analyzed using flow cytometry. The PBMCs were incubated with anti-CD3/CD28 beads, supplemented with transforming growth factor-β and interleukin-2 to induce differentiation of Tregs, with or without tunicamycin for 36 hours.

Results The percentage of Tregs in the PBMCs of SLE patients was lower than that in the HCs (1.8 ± 0.9 versus 2.6 ± 0.7%, p=0.02). The induced differentiation of Tregs increased in both groups, and the increased proportion was greater in the SLE group (600 ± 351 versus 252 ± 95%, p=0.01). Incubation with tunicamycin in the Treg differentiation process also increased the proportion of Tregs in both groups (385 ± 259 versus 166 ± 105%, p=0.006), and the increased proportion was higher in the SLE group.

Conclusions The baseline percentage of Tregs was lower in SLE patients than in HCs. However, when Treg differentiation was induced, the differentiation of Tregs was more pronounced in the SLE group. This exaggerated differentiation may reflect the paradoxical response to the diminished suppressive capacity of Tregs in SLE patients.

46 CD11C+T-BET+ B CELL IS CRITICAL FOR ANTI-CHROMATINTIN IGG2A PRODUCTION IN THE DEVELOPMENT OF LUPUS

D Dai*, Z Shiyu, S Nan. *Renji Hospital- School of Medicine- Shanghai Jiaotong University, Department of Rheumatology, Shanghai, China*

10.1136/lupus-2017-000215.46

Background and aims A hallmark of systemic lupus erythematosus is high titers of circulating autoantibodies. Recently a novel CD11c+ B cell subset has been identified in aged female mice that is critical for the development of autoimmunity.Transfer of MHC II-mismatched splenocytes from Bm12 mice into B6 mice causes a chronic graft versus host reaction (cGVHD), which is characterised by the production of high titers of autoantibodies and immunopathology that closely resemble SLE. The aim of this study was to figure out the role of CD11c+ B cell in the production of autoantibodies during the development of lupus induced by cGVHD.

Methods We developed and validated cGVHD model by splenocytes transfer of Bm12 mice into B6 mice and identified CD11c+ B cell by flow cytometry and examined anti-chromatin antibody by ELISA. We also identified CD11c+T-bet+ B cell of peripheral blood mononuclear cells obtained from SLE patients and healthy controls.

Results CD11c+T-bet+ B cell was significantly increased in the development of lupus induced by cGVHD. CD138 +CD11c+ B cell produced large amounts of anti-chromatin IgG2a upon *in vitro* stimulation. Depletion of CD11c+ B cells significantly ameliorated anti-chromatin IgG2a production *in vivo*. T-bet deficiency impaired the expression of CD11c in B cells and anti-chromatin autoantibodies production in the process of cGVHD. The accumulation of T-bet+CD11+ B cell was found in lupus patients.

Conclusions Our data demonstrated the aberrant activation and differentiation of CD11c+T-bet+ B cell, which produced large amounts of anti-chromatin IgG2a in lupus murine model and patients.

47 THE MEMBRANE-CYTOSKELETON LINKER EZRIN AND SRC FAMILY KINASE LYN COLLABORATE TO MAINTAIN OPTIMAL B CELL ACTIVATION AND PREVENT THE DEVELOPMENT OF AUTOIMMUNITY

N Gupta*, D Pore, N Parameswaran, E Huang, D Dejanovic, M Upadhyay, N Desai. *Cleveland Clinic Lerner Research Institute, Immunology, Cleveland, USA*

10.1136/lupus-2017-000215.47

Background and aims Systemic lupus erythematosus (SLE) is characterised by hyperactive B cell antigen receptor (BCR) signalling, autoantibody production and glomerulonephritis. Human GWAS studies have shown a strong association between alterations in the Src family kinase Lyn and incidence of SLE. Mice with genetic deletion of Lyn lose peripheral B cell tolerance and display all the hallmark symptoms associated with human SLE. Therefore, $Lyn^{-/-}$ mice represent a clinically relevant model to investigate the molecular regulation of B cell autoimmunity in SLE. We have previously reported that the membrane-cytoskeleton linker protein Ezrin regulates various facets of B cell function through its dynamic phosphorylation and dephosphorylation. Interestingly, we observed that Ezrin is hyperphosphorylated in $Lyn^{-/-}$ B cells, leading to the hypothesis that Ezrin facilitates B cell autoimmunity in $Lyn^{-/-}$ mice.

