Although SLE causal variants at the *FAM167A-BLK* locus are thought to reside in the *BLK* promoter region, our results reveal that genetic variants at distal regulatory elements modulate promoter activity, changing *BLK* and *FAM167A* gene expression and disease risk. Our results suggest that global haplotype-specific 3-dimensional chromatin looping architecture has a strong influence on local allelic *BLK* and *FAM167A* gene expression, providing mechanistic details for how regional variants controlling the *BLK* promoter may influence disease risk.

1001 A NEW MODEL OF SPONTANEOUS ANTI-PHOSPHOLIPID ANTIBODY INDUCED PREGNANCY LOSS IN MICE OVER-EXPRESSING HUMAN TLR8

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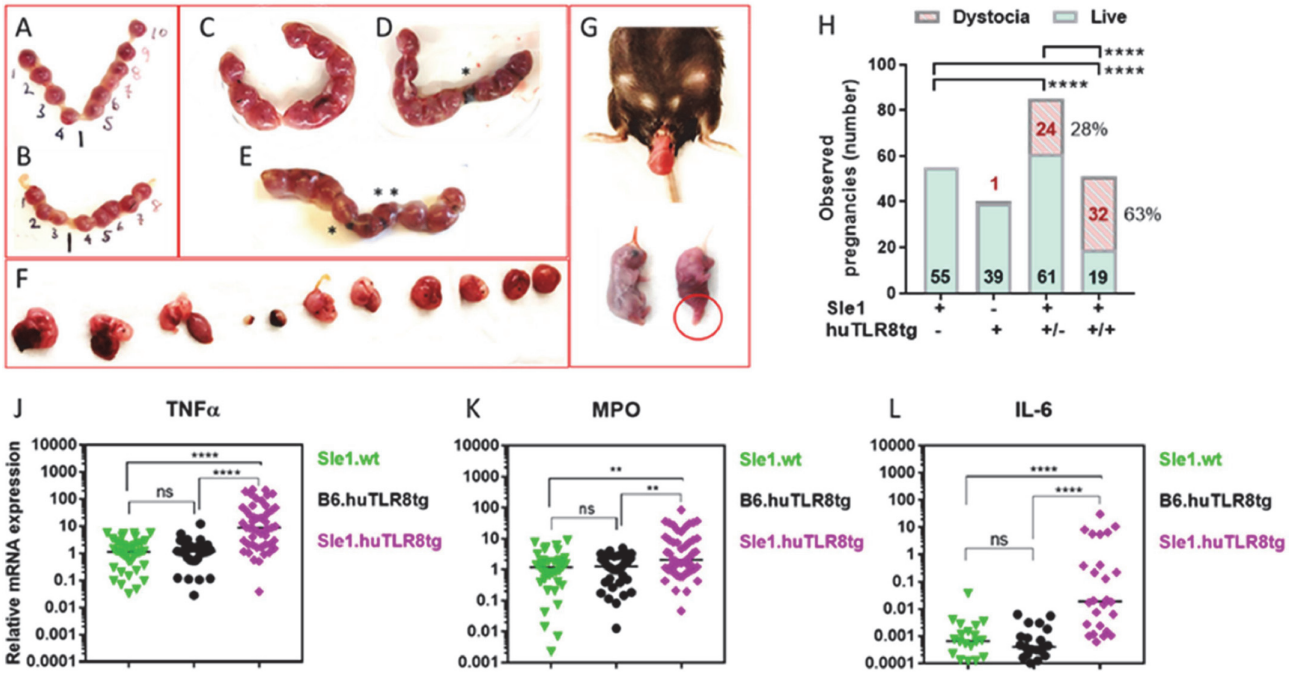
Background Systemic Lupus Erythematosus (SLE) predominantly affects women of childbearing age and is associated with adverse pregnancy outcomes including pre-eclampsia, placental abnormalities and fetal mortality, particularly in the presence of antiphospholipid antibodies (aPL). Pathogenic mechanisms damaging the fetal-maternal unit, however, remain poorly understood. Recent studies have implicated endosomal Toll-like receptor 8 (TLR8) in mediating pregnancy loss by amplifying the release of pro-inflammatory cytokines from human placental trophoblasts exposed to APL antibodies in vitro. However, to date there has been no reliable spontaneous model of APL antibody induced placental injury and the role of TLR8 in either systemic autoimmunity or placental injury has remained elusive as TLR8 function differs from mouse to man. We report here the development of a novel model of spontaneous fetal loss due to expression of human TLR8 (huTLR8) in Sle1 mice with APL autoantibodies. Our work indicates that this is due to a placental developmental defect that is associated with loss of the placental junctional zone and attenuated development of placental vasculature leading to placental ischemia, placental inflammation and fetal loss.

