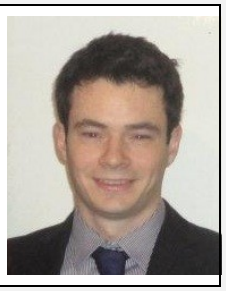
ImmunoTools IT-Box-139 Award 2012



Eanna Connaughton

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Phenotypic and functional analysis of novel monocyte subpopulations.

Blood monocytes represent immature myeloid cells produced in the bone marrow transiting to organs where they differentiate into specific cell types. Importantly, they have been implicated in atherosclerosis. By their expression of chemokine and pathogen-recognition receptors, monocytes represent a key link between the innate and adaptive immune systems. Following a recent international agreement, monocytes are classified into 3 distinct subsets, namely $CD14^{++}CD16^{-}$ "Classical", $CD14^{++}CD16^{+}$ "Intermediate" and $CD14^{+}CD16^{++}$ "Non-Classical". Functional differences between these subsets are still poorly defined and further distinct populations may exist. This PhD will entail further functional and phenotypic characterisation of monocyte subpopulations, with emphasis on the intermediate monocyte subpopulation, which we have been able to subdivide using a panel of additional cell surface markers. Functional studies will involve co-culturing monocyte subsets with human umbilical vein endothelial cells (HUVEC) to mimic monocyte-endothelial interactions, with a view of creating an *in-vitro* atherosclerotic model.

Fresh human monocytes will be isolated from blood using density gradient centrifugation. Using the antibodies provided by the ImmunoTools IT-Box-139, flow cytometric phenotypic analysis will require a combination of CD45, CD14, CD16 and additional markers which will allow distinction of the three major monocyte subsets. Further antibodies will screen for known monocyte markers such as CD86 (co-stimulatory signal for T-cell activation) and CD56 (cell-cell adhesion). Functional studies will involve sorting the various monocyte subsets based on combined marker expression followed by culture with HUVEC to determine their angiogenic potential. After culture, cells will be harvested and analysed by flow cytometry, using CD45, CD14, CD16 and additional markers such as CD31 (involved in cell migration and angiogenesis), CD36 (scavenger receptor), CD62p (cell-cell adhesion) and CD54 (cell adhesion). We would also screen samples with a panel of markers available in the ImmunoTools IT-Box-139.

ImmunoTools IT-Box-139 for Eanna Connaughton include 100 antibodies

FITC - conjugated anti-human CD1a, CD3, CD4, CD5, CD6, CD7, CD8, CD14, CD15, CD16, CD19, CD21, CD25, CD29, CD35, CD36, CD41a, CD42b, CD45, CD45RA, CD45RB, CD45RO, CD49d, CD53, CD57, CD61, CD63, CD80, CD86, HLA-DR, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V
PE - conjugated anti-human CD3, CD4, CD8, CD11b, CD15, CD14, CD18, CD19, CD20, CD21, CD22, CD31, CD33, CD38, CD40, CD45, CD45RB, CD50, CD52, CD56, CD58, CD62p, CD72, CD95, CD105, CD147, CD177, CD235a, HLA-ABC, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V
PE/Dy647 -tandem conjugated anti-human CD3, CD4, CD8, CD14, CD19, CD20, CD25, CD54
APC -conjugated anti-human CD2, CD3, CD4, CD8, CD10, CD11a, CD11c, CD14, CD16, CD27, CD37, CD42b, CD44, CD45, CD59, CD62L, CD69, CD71, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

DETAILS