

AI-11 ISOLATING AND CHARACTERISING AUTOACTIVE T CELLS IN LUPUS

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Background Activation of autoreactive T cells is a critical step in the pathogenesis of lupus. These T cells help autoreactive B cells, make inflammatory cytokines, and infiltrate tissues. Yet, compared to autoreactive B cells, little is known about the specificity, identity, origins, or functions of autoreactive T cells. Therefore, we sought to isolate, clone and characterise T cells that recognise peptides derived from the targets of anti-nuclear antibodies/B cells.

Materials and methods Using anti-IgG2a (“rheumatoid factor”, RF) B cells from site-directed transgenic mice as “universal APC” for the contents of dying cells that are in turn bound by IgG2a anti-nuclear antibodies (ANAs). We relied on a monoclonal IgG2a anti-chromatin to stimulate the T cells initially and then a panel of monoclonal ANAs to test them. These ANAs naturally form immune complexes (ICs) with material released from dead cells either in vitro or in vivo. We used these tools to serially stimulate primary polyclonal T cells and make autoreactive T cell hybridomas. We then cloned the TCRs of these hybridomas into retroviral vectors to make “retrogenic mice”, a source of primary T cells.

Results We isolated multiple clones of T cells that could help RF B cells make proliferate and differentiate both in vitro and in vivo and characterised two in detail. These T cells were activated by the combination of RF B cells and either IgG2a anti-chromatin or anti-RNA, indicating that the T cells recognised peptides derived from the ICs formed by the ANAs used to stimulate them. We further found that while TLR7 and 9 were required to stimulate the RF B cells in the absence of T cells, the requirement for these molecules was bypassed by the presence of autoreactive T cells. Reciprocally, the APC function of B cells for T cells also did not require TLR7/9 expression in B cells

Conclusions We used a novel method to clone and characterise autoreactive T cells that help autoreactive B cells. These T cells were isolated from normal animals without use of adjuvant or foreign Ag, confirming that normal primary repertoires contain relevant autoreactive T cells. These cells enhanced multiple modes of B cell activation and differentiation in vivo and themselves were activated and differentiated in divergent ways. Most importantly, because they could bypass in large part the need for B cell-intrinsic TLR stimulation, they support the idea that TLRs may be more important for initiation of autoimmunity rather than propagating it once it is well-established.

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AI-12 GLOMERULAR-TARGETED INHIBITION OF PROTEIN KINASE C- α AMELIORATES ANTIBODY-MEDIATED NEPHRITIS BY RESTORING MITOCHONDRIAL DYSFUNCTION IN ENDOTHELIAL CELLS

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Background Lupus nephritis is a major cause of morbidity and mortality that affects over half of the SLE population during the course of disease. Multiple factors contribute to the diverse nature of nephritis. Nevertheless, PKC- α activation, with consequent synthesis of TGF- β and matrix constituents, are common mediators of disease progression. In a murine model of antibody-mediated nephritis, nephrotic nephritis (NTN), that simulates the effector phase of lupus nephritis, we investigated the role of targeted PKC α inhibition on progressive renal injury.

Materials and methods After induction of nephritis, on day 2, mice were treated with either: (1) nothing, (2) the PKC α inhibitor (Ro-32-0432) i.p., or (3) the PKC α inhibitor conjugated to F.1.1, a human monoclonal α 3(IV) collagen antibody that targets glomeruli. Resolution of disease was determined serologically and by histology. Comparative proteome analysis, from kidney cortices, was performed using quantitative mass spectrometry.

