PTMs are known to alter both immune tolerance to self proteins (such as citrulline PTMs that are diagnostic in RA) as well as intracellular metabolic and signalling pathways. In particular, isoaspartyl (isoAsp) modification is one intracellular PTM previously demonstrated to be increased by cellular stress and inflammation. The present study examined T cell biology that is altered by PTMs in lupus.

Materials and methods Isoaspartyl PTMs were characterised in lymphocytes from both human SLE and in murine models. We specifically examined ZAP70 for PTMs to determine effects on intracellular signalling and cytokine production. We also examined ZAP70 amino acids sequences prone to isoaspartyl modification under inflammatory stress and their role in p-tyr signal transduction, effects on downstream functional domains, and binding to cbl-b.

Results PBMCs from SLE patients and from MRL lupus both have elevated levels of intracellular isoaspartyl modifications and hyperproliferative T cell responses. We identified 4 specific sites of isoAsp modification (Figure 1), two within the I-B functional domain of ZAP70, including the c-Cbl, Vav and Lck binding domain. IsoAsp modified ZAP70 reduces c-Cbl binding, upregulates TCR and T cell hyperplasia. Enzymatic repair of intracellular isoAsp modification corrects T cell hyperproliferative defects that are characteristic of murine and human SLE.

Conclusions This study has examined mechanisms of altered T cell autoimmunity in SLE. In particular, abnormal T cell hyperproliferation was found to be a result of isoaspartyl modification at 4 specific sites within ZAP70. Only a small number of PTMs are known to arise in the context of inflammation. Our study suggests that SLE is characterised by an inability to control or repair excessive production of PTMs due to inflammation, leading to altered cell biologic functions, specifically T cell hyperproliferation. Physiologic repair of intracellular isoAsp modifications reversed abnormal proliferative T cell responses and may provide one therapeutic pathway for intervention.

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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 autoimmune 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 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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