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 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.

Conclusions 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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AI-16 THE ROLE OF FC IN THE BINDING OF ANTI-DNA ANTIBODIES TO DNA

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Background Antibodies to DNA (anti-DNA) are the serological hallmark of systemic lupus erythematosus (SLE) and mediate pathogenesis via the formation of immune complexes. While the avidity of these antibodies is high, it depends on monomeric bivalency, a mode of antibody binding in which both IgG combining sites interact with an extended piece of DNA. In the current study, we investigated this interaction further by assessing the activity of Fab and F(ab’2) preparations of IgG from plasmas of SLE patients.

Materials and methods Using purified IgG, Fab fragments were generated by papain digestion while F(ab’2) fragments were prepared with pepsin. The binding to native calf thymus (CT) DNA was assessed by ELISA using an anti-human IgG (Fab specific) peroxidase reagent. In these experiments, the concentrations of IgG and fragments were determined on the basis of an equivalent number of binding sites. Control antigens were tetanus and an EBV antigen preparation. IgG and fragments from normal human subjects were used as controls for binding to foreign antigens.

Results For each of the SLE IgG preparations studied, Fab and F(ab’2) fragments failed to bind significantly to DNA in the ELISA (Figure 1). In contrast, the Fab and F(ab’2) fragments were active against the tetanus and EBV antigens. The binding of the fragments from SLE patients to the foreign antigens was similar to that of normal human subjects.

Conclusions These results define a new pattern of anti-DNA binding. Since a Fab fragment can bind monovalently, a lack of...