Abstracts

DECREASED INTRACELLULAR CALCIUM FLUX IN FOLLICULAR HELPER T CELLS AFTER T CELL RECEPTOR STIMULATION

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Background Follicular helper T (Tfh) cells are a specialised subset of CD4+ helper T cells that are required for B cell maturation in germinal centres and subsequent antibody formation following infection or immunisation with thymus dependent antigens. Tfh cells have also been implicated in mediating pathogenic autoantibody production in lupus and modulation of their function has been shown to ameliorate end organ disease in murine models of lupus. Understanding the molecular determinants of Tfh cell function may allow for the development of specifically targeted immunomodulating therapies for lupus and other autoantibody mediated diseases. In this study, we have systematically characterised the ability of Tfh cells to flux calcium in response to T cell receptor stimulation.

Methods B6 mice were immunised in bilateral foot pads with a mixture of papain and 4-Hydroxy-3-nitrophenylacetyl conjugated to ovalbumin (NP-OVA). After 5 days, inguinal and popliteal lymph nodes were harvested and lymphocytes were labelled with fluorophore-conjugated antibodies to allow identification of different T cell subtypes by flow cytometry. Cells were loaded with the calcium sensitive dyes Fluo4 and FuraRed to allow ratiometric imaging of intracellular calcium. Cells were stimulated with anti-CD3 antibodies to initiate T cell receptor (TCR) signalling and the intracellular calcium concentration was monitored in naïve T cells, Tfh cells and other effector T cell subtypes. Similar experiments were conducted using T cells obtained from the spleens of 1) B6 mice infected with the helminth Nippostrongylus brasiliensis, 2) B6 mice acutely infected with lymphocytic chorio meningitis virus or 3) 6 month old lupus-prone, B6.Sle1.Yaa, mice.

Results Tfh cells, relative to naïve T cells or to their Th1 or Th2 counterparts, exhibit significantly reduced calcium flux upon TCR stimulation in the context of NP-OVA immunisation (2.4-fold reduction, p < 0.0001), helminth infection (3.7-fold reduction, p < 0.0001), viral infection (2.3-fold reduction, p < 0.0001) or autoimmune activation in lupus-prone mice (3.3-fold reduction, p < 0.0001). These findings are not due to generalised defects in signalling as Tfh cells retain the ability to activate MAP kinases following TCR stimulation, suggesting a specific alteration in the ability of Tfh cells to handle calcium.

Conclusion Our results demonstrate that Tfh cells have a selective defect in calcium mobilisation upon TCR stimulation. The altered calcium handling profile of Tfh cells likely contributes to the unique molecular program of these specialised cells. These results have important implications for designing therapeutic strategies to selectively target Tfh cells in autoimmune disease.

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