expressed ISG gene set in IGHM+, IGHD+, and IGHG +B cells in AA patients with autoantibodies and renal disease. Further, ISG highly expressing SLE B cells exhibited unique heavy- and light-chain repertoires including expression of the autoreactive IGHV4-34 gene, targeted with the 9 G4 anti-idiotypic antibody that recognizes DNA- and RBP-autoreactive B cells.

Conclusions (i) B cells are an important source of type I IFNs in modulating TLR and BCR responses in SLE; (ii) there are well-orchestrated distinct programs in type I IFN expression and response genes in subsets of B cells, (iii) distinct pathways of autoreactive B cell survival and activation are effected by combined signaling through BCR, TLR, and IFNAR with resultant distinct BCR heavy- and light-chain repertoire.

Funding Source(s): R01-AI-077110, R01 AI134023, LRA Distinguished Innovator Award and Novel Research Award, VA Merit Review Award I01B × 004049, Immunology T32 Training Grant 2T32AI007051-39, the LFA Finnzi Summer Fellowship.

THE ENVIRONMENT AND LUPUS: IMPACT OF INFLAMMATION AND DIET ON T CELL EPIGENETICS

Bruce C Richardson, University of Michigan

10.1136/lupus-2019-lsm.193

Background Lupus flares when genetically predisposed people encounter environmental agents that trigger the flares such as UV light and infections which cause oxidative stress, but the mechanisms by which environmental agents induce flares are unclear. Our group has shown that lupus-inducing drugs such as procainamide and hydralazine inhibit DNA methylation in dividing CD4 +T cells, converting normal antigen specific T cells into autoreactive, cytotoxic pro-inflammatory cells that are sufficient to cause lupus in mice, and that similar epigenetically altered T cells are found in patients with active lupus. The mechanism(s) by which environmental agents alter the T cell epigenome to create the pathogenic cells was unclear. The enzyme DNA methyltransferase 1 (Dnmt1) is upregulated as T cells enter mitosis by signals transmitted through the ERK pathway, then binds the replication fork where it copies methylation patterns from the parent strand to the daughter strand by transferring the methyl group from S-adenosylmethionine (SAM) to dC bases in the daughter strand. This suggests that environmental agents which inhibit Dnmt1 upregulation or decrease SAM levels may inhibit T cell DNA methylation to trigger lupus flares.

Methods CD4 +T cells from lupus patients and controls were stimulated with PHA then cultured in custom media with normal or low transmethylation micronutrient levels. Oxidative stress was induced by treating the normal CD4 +T cells with peroxynitrite (ONOO-) prior to culture or injection into SJL mice. Methylation sensitive gene expression (CD70, KIR, and perforin) was measured by RT-PCR and flow cytometry

Results PHA stimulated CD4 +T cells from healthy controls expressed higher levels of CD70, KIR, and perforin mRNA and protein when cultured in media with low transmethylation micronutrient levels relative to cells cultured in complete media. Similar increases were seen in cells cultured in media with low or normal media methionine levels. PHA stimulated CD4 +T cells from lupus patients also overexpressed KIR, CD70 and perforin relative to PHA stimulated T cells from controls when similarly cultured. Treating PHA stimulated normal CD4 +T cells with ONOO- also increased methylation sensitive gene expression in normal CD4 +T cells, and low methionine or folate levels further increased gene expression relative to untreated T cells and T cells cultured in complete tissue culture media.

Conclusions Inflammation and transmethylation micronutrient deficiencies synergize to inhibit T cell DNA methylation, contributing to the onset of lupus flares.

Funding Source(s): Lupus Insight Prize from the Alliance for Lupus Research, the Lupus Research Institute and the Lupus Foundation of America
Programmed death-1 (PD-1), a negative regulator in T cells, limits helper T cell (Th) activation, restores regulatory T cell (Treg) suppression, and reinstates immune cell function. We have also shown that PD-1 expression in Treg down-regulates OX40L, which helps restore the suppressive capacity of Treg. However, concurrent administration of different immunotherapies may negate positive outcomes. We hypothesize that the sequence of blocking PD-1 and OX40L influences the induction and sustainability of Treg suppressivity.

Methods We treated 8-week-old BWF1 mice with a neutralizing Ab against PD-1 or OX40L intraperitoneally. At age 25 weeks when anti-dsDNA began to rise, mice were treated with either anti-PD1 or anti-OX40L that they had not received at age 8 weeks. OX40L, Foxp3 and PD-1 expression on CD4+CD25+Treg from spleens, apoptosis of Treg and CD4+CD25 Th were measured by flow cytometry. Serum production of IFN (Th1), IL4 (Th2), IL17a (Th17) and TGF-(Treg), and anti-dsDNA (B cells) were measured by ELISA. The survival of these mice were compared to those treated with anti-PD1 alone at 8 weeks, which we previously demonstrated prolonged survival with delayed onset of proteinuria.

Results Anti-OX40L suppressed Th function and proliferation independent of Foxp3 expression in Treg with decreased anti-dsDNA production. Subsequent blockade of PD-1 in anti-OX40L-treated mice generated more PD1hiTreg with increased TGF-β production; it sustained Treg suppressivity and delayed onset of proteinuria when compared to mice treated with anti-PD1 alone. Conversely, sequential blockade of anti-OX40L in anti-PD1-treated mice did not promote Treg survival and their disease inconsistently progressed: these mice had predominantly PD1hi or PD1-Treg, and antagonistic OX40L could not restore their suppressivity.

Conclusions Effective induction of Treg is associated with low expression of PD-1 and OX40L, which permits Treg to survive and perform cell suppressive function. Combination of Abs targeting OX40L and PD-1 can improve Treg function and survival outcomes, but it is determined by the timing and sequence of Ab administration: blocking OX40L followed by PD-1 has an additive effect which is not observed when the order of Abs given was reversed. OX40L and PD-1 signaling communicate sequentially with Treg to regulate its suppressive capacity and survival to achieve peripheral tolerance in SLE, suggesting that treatment with one immunotherapy could change the biology of T-cell signaling such that another immunotherapy may lose its efficacy or has unexpected negative outcome.

Funding Source(s): None

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SEQUENTIAL BLOCKADE OF OX40L FOLLOWED BY PD-1 ALTERED THE SUPPRESSIVE FUNCTION AND PROLIFERATION OF CD4+ REGULATORY T CELLS IN LUPUS MICE

Maida Wong*, Bevra H Hahn. University of California Irvine/VA Healthcare System Long Beach; University of California, Los Angeles

10.1136/lupus-2019-lsm.195

Background Naïve T cells constantly see self, need to avoid spontaneous immunity, yet be primed to react to foreign antigens when an infection occurs. Both CD4+ and CD8+ primary T cells exhibit tonic signaling (Myers et al., 2017b) and continuous interactions of the TCR with self-p/MHC generate tonic signals (Stefanova et al., 2002). T cells lacking the central adapter molecule LAT cause a spontaneous lymphoproliferative T helper 2 (TH2) cell syndrome in mice. Thus, LAT