

analyzed using flow cytometry. The PBMCs were incubated with anti-CD3/CD28 beads, supplemented with transforming growth factor- $\beta$  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 ( $1.8 \pm 0.9$  versus  $2.6 \pm 0.7\%$ ,  $p=0.02$ ). The induced differentiation of Tregs increased in both groups, and the increased proportion was greater in the SLE group ( $600 \pm 351$  versus  $252 \pm 95\%$ ,  $p=0.01$ ). Incubation with tunicamycin in the Treg differentiation process also increased the proportion of Tregs in both groups ( $385 \pm 259$  versus  $166 \pm 105\%$ ,  $p=0.006$ ), 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-CHROMATIN IGG2A PRODUCTION IN THE DEVELOPMENT OF LUPUS

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**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+CD11c+ 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

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**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<sup>-/-</sup> 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<sup>-/-</sup> B cells, leading to the hypothesis that Ezrin facilitates B cell autoimmunity in Lyn<sup>-/-</sup> 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

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**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