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Microarray analysis of primary endothelial cells challenged with different inflammatory and immune cytokines

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Abstract

To investigate the potential molecular mediators of tissue-specific recruitment, we explored the influence of different cytokine challenges on gene expression regulation in five primary endothelial cells (ECs), representing two different phenotypes: iliac artery and aortic (macrovascular); lung, colon and dermal (microvascular). We challenged ECs with cytokines that elicit different patterns of inflammatory and immune responses in immune cells: tumor necrosis factor (TNF-α), interferon-γ (IFN-γ) or interleukin-4 (IL-4), and used microarrays containing approximately 40,000 unique cDNAs, to assess changes in differential gene expression relative to untreated cells. Five hundred and sixty three sequences changed by at least 2.5 fold in one or more of the 15 possible EC /cytokine combinations. The list included highly regulated adhesion molecules, chemokines, cytokines, metalloproteases, and IFN-γ-induced genes. Overall, IFN-γ caused the largest number of gene expression changes and its profile was least correlated with IL-4. In addition to clusters that were predominantly EC/cytokine specific, we also observed several clusters that were regulated by more than one cytokine across several ECs. Furthermore, we identified genes that were reciprocally expressed in response to different cytokines that could serve as markers of inflammatory and immune expression. These results confirm the importance of microenvironment in primary ECs that could have important applications in developing targeted therapies for vascular diseases.

Keywords: Cytokines; Endothelium; Immunology; Inflammation; Vascular biology

1. Introduction

Several previous studies have suggested the importance of inflammatory mechanisms in vascular pathophysiology. Pro-inflammatory cytokines, such as TNF-α, IFN-γ and IL-4, are thought to be important in coordinating the cell-to-cell interactions between immunocytes, endothelial and smooth muscle cells. However, the differential effects of these cytokines on endothelial gene expression are not completely understood. Contrary to the early considerations, ECs lining the lymphatic and blood vessel walls are not simply a passive barrier, but an active participant in the recruitment process. Moreover, while it has long been recognized that ECs from various organs exhibit phenotypic specialization [1], the influence of microenvironment is only beginning to be understood at the molecular level. There is increasing evidence showing that ECs cultured from different vascular beds display specific patterns of

Abbreviations: EC, Endothelial cell; EST, Expressed sequence tag.

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gene expression and associated markers [2], and that the surface molecules on the endothelium are important in determining which circulating cell subsets can bind and cross the vessel wall [3]. Differential chemokine expression causes recruitment of distinct cell subsets (such as T cells and B cells) at specific positions along a single vessel within a given lymph node [4]. Furthermore, we have previously shown that lymph node microenvironment can dictate the nature of molecules expressed on high endothelial venule (HEV) subsets in a TNF-dependent fashion and that inflammation-induced MIG expression by HEVs can subsequently mediate monocyte recruitment [5]. Hence, HEV respond to the surrounding cytokines and chemokines in the local microenvironment and recruit very specific immune cell subsets. Cytokines can also participate as autocrine/paracrine mediators of pathophysiological processes such as atherogenesis. For example, cells in the atherosclerotic plaque can both produce and respond to these mediators [6]. Vascular cells (endothelial cells, fibroblast and smooth muscle cells) are a significant source of cytokines that regulate the vascular functions in an autocrine/paracrine manner: up-regulation of cytokine TNF production in vascular cells has been shown in a several pathophysiological conditions [6,7].

The study of global gene expression patterns of endothelium in disease tissues presents unique challenges. Primary cells offer the advantage of a controlled culture environment where the relative degree of gene activation in response to various cytokine challenges can be gauged. In contrast to prior studies, which have examined cytokine stimulation in human umbilical vein EC (HUVEC) [8,9], we examined gene expression in five different commercially available primary ECs that are thought to represent macrovascular-type (iliac artery and aortic) and microvascular-type (lung, colon and dermal) ECs. IFN-γ and IL-4 are primarily associated with T helper cell subsets, whereas TNF-α, a pleiotropic cytokine with a critical function in both inflammatory and immunological responses, is thought to model a "general" inflammatory response. Since Th1 and Th2 cellular responses have critical roles in the inflammatory diseases, their contrasting cytokine profiles offer an excellent means to test for cytokine specific differential responses by ECs, and ultimately perhaps shed further insights into the pathophysiology of inflammation in ECs. We challenged each of the five ECs with three different cytokines for a total of 15 combinations. This study used high density DNA microarrays as a preliminary step in determining whether individual cytokine induced primary ECs differentially regulate particular functional classes of genes that might offer insights into the specificity of ECs in recruiting distinct cell subsets, and furthermore, whether a clear distinction between EC phenotypes could be observed. In summary (A) we generate important knowledge and add to the understanding of cytokine induced EC variability along the vascular tree, (B) identify cytokine specific marker genes that are differentially expressed in a particular EC type and/or phenotype, and (C) demonstrate that IFN-γ and IL-4 have contrasting effects in ECs on the number and ratio of induced to repressed genes.

2. Results and discussion

2.1. Overview of differential gene expression patterns

The expression of 940 genes and ESTs, out of approximately 40,000 unique sequences, changed by at least 2.5 fold in one or more of the cytokine challenged EC vs unchallenged EC pair-wise comparisons. After removal of ESTs of unknown function, 563 genes remained that had associated GenBank IDs. Hierarchical cluster analysis partitioned the genes into clusters that were organized based on the similarities of expression patterns (Fig. 1A and B). The genes exhibited a wide range of color intensities that represented fold differences according to the Incyte convention, with increased expression in cytokine challenged ECs (from −2.5 to −57 fold) colored in red and genes with reduced expression (from 2.5 to 9 fold) colored in green.

Hierarchical cluster analysis revealed several compelling patterns of gene expression changes in ECs that were associated with both the nature of cytokine and EC type. Overall, we did not observe a significant contribution by these cytokines in differentiating the responses of microvascular from macrovascular ECs (Fig. 1A and B). This stands in contrast to the distinct gene expression profiles that have previously been reported between these phenotypes in the unstimulated state. Several prominent clusters distinguished the majority of ECs on the basis of cytokine challenge. The most striking difference was observed between cluster 14, which grouped genes on the basis of ECs response to IFN-γ, and clusters 2, 4 and 12, containing TNF-α induced genes (Fig. 1A). Many genes, however, clustered on the basis of a characteristic expression pattern associated with a single cytokine/EC combination. Cluster 1 represents a prominent example of IFN-γ stimulated lung ECs (Fig. 1A), while cluster 10 contains a smaller group of IL-4 induced genes in iliac artery ECs (Fig. 1B). Indeed, ECs were overall far less responsive to IL-4, which may partly explain the observation that there were very few instances (such as cluster 27) where genes demonstrated a reciprocal differential gene expression pattern, by the same EC type, in response to the two different biological classes of cytokines (Fig. 1B). Our results also suggest that IFN-γ and IL-4, immune cytokines that are known to elicit contrasting biological