activation-induced apoptosis, and promotes B cell survival. MIF antagonists show auspicious activity in mouse models of autoimmunity and both anti-MIF (*Imalumab*) and anti-MIF receptor antibodies (*Milatuzamab*) have advanced into phase II human clinical testing.

The *MIF* promoter polymorphism comprises a unique four-nucleotide microsatellite repeat ($CATT_{5-8}$), with higher repeat number producing increased *MIF* expression. Because there is no information about the transcriptional regulation of these common alleles, we sought to identify the nuclear protein(s) regulating expression at this functional promoter polymorphism.

**Materials and methods** We utilised DNA affinity chromatography and liquid chromatography-mass spectrometry analysis to identify unique nuclear proteins that interact with the −794 $CATT_{5-8}$ *MIF* promoter polymorphism. Functional knockout, ectopic expression, and −794 CATT-length dependent transcriptional assays and tissue microarray studies confirmed findings.

**Results** Proteomic analysis identified the transcription factor ICBP90, previously implicated in oncogenesis, as a unique −794 $CATT_{5-8}$ microsatellite interacting protein. Phosphorylated ICBP90 bound to the *MIF* promoter in a CATT-length dependent manner and upregulated *MIF* expression in monocytes, and B and T lymphocytes. Strong correlation was observed between ICBP90 and MIF expression in human inflammatory tissue, with a noteworthy overlap between downstream transcripts regulated by ICBP or MIF.

**Conclusions** ICBP90 regulates *MIF* transcription at the −794 *MIF* $CATT_{5-8}$ susceptibility locus. Pharmacologic targeting of the ICBP90:$CATT_x$ interaction is underway to inhibit *MIF* promoter overactivity and provide for a structurally-defined, pharmacogenomic approach to treatment.

## II-03 PATHOGENESIS OF DIFFUSE ALVEOLAR HAEMORRHAGE (DAH) IN LUPUS

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**Background** Diffuse alveolar haemorrhage (DAH) in lupus patients carries a mortality rate of over 50%. C57BL/6 mice with pristane-induced lupus develop DAH closely resembling the human disease. The role of cell death, complement, immunoglobulin, Toll-like receptors, and myeloid cells was examined in pristane-treated mice with DAH.

**Materials and methods** Clinical/pathological and immunological manifestations of pristane-induced lupus in gene-targeted vs. wild type mice were compared with the manifestations in SLE patients. Tissue distribution of pristane was examined histologically and by mass spectrometry. The cell types responsible for disease were examined by *in vivo* depletion using clodronate liposomes (CloLip) and anti-neutrophil monoclonal antibodies (GR1). The effect of treatment with the C3b-analogue cobra venom factor (CVF) was examined.

**Results** After peritoneal injection, pristane was detected in the lung by mass spectrometry and oil red staining, and was found to induce cell death, phagocytosis of the dead cells and erythrocytes by alveolar macrophages, consolidation of the alveolar spaces by erythrocytes and inflammatory cells, thickening of the alveolar wall, and extensive cellular proliferation (Ki-67 staining) within the alveolar septa. Small vessel vasculitis characterised by perivascular neutrophils and $F4/80^+$ macrophages was present. Lung tissue from SLE patients with DAH had a similar appearance. B-cell-deficient (μMT) mice were resistant to the induction of DAH, but susceptibility was restored by infusing IgM. C3-deficient and CD18-deficient mice also were resistant, and DAH could be prevented in wild-type mice by depleting complement with CVF. Induction of DAH was independent of MyD88, TRIF, TNFα, and type I interferon, but mortality was increased in IL-10-deficient mice. *In vivo* neutrophil depletion had no effect on susceptibility, whereas treatment with CloLip depleted both resident alveolar macrophages and presumptive bone marrow-derived $F4/80^+$ macrophages while preventing DAH, suggesting that macrophages are central to DAH pathogenesis.

**Conclusion** Induction of DAH in pristane-lupus is likely to involve opsonization of dead cells in the lung by natural IgM and complement followed by complement receptor 3 (CD11b/CD18) and/or CR4 (CD11c/CD18)-mediated phagocytosis, resulting in lung inflammation. Disease is macrophage-dependent and independent of type I interferon, TNFα, MyD88, and neutrophils. Complement inhibition and/or macrophage-targeted therapies may be attractive candidates for treating SLE-associated DAH.