Methods huTLR8 transgenic (tg) Sle1 mice were generated and followed clinically. Non- transgenic littermates were used as controls. huTLR8 DNA copy number and mRNA expression was confirmed by qDigital- and qRT-PCR and huTLR8 function was confirmed by measuring BM derived macrophage responses to TLR8 agonists. Timed pregnancies were closely followed and placentas were harvested at Days 8.5, 13.5 and from mice exhibiting signs of dystocia/prolonged labor at Day 20-21. Pup and placental weight was assessed. Placental tissues were characterized by H&E and myeloperoxidase (MPO) staining to identify neutrophils. Inflammatory cytokine expression was assessed in placental tissue by qPCR. Splenic and placental immune populations were analyzed using flow cytometry. Neutrophil netting was evaluated after exposure to PMA or TLR8 agonists. Bulk RNASeq was performed on Day 8.5 fetal-placental units and Day 13.5 placentas.

Results We observed a high frequency of maternal and fetal death in both huTLR8tg heterozygous and homozygous dams. Maternal death was due to dystocia as a result of intrauterine growth retardation and failed delivery of non-viable pups. Genotyping of resorbed pups revealed no difference in the percentage of resorbed males and females. Fetal resorptions did not occur in wild type Sle1 or huTLR8tg. C57BL/6 females. Litter size was also smaller in successful huTLR8tg pregnancies than in control pregnancies with no difference in the ratio of male:female pups. IL-6, TNF? and MPO mRNA expression was significantly increased in



Abstract 1001 Figure 1 A-E: Uteri from Sle1 (A, C) and Sle1.huTLR8 (B, D, E) mice at Day 8.5 (A, B) and 13.5 (C-E). F: Pups and placentas from an affected pregnancy at Day 13.5. G-H: Dystocia at term with affected pups. I: Percent live and affected pregnancies in Sle1 and Sle1.huTLR8 mice. J-L: Expression of inflammatory cytokines in term placentas measured by qPCR

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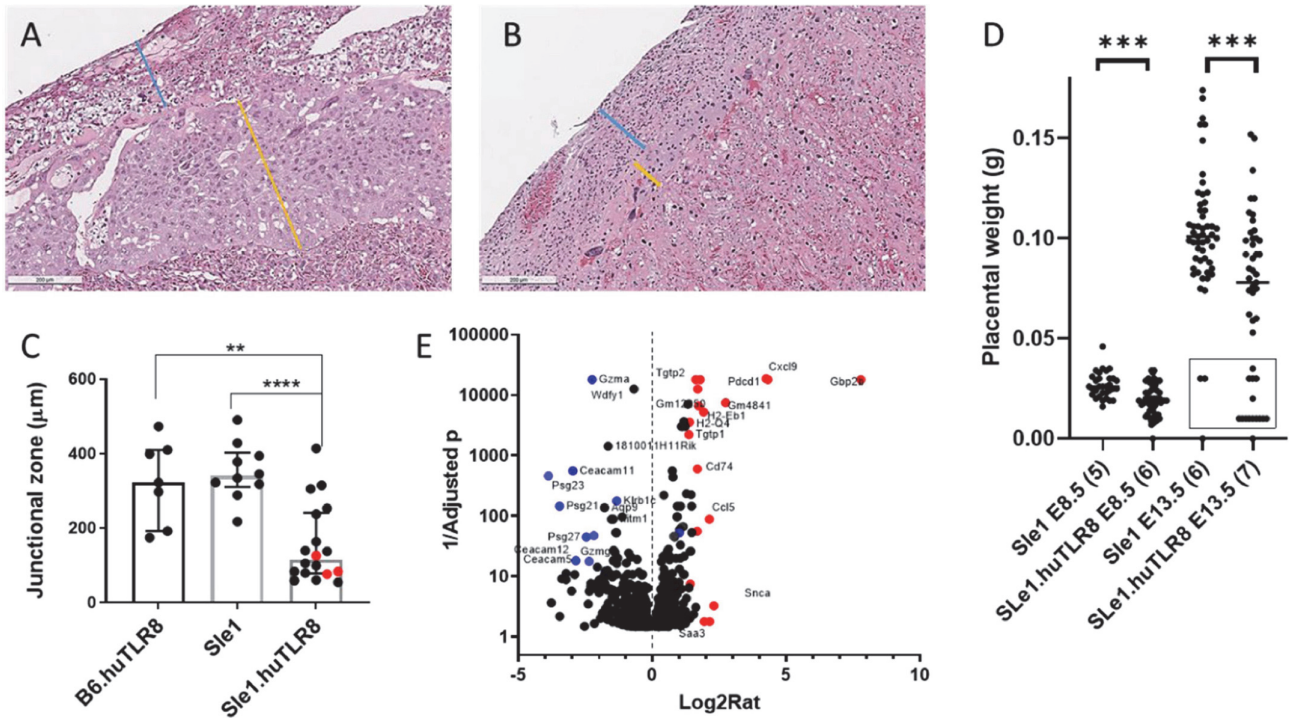

