Analysis of B Lymphocyte Surface Receptor Mobility and Behaviour

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1 Introduction and Background

This summer I worked on a biomathematics project under the supervision of Professor Daniel Coombs. Results from this project will be published later this year. This is a brief overview of the techniques used in this project and a general statement of our results.

1.1 B Lymphocytes and Imaging Techniques

B lymphocytes, or B cells, are an immune system constituent responsible for the production of antibodies. B cell membranes contain thousands of transmembrane proteins known as B Cell Receptors (BCRs), which bind to antigens and play a central role in B cell activation. Sufficient numbers of antigen-bound BCRs lead to B cell activation and antibody production.

A better understanding of B cell activation is important for the treatment of disease involving overactive or underactive immune systems. Surpressing B cell activation is a potential basis for the treatment of antibody-based autoimmune diseases, such as arthritis. Conversely, stimulating B cell activation may be helpful for patients with unresponsive immune systems, or as a potential treatment for other conditions, such as some cancers.

Tracking BCR movement is possible through Total Internal Reflection Fluorescence (TIRF) microscopy. Small numbers of BCRs are tagged with antigen-bound fluorescent molecules (fluorophores), and high speed video of the cell's surface is taken. To allow for analysis on small spacial scales, two fluorescent dyes with differing emission spectra are used alongside two cameras, which each pick up a single dye colour. In single-colour microscopy, when the distance between two BCRs is less than the Rayleigh Limit, there is significant overlap in the Gaussians produced by each fluorophore. This results in resolution issues when two BCRs come sufficiently close. By using two-colour microscopy, it is possible to compare BCR positions between channels, even when two particles are separated by a distance below the Rayleigh Limit.

1.2 BCR Localization and Track Analysis

Once data streams are collected for active and inactive B cells, quantitative analysis of BCR behaviour may be performed. In each channel, particles are detected and tracked using Single Particle Tracking (SPT) software.

To compensate for alignment differences between the two cameras, static TetraSpeck beads, which appear in both channels, are also imaged. Bead location in each channel is compared, and an optimal set of transformation parameters (rotation, translation, shear) is generated. These transformations are then applied to the track detection output for a single channel to ensure correct alignment between channels.

Once tracks are produced and aligned, a variety of quantitative techniques for track analysis are possible. Diffusivity (D), for example, is a general measure of how quickly the BCRs are moving:

$$D = \frac{\Sigma_{i=1}^{k} ||r_i||^2}{2n\Delta t}.$$

Here, r_i is the vector between the BCR's location in the ith and $i+1$th frames, n is the dimension ($n = 2$ in our case), and Δt is the time duration of the entire track.

Using the diffusion coefficient, exit time analysis may be performed. For a disc of radius R and a particle undergoing a random walk with diffusivity D from a starting radius within the disc r, the average exit time is $T = \frac{R^2 - r^2}{4D}$. Theoretical and experimental exit times may be compared to determine if there is a "stickiness" between BCRs in activating B cells, as predicted by the Collision Coupling Model.

Many other SPT analysis techniques are available and were used to quantify BCR behaviour. Some of these techniques involve examining BCRs which are within close proximity, and then analyzing the path of each BCR to draw conclusions about the effects of proximity on displacement direction or diffusivity. Other techniques involve tracking a single BCR through areas of high and low receptor density, to see if higher collision frequency results in lower diffusivity.

2 Results and Discussion

2.1 Statement of Results

Many aspects of BCR behaviour were examined. In general, BCRs found in activated B cells have higher diffusivity and undergo slightly longer colocalizations than BCRs in inactive B cells. These observations support the Collision Coupling Model. Furthermore, it seems probable from our data that small BCR separations are correlated with smaller displacement angles between BCRs. In other words, the closer two particles are to each other, the more likely they are to travel together. A full analysis of this will be performed when more data is available.

A recent analysis has also found that BCRs in active B cells are likely to have similar speeds to their nearest neighbours, while BCRs in inactive cells show no relationship between proximity and difference in displacement speed. This tentatively indicates that BCRs in active B cells do in fact have the "stickiness" hypothesized by the Collision Coupling Model.

Our analyses also found significant issues with particle tracking at small spatial scales. This result lead to alternative analysis techniques which focus on lower temporal resolution (30FPS) to allow for higher image quality, and which examine BCR behaviour at a larger spacial scale.

Mask analysis is an example of such a technique. For this analysis, two receptor types (IgM and CD19) are stained at different densities, so that single particle motion may be compared to receptor density in the other channel. This analysis has yielded promising results, which seem to indicate that for certain receptor types, in active B cells, BCRs have lower diffusivity when they pass through areas of high receptor density. In inactive B cells no relationship between BCR density and single particle diffusivity is found, as is expected.

2.2 Discussion

Several differences were found between BCR behaviour in active and inactive B cells, and in BCR colocalization behaviour between different receptor types. These results help to better understand the process of B cell activation. Software to examine BCR motion has also been developed and will be made public upon completion of this project.

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