## II-04 IMMUNE COMPLEX-MEDIATED TLR8 ACTIVATION REGULATES NEUTROPHIL SHEDDING OF FCGRIIA

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**Background** Neutrophils participate in host defence through mechanisms including phagocytosis and formation of neutrophil extracellular traps (NETs), a neutrophil cell death process in which DNA is extruded together with cytoplasmic and granular content to trap and eliminate pathogens. Immune complex (IC)-mediated NET formation has emerged as a mechanism that may increase the autoantigenic burden as well as promote type I interferon production in patients with the autoimmune disease systemic lupus erythematosus (SLE). Although TLR agonists, such as nucleic acids, have been shown to enhance phagocytosis by macrophages and dendritic cells, the role of TLR signalling in neutrophil phagocytosis of RNA-containing SLE ICs has not been extensively studied. The aim of the current study was to explore the cross-talk between TLRs and FcgRs in the regulation of IC-mediated phagocytosis and NETosis.

**Materials and methods** Neutrophils, isolated from healthy individuals were incubated with RNA-containing ICs and analysed for phagocytosis and NETosis by flow cytometry and fluorimetry, respectively, in the presence of blocking antibodies or TLR8 inhibitors (oligodinucleotides, RNase). Neutrophils from healthy controls ($n = 7$) and SLE patients ($n = 19$) were analysed for FcgRIIA expression by flow cytometry, using two antibody clones, recognising full-length or shed FcgRIIA, and the results related to clinical data.

**Results** Both FcgRIIA- and TLR8-engagement were required for induction of NETosis by RNA-ICs, as demonstrated by FcgR blocking antibodies as well as RNase treatment. Although degradation of RNA inhibited NETosis, removal of the TLR ligand by RNase markedly increased the phagocytosis of RNA-ICs by neutrophils ($p < 0.0001$), suggesting that TLR activation suppressed phagocytosis. Consistent with this hypothesis, addition of TLR8 agonist (R848) inhibited phagocytosis of ICs ($p < 0.0001$), but not beads, in neutrophils. Mechanistically, TLR8 activation mediated furin-dependent proteolytic cleavage of the most N-terminal

part of FcgRIIA reducing the phagocytic capacity while promoting progression into NETosis. Importantly, ex vivo isolated neutrophils from SLE patients demonstrated increased shedding of neutrophil FcgRIIA ($p < 0.0001$), which was correlated with neutrophil activation ($r = -0.73$, $p = 0.003$) and the presence of anti-Sm/RNP antibodies ($p < 0.001$).

**Conclusions** Neutrophils are not terminally differentiated cells but could shift into phagocytic or NETosing cells, partly regulated by a cross-talk between TLR8 and FcgRIIA. SLE patients have ongoing shedding of neutrophil FcgRIIA related to neutrophil activation and anti-RNA antibodies, demonstrating the in vivo relevance of our observation. Therapeutic approaches aimed at degrading the TLR8 ligand would be predicted to increase uptake of circulating ICs, while disarming their inflammatory potential and ability to induce NETs.

## II-05 INTERFERON REGULATORY FACTOR 5 PROMOTES THE EFFECTOR PHASE OF IMMUNE COMPLEX-MEDIATED GLOMERULONEPHRITIS

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**Background** Lupus nephritis is a serious manifestation of lupus for which treatment is only partially effective. It is characterised by the deposition of immune complexes in the kidney, activation of the complement cascade, cellular injury with the release of necrotic cell debris and the development of glomerular inflammation. However, the signalling pathways leading to the glomerular inflammation are incompletely defined. Interferon regulatory factor 5 (IRF5) polymorphisms are strongly associated in human genetic studies with an increased risk of developing lupus and, in mouse models, IRF5 has been shown to play an important role in the early phases of lupus pathogenesis including B cell autoantibody production and T cell activation by dendritic cells. IRF5 is a transcription factor that acts downstream of Toll-like receptors (TLRs) and other innate immune receptors to induce inflammatory responses. As necrotic cell debris has the potential to activate innate immune receptors, we hypothesised that IRF5 may also play a role in the later phases of lupus pathogenesis by promoting glomerular inflammation and lupus nephritis.

