within atherosclerotic lesions are monocytes, which differentiate into macrophage like lipid-laden foam cells within the lesion. Early studies in vitro showed the rapid and efficient migration of monocytes into the subendothelial space in a layered aortic construct of ECs on a collagen gel, on top of SMCs cultured on a filter [125]. Migration could be enhanced by addition of fMLP beneath the filter of coculture constructs but not monocultures [125]. Coculture of aortic ECs and SMCs on opposite sides of a filter also enhanced monocyte migration through the filter and accelerated their subsequent differentiation into macrophages [126]. Similarly, migration of MonoMac6 cells (a monocyte cell line) through an intima-like structure (ECs cocultured with SMCs on a fibrin/thrombin polymer) was reported to induce their differentiation into macrophages or foam cells in the presence of LDLs, over 4 days [127]. Thus, in these static models, secretory SMCs appeared to stimulate the overlying endothelium enhancing monocyte migration and potentially altering their phenotype within the "tissue".

Considering the capture process, we showed that coculturing ECs and secretory SMCs on opposite sides of a porous filter augmented the response of the ECs to the TNF greatly, so that leukocytes (neutrophils, monocytes, and lymphocytes) were captured at a much lower dose [124]. This priming effect occurred through the release and activation of TGF-$\beta$ during coculture, leading to an increased expression of endothelial E-selectin and VCAM-1 [124]. Despite this priming capacity, secretory SMCs did not activate endothelium to capture leukocytes in the absence of exogenous cytokine. In a subsequent study, ECs were seeded onto a collagen gel containing SMCs and incorporated into a perfusion chamber with a vertical step to mimic disturbed flow at vessel bifurcations [128]. Under these conditions, SMCs increased the ability of ECs to support leukocyte (neutrophil, monocyte, and lymphocyte) capture from flow and promoted subsequent transendothelial migra-

tion [128]. The presence of SMCs augmented the surface expression of E-selectin, ICAM-1, and VCAM-1, and several chemokines (e.g., CXCL8, CCL2, and CXCL12) were secreted into the collagen gel, each of which was reported to a play role in the recruitment and migration of the different leukocyte subsets [128]. The latter studies did not use exogenous cytokine. In more recent studies, we found that secretory SMCs also caused ECs to support adhesion of flowing platelets through TGF-$\beta$-induced presentation of the von Willebrand factor [25]. Importantly, this process caused selective recruitment of monocytes from flowing blood, through adhesive platelet "bridges" and through platelet factor-4 acting to upregulate production of CCL2 by the ECs [129]. Although the above studies do not all agree on the direct activating or priming role(s) of SMCs, they clearly illustrate that secretory SMCs have a "proinflammatory" phenotype that promotes leukocyte recruitment from flow in a TGF-$\beta$-dependent manner (Fig. 3C).

Direct contact of leukocytes with SMCs in the absence of prior migration has also been studied. For example, neutrophils, monocytes, and T cells have been shown to bind SMCs treated with IL-1 alone or in combination with TNF, where adhesion could be inhibited partially with antibodies against VCAM-1, ICAM-1, or their leukocyte counter-receptors ($\alpha_4\beta_1$- or $\alpha_L\beta_2$-integrins) [130–132]. Similarly, combined inhibition of $\beta_2$- and $\beta_1$-integrins and CD44 significantly reduced TNF-induced T cell adhesion to SMCs [133]. In another study, SMCs cultured from injured arteries of rat were shown to capture flowing monocytes and T cells in vitro through their abnormal surface expression of P-selectin and chemokines (e.g., CXCL1 and CXCL12) [134].

The foregoing illustrates that transformed SMCs are capable of modulating endothelial responses and promoting leukocyte migration. In contrast to the studies with fibroblasts, TGB-$\beta$ was proinflammatory, and this again raises questions about the cofactors present in a complex stromal milieu, which combine to regulate recruitment of leukocytes. Interaction with SMCs influenced development of the monocyte-macrophage phenotype, but it remains to be clarified whether this depended on direct adhesive interactions and whether SMCs can modulate responses or a phenotype of other recruited leukocytes, such as T cells. Thus, further studies of leukocyte fate in coculture models, possibly incorporating platelets, as well as SMCs and ECs, appear warranted.

