Adaptive Immunity

AI-01 ALTERED RECRUITMENT OF LYN AND SYK INTO LIPID RAFTS OF B CELLS IN *SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)*: A BALANCE BETWEEN ACTIVATION AND INHIBITION

[1]Ana Vásquez, [1]Andres Baena, [2]Luis A Gonzalez, [2]Mauricio Restrepo, [2,3]Carlos H Muñoz, [2,3]Adriana Vanegas, [1]Blanca Ortiz, [4]Nursamaa Abdoel, [1,2]**Gloria Vásquez***. [1]*Grupo de Inmunología Celular e Inmunogenética (GICIG), Facultad de Medicina, Universidad de Antioquia. Medellín Colombia*; [2]*Grupo de Reumatología, Facultad de Medicina, Universidad de Antioquia. Medelllín, Colombia*; [3]*Sección Reumatología, Hospital Universitario San Vicente Fundación. Medellín, Colombia*; [4]*Centro Nacional de Enfermedades Reumáticas, Hospital Universitario de Caracas, Venezuela*

10.1136/lupus-2016-000179.1

Background Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by autoantibody production, immune complex deposition and B and T cell infiltrates in different organs. B cells from SLE patients have been reported to exhibit signalling alterations. Signalling through the BCR is initiated upon antigen induced crosslinking. These early events occur in cell membrane areas called lipid rafts (LR). Syk is an important PTK in the BCR-signalling pathway. Prior studies showed that in chronic lymphocytic leukaemia (CLL) cells, the extent to which Syk underwent tyrosine phosphorylation appeared to be associated with the ability of the leukaemia cells to respond to BCR ligation. the leukaemia cells that were better able to respond to BCR ligation expressed ZAP-70 in addition to Syk.

In order to characterise possible alterations in kinase recruitment in SLE B lymphocytes, this study aimed to determine the levels of Lyn, Syk and ZAP-70 in LR after BCR engagement.

Materials and methods Fifteen patients with SLE and ten healthy controls (all women) were included. The patients were recruited at Hospital Universitario San Vicente Fundación and controls at Sede de Investigación Universitaria (SIU), Universidad de Antioquia. Circulating B cells were isolated by negative selection and stimulated with goat Fab'2 anti-human IgM/IgG. LR were isolated with a non-ionic detergent and ultracentrifuged on 5–45% sucrose discontinuous gradients. Proteins from each fraction were precipitated and analysed by Western Blot.

Results Total levels of Lyn and Syk in resting B cells from SLE patients were similar to healthy controls. However, in resting B cells the presence of ZAP-70 in LR was detected and it was higher in patients than in controls. Upon BCR activation, only 64% and 47% of SLE patients recruited Lyn and Syk, respectively, compared to controls (100%). Also, BCR stimulation induced a significant increase of ZAP-70 levels into LR in the group of patients but the higher frequency of recruitment of ZAP-70 was observed in patients with a Syk decreased recruitment in activated B cells.

Conclusions Our findings suggest that signalling through the BCR and the composition of B cell LR are dysregulated in SLE patients. The finding of a reduced recruitment of the negative regulator Lyn, coupled with the absence of Syk and presence of ZAP-70 in LR support that in SLE, B cells are under constant activation through BCR signalling, as has been proposed in SLE

Acknowledgements This study was supported by Colciencias, Colombia, Grant RC 461–2012, Code 111556933414

AI-02 TRANSCRIPTION FACTOR IRF5 REGULATES HUMAN PLASMABLAST DIFFERENTIATION

[1,2]Saurav De, [3]Baohong Zhang, [2]Bryan Ciccarelli, [2]Sukwinder Singh, [3]Aaron Winkler, [1,2]**Betsy J Barnes***. [1]*The Feinstein Institute for Medical Research, Northwell Health, Manhasset, NY, USA*; [2]*Rutgers Biomedical and Health Sciences, Newark, NJ, USA*; [3]*Pfizer Biotherapeutics RandD, Cambridge, MA, USA*

10.1136/lupus-2016-000179.2

Background The transcription factor interferon regulatory factor 5 (IRF5) is primarily expressed in human monocytes, B cells, and dendritic cells. Polymorphisms in the *IRF5* gene have been significantly associated with susceptibility to the autoimmune disease systemic lupus erythematosus (SLE). In peripheral blood mononuclear cells (PBMC) from SLE patients, IRF5 expression was significantly elevated as compared to expression in healthy donor PBMC. The specific immune cell subset that IRF5 is (dys)functioning in to contribute to SLE pathogenesis is not known. SLE patients are characterised by increased levels of auto-reactive B cells and autoantibodies, and *Irf5*$^{-/-}$ mice were previously shown to have drastically reduced numbers of antibody secreting cells. Conclusions made from these mice, however, were compromised by the discovery of a secondary mutation in *DOCK2*. Important, multiple murine models of lupus show *Irf5*$^{-/-}$ mice are protected from pathogenic autoantibodies. To determine if IRF5 has similar function(s) in human primary B cells, we sought to characterise the role of IRF5 in plasmablast differentiation, antibody secretion, and B cell activation.

Materials and methods Knockdown of IRF5 was performed in primary human B cells using an Amaxa 4 D nucleofector, followed by *in vitro* culture to assay differentiation of naïve B cells to plasmablasts. B cell receptor cross-linking antibody and the TLR9 ligand CpG-B were used to activate B cells. Identification of IRF5 transcriptional targets was done through chromatin immunoprecipitation combined with next generation sequencing. B cell proliferation was assessed through reactive dye dilution.

Results Upon IRF5 knockdown in primary human B cells, a marked decrease in plasmablast differentiation was seen (Figure 1). Secreted levels of IgG1, IgG2, and IgG3 were severely decreased. Interestingly, upstream B cell activation pathways were also found to be decreased in B cells with the IRF5 knockdown. B cell proliferation was also found to be consistently decreased following IRF5 knockdown. ChIP-seq experiments identified IRF5 enrichment on the promoter regions of several plasmablast-associated genes as well as several genes known to impact B cell proliferation.

Conclusions These findings demonstrate for the first time that IRF5 is a critical regulator of human plasmablast differentiation. Furthermore, our work suggests that IRF5 knockdown impacts early stage B cell activation and proliferation. IRF5 ChIP-Seq results indicate that upon activation, IRF5 acts as a transcriptional regulator for a large group of genes that regulate both plasmablast differentiation and B cell function. Given that plasmablasts are over-represented in SLE, the therapeutic potential of IRF5 inhibition is supported.

Acknowledgements This work was supported by the Alliance for Lupus Research and NIAMS grant AR065959 to B.J.B. S.D. was supported in part by a predoctoral fellowship from the New Jersery Commission on Cancer Research.