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

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**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.

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