

CO-CULTURE WITH ENDOTHELIAL CELLS DOES NOT ENHANCE PROPLATELET FORMATION IN PRIMARY BONE MARROW DERIVED RAT MEGAKARYOCYTES

Spencer, N B, Savill, J, Brown, S

University of Edinburgh

PROBLEM: Aberration in platelet numbers is a feature of renal disease and platelets have been shown to play a role in immune complex nephritis. Apart from transfusion, no effective therapy exists for regulating platelet numbers in humans. Although highly efficient *in vivo*, proplatelet formation (PPF) *in vitro* is highly inefficient, making mechanistic studies difficult. The bone marrow microenvironment may be critical to understanding PPF. Oestradiol (E₂), nitric oxide (NO) and Fas ligand (FasL) are all known to modulate thrombopoiesis either *in vitro* or *in vivo* in which oestradiol is reported to upregulate Fas ligand expression by endothelial cells in an NO-dependent manner.

PURPOSE: This study aims to model the bone marrow environment by co-culturing megakaryocytes with endothelial cells.

DESIGN: Mature primary CD41⁺ megakaryocytes were expanded *ex vivo* from rat femoral bone marrow and co-cultured with a rat microvascular endothelial cell line (GPNT) which had been pretreated for 24 hours with IL-1 β (activates endothelial cells), oestradiol or GSNO (a long-acting NO donor). The number of megakaryocytes bearing PPF (Figure 1) was measured after 24 hours using light microscopy. GPNT cells were assessed for Fas ligand by RT-PCR.

FINDINGS: 3.5% \pm 0.8% (n=5, S.D.) of megakaryocytes formed proplatelets when cultured in the absence of GPNT cells. Co-culturing with GPNTs, regardless of their pretreatment with IL-1 β , oestradiol or GSNO did not enhance the development of PPF (Figure 2). Furthermore, we found no evidence that GPNT constitutively or inducibly expressed Fas ligand.

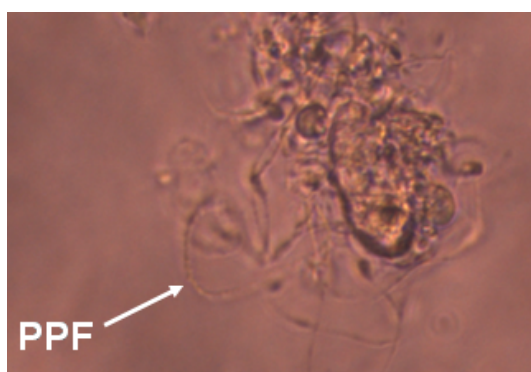


Figure 1. Megakaryocytes bearing PPFs

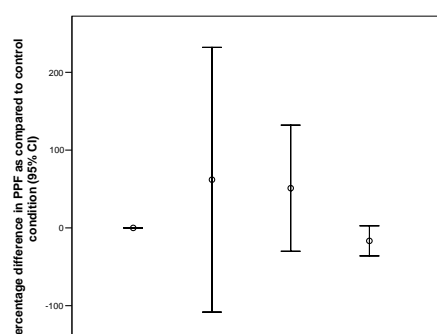


Figure 2. Percentage difference in PPF in the co-cultured megakaryocytes compared to the control condition (n=5).

CONCLUSION AND RELEVANCE: Although megakaryocytes are in close proximity to endothelial cells in the bone marrow *in vivo*, we have so far found no evidence that endothelial cells enhance platelet production *in vitro*. The interaction with other bone marrow stromal cells may also be important in promoting platelet generation.