ImmunoTools IT-Box-139 Award 2012

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Plasmacytoid Dendritic Cell Responses to Apoptotic Cells.

In health, tolerance to apoptotic cells is maintained by rapid clearance and immunoregulation. Systemic lupus erythematosus (SLE) is an autoimmune disease thought to be caused by defective clearance of apoptotic cells. Characteristics of SLE include the formation of immune complexes consisting of IgG autoantibodies interacting with nuclear components of apoptotic cells, and a high concentration of IFN-α in patients' serum. Plasmacytoid dendritic cells (pDC) are important in anti-viral immunity due to their rapid ability to produce vast quantities of IFN-α following activation. For that reason, pDC are considered to be the source of IFN-α associated with SLE pathogenesis, occurring in response to activation by nucleic acids found in immune complexes. Conversely, pDC also function to regulate immune responses. However, it is not clear if they promote tolerance to apoptotic cells in health.

Results previously generated by the lab suggest interactions with apoptotic cells induce pDC to become regulatory. However, this regulatory response is diminished when pDC are stimulated with an inflammatory compound. My project will involve further investigations in to the loss of tolerance to apoptotic cells by pDC from healthy donors and SLE patients. Successful induction of apoptosis in the apoptotic cell feed will be measured using Annexin V, and antibodies specific for apoptotic cell lineage markers.

It is important to assess the purity of the pDC population prior to co-culture to eliminate the possibility that the responses observed are caused by other cell types interacting with apoptotic cells. This would be achieved by flow cytometric analysis using a panel of antibodies specific for lineage markers from IT-Box-139 to distinguish other peripheral blood mononuclear cells, including B cells (CD19, CD21), T cells (CD3, CD4, CD8), conventional dendritic cells (CD11c), monocytes (CD14), and NK cells (CD56).

An additional aim of my project is to determine the method used by pDC to recognise apoptotic cells. Antibodies provided from ImmunoTools IT-Box-139 would be used to assess cell-surface expression of molecules that may potentially be involved in pDC recognition and uptake of apoptotic cells. CD86 could also be used to analyse the activation status of pDC following interactions with apoptotic cells.

ImmunoTools IT-Box-139 for Joanne Elizabeth Simpson includes 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