AI-11 ISOLATING AND CHARACTERISING AUTOREACTIVE 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-ß 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 PKCα inhibition on progressive renal injury.
Materials and methods After induction of nephritis, on day 2, mice were treated with either: (1) nothing, (2) the PKCα inhibitor (Ro-32–0432) i.p., or (3) the PKCα inhibitor conjugated to F.1.1, a human monoclonal α3(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 PKCα 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 PKCα inhibitor and F1.1- PKCα 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 PKCα 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 PKCα 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). PKCα inhibition normalised OCR and ECAR levels, and this was associated with restoration of celluar function.
Conclusions The results suggest that PKCα 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.