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 cytoplastic 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 anti-phospholipid syndrome.

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/cDNA 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.

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

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

1Ryan J. Pachucki, 1,2Xinyan Zhang, 1Lynne Kohler, 3Sarah Tursi, 3Lauren NiCastro, 4Laurie Kilpatrick, 3Çagla Tükel, 3,5Stefania Gallucci, 1,2†Roberto Caricchio*.

1Division of Rheumatology, Department of Medicine; 2Currently Division of Rheumatology, Department of Medicine; 3Department of Microbiology and Immunology; 4Department of Thrombosis, Lewis Katz School of Medicine, Temple University, Philadelphia PA USA; 5Currently Division of Innate Immunity, UMass Chan Medical School, Worcester MA USA

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/cDNA 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.
Neutrophil extracellular traps (NETs) are part of the innate immune system and are pathogenic in SLE. We therefore investigated 56 lupus patients who met at least 4 SLICC criteria. Results were compared to 20 age, sex, and race matched healthy controls. We found that curli/eDNA induced more NETs in SLE PMNs compared to healthy controls. In SLE, patients who were high inducers of NETs triggered by curli/eDNA complexes were also a high inducer of NETs triggered by LPS and PMA. Interestingly, patients who were anti-dsDNA positive made more NETs in response to curli/eDNA complexes and LPS. We did not observe this in patients who were anti-dsDNA negative. Mechanistically, we found that curli/eDNA induce NETs via NADPH oxidase. Finally, we found patients who had bacteriuria had a higher amount of NET production in response to curli/eDNA complexes and PMA compared to patients with no bacteriuria. We conclude

1) that lupus PMNs are in a chronic inflammatory state. And 2) that curli/eDNA complexes can activate neutrophils and exposure to UPECs could be a mechanism to sustain autoantigens in the form of neutrophil extracellular traps.

1003 IMPAIRED INTRACELLULAR PROTEIN TRANSPORT AND AN ENDODOTHelial STRESS RESPONSE REMINISCENT OF COPA SYNDROME IN SLE-ASSOCIATED DIFFUSE ALVEOLAR HEMORRHAGE
Haoyang Zhuang, Erin Hudson, Shuhong Han, Rawad Daniel Arja, Li Lu, Westley Reeves*.
Division of Rheumatology, Allergy, and Clinical Immunology, University of Florida, Gainesville, Florida, USA
10.1136/lupus-2022-lupus21century.63

Background Human mutations of the coatomer coat protein alpha subunit (COPA) affect retrograde Golgi to endoplasmic reticulum (ER) protein transport, resulting in endoplasmic reticulum (ER) stress and a clinical syndrome consisting of polyarthritits and diffuse alveolar hemorrhage (DAH) with autoimmune features. Some SLE patients also develop DAH and C57BL/6 (B6) mice with pristane-induced lupus develop monocyte-dependent DAH closely resembling the human disorder. In contrast, BALB/c mice are resistant to DAH. We examined the role of COPA and ER stress in lupus mice with DAH.

Methods B6 and BALB/c mice were treated with pristane. Expression of Copa and markers of ER stress and vascular injury were assessed by quantitative PCR and immunohistochemistry.

Lung tissue was disrupted by Gentle MACS and ER stress was assessed in CD45-CD146- bone marrow-derived cells and CD45+CD146+ lung microvascular endothelial cells by flow cytometry. COPA transcripts were quantified in peripheral blood from 54 SLE patients and 22 controls.

Results DAH in B6 mice was associated with impaired Copa mRNA and protein expression and evidence of ER stress (increased Ddit3 and CHOP protein, Hspa5 and BIP protein, and other markers). Although DAH did not develop in BALB/c mice treated with either pristane or the ER stress inducer thapsigargin, DAH with impaired Copa expression and evidence of ER stress was induced when BALB/c mice were treated with pristane plus low dose thapsigargin. (BALB/c X B6)F1 mice did not develop DAH or ER stress, suggesting that susceptibility was recessively inherited. Flow cytometry of single cell suspensions of lung tissue revealed increased expression of the ER stress protein BiP in CD45+CD146+ pulmonary endothelial cells and CD45+CD146+ myeloid cells from pristane-treated B6 mice and also in CD45- cells from BALB/c mice treated with pristane + thapsigargin. Von Willebrand factor (VWF), a marker of endothelial injury, and the monocyte-attractive chemokine Ccl2 increased in lung from B6 mice with DAH, but not in lung from BALB/c mice treated with pristane (without thapsigargin). These data suggest that pristane-induced ER stress and impaired Copa expression promote lung microvascular injury (indicated by increased VWF expression) and the production of monocyte-attractive chemokines, such as Ccl2. COPA expression also was low in SLE patients with interstitial lung disease or nephritis, suggesting that increased susceptibility to ER stress and impaired retrograde Golgi-to-ER transport also may be associated with a subset of human lupus.

Conclusion DAH in a mouse lupus model appears to be initiated by genetically-regulated susceptibility of the lung microvasculature endothelium to pristane-induced injury, resulting in an ER stress response and culminating in a monocyte-dependent inflammatory response. Lupus-associated DAH in mice and possibly humans may represent an acquired form of COPA syndrome.

Acknowledgments This work was supported by the National Institutes of Health (NIAMS) Grant number R01-AR44731.

Lay summary Lung hemorrhage is a severe complication of SLE. Patients with mutations affecting intracellular protein transport ("COPA syndrome") also develop lung hemorrhage along with other clinical features of reminiscent of lupus (arthritis, autoantibodies, and abnormal regulation of interferon production). Using a mouse model, we found that lung hemorrhage in lupus replicates some of the clinical features of COPA syndrome, including low expression levels of COPA mRNA and protein and evidence of a stress response in lung microvascular endothelial cells. Additionally, we found that COPA expression was low in a subset of SLE patients, raising the possibility that these individuals might be at increased risk to develop lung hemorrhage.

Innate Immunity

1004 ALTERED ERα LOCALIZATION DIFFERENTIALLY MODULATES IMMUNE CELL SUBSETS
1Cameron Meyers, 2Jena Wirth, 3Melissa Cunningham. 1Department of Microbiology and Immunology, Graduate Studies, Medical University of South Carolina, Charleston SC 29407; 2Department of Medicine, Division of Rheumatology and Immunology, Medical University of South Carolina, Charleston SC 29407
10.1136/lupus-2022-lupus21century.64

Background Estrogen is anti- or pro-inflammatory depending on milieu and plays a role in the increased incidence of lupus in reproductive age women. Estrogen’s pleiotropic effects are in part due to estrogen receptors (ER) and their variants that localize to different regions in the cell. To understand the role of ERα localization in immune responses, we investigated the effects of altered ERα localization on Toll Like Receptor (TLR7)-stimulated endpoints, often dysregulated in lupus.

Methods Membrane-only ERα (MOER) or Nuclear-only ERα (NOER) mice were used to isolate and culture spleen cells, ex vivo bone marrow (BM), and BM-DCs. Cells were phenotyped...