Methods To test our hypothesis we generated double knockout mice (DKO) bearing systemic deletion of Lyn and conditional deletion of Ezrin in the B cell lineage. B cell activation, lupus-associated autoantibodies and kidney pathology were investigated.

Results Compared to Lyn-deficient mice, the DKO mice displayed reduced germinal centre B cell and plasma cell differentiation, and decrease in autoantibody levels and glomerulonephritis. Further, an increase in BCR repertoire diversity and inhibition of BCR signalling pathways was observed in DKO B cells.

Conclusions Investigation of proteins that drive B cell hyperactivation in SLE is important for the development of effective and novel therapies. Our data demonstrate that ezrin is an important regulator of B cell activation in the absence of Lyn, and thus a potential molecular target in SLE.

48 IXAZOMIB, AN ORAL PROTEASOME INHIBITOR, REDUCES ANTIBODY PRODUCTION BY DEPLETING PLASMA CELLS IN A T CELL DEPENDENT ANTIGEN RESPONSE MODEL

[1]Y Itomi*, [1]T Tanaka, [1]M Sagara, [2]T Kawamura, [1]T Sato. *[1]Takeda Pharmaceutical Company, Immunology Unit, Fujisawa- Kanagawa, Japan; [2]Takeda Pharmaceutical Company, Regenerative Medicine Unit, Fujisawa- Kanagawa, Japan*

10.1136/lupus-2017-000215.48

Background and aims Pathogenic auto-antibodies produced by plasma cells are key drivers of many auto-immune diseases such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), and Sjogren's Syndrome (SS). In addition, solid organ transplant rejection is also mediated by antibodies produced against the donor organ. Plasma cells are highly metabolically active antibody factories and thus sensitive to depletion by proteasome inhibitors. Ixazomib, an oral

proteasome inhibitor, was recently approved in the US and Canada for use in combination with lenalidomide and dexamethasone in patients with multiple myeloma who have received at least 1 prior therapy. The Keyhole limpet hemocyanin (KLH) model of T cell-dependent antigen response was used to determine if ixazomib depletes plasma cells resulting in a reduction of antibodies.

Methods Briefly, rats were immunised with KLH and TiterMax adjuvant then treated with ixazomib twice weekly until study termination.

Results Treatment with ixazomib significantly inhibited anti-KLH antibodies by 34% ($p<0.05$) versus vehicle. Additionally, KLH plasma cells quantified by ELISpot were decreased 78% ($p<0.01$) in the spleen and 53% ($p<0.01$) in the bone marrow compared to control. To gain some understanding of the selectivity of plasma cell depletion total White Blood Cells, Red Blood Cells (RBC) Platelets, Neutrophils, and total Lymphocytes were quantified with small a reduction only seen in RBCs and platelets.

Conclusions Ixazomib depleted plasma cells resulting in reduced antibodies suggesting further preclinical studies are warranted in diseases with pathogenic antibodies such as SLE, RA, SS and solid organ transplant rejection.

49 ABSENCE OF HOST CD137 SIGNALLING CONVERTS CHRONIC GVHD TOWARD ACUTE GVHD

J Kim*, UJ Moon, H Kim, HR Cho, B Kwon. *Ulsan University Hospital, Biomedical Research Centre, Ulsan, Republic of Korea*

10.1136/lupus-2017-000215.49

Background and aims CD137 functions mainly as a costimulatory molecule for T cell activation. However, its functions have been found in a variety of other immune and nonimmune cells. Transfer of BM12 $CD4^+$ T cells into unirradiated, MHC II-mismateched C57BL/6 mice induces lupus-like chronic GVHD, which occurs because donor CD4+ T cells break host B cell tolerance with help from host $CD4^+$ T cells.