Results On day 7, the untreated NTN mice had severe nephritis (by BUN, proteinuria and, histology), whereas mice that received PKC α inhibitors, in either form, had minimal evidence of kidney injury: BUN was reduced to 32.29 ± 1.70 mg/dL and 36.07 ± 4.51 mg/dL in PKC α inhibitor and F.1.1- PKC α inhibitor injected mice vs. 99.84 ± 0.33 mg/dL in NTN mice, and there was a parallel normalisation of histology. Serum cytokine levels confirmed reduction of systemic inflammation. To further define the role PKC α inhibition, comparative proteome analysis from kidney cortices were performed. The functional protein groups most affected by NTN were mitochondrial proteins associated with respiratory processes; proteins of all four complexes of the respiratory chain were down regulated in NTN mice. By contrast, their expression was restored by PKC α inhibition, suggesting a role for proteins that regulate oxidative phosphorylation in recovery. This hypothesis was further evaluated in glomerular endothelial cells by measuring respiration (oxygen consumption rates, OCR), and glycolytic lactic acid production (ECAR) with injury and recovery. NTS reduced OCR (225 pmol/min to 185 pmol/min) and increased ECAR (42 to 62 mpH/min). PKC α inhibition normalised OCR and ECAR levels, and this was associated with restoration of cellular function.

Conclusions The results suggest that PKC α is an important mediator of glomerulonephritis. Furthermore targeted inhibition of this enzyme protects the kidneys from progressive damage associated with severe inflammation by restoring oxidative phosphorylation. This has therapeutic implications for treatment of human glomerular diseases, including lupus nephritis.

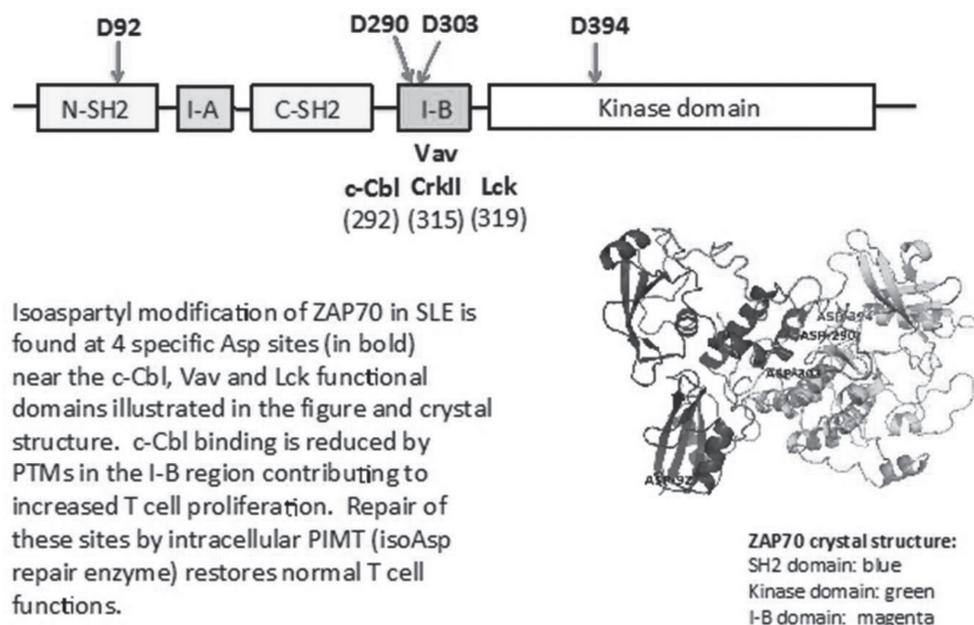
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AI-13 PROTEIN MODIFICATIONS THAT TRIGGER T CELL AUTOIMMUNITY

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Background T cell responses are known to be amplified in SLE, via TCR pathways of signal transduction. In particular, increases in intracellular tyrosine phosphorylation, calcium flux and actin polymerization are among the abnormal responses found in SLE T cells. An imbalance of how T cells signal through both Syk and ZAP70 shape the activation and cytokine responses in human SLE. The present study examines posttranslational protein modifications (PTMs) that arise in the context of SLE inflammation.



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

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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 IFN γ -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 TLR9^{KO} 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