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 ± 259.1, BM + necrotic: 12091 ± 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 ± 90, serum + necrotic: 114292 ± 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-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 FcγR-IIA 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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