## EPITHELIAL CELLS

Epithelial cells are close neighbors to vascular endothelium in several organs, including the lung, kidney, gut, and liver, and might be expected to modulate inflammatory responses. Their effects on inflammatory cell recruitment have been examined in various experimental models. Activation of colonic epithelial cells (Caco-2 carcinoma line) with TNF + IFN induced ICAM-1 and VCAM-1 surface expression on otherwise resting ECs following coculture in a process partially dependent on the release of TNF [135]. Such evidence suggests that epithelial cells might activate neighboring ECs to support leukocyte recruitment. When renal tubular epithelial cells and ECs were cultured on opposite sides of filters and treated with TNF or LPS, less neutrophils were able to transmigrate than for ECs alone but more than for epithelial cells alone [136]. In another study, culture of mesothelial cells (peritoneal lining epithelium) with ECs did not modify neutrophil chemotaxis through the cultures to TNF or C5a [137]. Coculture of transformed lung epithelial cells (H292 lung adenocarcinoma cell line) or bronchial epithelial cells with ECs tended to enhance neutrophil chemotaxis to CXCL8 but did not promote migration without addition of chemokine [138]. Transmigration through epithelial cells alone was not efficient. There was evidence of crosstalk between the cultured cell types involving epithelial IL-6, but as the two stages of migration through the ECs and epithelial cells were not separated, it is not certain if there was an effect on the endothelial migration stage itself. Another lung epithelial cell line (A549) differentially modified endothelial responses to different stimuli, inducing a reduction in neutrophil transendothelial migration when cocultures were treated with LPS, but not TNF [139, 140]. The reduction with LPS was attributed to decreases in the production of E-selectin and CXCL8 in the cocultured endothelium [140]. Impaired recruitment was specific to the transendothelial stage of migration, which was observed directly; epithelial transmigration was not possible, as 0.4 $\mu$m pore filters were used to separate the two cell types.

An in vivo study attempted to dissect the kinetics of neutrophil migration into lung using a murine model of acute lung injury [141]. Following LPS inhalation, murine neutrophils exited the vasculature and migrated into the lung interstitium within 1 h [141]. Interestingly, onward movement across alveolar epithelium was a much slower process, as recruited cells appeared to be retained within the interstitial space for several hours [141]. In PI3K$\gamma$-deficient mice, neutrophil migration into the lung interstitium was enhanced, whereas transepithelial migration into the alveolar space was reduced substantially [142]. Moreover, neutrophil chemotaxis across human pulmonary epithelial monolayers but not endothelial monolayers was also reduced when monolayers were pretreated with a PI3K$\gamma$ inhibitor [142]. Recent evidence indicates that alveolar epithelial cells can enhance infection-induced leukocyte recruitment by shedding ICAM-1 into the alveolar space [143]. In this format, soluble ICAM-1 was reported to act in synergy with pathogen-derived LPS to amplify the response of alveolar macrophages, which was speculated to encourage the transepithelial migration of leukocytes [143].

For isolated epithelial cells without endothelium, neutrophils migrated through intestinal crypt T84 cells toward fMLP in a $\beta_2$-integrin-dependent manner [144]. Migrating neutrophils have been reported to facilitate their onward transit across intestinal epithelial barriers in vitro by inducing the activation of epithelial protease-activated receptors 1 and 2 [72]. Moreover, viral infection of primary alveolar epithelial cells induced the release of CCL2 and CCL5, promoting integrin-dependent monocyte transepithelial migration in vitro [145]. In addition, TNF stimulation of A549 alveolar epithelial cells induced CXCL8-dependent neutrophil chemotaxis [146].

In summary, epithelial cells present a distinct barrier to leukocytes, and it appears that transepithelial migration is an actively regulated process, as well as transendothelial migration. However, there is a difficulty in interpreting studies where the stromal cells, such as epithelial cells, form a confluent physical barrier beyond ECs. In general, the combined layers may be more permissive than the epithelial cells alone, suggesting that the epithelial cells may have modulated endothelial responses (or vice versa). However, current studies have not assessed how the two cell types are affecting migration through the separate layers. To answer the question of "Who is doing what to whom?" will require use of integrated models, which allows the evaluation of the separate stages of migration in sequence. It may then be possible to dissect the crosstalk between cells, which modifies entry into interstitium and exit into epithelial-lined spaces. Moreover, there are yet to be studies that directly compare diseased with healthy epithelial cells of a specific type and thus, address what role abnormal epithelium may play in generating inflammatory infiltrates.

