

**Background** SLE is characterised by the inappropriate activation of type I Interferon (IFN) and increases in apoptosis and NETosis by neutrophils, which in combination with defective apoptotic cell and NET clearance provides an ongoing source of self-antigen. IFN can further propagate the disease process by promoting B cell activation, survival, and differentiation into plasma cells (PC). PC produces autoantibodies that can form immune complexes (IC) to further stimulate IFN production creating a vicious cycle. Important questions that remain unclear are the site and mechanisms of IFN activation. We have recently demonstrated a prominent IFN signature in the bone marrow (BM) of SLE patients that is more pronounced than paired peripheral blood and correlated with higher serum autoantibodies and disease activity. We hypothesise that BM is a key site of IFN activation in SLE and better understanding of signals in the BM that regulate IFN activation may lead to discovery of new treatment targets.

**Materials and methods** BM supernatant and serum were obtained from SLE patients (n = 11 IFN high, n = 11 IFN low). Plasmacytoid dendritic cells (pDC) were purified from healthy donor blood. To determine if BM supernatant and serum induce IFN production, pDC was cultured with BM supernatant or serum with and without necrotic cell material. Necrotic cell material was generated by repeat freeze-thawed U937 cells. Culture supernatants were collected and IFN was measured by ELISA.

**Results** We found that BM supernatant from SLE patients with high level of multiple serum autoantibodies was able to induce pDC to produce type I IFN (BM:  $12481 \pm 259.1$ , BM + necrotic:  $12091 \pm 68.50$  pg/ml). The serum from the same patient also induced pDC to produce IFN that was greatly enhanced by necrotic cell material (serum:  $12244 \pm 90$ , serum + necrotic:  $114292 \pm 6998$  pg/ml). BM supernatant and serum from SLE patients with low level of serum autoantibodies did not induce pDC to produce IFN even in the presence of necrotic cell material. The relationship to the IFN signature and NETosis is under evaluation. Additionally, we are examining ICs as interferonogenic factors in the BM.

**Conclusions** These data suggest that the BM microenvironment of SLE patient contains factor(s) that promote type I IFN production by pDC that may correlate with the presence of serum autoantibodies. However, the mechanisms of IFN production in the BM appear to be different from that of the serum.

II-10

#### HO-1 EXPRESSION IN MONOCYTES MIGHT CONTRIBUTE TO INCREASED ROS LEVELS DURING PHAGOCYTOSIS IN LUPUS NEPHRITIS PATIENTS

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**Background** Systemic lupus erythematosus (SLE), an autoimmune disorder, is associated with autoantibody synthesis and inflammation. Although Lupus Nephritis (LN) is a complex manifestation of SLE that affects about 50% of patients, the cascade of events leading to glomerular damage develops its own dynamic of progression. Our group has studied the potential role of Heme Oxygenase-1 (HO-1) in the modulation of innate immune cells during SLE onset and the progression of disease, and its therapeutic potential. We have recently shown that HO-1 mRNA and protein are decreased in CD14+ monocytes from SLE patients.

Also, we showed that CO exposure reduces CD11b+ cells in the spleen of FcγRIIb knockout mice, and that both CO and CoPP, a HO-1 inducer, delays the onset of proteinuria in these mice. Here we have evaluated HO-1 expression, phagocytosis levels and reactive oxygen species (ROS) production in purified monocytes from peripheral blood of LN patients and healthy controls.

**Materials and methods** SLE patients with proliferative LN confirmed by renal biopsy (Class III, IV or V ISN/RPS) were recruited at Hospital Clínico of PUC. All individuals signed an informed consent form. This study was approved by the Research Ethics Committee of PUC, School of Medicine. Monocytes were purified using pan-monocytes MACS kit. HO-1 expression was measured by FACS and the mean intensity of fluorescence (MFI) was determinate. The phagocytic ability was measured by FACS and the total phagocytosis was calculated as the percentage of cells with engulfed beads. ROS was measured using CellRox Kit and the MFI was calculated.

**Results** We found that monocytes purified from LN patients show significant differences as compared to healthy controls in all the parameters analysed. HO-1 expression was decreased in monocytes from LN patients. The phagocytosis level was increased in monocytes of LN patients independently of the serum used to opsonize the beads (Control or autologous serum). The most important difference was observed in the percentage of monocytes that phagocyte 4 or more beads. The basal ROS level was higher in monocytes of LN patients, reaching a value similar to the monocytes of healthy controls treated with TBHP, a ROS inducer.

**Conclusions** Although our preliminary findings show that LN monocytes display increased phagocytosis, the basal levels of ROS are elevated in LN when compared to healthy controls. We propose that this increment could be modulated by HO-1 levels, which are decreased in LN monocytes. FONDECYT N° 1150173.

II-11

#### PLATELET ACTIVATION AND MITOCHONDRIAL RELEASE IN SYSTEMIC LUPUS ERYTHEMATOSUS

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**Background** Mitochondria are the powerhouses of the cell, providing energy to the cell through oxidative phosphorylation. Possibly owing to their similarities with bacteria, however, mitochondria extruded from cells promote inflammation. Platelets are anucleated elements highly abundant in blood and are activated in rheumatic diseases. As platelets represent a major reservoir of mitochondria in blood circulation, we hypothesised that activated platelets could release their mitochondria in rheumatic diseases.

