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