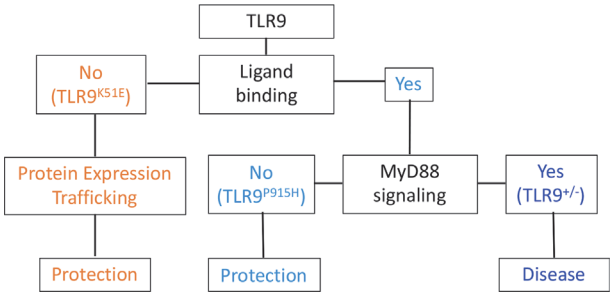


**Abstract 402 Figure 1** Model explaining the implications of the TLR9 mutant disease phenotypes

the restoration of TLR9 expression induces protection. To assess the effect of TLR9-MyD88 signaling, we compared $TLR9^{P915H/P915H}$ and TLR9-sufficient cohorts of MRL/lpr mice. Kidney disease, survival and immune activation were significantly more severe in $TLR9^{\pm}$ mice. Thus, there is a TLR9-MyD88 dependent pathway that promotes disease. Moreover, $TLR9^{K51E/-}$ mice had increased glomerulonephritis and immune activation compared to $TLR9^{P915H/P915H}$ mice, suggesting that TLR9 could regulate disease through a ligand binding-dependent but MyD88-independent mechanism (figure 1).

Using a 3 way ($TLR9^{WT}$, $TLR9^{P915H}$ and $TLR9^{-/-}$)-mixed bone marrow chimera, we found that TLR9 inhibits B cell development and differentiation in a B cell-intrinsic fashion and that the absence of TLR9 ($TLR9^{-/-}$) was very different from the inability of TLR9 to signal ($TLR9^{P915H}$). RNA seq analysis of sorted age-associated B cells (ABC) revealed that $TLR9^{WT}$, $TLR9^{P915H}$ and $TLR9^{-/-}$ ABC exhibit different transcriptional programs. Notably, the absence of TLR9 did not lead to an increase in genes that are induced by TLR7, arguing against the idea that TLR9 simply restrains TLR7 signaling.

**Conclusion** This in vivo genetic dissection of TLR9 reveals how it both promotes and regulates lupus. Inability of TLR9 to signal via MyD88 is different from absence of TLR9 and also is different from inability of TLR9 to bind ligand. These findings shed light on the basic biology of endosomal TLR signaling and are relevant to the design of TLR-targeted therapy.

**Acknowledgement** Funded by R37-AI118841 (M. Shlomchik).

## 403 BACTERIAL BIOFILM PRODUCT CURLI/EDNA INDUCES NEUTROPHIL EXTRACELLULAR TRAPS AND SERUM ANTI-CURLI/EDNA LEVELS CORRELATE WITH BACTERIURIA AND LUPUS ACTIVITY

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10.1136/lupus-2021-lupus21century.16

**Background** Infections are a major contributor to lupus disease. We have previously demonstrated that bacterial amyloid curli, produced by E.coli, can accelerate disease in mouse models of lupus. Interestingly curli incorporates extracellular DNA, which in turn can be both adjuvant and a self-antigen in lupus. Uropathogenic E. coli (UPEC) is responsible for the majority of urinary tract infections in SLE. Based on our previous results, we hypothesize that exposure to UPEC triggers anti-curli/eDNA antibodies and curli/eDNA complexes can trigger the innate immune system.

**Methods** We investigated 98 lupus patients who met at least 4 SLICC criteria. Results were compared to 54 age, sex and race matched healthy controls. We tested the production of anti-curli/DNA complex for both IgG and IgA subclasses. We than correlated the levels of anti-curli/DNA antibodies with clinical parameters. Finally, we treated human neutrophils with curli/eDNA complexes.

**Results** We found that curli/eDNA induces neutrophil extracellular traps in a ROS-dependent manner. Anti-curli/eDNA IgG levels were detected in lupus and controls plasma and the levels correlated with persistent bacteriuria ($p<0.05$) and disease flares in lupus patients. In addition, anti-dsDNA could bind to anti-curli/eDNA complexes.