**Materials and methods** Human platelets were activated using synthetic immune complexes (IC) and mitochondrial extrusion was determined using electron microscopy and high sensitivity flow cytometry. To determine whether mitochondrial release could occur in vivo, the presence of extracellular mitochondria in blood of systemic lupus erythematosus (SLE) patients was monitored concomitantly with platelet activation by the assessment of surface P-selectin and of the activated form of glycoprotein IIb/IIIa. As mice naturally lack the expression of the immunoglobulin G (IgG) Fc receptor FcγRIIA, the unique platelet receptor

for IgG in humans, we included transgenic mice expressing FcγRIIA to our *in vivo* mechanistic investigations. Platelet activation and mitochondrial release were monitored upon intravenous injection of synthetic ICs in wild type (WT) and transgenic (Tg) FcγRIIA mice.

**Results** We found that on activation, platelets relocate their mitochondria toward the cell membrane and then release respiratory-competent mitochondria into the extracellular milieu, both as free organelles and encapsulated within vesicles called microparticles (MP). Extracellular mitochondria were internalised by bystander leukocytes and, importantly, induced leukocytes rolling and adhesion to the blood vessel wall, suggesting that neutrophils and/or endothelial cells are activated by extracellular mitochondria. Activated platelets were more abundant in SLE patients than control subjects, and were associated with IgG. Extracellular mitochondria, both encapsulated in platelet MPs or naked, were also observed in blood circulation in SLE, and were frequently associated with IgG. Mechanistically, ICs present in blood induce profound cell activation, which is dependent on platelet FcγRIIA and its signalling cascade.

**Conclusions** Platelets represent an important source of mitochondria, which release in blood upon stimulation of FcγRIIA, might promote systemic inflammation in SLE. Whether the blockade of FcγRIIA might represent an attractive avenue in SLE research, and whether platelet activation markers and extracellular mitochondria might be utilised as potential biomarkers for the stratification of lupus patients needs to be further considered.

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#### 11-12 CSF-1 AND IL-34: DISTINCT POTENTIAL BIOMARKERS FOR LUPUS

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**Background** A noninvasive means to predict the onset and recurrence of lupus is needed to optimise and individualise treatment. Macrophages (M $\phi$ ) are prominent in inflamed tissues targeted for destruction in SLE. We hypothesised that the principal molecules required for M $\phi$  survival and proliferation are biomarkers for SLE. CSF-1 and IL-34 are promising candidates as both (i) bind to cFMS expressed by M $\phi$ , thereby promoting M $\phi$  survival and proliferation and (ii) promote destructive inflammation. However, IL-34 and CSF-1 have differing functions, which may be related to IL-34, not CSF-1, binding to a second receptor and distinct spatial temporal expressions.

**Materials and methods** We analysed serum and urine CSF-1 and IL-34 levels in SLE patients with nephritis (LN), arthritis (LA), cutaneous and serositis compared with healthy controls in two large cohorts (ELISA). While serum and urine CSF-1 expression is elevated in each manifestation, CSF-1 is notably higher in LN. In contrast, serum IL-34 expression is dramatically higher in LA, not LN. Thus, we probed for CSF-1 and IL-34 expression in LN (kidney) and LA (synovium). Moreover, we longitudinally tracked serum CSF-1 and IL-34 prior to LN (biopsy proven), with disease activity including flares and during LA in comparison to disease activity

#### Results

- **LN.** CSF-1 and IL-34 are expressed in the same and different renal tubular epithelial cells in LN. Elevated serum or urine CSF-1, not IL-34, levels correlate with increasing intra-renal CSF-1 expression and histopathology index. Longitudinally tracking serum CSF-1, not IL-34, levels heralds the initial onset of nephritis and a rise in serum or urine CSF-1 predicts LN recurrences before clinical evidence of renal dysfunction and conventional serologic measures.
- **LA.** IL-34, not CSF-1, expression is higher in synovial fluid and synovium in LA compared to osteoarthritis and healthy controls and correlates with magnitude of intra-synovial leukocytes. Moreover, intra-synovial IL-34 expression is similar in LA and rheumatoid arthritis. Longitudinally monitoring serum IL-34, not CSF-1, levels track with clinical disease activity in LA and RA.

**Conclusions** Serial monitoring a rise in serum or urine CSF-1, not IL-34, in SLE reflects renal histopathology and clinical disease activity and the onset and reoccurrences of LN more accurately than conventional laboratory measures. While serial monitoring a rise in serum IL-34, not CSF-1, reflects clinical disease activity in LA. Thus, CSF-1 and IL-34 are inexpensive and accurate potential biomarkers for LN and LA, respectively.

#### 11-13 SUPPRESSION OF SYSTEMIC AUTOIMMUNITY BY THE INNATE IMMUNE ADAPTOR STING

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**Background** Cytosolic DNA-sensing pathways that signal via the adaptor Stimulator of Interferon Genes (STING) mediate immunity to pathogens and have also been known to promote autoimmune pathology in DNaseII/III-deficient mice. However, the role of these pathways in systemic models of autoimmunity is unexplored. We hypothesised that cytosolic DNA sensing pathways contribute to the pathogenesis of autoimmune disease. Surprisingly, we report here that STING *potently* suppresses inflammation in several models of systemic lupus erythematosus (SLE).

**Materials and methods** A controlled F2 intercross between heterozygote STING<sup>±</sup> lpr<sup>±</sup> littermates generated STING-deficient lupus-prone mice homozygous for deficiency in Fas as well as STING (STING/lpr, n ≥ 10) or wild type for STING (WT/lpr, n ≥ 10). Mice were analysed at 16 wk of age. A similar F2 cross was set up for IRF3<sup>-/-</sup> and MRL/lpr mice as well as STING<sup>-/-</sup> and C57Bl/6<sup>lpr/lpr</sup> and analysed as above for STING/lpr mice (n ≥ 10 per group). C57Bl/6, cGAS<sup>-/-</sup>, Unc93b<sup>3d/3d</sup>, and STING<sup>-/-</sup> mice were injected i.p. with TMPD and evaluated at day 14 and 6 months post injection.