

P071 Using Lentivirus as a Tool for the Study of Neutrophil Biology

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Investigation of human neutrophil biology is restricted by the inability to transfect and genetically manipulate these cells. As a result there are mechanisms behind the cellular response to pro-inflammatory factors that still remain unknown, however, here we demonstrate some early evidence that the modulation of human neutrophils using lentiviral transduction is an effective and novel method for studying signalling. Incubation of highly purified peripheral blood neutrophils (PBN) with GFP and GFP-Bid encoding lentiviruses resulted in cell transduction and protein expression over short time courses detected by flow cytometry and western blot. PBN stimulated with lipopolysaccharide (LPS) and granulocyte-macrophage colony stimulating factor (GM-CSF) showed increased survival, determined by morphological analysis. LPS-, but not GM-CSF-, induced survival was abolished by prior transduction with lentiviruses encoding dominant-negative (dn) TLR4, MyD88 and TRIF. Conversely, transduction with a kinase dead IRAK-1 lentivirus led to an enhancement in neutrophil survival. These data imply that TLR4 signalling may be able to employ both pro- and anti-apoptotic pathways with differential adapter use, though the primary LPS response is cell survival mediated by TLR4 and MyD88. These data show for the first time that lentiviral technology signifies a useful tool for the study of human neutrophil function, and help develop a clearer understanding of TLR4 signalling in these cells.