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# GENERATION OF CELL DISTANCE MAPPING PLUS (CDM<sup>+</sup>): 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 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.

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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 µg), 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 auto-reactivity, 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.

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# SLE BONE MARROW CONTAINS FACTORS THAT PROMOTE TYPE I INTERFERON ACTIVATION

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