**Conclusions** We conclude curli/eDNA complexes can activate the innate and adaptive immune system and could be a mechanism to sustain disease in lupus.

**Acknowledgments** We thank Drs. Marc Monestier and Philip Cohen for their insightful suggestions. We also thank the generosity of the lupus patients from the Temple Lupus Program.

## 404 PLATELETS ARE A SOURCE OF EXTRACELLULAR MITOCHONDRIA AND MITOCHONDRIAL DNA IN SYSTEMIC LUPUS ERYTHEMATOSUS

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10.1136/lupus-2021-lupus21century.17

**Background** The accumulation of DNA and nuclear components in blood and their recognition by autoantibodies play a central role in the pathophysiology of systemic lupus erythematosus (SLE). Despite the efforts, the sources of these circulating autoantigens in SLE are still unclear. While damaged organs and activated cells are generally considered as potential sources of autoantigens, platelets are often overlooked given that they are anucleated and thereby cannot release genomic DNA. However, accumulating findings suggest that mitochondria are also targeted by antibodies in SLE.

**Methods** We examined the presence of extracellular mitochondria in blood of patients with SLE and determined correlations with platelet activation. Because mice lack FcγRIIA and murine platelets are completely devoid of receptor capable of binding IgG-containing immune complexes, we generated transgenic lupus mice expressing FcγRIIA for our in vivo investigations. We used a reporter mouse with red fluorescent protein targeted to the mitochondrion to identify the cellular source of the extracellular mitochondria.

**Results** We found extracellular mitochondrial DNA (mtDNA) and mitochondrial organelles in the plasma of patients with SLE. The concentrations of mtDNA and extracellular mitochondria were higher than in healthy individuals, and mtDNA levels correlated with that of platelet factor 4 (PF4), a marker of platelet degranulation. The majority of the detected mtDNA was associated with the extracellular mitochondrial organelle. In our in vitro and in vivo investigations, mitochondrial release by platelets required the stimulation of platelet FcγRIIA, a receptor for immune complexes. FcγRIIA expression in lupus-prone mice accelerated nephritis, led to the recruitment of platelets in kidneys and to the release of mitochondria in vivo. Using our mouse model with fluorescently labeled mitochondria, we confirmed platelets as a source of extracellular mitochondria driven by FcγRIIA and its co-signaling by the fibrinogen receptor α2bβ3 in vivo.

**Conclusion** Our findings suggest that platelets might be a key source of mitochondrial antigens in SLE and might be a therapeutic target for treating SLE.

**Acknowledgments** This work was supported by a Foundation grant from the Canadian Institutes of Health Research (CIHR) (to E.B.). E.B. is recipient of a new investigator award from the CIHR and the Fonds de Recherche en Santé du Quebec (FRQS) PRF is recipient of a tier 1 Canada Research Chair on Systemic Autoimmune Rheumatic Diseases.

## 405 DOES ANTIMALARIAL ADHERENCE DECREASE THE RISK OF CARDIOVASCULAR EVENTS AND MORTALITY AMONG PATIENTS WITH INCIDENT SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS? A POPULATION-BASED STUDY

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10.1136/lupus-2021-lupus21century.18

**Background** There is an evidence of poor adherence (ranging from 25% to 57%) to antimalarial (AM) medications in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). We examine the association between AM adherence and cardiovascular (CVD) events and mortality among incident SLE and RA patients, and assess if the association in SLE differs from that in RA.

**Methods** All patients with incident SLE or RA and incident AM use in British Columbia, Canada, between January 1997 and March 2015 were identified using provincial administrative databases. The outcomes were incident CVD events and mortality attributed to myocardial infarction, stroke or venous thromboembolism. We used marginal structural models (MSM) to estimate the effect of AM adherence on incident CVD events and mortality, accounting for potential confounders and competing events due to death unrelated to CVD. In the analysis, for each 90-days window of follow-up time, the proportion of days covered (PDC), a measure of adherence, was calculated and categorized as adherence (PDC≥0.90), partial adherence (0<PDC<0.90), and non-taking (PDC=0) for AM use. The analyses were controlled for baseline demographics as well as the following sets of baseline and time-varying covariates: medication use, health resource utilization, comorbidities, and Romano adaptation of Charlson comorbidity index. Also, SLE and RA patients were analyzed separately.

