

# Heterogeneity in Permeability of Human Endothelial Cell Monolayers

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## Introduction

A major consequence during anaphylaxis is increase in permeability of the blood vessel walls due to anaphylactic mediators such as histamine and platelet-activating factor (PAF). This permeability is highly controlled by endothelial cells (ECs) that constitute the inner lining of all blood vessel walls.

Mouse studies have shown that there is a heterogeneous response in permeability of different types of blood vessels in response to stimuli. Whether this heterogeneity is also found between different human EC phenotypes is however not known.

## Methods

ECs were isolated and cultured from human saphenous vein (HSVEC, n=6) and aorta (HAEC, n=5).

Primary ECs from lung microvessel (HMLEC, n=6) were commercially bought and cultured.

The cultured ECs were added to transwell inserts (pore size 0.4  $\mu\text{m}$ , 6.5 mm diameter) at a concentration of  $10^5$  ECs per transwell and grown into a confluent monolayer. Cells were then treated on the apical site (upper chamber) with 100  $\mu\text{l}$  FITC-Dextran (40 kDa, 1 mg/ml) combined with either stimulation (10  $\mu\text{M}$  histamine or 10  $\mu\text{M}$  PAF) or culture medium (control). At different timepoints (30 and 60 minutes) samples of medium was taken from the basal side (lower chamber) and the level of passed fluorescence was measured.

## Results

At steady-state conditions (i.e. control) a difference in permeability of the EC monolayers from different EC phenotypes was observed; at 60 min, HMLEC monolayers were found to be the least permeable followed by HAEC (9.5% more permeable) while HSVEC monolayers were much more permeable (73.7% more permeable).

When activated, high variation was observed between ECs from different patients. Despite this, a tendency of increase in permeability when stimulated by PAF was observed with HMLECs (foldchange compared to control at 30 min;  $1.45 \pm 0.34$ , 60 min;  $1.43 \pm 0.49$ ) compared to both HSVEC (30 min;  $1.13 \pm 0.20$ , 60 min;  $1.11 \pm 0.13$ ) and HAEC (30 min;  $1.10 \pm 0.12$ , 60 min;  $1.07 \pm 0.08$ ).

Surprisingly, histamine did not lead to increase in permeability in any of the ECs (at 60 min: HSVEC  $1.02 \pm 0.21$ ; HAEC  $0.87 \pm 0.08$ ; HMLEC  $1.15 \pm 0.21$ ). This might be related to expression of the histamine degrading enzyme Diaminoxidase by ECs. This is currently under investigation.

## Conclusion

In steady-state condition, EC phenotypes clearly have a different permeability with HMLEC and HAEC being the least permeable and HSVEC the most. Stimulation with PAF only induced permeability in HMLECs. This illustrates a heterogeneous pattern in permeability between different human EC phenotypes.