## HEPATOCYTES

Liver sinusoidal ECs exhibit a distinct morphology lacking tight junctions and associated BM, resulting in their close proximity to the underlying hepatocytes (reviewed in ref. [147]). It has been reported that rat hepatocytes were able to transform quail microvessels into the sinusoidal phenotype associated with the endothelium of the liver [148], supporting the concept that the endothelial phenotype may be derived from the local stroma. During coculture, human hepatocytes triggered the up-regulation of VCAM-1 and E-selectin upon the surface of human hepatic sinusoidal endothelium, enabling lymphocyte capture from flow [149]. Moreover, if lymphotoxin $\beta$ were added, the presence of hepatocytes further enhanced lymphocyte adhesion to hepatic sinusoidal endothelium by augmenting the expression of additional adhesion receptors (vascular adhesion protein-1, ICAM-1, and CD31) [149]. The "modified" state observed in untreated cocultures suggests that a basal level of endothelial activation could exist in vivo (similar to that noted earlier for the bone marrow). Consequently, the endothelium in a specialized organ, such as the liver, might support constitutive lymphocyte recirculation as part of immune surveillance, whereas during inflammation, the level of endothelial activation could be substantially amplified to maximize leukocyte infiltration.

## ASTROCYTES AND MICROGLIAL CELLS

The BBB is formed by the interactions between microvascular ECs with neighboring astrocyte processes and microglial cells. Under normal conditions, the BBB functions to restrict the passage of cells and macromolecules into the CNS. Indeed, coculturing ECs with astrocytes dramatically reduced their permeability when compared with endothelial monocultures, indicating the ability of astrocytes to induce barrier properties in neighboring ECs [150]. These regulatory responses appear to be lost during diseases such as MS, where the BBB becomes leaky and may permit or facilitate the passage of leukocytes into the CNS.

Most of the evidence surrounding leukocyte trafficking into the CNS has been obtained using animal models, where it is difficult to attribute effects to particular stromal cells (reviewed in ref. [10]). However, a few studies have attempted to dissect these processes in vitro using primary cells. Early studies reported that CCL2 induced monocyte chemotaxis across resting endothelial-astrocyte cocultures in a dose-dependant manner [151]. In response to inflammatory cytokines (TGF-$\beta$, TNF-$\alpha$, IFN-$\gamma$, or IL-1$\beta$), cocultures supported greater levels of monocyte and lymphocyte migration, an effect that was dependent on astrocyte-derived, soluble CCL2 [151]. Monocyte migration was supported by ICAM-1 but not VCAM-1 and was also partially attributed to E-selectin [151]. The importance of E-selectin within this study was presumably during the initial binding rather than contributing to migration per se. In a study incorporating feline cells, greater numbers of mononuclear leukocytes were observed adhering to endothelium cocultured with astrocytes compared with monocultures [152]. Coculture with microglial cells had no effect on the overall level of mononuclear leukocyte adhesion to endothelium. Subsequently, a triculture was established, where microglial cells were cultured on the bottom of a well underneath ECs and astrocytes cultured on opposite sides of a filter [152]. Under these conditions, the microglial cells dramatically reduced mononuclear cell adhesion to the endothelium [152]. This suggested a suppressive regulatory effect of microglial cells under resting states. Whether similar effects might be observed under exogenous inflammatory stimuli was not determined in this study. The phenotypes of astrocytes, pericytes, and other glial cells are altered in chronic diseases, including MS, but whether these actively impact leukocyte recruitment in conjunction with disrupting BBB integrity remains unclear from current studies (e.g., reviewed in ref. [153]).

