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

1Vladimir Liarski*, 2Adam Sibley, 4Nicolas van Panhuys, 5Ronald Germain, 1Maryellen Giger, 2Marcus Clark. 1Section of Rheumatology and Gwend Knapp Centre for Lupus and Immunology Research, University of Chicago, Chicago, IL 60637; 2Department of Radiology, University of Chicago, Chicago, IL 60637; 3National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892

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 utilised 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 variable 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.

Acknowledgements Vladimir Liarski is supported by NIH NIAMS K08 AR068421. Marcus Clark is supported by NIH grants U19 AI082724 and AR55646.
Abstracts

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 cultures. 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

Loreto Cubino, Natalia Crisostomo, Fabian Vega, Camila B Schmidt, Andy Torres, Carolina Llanos.
Departamento de Inmunología Clínica y Reumatología, Escuela de Medicina, Chile; Millennium Institute on Immunology and Immunotherapy, PUC, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Chile.

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 FcRRIb 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 de 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 determined. 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).

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 No 1150173.

II-11 PLATELET ACTIVATION AND MITOCHONDRIAL RELEASE IN SYSTEMIC LUPUS ERYTHEMATOSUS

Eric Boilard*, Imene Melki, Nathalie Cloutier, Isabelle Allaeys, Paul R Fortin. Centre de recherche du CHU de Québec, Faculté de médecine, Université Laval, Quebec City, Quebec, G1V 4G2, Canada; Division of rheumatology, CHU de Québec – CHUL, Quebec City, Quebec, G1V 4G2, Canada. 10.1136/lupus-2016-00179.41

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 IIbIIa. As mice naturally lack the expression of the immunoglobulin G (IgG) Fc receptor FcγRIIA, the unique platelet receptor...