**Results** We identified 21,114 individuals with incident SLE or RA (3,496 SLE; 17,618 RA patients, mean age 55.8 years, 75.9% female) with ≥1 filled AM prescription. Over the mean follow-up of 8.8 and 8.6 years, 2759 (14.3%) and 900 (4.3%) patients experienced incident CVD events and CVD mortality, respectively. The incidence rates of CVD mortality for AM adherence, partial adherence, and non-taking were 3.10, 4.68, and 5.65 per 1000 person-years. Using MSM, the adjusted hazard ratios (aHRs) of CVD mortality obtained for AM partial adherence and adherence in SLE or RA patients were 1.08 (95% CI: 0.91-1.31) and 0.51 (95% CI: 0.42-0.62), respectively, relative to non-taking (table 1). Also, the aHR for adherence compared to partial adherence was 0.47 (95% CI: 0.37-0.60). Findings were similar for CVD events (table 1). There was no significant difference in risk estimates between SLE and RA patients (Wald test p-values, table 1) though SLE patients

**Abstract 405 Table 1** Overall risk of incident CVD events and mortality in incident SLE and RA patients during follow-up

| | Both SLE and RA | | | | SLE patients | | RA patients | | Wald test p-values comparing SLE and RA |
|---|---|---|---|---|---|---|---|---|---|
| Adherence Levels | Outcome counts | IR per 1000 person-years | IR Ratios (95% CI) | MSM aHRs (95% CI) | Outcome counts | MSM aHRs (95% CI) | Outcome counts | MSM aHRs (95% CI) | |
| **Outcome: Incident CVD events** | | | | | | | | | |
| Non-taking [Reference] | 1,706 | 18.40 | 1.00 | 1.00 | 136 | 1.00 | 1,570 | 1.00 | |
| Partial adherence | 527 | 16.47 | 0.90 (0.81-0.99) | 1.02 (0.92-1.13) | 80 | 1.17 (0.88-1.56) | 447 | 1.01 (0.91-1.13) | 0.37 |
| Adherence | 526 | 14.00 | 0.76 (0.69-0.84) | **0.72 (0.65-0.80)** | 83 | 0.90 (0.68-1.20) | 443 | **0.70 (0.63-0.78)** | 0.14 |
| Contrast: Adherence vs. Partial adherence | | | 0.85 (0.75-0.96) | **0.70 (0.62-0.80)** | | 0.77 (0.56-1.06) | | **0.69 (0.60-0.79)** | 0.55 |
| **Outcome: CVD mortality** | | | | | | | | | |
| Non-taking [Reference] | 602 | 5.65 | 1.00 | 1.00 | 30 | 1.00 | 572 | 1.00 | |
| Partial adherence | 165 | 4.68 | 0.82 (0.70-0.98) | 1.08 (0.91-1.31) | 9 | 0.61 (0.29-1.28) | 156 | 1.13 (0.94-1.37) | 0.06 |
| Adherence | 133 | 3.10 | 0.55 (0.45-0.66) | **0.51 (0.42-0.62)** | 16 | 0.66 (0.35-1.26) | 117 | **0.50 (0.40-0.61)** | 0.46 |
| Contrast: Adherence vs. Partial adherence | | | 0.66 (0.53-0.83) | **0.47 (0.37-0.60)** | | 1.08 (0.46-2.52) | | **0.44 (0.34-0.56)** | 0.17 |

Non-taking: PDC=0, Partial adherence: 0<PDC<0.90, Adherence: PDC≥0.90. Abbreviations: **IR**, incidence rate; **aHR**, adjusted hazard ratio; **MSM**, marginal structural model; **CI**, confidence interval; **PDC**, proportion of days covered.