**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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10.1136/lupus-2016-000179.37

**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 data 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.