**Materials and methods** We developed a novel model of immune complex-mediated glomerulonephritis in which glomerulonephritis can be induced without exogenous adjuvant, making it possible to study the role of innate immune system activation by endogenous ligands. We induced nephritis using an endotoxin-free IgG1 fraction of sheep nephrotoxic serum (NTS) administered intravenously to wildtype (WT), IRF5-deficient ($IRF5^{-/-}$) and TLR7-deficient ($TLR7^{-/-}$) Fc gamma receptor IIB-deficient mice. Five days later, we euthanized the mice and measured nephritis severity.

**Results** WT mice developed severe glomerulonephritis characterised by glomerular necrosis and crescents in 28 ± 6% of glomeruli and a marked mononuclear cell infiltrate. $IRF5^{-/-}$ mice developed significantly less severe disease with glomerular crescents and necrosis seen in only 6 ± 2% of glomeruli ($p < 0.01$) and a substantial reduction in mononuclear cell infiltration. $TLR7^{-/-}$mice exhibited an intermediate phenotype. All mice had similar amounts of IgG and complement deposition in the kidney indicating that the differences in disease severity observed were not due to differences in the initial deposition of IgG1 NTS.

**Conclusions** IRF5 signalling plays an important role in the pathogenesis of the effector phase of immune complex-induced glomerulonephritis. This is likely mediated, at least in part, by the role of IRF5 downstream of innate immune receptors involved in the sensing of endogenous ligands released from injured cells. The reduction in disease in $TLR7^{-/-}$ mice suggests that RNA may be one such endogenous ligand involved.

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## II-06 THERAPEUTIC BLOCKADE OF IMMUNE COMPLEX-MEDIATED GLOMERULONEPHRITIS BY HIGHLY SELECTIVE INHIBITION OF BRUTON'S TYROSINE KINASE

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**Background** Lupus nephritis (LN) is a potentially dangerous end organ pathology that affects upwards of 50% of patients with systemic lupus erythematosus (SLE). Classical treatments for this condition have targeted the adaptive immune response and/or autoantibodies, rather than the inflammatory process itself. Besides its role in B cell development, Bruton's tyrosine kinase (BTK) is important for Fc receptor signalling and macrophage polarisation. Furthermore, increasing evidence points to the role of the innate immune system, and particularly macrophages, in the pathogenesis of lupus nephritis.

**Materials and methods** In this study, we investigated the effects of a novel, highly selective and potent BTK inhibitor, BI-BTK-1, in an inducible model of LN in which female 129/SvJ mice receive nephrotoxic serum (NTS) containing anti-glomerular antibodies. Mice were treated once daily with vehicle alone or BI-BTK-1 (0.3–10 mg/kg, n = 16/group), either prophylactically or therapeutically.

**Results** When compared with control treated mice, NTS-challenged mice treated prophylactically with BI-BTK-1 exhibited significantly attenuated disease which was dose dependent, as measured by proteinuria, serum creatinine, and serum BUN. Histological assessment confirmed marked renal protection in the BI-BTK-1 treatment groups. BI-BTK-1 treatment resulted in decreased recruitment of inflammatory monocytes from the splenic reservoir, and a decrease in infiltrating IBA-1+ cells as well as C3 deposition within the kidney. RT-PCR on whole kidney RNA and serum profiling indicated that BTK inhibition significantly decreased levels of LN-relevant inflammatory cytokines and chemokines. Renal RNA expression profiling by RNA-seq revealed that BI-BTK-1 dramatically modulated pathways related to inflammation and glomerular injury. Importantly, when administered therapeutically, BI-BTK-1 reversed established proteinuria and improved renal histopathology. Moreover, preliminary results confirm the efficacy of BI-BTK-1 in the spontaneous MRL-lpr/lpr murine lupus model as well.

**Conclusion** Our results highlight the important role for BTK in the pathogenesis of immune complex-mediated nephritis. These results, together with additional studies by our group showing comparable efficacy with other small molecule macrophage inhibitors in nephrotoxic serum nephritis and spontaneous lupus, point to macrophage modulation as a promising therapeutic target for LN and possibly other immune related glomerulopathies.