In the context of infection, feline immunodeficiency virus infection tended to increase leukocyte adhesion to astrocytes, a phenomenon that was suppressed by cocultured microglial cells [152], indicating the ability of microglial cells to regulate the inflammatory activity of neighboring astrocytes. In a similar study using human cells, HIV infection of microglial cells enhanced monocyte migration through cocultures [154], suggesting that the suppressive effect was modulated during the activation/infection process. Moreover, infection with HIV increased CCR2 expression on monocytes and lymphocytes, enhancing CCL2-directed migration through uninfected, endothelial-astrocyte cocultures [155]. Similarly, pretreating cocultures with individual HIV proteins (Tat B or Tat C) also enhanced monocyte migration in the absence of exogenous CCL2 [156]. Of note, these HIV proteins were shown to alter the expression of tight-junction proteins [156]. Thus, changes in leukocyte migration during HIV infection may include a contribution from physical disruption of the integrity of the BBB, as well as modulation of stromal cells.

In summary, astrocytes may encourage leukocyte migration, but this response may be modulated by microglial cells. Whether these regulatory effects are disrupted in inflammatory disorders, the mechanisms by which microglial cells have their effects, and whether they occur only through astrocytes require further investigation.

## STROMAL MICROENVIRONMENT IN TUMORS

The stroma in tumors may have many effects on development of disease, including regulation of local inflammation. As in chronic inflammatory diseases, tumor stroma has the capacity to communicate with its surrounding vascular endothelium, healthy stromal cells, or infiltrated leukocytes to modulate its behavior (see reviews in refs. [157, 158]). For example, tumor-induced endothelin B receptor signaling in endothelium suppressed recruitment of tumor-infiltrating lymphocytes, a response attributed to alterations in ICAM-1 expression [159]. This may be considered an example of immune evasion that could shield tumors from attack. On the other hand, extensive evidence indicates that infiltrating leukocytes can also have

**TABLE 1. Overview of Effects of Stroma on Leukocyte Recruitment in Inflammation and Outstanding Questions Regarding Functions**

| Stromal component | Origin/phenotype | Effects or changes in inflammation | Outstanding questions |
|---|---|---|---|
| BM | Endothelial culture, murine venules | Modulates EC responses | Roles of specific constituents |
| | Inflammatory diseases | Barrier to migrating leukocytes | Impact of changes in content on EC or leukocyte migration |
| | | Changes in constituents and thickness | Link between changes in constituents and function |
| Fibroblast | Healthy skin | Modulates EC response to cytokines | How do IL-6 and TGF-$\beta$ combine to regulate an endothelial response? |
| | Bone marrow (and other tissues?) | Induces PBL adhesion | Role in tissue-specific or constitutive recruitment |
| | RA or OA | Activate ECs and induce leukocyte adhesion | When does change in phenotype occur during pathogenesis? |
| | | | Cause or effect of inflammation |
| | | | Cofactors operating with IL-6, and are mechanisms similar for different arthritides? |
| | | | Transformation reversible or inhibitable for therapy |
| | Generic | | Role in onward migration, leukocyte phenotype, and egress |
| SMCs | Secretory | Prime/activate EC to increase leukocyte recruitment | What cofactors underlie the proinflammatory effect of TGF-$\beta$? |
| | | Modify monocyte/macrophage development | Are phenotypes of other recruited leukocytes modified? |
| Epithelial cells | | Barrier to migrating leukocytes | Can epithelial cells modulate EC responses? |
| | | Possible crosstalk with EC to modify transmigration | Can epithelial cells regulate interstitial leukocyte migration? |
| | | | Do changes in epithelial cells influence recruitment in disease? |
| Hepatocytes | | Initiate or augment PBL recruitment | Role in constitutive traffic in liver |
| Astrocytes and microglia | | Astrocytes increase leukocyte migration. | Are effects through modulation of EC responses? |
| | | Microglia modulate effects of astrocytes. | Do microglial cells impact on EC, astrocytes, or both? |
| | | | What are the soluble mediators? |
| | | | Are effects modified in disease (e.g., MS)? |
| Mast cells and macrophages | | Generally act as sensors of inflammatory insults and in turn, instigate leukocyte recruitment | To what extent can cells derived from blood monocytes be considered as stromal cell regulators of inflammation? |
| Other | | | Do pericytes modulate recruitment outside of a barrier function? |
| | | | What cofactors regulate the pro- and anti-inflammatory effects of ubiquitous mediators, such as IL-6 and TGF-$\beta$? |

tumorgenic roles (see review in ref. [160]), leaving open the question of whether stromal effects on recruitment would be harmful or beneficial. Such effects of tumor stroma may be considered as special cases of effects of fibroblasts, epithelial cells and others, and are not reviewed here further.

