PROTECTION OF LUPUS NEPHRITIS BY IrHOM2 DEFICIENCY IN FcRRIIB–/– MICE

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Background Lupus nephritis (LN) is a major cause of morbidity and mortality in lupus. A disintegrin and metalloprotease 17 (ADAM17), is a principal membrane-anchored metalloprotease that cleaves a large spectrum of membrane-bound proteins into their soluble forms. Inactive rhomboid protein 2 (iRhom2), a newly identified regulator of ADAM17, controls maturation and function of ADAM17. Interestingly, in iRhom2–/– mice, loss of ADAM17-dependent shedding activity is limited to the immune organs. Accumulating evidence has shown increased protein shedding and possibly activation of ADAM17 in lupus. Among ADAM17 substrates, tumour necrosis factor α (TNF-α) and heparin-binding EGF (HB-EGF) have been reported to play important roles in mediating renal damage in lupus. We hypothesised that the activation of iRhom2/ADAM17 pathway plays a role in the pathogenesis of lupus nephritis.

Materials and methods We crossed iRhom2–/– mice with the well-established FcRRIIB–/– lupus-prone mice, and assessed development of lupus-like syndrome in these mice.

Results We found that iRhom2 deficiency protects FcRRIIB–/– mice from severe kidney damage (Figure 1), with minimal impact on the production of anti-double stranded (ds) DNA antibodies and renal deposition of immune complex and complement C3. In the absence of iRhom2, glomerular and tubulointerstitial structures were preserved, and massive inflammatory infiltrates including myeloid and CD4+ T cells were alleviated in the lupus kidneys. Protection of kidney injury by iRhom2 deficiency is associated with reduced EGFR signalling and ERK1/2 activation in the kidneys of FcRRIIB–/– mice. Transcriptome analysis of the whole kidneys as well as kidney macrophages from FcRRIIB–/– mice identified genes encoding pro-inflammatory cytokine/chemokines, fibrosis and tissue remodelling highly upregulated, and many of these genes were significantly reduced in the absence of iRhom2. In addition, kidney biopsies from patients with lupus nephritis show intense staining for HB-EGF, an EGFR ligand, in areas of crescents.

Conclusions Our findings here provide the first evidence that iRhom2, a major regulator of ADAM17, plays a critical role in the pathogenesis of LN. The role of iRhom2 in a spontaneous chronic mouse model of LN, FcRRIIB–/– mice, appears to be targeting at the effector arm of the disease, rather than affecting the process of autoimmunity development. iRhom2 may be a potential therapeutic target in LN.

Abstract II16 Figure 1 iRhom2 deficiency protects FcRRIIB–/– mice from developing severe kidney injury. FcRRIIB–/– mice crossed with iRhom2–/– mice were assessed for survival and kidney injury. Survival (A) and proteinuria (B) were plotted at each age. Pathological scores were illustrated for glomerular (C) and tubular-interstitial area (D) respectively. (A) survival, Chi square test. (B) proteinuria, student t-test. (C, D) pathological scores, non-parametric Mann Whitney test. ** P < 0.005, **** P < 0.0001

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CELL-BASED THERAPY IN SYSTEM LUPUS ERYTHEMATOSUS (SLE): EFFECTS ON NEUTROPHIL NETTING

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Background Evidence that mesenchymal stem cells (MSCs) derived from bone marrow, fat and umbilical cord can be used to treat refractory SLE and SLE nephritis is growing. MSCs were originally described as cells from bone marrow that have the capacity to differentiate into bone, cartilage and fat. More recently it has been recognised that all MSCs are peri-cytes and that their greatest potential is because they are pleiotropic and can both sense and repair their environment. We hypothesised that MSCs can reduce neutrophil activation in SLE by inhibiting neutrophil netting thus reducing induction of T-helper follicular cells that promote the development of long-living plasmablasts that can secrete autoantibodies.

Materials and methods We studied neutrophils derived from healthy donors and patients with SLE. Neutrophils were isolated using MACS™ Neutrophil Isolation Kit (Miltenyi) and onto coverslips in 24-well plates and incubated for 1–2 hours with conditioned medium derived from MSCs or control medium. Netting was induced by culture ex vivo with 20 nM PMA for 2 hours. Coverslips were fixed in 4% paraformaldehyde and NETs were quantified using anti-human antibody directed against neutrophil elastase colocalizing with extracellular DNA using Hoechst 33342.

Results To date we have optimised the conditions of our assay. Studies are ongoing to determine the effect of MSCs and/or their products on neutrophil netting. Figure 1: seen below are netting neutrophils induced as described above. Assays are underway to determine the effect of MSCs on SLE netting neutrophils ex vivo.

Conclusion The possibility that MSCs and/or their products could act both on innate and adaptive immune responses in SLE is appealing. Demonstration of the effect of MSCs on neutrophils is critical in understanding the potential therapeutic role of MSCs in SLE and SLE related organ damage.

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