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. Moreover, we found patients who are anti-dsDNA positive responded highly 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 ENDOTHELIAL STRESS RESPONSE REMINISCENT OF COPA SYNDROME IN SLE-ASSOCIATED DIFFUSE ALVEOLAR HEMORRHAGE

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**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 polyarthritis 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.

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**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

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**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

via flow cytometry to identify immune cell subsets. Spleen cells were treated with vehicle or TLR7/8 agonist overnight prior to supernatant analysis. In a parallel experiment, mice were treated for two weeks with a topical TLR7 agonist (R848) to assess effects on immune cell populations.

**Results** Immune cell subsets in spleen were similar in all mice. Cell counts of *ex vivo* and Flt3L- cultured BM-DCs were reduced in NOER mice. Conventional DCs (cDCs) were increased in MOER mice, and NOER mice trended higher in CD19+ cells. Other immune populations remained similar. NOER mice trended lower in IL-6 response after overnight R848 stimulation.

**Conclusion** Preliminary results suggest membrane ERα-initiated events are required to develop certain innate immune cell subsets and for robust expansion of DCs. Membrane and nuclear functions of ERα may compensate for each other in some cases. More studies are needed to clarify the role of ERα localization in modulating immune cell development and function.

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## 1005 CHARACTERIZATION OF REGULATORY RECEPTORS ON PLASMACYTOID DENDRITIC CELLS IN LUPUS

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Regulatory or suppressive receptors on plasmacytoid dendritic cells (PDCs) are an attractive therapeutic target in systemic lupus erythematosus (SLE), given the role type I interferon (IFN) plays in this disease. In this study, we determine whether SLE patient PDC regulatory receptor expression and function associates with disease features in SLE. We used quantitative multicolor flow cytometry to measure regulatory receptors on PDCs from SLE patients and control subjects, including immunoglobulin like transcript 7 (ILT7), bone marrow derived antigen 2 (BDCA2), ILT3, leukocyte-associated immunoglobulin-receptor 1 (LAIR1), natural killer cell P44-related protein (NKp44), bone marrow stromal cell antigen 2 (BST2), and dendritic cell (DC) immunoreceptor (DCIR). For functional studies, cells from 9 SLE patients and 9 controls were treated with ILT7 and BDCA2 crosslinking antibodies followed by TLR9 agonists. ILT7 and BDCA2 expression on SLE patient PDCs were inversely correlated with disease activity by SLEDAI score. High IFN SLE patients had increased levels of the ILT7 ligand BST2, and at the same time reduced ILT7 expression. BDCA2 levels were 5-fold higher than ILT7 levels, and crosslinking ILT7 only weakly inhibited IFN secretion. Crosslinking BDCA2 significantly reduced IFN production in SLE patient cells, but this effect on IFN was much greater in patients with low SLEDAI scores than those with high SLEDAI scores. In conclusion, we identify associations between PDC regulatory receptor expression and clinical disease in SLE, and dominant inhibitory function of BDCA2 over ILT7 in PDC type I IFN secretion with dependency upon disease activity.

## 1101 URINE COMPLEMENT ACTIVATION PRODUCTS IN LUPUS NEPHRITIS

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**Background** Complement activation plays a critical role in the development of kidney injury during lupus nephritis (LN). Clinical trials targeting the complement pathway are now underway in LN. It is therefore important to understand the

**Abstract 1101 Table 1**

| Parameter | Spearman r | | | P Value | | | 95% Confidence Interval | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ba | C5a | C5b-9 | Ba | C5a | C5b-9 | Ba | C5a | C5b-9 |
| **Activity Index** | 0.31 | 0.25 | 0.37 | 0.0004 | 0.005 | <0.0001 | 0.14,0.47 | 0.07,0.41 | 0.20,0.52 |
| •Endocapillary Hypercell | 0.25 | 0.23 | 0.36 | 0.005 | <0.01 | <0.0001 | 0.07,0.41 | 0.05,0.39 | 0.19, 0.51 |
| •Hyaline Deposits | 0.19 | 0.23 | 0.23 | 0.036 | 0.018 | 0.0094 | 0.008,0.36 | 0.031,0.38 | 0.05,0.39 |
| •PMN/Karyorrhexis | 0.18 | 0.18 | 0.32 | 0.044 | 0.044 | 0.0003 | 0.0005,0.35 | 0.0004,0.35 | 0.15,0.47 |
| •Necrosis | 0.19 | | 0.21 | 0.027 | 0.42 | 0.017 | 0.018,0,37 | | 0.034,0.38 |
| •Crescents | 0.21 | | 0.21 | 0.019 | 0.06 | 0.004 | 0.03,0.38 | | 0.08,0.42 |
| •Interstitial Inflammation | 0.35 | 0.26 | 0.27 | <0.0001 | 0.003 | 0.002 | 0.18,0.50 | 0.08,0.42 | 0.097,0.43 |
| **Chronicity Index** | 0.29 | 0.19 | | 0.0005 | 0.022 | 0.22 | 0.13,0.44 | 0.02,0.35 | |
| •Glomerulosclerosis | | | | 0.23 | 0.36 | 0.73 | | | |
| •Fibrous Crescents | | | | 0.94 | 0.37 | 0.83 | | | |
| •Tubular Atrophy | 0.31 | 0.24 | | 0.0002 | 0.031 | 0.17 | 0.15,0.45 | 0.079,0.39 | |
| •Interstitial Fibrosis | 0.31 | 0.24 | | 0.0002 | 0.031 | 0.16 | 0.15,0.45 | 0.079,0.39 | |
| **Proteinuria** | 0.41 | 0.54 | 0.42 | <0.0001 | <0.0001 | <0.0001 | 0.25,0.54 | 0.42,0.66 | 0.27,0.55 |
| **Serum Creatinine** | 0.51 | 0.31 | | <0.0001 | 0.0001 | 0.61 | 0.38,0.63 | 0.15,0.46 | |
| **Complement C3** | -0.31 | | | 0.0002 | 0.06 | 0.16 | -0.46,-0.15 | | |
| **Complement C4** | | | | 0.09 | 0.18 | 0.25 | | | |
| **Urine Ba** | | 0.71 | 0.46 | | <0.0001 | <0.0001 | | 0.62,0.78 | 0.31,0.58 |
| **Urine C5a** | | | 0.68 | | | <0.0001 | | | 0.59,0.76 |