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**ISOLATION AND INITIAL CHARACTERIZATION OF MICROVASCULAR ENDOTHELIAL CELLS DERIVED FROM THE RETROORBITAL CONNECTIVE TISSUE OF PATIENTS WITH GRAVES' OPHTHALMOPATHY.**

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Activation of certain adhesion molecules in vascular endothelial cells and in the surrounding extravascular tissue likely plays an central role in the site-specific recruitment of immunocompetent cells in Graves' ophthalmopathy (GO). Transendothelial migration of lymphocytes into the extravascular retroorbital space depends upon the expression of a finely tuned cascade of cytokine-activated adhesion receptors by endothelial cells of the retroorbital microvasculature. Systematic investigation of the cellular mechanisms involved in these interactions requires carefully controlled in vitro experiments, using purified populations of the different cell types involved. To characterize in detail the interactions between orbital endothelial cells (OEC) and immunocompetent cells in vitro, we designed a procedure that allows isolation of microvascular endothelial cells from small volumes of orbital connective tissue. Of the various techniques tested, an indirect approach using successive rounds of incubation with anti-Ulex europaeus I (UEA I) lectin and anti-human endothelial cell (CD31) monoclonal antibody coupled to immunomagnetic polystyrene beads resulted in the recovery of viable OEC with high yield and purity (>99%). Following isolation of OEC, rosetted magnetic beads were detached using excess fucose (0.01 M). Endothelial origin of the resulting cell population was demonstrated by the characteristic cobblestone-like appearance of OEC monolayers, and by specific positive immunoreactivity when using antibodies directed against von Willebrand factor, thrombomodulin, and human endothelial cells (CD31). Following exposure to tumor necrosis factor-α (100 U/ml), immunoperoxidase staining of primary cell monolayers revealed positive immunoreactivity for endothelial cell-specific adhesion molecule-1 (ELAM-1) and vascular cell adhesion molecule (VCAM-1). Negative immunostaining using monoclonal antibodies directed against cytokeratin, vimentin and fibroblast antigen further confirmed the homogeneity of the OEC population obtained. In conclusion, this technique allows highly purified OEC to be isolated from small volumes of orbital connective tissue with high yield and reproducibility. Availability of OEC provides an important tool for the analysis of the cellular mechanisms involved in the site-directed recruitment of lymphocytes in GO. Further, experiments using OEC in vitro may help to explore the potential benefits of therapy specifically designed to block lymphocyte access to the retroorbital space at the level of the endothelial cell.

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