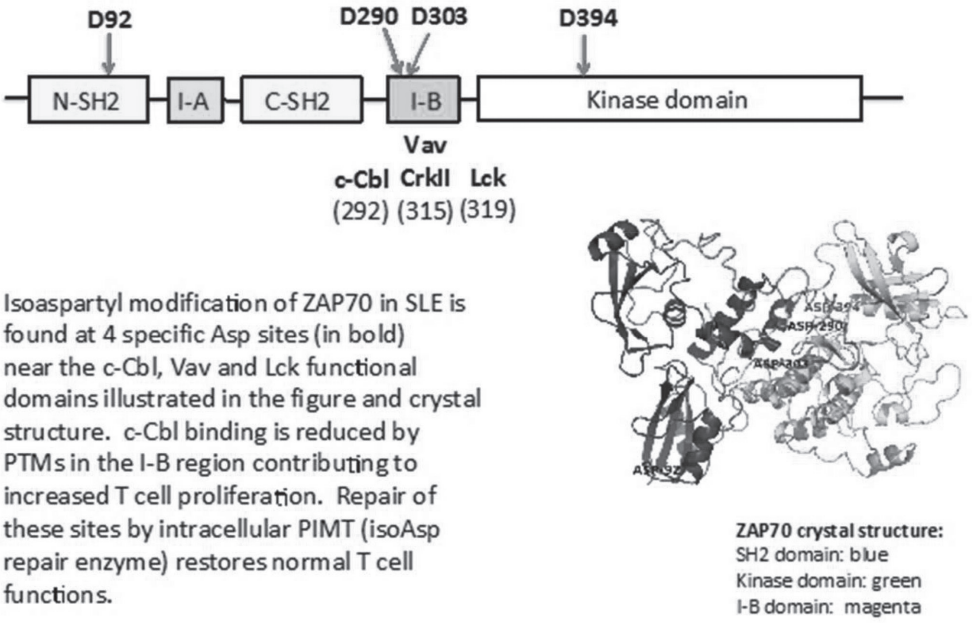

Abstract AI-13 Figure 1 Posttranslational Protein Modifications in ZAP70 in SLE

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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AI-14 AUTOANTIBODY PRODUCTION IN AN INDUCIBLE MODEL OF CUTANEOUS LUPUS

[1]Purvi Mande, [1]Tia Brodeur, [2]Karen Lee, [1]**Ann Marshak-Rothstein***. *[1]University of Massachusetts Medical School, Worcester, USA; [2]Children's Hospital of Pennsylvania, Philadelphia, PA USA*

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Background Despite the numerous murine models of SLE, models that accurately reflect the central features of CLE are much more limited. The MRL/lpr line is commonly studied in this context, but the onset of cutaneous disease in MRL/lpr mice is highly variable, colony dependent, and takes 6 months or more to develop. Endosomal TLRs play a key role in the development of murine models of SLE and mice lacking all endosomal TLR function have markedly attenuated disease. However autoimmune-prone mice deficient for only TLR9 invariably develop more severe SLE.

Materials and methods We have generated $TLR9^{WT}$ and $TLR9^{KO}$ mice that express a membrane-bound OVA fusion protein on MHC class II+ cells under the control of a doxycycline (DOX) inducible promoter. These mice were given DOX chow, sublethally irradiated and injected with activated OVA-specific DO11 T cells and then monitored for indications of systemic autoimmune disease.

Results 3–4 weeks following T cell transfer, the $TLR9^{KO}$ recipients develop cutaneous manifestation of SLE characterised by a mononuclear interface dermatitis associated with mucin deposition, absence of skin-associated Tregs, accumulation of IFNγ-producing DO11 T cells, elevated MHC class II expression by LCs and keratinocytes, and excessive keratinocyte death. Many more DO11 T cells are found in the epidermis of the TLR9KO recipients compared to the $TLR9^{WT}$ recipients, and the $TLR9^{WT}$ recipients have a higher frequency of DO11 Tregs. Importantly, the $TLR9^{KO}$ recipients have more germinal centre B cells in the spleen and more ELIspot+ cells in the bone marrow, and make autoantibodies specific for Ro52, a self-reactivity commonly detected in human CLE patients. An additional key feature of the

model is our ability to turn disease on and then off, simply by providing, or not providing, DOX. In mice on DOX for 4 wks and then off DOX for 2 wks, autoantibody titers markedly decrease and skin lesions resolve with minimal if any residual scarring. Subsequent DOX re-administration, without the transfer of additional T cells or any additional irradiation, leads to the rapid recurrence of autoantibody production and skin disease, thereby recapitulating lupus flares.

Conclusions We have now leveraged the hyperactivity of TLR9-deficient mice to develop a novel T cell dependent model of cutaneous inflammation that is strikingly similar to human CLE. This model provides a means for characterising both T and B cell memory responses elicited by autoantigens, and determining to what extent the primary vs secondary responses can be limited by TLR antagonists.

AI-15 DECREASED INTRACELLULAR CALCIUM FLUX IN FOLLICULAR HELPER T CELLS AFTER T CELL RECEPTOR STIMULATION

[1]**Abhinav Seth***, [2]Edward I Herman, [1]Jason S Weinstein, [1]Jin-Young Choi, [1,2]Joseph E Craft. *[1]Departments of Internal Medicine (Rheumatology); [2]Immunobiology, Yale University School of Medicine, New Haven, CT 06520, USA*

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

David S Pisetsky*, Nancy A Stearns. *Division of Rheumatology and Immunology, Department of Medicine, Duke University Medical Centre Medical Research Service, VA Medical Centre*

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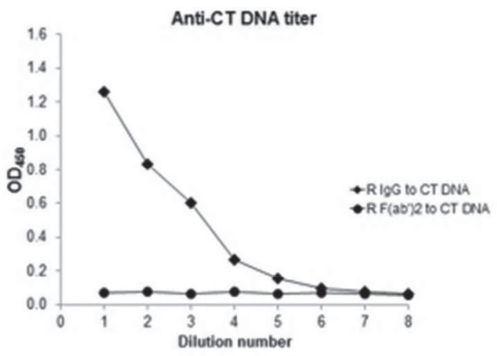
Background Antibodies to DNA (anti-DNA) are the serological hallmark of systemic lupus erythematosus (SLE) and mediate pathogenesis via the formation of immune complexes. While the avidity of these antibodies is high, it depends on monogamous bivalency, a mode of antibody binding in which both IgG combining 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



Abstract AI-16 Figure 1 The binding of intact IgG and F(ab')2 fragments to DNA was determined by ELISA using calf thymus DNA as antigen.