Abstract 1001 Figure 2 A-B: Placentas from Sle1 (A) and Sle1.huTLR8 (B) term pregnancies showing the width of the decidua (blue bar) and junctional zone (yellow bar). C: Junctional zone width in placentas from transgenic mice and controls - red indicates resorbed or dead pups. D: Placental weight at Days 8.5 and 13.5 (resorption frequency 0% at Day 8.5 and 32.1% in Sle1.TLR8 mice at Day 13.5 vs. 3.8% in Sle1 mice). E: Volcano plot of differentially expressed genes in Sle1.huTLR8 compared with Sle1 fetal-placental units at Day 8.5

placentas of affected litters and negatively correlated with placental weight. TNF deficiency did not prevent dystocia in transgenic dams. Abnormal placental morphology included loss of the placental junctional zone, placental infarcts, loss of placental lakes, arterial wall thickening and increased MPO+ netting neutrophils. In vitro neutrophil netting in response to TLR8 agonists was not increased. Observation of timed pregnancies showed that implantation was successful at Day 8.5 but that fetal resorptions began to occur between Day 8.5 and Day 13.5. RNA sequencing of the fetal-placental unit at Day 8.5 revealed a significant decrease in expression of pregnancy associated glycoproteins from the CEACAM family; these are synthesized by junctional zone trophoblasts and mediate placental angiogenesis. In addition, there was a decrease in NK cell markers, suggesting a failure of recruitment of these cells to the maternal decidua. By contrast, expression of inflammatory markers was upregulated. These differences persisted in Day 13.5 placentas with a decrease in cytolytic programs. Upregulated genes of interest at Day 13.5 included Prl4a1 that negatively regulates NK cell cytolytic function and Phlda2 that negatively regulates the size of the junctional zone.

Conclusions Here we identify a new model to study adverse pregnancy complications in SLE and APS. In the presence of both Sle1 and huTLR8, we observed early onset of aPL autoantibodies and spontaneous pregnancy loss due to fetal resorption/dystocia, resulting in both fetal and maternal death attributed to placental developmental abnormalities already present by Day 8.5 that caused decreased placental vascularization, placental infarcts and inflammation. This new model may be useful to study mechanisms of pregnancy loss in antiphospholipid syndrome.

1002 BACTERIAL AMYLOID CURLI/EDNA COMPLEXES INDUCE NETOSIS IN LUPUS PATIENTS POSITIVE FOR ANTI-DSDNA

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Infections are a major contributor to lupus disease. Uropathogenic E. coli (UPEC) is responsible for the majority of urinary tract infections in both healthy individuals and lupus patients. We have previously demonstrated that bacterial amyloid curli complexes of curli/DNA, produced by E.coli, can accelerate disease in mouse models of lupus. Moreover we have extended these findings to human lupus and demonstrate that curli/DNA complexes mimic lupus autoantigens and that patients with chronic bacteriuria and high levels of anti-curli/DNA have higher levels of anti-dsDNA, more flares and a proinflammatory profile. These findings suggest that curli/DNA complexes and subclinical chronic urinary bacterial infections might be a trigger and a propagator of autoimmunity via activation of the innate and adaptive immune system. Based on our previous results, we hypothesize that exposure to UPEC containing curli/eDNA complexes could also activate neutrophils, the first responders to bacterial infections, and specifically via generation of neutrophil extracellular traps (NETs), a fundamental mechanism to clear bacteria and a recently appreciated pathogenic mechanism in lupus.

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