Transwell co-culture model with MAdCAM-1-expressing endothelial cells to study vedolizumab response in Crohn's disease

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INTRODUCTION

Crohn's disease (CD) is a chronic immune-mediated inflammatory disorder of the gastrointestinal tract. Vedolizumab (VDZ) blocks α4β7 integrin-mucosal addressin cell adhesion molecule-1 (MAdCAM-1) interactions, preventing peripheral blood mononuclear cell (PBMC) trafficking to the gut and thus excessive inflammation. However, up to 40% of patients show primary non-response to VDZ therapy [1].

We aim to develop a transwell immune-endothelial co-culture model with MAdCAM-1–expressing endothelial cells to mimic intestinal microvasculature in CD, enabling prediction of VDZ response, exploration of underlying mechanisms, and biomarker discovery (Fig. 1).

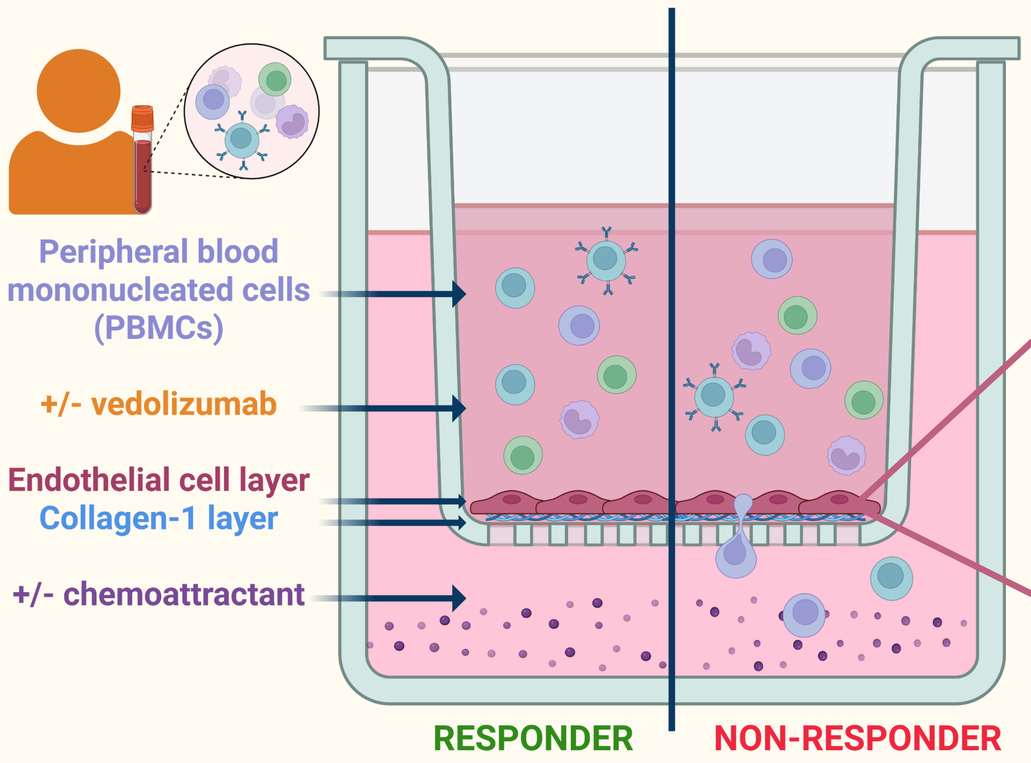


Figure 1. Schematic of a transwell immune-endothelial co-culture model.

MATERIALS AND METHODS

The model is based on a transwell system with collagen-coated porous inserts seeded with human microvascular endothelial cells-1 (HMEC-1), transiently transfected to express MAdCAM-1, where integrity of the collagen-supported endothelial layer—verified by zonula occludens-1 (ZO-1) staining and FITC-Dextran permeability assay—ensures a selective barrier enabling active pasage while preventing passive PBMC transmigration. The insert simulates a blood vessel environment where PBMCs from CD patients are added, while chemoattractants in the lower chamber mimick gut inflammation. Transmigration (+/- VDZ) is quantified and cell types identified using TruCount™ tubes (BD Biosciences), immunofluorescence, and flow cytometry (Fig. 3). VDZ effects on PBMC transmigration are assessed, with responders expected to show fewer PBMCs in the bottom chamber than non-responders. Molecular pathways associated with response are analysed by qRT-PCR, RNA-seq, immunofluorescence microscopy, and Western blot.