## TISSUE-RESIDENT CELLS DERIVED FROM LEUKOCYTES

In addition to the structural stromal components outlined above, there are long-lived leukocytes that reside within the tissue (e.g., perivascular mast cells or tissue-resident macrophages) with the clear capacity to modulate the leukocyte recruitment cascade. Whether these can be considered as "stromal" effectors is open to question. However, perivascular mast cells house a reservoir of proinflammatory mediators (cytokines, chemokines, LTs, and proteases) in preformed intracellular granules. Therefore, it is not surprising that mast cell activation and the resulting degranulation actively favor leukocyte recruitment (reviewed in ref. [161]). For example, depletion or stabilization of mast cells reduced neutrophil accumulation in LPS and zymosan-induced peritonitis [162] and collagen-induced arthritis [163]. In vitro, MIP2 stimulation of murine mast cells cultured with ECs augmented neutrophil adhesion when compared with unstimulated cocultures [164]. The immunomodulatory functions of mast cells have largely been elucidated in vivo using animal models of inflammation (see review in ref. [165]). As far as we are aware, no studies have directly examined capabilities of human cells in vitro, but the fact that they contain agents known to induce expression of adhesion molecules makes it likely that they can induce recruitment.

The capacity of monocyte/macrophages to modulate inflammation has been well-documented (see reviews in refs. [166, 167]). For instance, in vitro coculture of newly recruited monocytes with ECs caused activation of the latter within hours, inducing the expression of VCAM-1 and P- and E-selectin, which in turn, supported the binding of neutrophils and lymphocytes from flow [168, 169]. In vivo, tissue-resident macrophages displayed a stimulus-specific immunomodulatory role; their depletion augmented neutrophil infiltration in response to zymosan-induced peritonitis but reduced neutrophil migration in LPS-induced peritonitis [162]. These studies suggest that recruited, monocyte-derived cells can be considered as regulators of the outcome or progress of inflammation, as well as more obviously, the direct instigators responding to external stimuli.

## CONCLUDING REMARKS

The main effects of stromal components on leukocyte recruitment in inflammation are summarized in Table 1, where outstanding questions noted above are also highlighted.

An overarching theme is that ECs are pliable in their phenotype, at least where leukocyte recruitment is the readout, with marked changes induced by stromal and also the local hemodynamic environment (e.g., reviewed in ref. [8]). Stromal components may impose tissue-specific transcriptional repertoires on the endothelium dictating recruitment patterns (e.g., specificity of leukocyte subsets and order of ingress). Here, we have highlighted responses associated with healthy tissues, which can be transformed by stromal cells derived from sites of chronic inflammation. Physiological regulatory signals may maintain the vasculature and tissue in an inert state, preventing any unwanted leukocyte recruitment, while modulating the intensity of endothelial activation during protective inflammation to allow appropriate levels of recruitment. Other tissue-specific stroma may allow constitutive recirculation as part of immune surveillance by maintaining the endothelium in a partially activated state, which can be amplified in response to cytokines to maximize leukocyte infiltration. These regulatory mechanisms appear to be lost in chronic diseases, where stromal cells can maximally and continuously activate overlying ECs inducing and/or sustaining unwanted leukocyte infiltration.

As noted in Table 1, the mechanisms responsible for changes in stromal cell phenotype and their importance in disease initiation and progression require further investigation. Indeed, the mechanism by which basal tissue imprinting occurs also remains to be fully understood. Evidence to date suggests that in either case, there may be complex interplay between cytokines and growth factors, which is difficult to dissect and requires sophisticated experimental modeling. However, an ability to locally target the stroma to "turn off" recruitment or promote clearance of leukocytes would be a powerful therapeutic tool. This might require chronically inflamed stroma to be reprogrammed to revert to its stable physiological state or therapeutic mimicry of the effects of normal immunomodulatory stroma. In either case, improved understanding of the mechanisms by which stroma modulates recruitment and function of leukocytes will be essential.

## AUTHORSHIP

H..M.M, L.M.B., C.D.B., G.E.R., and G.B.N. contributed to the writing of this review.

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KEY WORDS:
endothelial cells · adhesion · migration · stroma