Modulation of endothelial responses by the stromal microenvironment: effects on leucocyte recruitment

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Abstract

Vascular endothelial cells play a pivotal role in regulating leucocyte recruitment during inflammation, and their responses can be modulated by their local environment, including cells of the tissue stroma. We have developed a model system to examine how the communication between endothelial cells and fibroblasts regulates the recruitment of leucocytes and their subsequent subendothelial fate. Here, we describe a novel co-culture filter-based flow assay and highlight the ability of synovial fibroblasts obtained from chronically inflamed tissue to promote leucocyte recruitment to otherwise ‘resting’ endothelial cells.

Introduction

A fundamental requirement for an inflammatory response is the recruitment of leucocytes from the circulation into the extravascular tissue. This process typically occurs in post-capillary venules after the vascular endothelium has become activated by pro-inflammatory cytokines such as TNFα (tumour necrosis factor α) or IL (interleukin)-1 (reviewed in [1]). In response to cytokines, the endothelium expresses receptors [e.g. selectins and VCAM-1 (vascular cell adhesion molecule-1)], which allow the capture of flowing leucocytes. Migration is further induced by the presentation on the endothelial surface of chemokines [e.g. CXCL8 (CXC chemokine ligand 8; IL-8)] that activate leucocyte integrins and induce their motility (reviewed in [1,2]). In vivo, the vascular endothelium is situated in close proximity to other stromal cells [e.g. pericytes, fibroblasts and SMCs (smooth-muscle cells)], which are likely to have a significant impact on endothelial responses during inflammation. Thus, although in vitro models using endothelial cells cultured in isolation have proved useful in elucidating the mechanism of capture and initial migration of leucocytes, they are too simple to allow investigation of the more complex events that occur as a result of the cross-talk between the endothelium and neighbouring stromal cells.

Key words: adhesion, endothelial cell, fibroblast, leucocyte, neutrophil, stromal cell.

Co-culture and flow-based adhesion models

We have developed co-culture methods to investigate the influence of normal and pathogenic stromal cells on recruitment of leucocytes across the vascular endothelium. For example, we have found that co-culturing HUVEC (human umbilical-vein endothelial cells) with secretory SMCs greatly sensitized the endothelial cells to exogenous TNFα, thereby amplifying the recruitment of neutrophils, monocytes and lymphocytes [3]. For such experiments, we seeded HUVEC and the desired stromal cell type (either SMCs or fibroblasts) on opposite sides of a porous Transwell filter, allowing cross-talk between each cell type for a desired period, typically 24 h. In initial experiments, the filter assemblies were incorporated whole into a flow chamber and we used fluorescence microscopy to study the capture of flowing leucocytes labelled with fluorochromes [3]. However, we could not easily visualize the adhesive or migratory behaviour of leucocytes in this model, or follow them through the filter. Adapting this model for phase-contrast microscopy, we designed a system where porous filters were cut out and mounted on to glass coverslips before incorporation into the flow chamber (Figure 1A) [4]. This novel filter-based model allowed us to directly visualize leucocyte capture from flow, subsequent transendothelial migration and then trans-filter migration out of the subendothelial space into the stromal layer. In initial experiments, we fixed and fluorescently stained the filters following the flow assay to confirm leucocyte location (Figures 1B–1D).

Role of chronically inflamed fibroblasts in modulating endothelial responses

Fibroblasts are a heterogeneous population that exhibit topographic and positional memory, even within a single tissue [5–7]. Recently, we proposed a ‘stromal post-code’ hypothesis for fibroblasts from different anatomical sites, which indicated their capacity to differentially modulate leucocyte recruitment and subsequent fate in inflammation [8]. Others have reported that dermal fibroblasts isolated from scleroderma patients can promote T-cell (Jurkat cell line) transmigration through an immortalized HUVEC line...
Figure 1 | Schematic representation of the flow chamber allowing the visualization of neutrophil location within the filter

(A) The filter was cut out directly on to the glass coverslip, covered with a Parafilm gasket containing a flow channel and placed into the receiving slot milled into the bottom plate. The plates were screwed together so that the inlet and outlet holes aligned with the flow channel, and leucocytes were perfused at a constant wall shear stress of 0.1 Pa. In addition to visualizing neutrophil adhesion and transendothelial migration (B), we could observe trans-filter migration (F) or trans-fibroblast migration (D).

[9]. Thus we hypothesized that changes in stromal post-code in fibroblasts could influence the transition from acute to chronic inflammation.

Rheumatoid synovial fibroblasts are believed to play a pivotal role in the progression and perpetuation of RA (rheumatoid arthritis). However, the mechanisms by which synovial fibroblasts influence leucocyte recruitment remain poorly understood. We recently demonstrated that synovial fibroblasts from RA patients were capable of activating co-cultured endothelial cells so that they supported capture and adhesion of flowing neutrophils in vitro [10]. Paracrine signals between rheumatoid synovial (but not dermal) fibroblasts and endothelial cells caused neutrophil adhesion in the absence of exogenous cytokines [10]. This adhesion was dependent on the presence of soluble IL-6 generated during co-culture (Figure 2) [10]. Furthermore, inhibition of P-selectin, CXCR2 (CXC chemokine receptor 2) and CXCL5 (ENA-78 [epithelial cell-derived neutrophil-activating factor (78 amino acids)]) reduced neutrophil adhesion [10]. We have now noted a similar ability of rheumatoid fibroblasts to induce lymphocyte recruitment, and we also investigated the ability of different types of fibroblasts to modulate responses driven by cytokines (results not shown). Thus we suggest that endothelial cells may be stimulated, or have their inflammatory responses modulated, by underlying stromal cells, and a pathologically altered stromal microenvironment appears capable of inducing and sustaining an activated endothelial state, which could contribute to perpetuation of chronic inflammation.

Figure 2 | Proposed model for synovial fibroblast activation of endothelial cells, allowing leucocyte recruitment

IL-6, secreted during endothelial cell co-culture with synovial fibroblasts, is believed to signal through the fibroblasts to generate unknown stimulatory signals, which in turn lead to chemokine trafficking to the endothelial cell surface and up-regulation of adhesion receptors.

Summary

We have developed in vitro co-culture models to provide new insights into the intricate communication between endothelial cells and underlying stroma that regulates the recruitment of leucocytes. A detailed understanding of inflammatory processes is vital for elucidating the mechanism responsible for the transition from transient to persistent inflammation. Multicellular in vitro models can play a role in furthering our knowledge of such issues and have the potential to reveal novel pathogenic pathways. Dissection of the cross-talk between endothelial cells and stromal cells in vitro may further our understanding of the development and progression of inflammatory diseases such as RA and potentially reveal targets for therapeutic interventions.

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References


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