II-07 GENERATION OF CELL DISTANCE MAPPING PLUS (CDM+): MAPPING COGNATE T CELL: DENDRITIC CELL INTERACTIONS AND THEIR RELATIONSHIP TO CELL SHAPE

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Background We developed Cell Distance Mapping (CDM) to study tubulointerstitial inflammation in human lupus nephritis biopsies. Using CDM, we were able to translate proximal distance measurements of T:B cell pairs to their functional state in human tissue. One criticism of our work was that it could be considered descriptive. To address this, we utilise an animal model, wherein the antigen specificity of cells can be controlled. Using this, we wanted to expand CDM to the study of innate and adaptive immune responses, chief among which are T cell:Dendritic cell (DC) interactions. We also wanted to incorporate measures of cell shape parameters to improve our ability to distinguish cognate from non-cognate interactions.

Materials and methods An adoptive triple transfer mouse model was utilised, with each population labelled with fluorescent cell trackers: pigeon cytochrome C-pulsed and LPS-activated dendritic cells (DCs), antigen-specific T cells, and wild type T cells. After transfer, cervical lymph nodes were subjected to two-photon excitation microscopy (TPEM) analysis, frozen at −80 °C, and further subjected to CDM analysis. A total of 79 images from the lymph nodes of 5 animals was used. The results were analysed with respect to global cell shape, as visualised by freely diffusible cell trackers for T cells. This was superimposed on CDM data for interactions between respective T cell subsets and dendritic cells.

Results Analysis of 512×512 pixel images, representing 640 ×magnification views, revealed significant differences at <0.27 μm (8.70 vs 3.22%, $p = 0.028$), <1 μm (11.7 vs 3.70%, $p = 0.01$), and <2 μm (13.1 vs 5.26%, $p = 0.031$) distance cutoffs comparing antigen specific T cell:DC interactions versus WT T cell:DC interactions. Our results compared favourably with arrest coefficient calculation performed on TPEM data (mean of 0.06 vs 0.26, respectively; $p < 0.01$). Global cell shape analysis did not reveal any additional statistically significant differences. Increasing acquisition resolution to 1024×1024 pixels revealed the following measurements that distinguished between the two T cell subsets: area ($p < 0.0001$), circularity ($p < 0.0001$), perimeter to area ratio ($p < 0.0001$), aspect ratio to area ratio ($p < 0.0001$). Each variables\ was controlled for area to ensure that observed findings were not due to global differences between the two respective T cell subsets or influenced by variances in wavelengths, utilised to visualise individual cell trackers.

Conclusions Our data shows that CDM is able to reliably identify cognate interactions on par with TPEM, using distance as the main measurement. The addition of global cell shape parameter measurements helped to further distinguish cognate from non-cognate interactions at the same distance measurements.

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II-08 THE ROLE OF NEUTROPHILS IN B CELL DYSREGULATION IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background SLE is an autoimmune disease involving pathological dysregulation of both innate and adaptive immune compartments and loss of B cell tolerance. Recent evidence showing that neutrophils are dysregulated in SLE has led us to hypothesise that this innate immune cell may contribute to loss of B cell tolerance, both as a producer of cytokines and of self-antigen, in the form of apoptotic debris or neutrophil extracellular traps (NETs). Indeed, in previously published work from our group we demonstrated that neutrophils contribute to a Type I interferon signature in the bone marrow (BM) of SLE patients and represent a significant source of cytokines known to affect B cell function such as BAFF and APRIL. In order to ask whether neutrophils promote loss of B cell self-tolerance and immune activation, we examined neutrophil function over the course of disease and the impact of neutrophil depletion in the NZB/W lupus model.

Materials and methods NZB/NZW F1 female mice were injected intraperitoneally every other day with anti-Ly6G antibody (500 ug), either from 25 to 30 weeks of age (established disease) or 14 to 26 weeks (disease onset). Proteinuria was monitored weekly. At the end of the therapy, mice were euthanized and spleen, BM and kidneys were collected to enumerate neutrophils and lymphocytes by flow cytometry and immunofluorescence and antibody secreting cells (ASC) by ELISpot.

Results BM neutrophils are increased in frequency in lupus, display increased apoptosis, and have elevated production of BAFF, APRIL and IFNα near developing B cells. We also find an enrichment of IFNα-producing neutrophils in the spleen in close proximity to B cells late in disease. Surprisingly, following neutrophil depletion early in disease, there was an acceleration of proteinuria and pronounced increases in germinal centre formation, anti-dsDNA titers, and anti-dsDNA ASCs in the spleen, BM, and kidney. However, neutrophil depletion after onset of overt disease pathology does not impact SLE progression. To elucidate the specific mechanisms underlying neutrophil effects on B cell autoreactivity, we further examined the cytokine profile and splenic localization of neutrophils over the course of disease. Early in disease splenic neutrophils were in closer proximity to T cells, whereas B cell interactions increased with disease progression.

Conclusions These data delineate a shifting balance of regulatory and activating roles for neutrophils during SLE progression, possibly dominated by suppressive effects on T cell activation and/or differentiation early and production of pro-inflammatory cytokines later in disease.

II-09 SLE BONE MARROW CONTAINS FACTORS THAT PROMOTE TYPE I INTERFERON ACTIVATION

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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-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 erythematous (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 Clinico 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 IIbIIIa. As mice naturally lack the expression of the immunoglobulin G (IgG) Fc receptor FcγRIIA, the unique platelet receptor