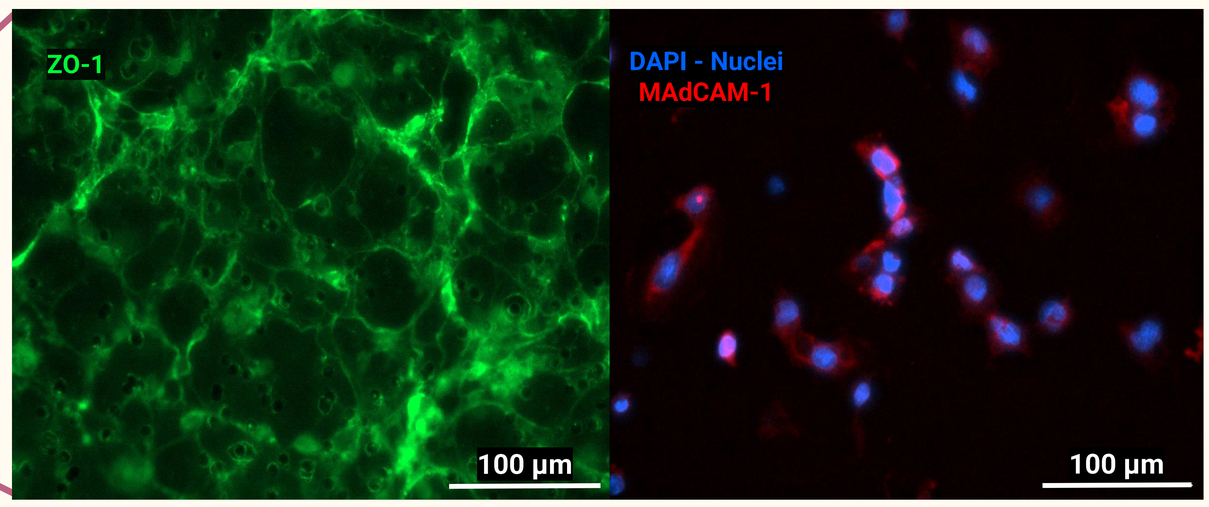


Figure 2. Immunofluorescent staining of zonula occludens-1 (ZO-1) in HMEC-1 cells, showing a tight, continuous endothelial layer (left), and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in transiently transfected HMEC-1 cells (right).

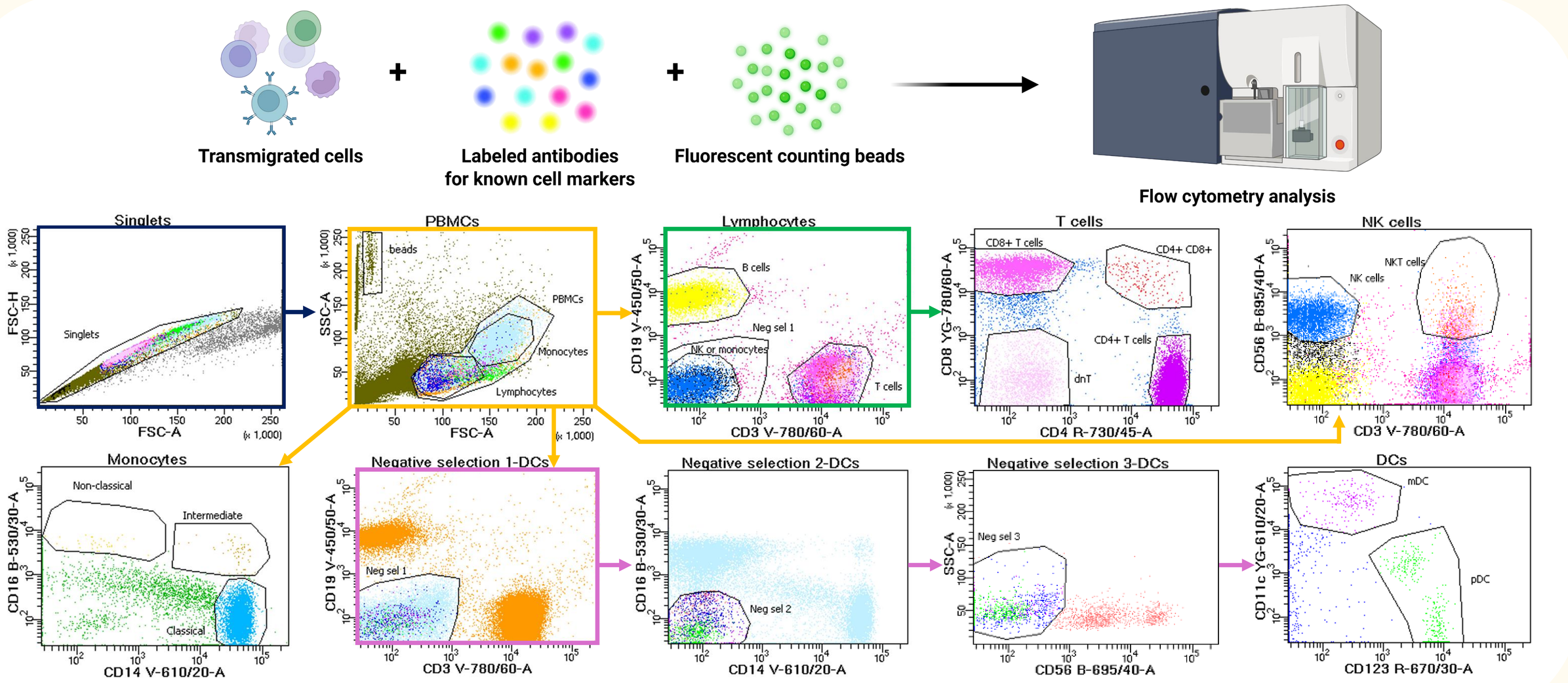


Figure 3. Workflow for quantification and phenotyping of transmigrated cells, including flow cytometry panel design.

RESULTS AND CONCLUSIONS

A continuous HMEC-1 monolayer with intact tight junctions and functional barrier integrity was established. Transient MAdCAM-1 transfection of HMEC-1 was optimized (Fig. 2). Currently, we are optimizing the transmigration protocol and collecting PBMCs from patients and healthy controls. While existing models assess only PBMC attachment to immobilized MAdCAM-1 and lack both transmigration and an endothelial context [2], our transwell model enables active PBMC transmigration and evaluation of VDZ response, thereby providing a more physiologically relevant platform for predicting treatment outcomes in CD.

REFERENCES

[1]G. Krajnc et al., BIOCELL, 0:1–10, 2025. [2] C. Allner et al., BMC Gastroenterol., 20:103, 2020. Figures were created using BioRender.com

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