Microrheological properties of mice skin draining and mesenteric lymph nodes using live tissue slicing

[1]Ann Ramirez, [1]Brooke Merwitz, [1]Dalia Kassass, [1]Katharina Maisel
[1]University of Maryland, College Park

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Statement of Purpose

The lymph node (LN) is a key site in shaping the adaptive immune response. It is a highly structured organ with specialized compartments for specific cell types and strict entry requirements. Foreign particles first enter the capsule of the LN through the afferent lymphatic[1]. Any particles >70kDa make their way around the capsule of the LN and exit through the efferent lymphatic[1]. Smaller materials are taken up by dendritic cells just past the basement membrane[1]. Materials that cross into the LN parenchyma make their way through deeper portions of the LN across a network of collagen fibers and fibroblast reticular cells, called the conduit system[2]. Materials can travel from the outer B cell filled cortex to the deeper T cell filled paracortex[2]. While this conduit system is well described, the effects of different physiological parameters and conditions on LN extracellular matrix spaces are not fully understood. Additionally, targeting LNs to enhance efficacy of immunotherapy has recently received significant attention and thus a better understanding of LN structure will help better direct therapeutic design.

The purpose of this study was to enhance our understanding of the rheological properties and structure of the extracellular spaces in the LN using multiple particle tracking. LN extracellular spaces consist of cells and ECM, resembling a hydrogel. We studied the extracellular tissue structure and microrheology using two different size nanoparticles (40 nm and 100 nm) with bio-inert surface chemistry. We used skin draining and mesentery LNs to determine if the location of the LN plays a role in the overall structure of the LN. Additionally, we correlated nanoparticle diffusion with the different regions of the LN, including B and T cell zones, to better understand the structure – rheology/diffusion relationship.

Methods

Lymph node collection and slicing

10-week-old female C57bl/6 mice were euthanized via CO_2 inhalation and LNs were collected immediately and placed in 1X PBS with 2% FBS: inguinal, brachial, and mesentery[3]. LNs were embedded in a 6% low-gelling agarose gel and removed using a biopsy punch[3]. A Leica VT1000 S Vibrating blade microtome was used to cut 300 µm thick LN slices, using a speed of 3.9 mm/s, frequency set to 0.3 Hz, and amplitude set to 0.6 mm [3]. Slices were then placed in RPMI media supplemented with FBS, L-glutamine, Pen/Strep, non-essential amino acids, HEPES, pyruvate, and beta-mercapto ethanol and let to rest for at least an hour before slicing[3].

Staining and multiple particle tracking

Slices were placed on microscope slides and an A2 stainless steel flat washer was placed around the LN. Slices were Fc blocked for 30 minutes and then stained with B220-FITC and anti-CD4 for one hour at 37°C and 5% CO_2. Slices were washed with 1X PBS for 30 minutes[3]. Fluorescent 40 nm and 100 nm polystyrene nanoparticles were densely PEGylated using EDC/NHS chemistry and delivered to slices[3]. Movies of particle diffusion was recorded using a Zeiss Axiovert fluorescent microscope.

Analysis

Mean squared displacement was calculated using a Matlab program. From this, material properties including diffusion coefficient pore size, G', G", G* were calculated.

Results

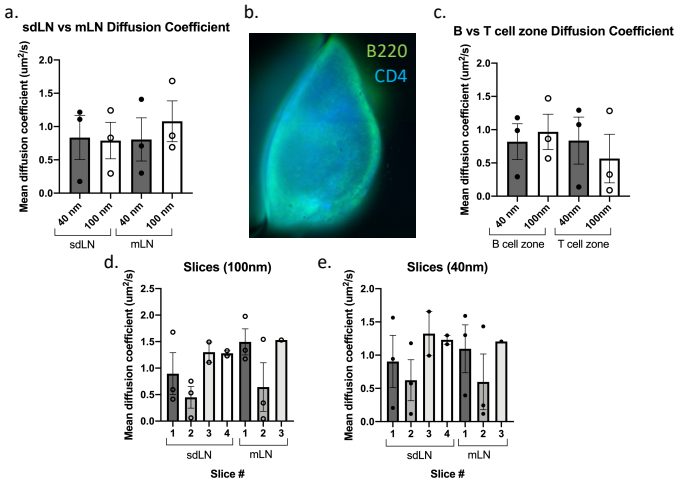

Figure 1. (a) Mean diffusion coefficient of 40nm and 100nm particles in skin draining and mesenteric lymph nodes (sdLN and mLN respectively). (b) B cell (B220) and T cell (CD4) staining of a mouse lymph node slice. B cells are located more on the outside of slice, while T cells are located more in the center of the slice. (c) Mean diffusion coefficient of 40nm and 100nm particles in B and T cell zones. (d) Mean diffusion coefficient of 40nm particles in differing slices collected (1 – 1st slice, 2 – 2nd slice, etc.). (d) Mean diffusion coefficient of 100nm particles in differing slices collected.

It was found that there was no difference in diffusion coefficient of either particle size when comparing between skin draining LN and mesenteric LN. However, when looking at B and T cell zones, the 100nm particles had a lower diffusion coefficient in the T cell zone compared to the B cell zone. There seems to be no difference between the B and T cell zones with the 40nm particles. When looking at individual slices within the lymph nodes, the second slice had a lower diffusion coefficient in all conditions including size and LN type.

Conclusion

Double staining for both B and T cells confirmed that any non-B-cell-stained area was indeed a T cell zone. Confirmation of this was useful in tracking particles in each of these two zones. With the 100nm particle, a lower diffusion coefficient in the T cell zone indicates that there is a more packed structure in this region. Differences in diffusivity through the depth of the LN suggests heterogeneity through the entire LN.

References

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