Methods cGVHD was induced by transferring 8×10^7 cells/mouse BM12 spleen/lymph node cells into C57BL/6 and CD137-/- mice. Serum samples were collected 2 wk after disease induction and assayed by ELISA for IgG1 anti-DNA autoantibody. Mouse body weight measured two times a week. Splenocytes were harvested 10 days after disease induction. After counting the number of total spleen and analysed by flow cytometry. Pathological scores of colons and livers. Colons and livers were harvested 10 days after disease induction.

Results We found that chronic GVHD was inhibited when $CD137^{-/-}$ mice were used as the host in this chronic GVHD model. Instead, they exhibited evident loss of body weight, indicating that they had acute GVHD. Indeed, their splenocytes were markedly depleted and they had severe intestinal and liver GVHD. Consistent with these phenotype changes, there were increased numbers of Th1 and Th17 cells but decreased numbers of Treg cells in the spleen of $CD137^{-/-}$ recipient mice 10 days after disease induction.

Conclusions Our results indicate that host CD137 signalling is a key factor to determine the fate of donor $CD4^+$ T cells during GVHD course.

50 EXPRESSION OF LY6C/6G DEFINES A NOVEL AIRE-DEPENDENT SUBSET OF MEDULLARY THYMIC EPITHELIAL CELLS WITH TOLEROGENIC FUNCTION

J Morimoto, Y Nishikawa, H Nishijima, M Matsumoto*. *Division of Molecular Immunology, Institute for Enzyme Research, Tokushima University*

10.1136/lupus-2017-000215.50

Medullary thymic epithelial cells (mTECs) are a heterogeneous population in terms of the spectrum of tissue-restricted Ags (TRAs) expressed from each cell for ensuring the elimination of autoreactive T-cells. Additionally, mTECs comprise cells at different developmental stages and/or in various activation conditions. Because of these heterogeneities, it is unclear whether mTECs are composed of any particular subsets possessing unique properties for their developmental pathway and/or immunological function. Here, we report a distinct mTEC subset characterised by expression of Ly6 family protein prior to and concomitant with Aire expression during its differentiation. Ly6C/6G+ mTECs, constituting 5%–15% of mature mTECs, were preferentially localised at the corticomedullary junction, and expressed high levels of TRAs and thymocyte-attracting chemokines. Remarkably, Ly6C/6G+ mTECs were absent in Aire-deficient mice, suggesting that this subset requires Aire and/or Aire+ mTECs for its production. Uniquely, Ly6C/6G+ mTECs lack a post-Aire stage because of a tendency to die after Aire had been expressed. With a TCR-transgenic model in mice, we found that *in vivo* depletion of Ly6C/6G+ mTECs frequently induced organ-specific autoimmunity. We suggest that Ly6C/6G+ mTECs serve as an important source of TRAs for efficient cross-presentation during establishment of self-tolerance.

51 INCREASED APOPTOSIS AND ABERRANT APOPTOSIS SIGNALLING PATHWAYS OF NATURAL CD4+CD25+FOXP3+ REGULATORY T CELLS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

[1]L Na*, [1]F Hao. [1]*Southwest Hospital Third Military Medical University, Dermatology, Chongqing, China*

10.1136/lupus-2017-000215.51

Background and aims Systemic Lupus Erythematosus(SLE) is a prototype of autoimmune disease.Decreased cell numbers and suppressive defects of naturally occurring $CD4^+CD25^+FOXP_3^+$regulatory T cells(Tregs) play an important role in the breakdown of SLE immune tolenrance.We have peviously observed significantly increased apoptosis of peripheral blood $CD4^+$T cells in SLE patients.Our objective here was to detect the apoptosis of Tregs in SLE patients to see if it could contribute to reduced suppressive activity of Tregs,and further elucidate the genes and signalling pathways which trigger the apoptosis in these cells.

Methods The cell number and apoptosis rates of Tregs was respectively evaluated in SLE patients and normal controls (NCs) by FACS.The suppressive activity of Tregs was measured by coculture with $CD4^+CD25^-CD127^{dim/-}$T cells.The relationship of abnormal Tregs apoptosis with clinical parameters was analysed by correlation analysis.Gene expression profiles of unstimulated Tregs from active SLE patients and NCs were generated by microarray analysis.Differential genes expression were verified by real time-PCR.