Rigidty-dependent Activation of CD4+ T Lymphocytes

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Statement of Purpose: *Ex vivo* expansion of T cells is a key step in adoptive immunotherapy. This is routinely carried out by exposing harvested T cells to beads presenting surface-immobilized, activating antibodies to CD3 and CD28, which are antigenic and costimulatory T cell surface receptors, respectively; this procedure induces T cell activation, proliferation, and population expansion. We explore in this report how the rigidity of the activation substrate influences T cell activation. The sensitivity of other cell types to substrate rigidity is well established, but is largely unexplored in the context of T lymphocyte signaling. However, T cell activation is dependent on actin organization and contraction [1,2], suggesting a role of such forces. We compare T cell activation by anti-CD3 and anti-CD28 antibodies attached to polydimethylsiloxane (PDMS) and polyacrylamide (PA) substrates of different rigidities, focusing on secretion of IL-2, the primary cytokine associated with naïve T cells.

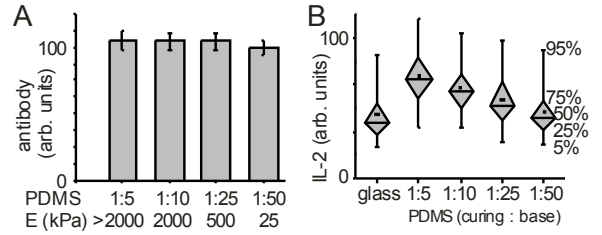

Figure 1. Rigidity-dependent activation of naïve CD4+ T cells. (**A**) Antibody adsorption onto PDMS. Data are mean ± s.d., n=3 independent experiments. No statistical difference was detected by ANOVA, $\alpha = 0.05$. (**B**) IL-2 secretion correlated with PDMS rigidity. These box plots from a representative experiment (of four independent experiments) include greater than 2000 cells for each substrate. Each condition is statistically different than all others, $\alpha = 0.05$, Kruskal-Wallis analysis.

Methods: PDMS elastomer substrates of varying rigidity were prepared by controlling the ratio of curing agent : base elastomer. The standard ratio of 1:10 provided a Young's modulus, E, of ~ 2MPa, while a 1:50 ratio resulted in E ~ 25 kPa. Young's modulus of these samples was measured using a custom-built, uniaxial compression apparatus. These substrates were coated with a mixture of 5 μg/mL each of anti-CD3 and anti-CD28 antibodies (eBiosceince), which activate their respective surface receptors. To quantify adsorption, PDMS was coated with 10 μg/ml of Donkey anti-Rabbit antibody. An HRP-conjugated rabbit antibody was subsequently captured and quantified using ELISA Turbo-TMB. PA gels were prepared following the methods of Yeung *et al*[3], using acryl-NHS as a protein linker. After the gel was cured, they were immediately coated with 25 μg/mL each of anti-CD3 and anti-CD28. Both types of substrates were seeded with primary mouse naïve CD4+ T cells at a density of 3 x 10^5 cells/cm^2. IL-2 secretion was determined after 6 hour incubation using a commercial surface-capture technique (Miltenyi Biotech) which provides a cell-by-cell measure of relative cytokine secretion[4].

Results: PDMS was chosen as a substrate of interest based on the ability to readily modulate elastic modulus, and its suitability as a replacement for polystyrene beads as an *ex vivo* expansion system. Mechanical testing indicated that under our standard curing conditions, the Young's modulus of these samples varied over two orders of magnitude (Figure 1A); the 1:5 sample was stiffer than the 1:10 sample, but beyond the range of linear, reliable interpretation on our apparatus. Quantification of adsorbed proteins indicated that the per-area amount of antibodies adsorbed onto PDMS substrates was independent of substrate rigidity (Figure 1). Focusing on cell activation, we found that secretion of IL-2 over a six-hour period correlated with substrate rigidity (Figure 1B), increasing by 60% between the softest and hardest surfaces. However, these PDMS substrates are of higher Young's modulus than that normally associated with tissues (< 10 kPa). For this reason, PA substrates are also being examined. A challenge in preparing these gels is immobilization of sufficient concentrations of activating antibody to the gels; while polymerizing ECM proteins are routinely attached to these gels[3], attachment of antibodies, which do not typically polymerize, remains a challenge. We have optimized the acryl-NHS method for use with these antibodies, and demonstrate activation of naïve CD4+ T cells, as evidenced by robust secretion of IL-2 on these surfaces (Figure 2).

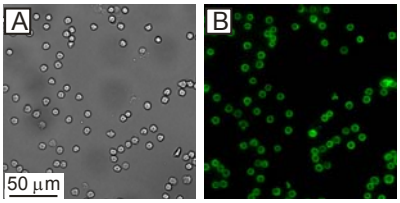

Figure 2. Activation of naïve T cells on PA gels. (**A**) DIC image of T cells adherent to PA of predicted modulus E = 10Pa (**B**) IL-2 secretion assay, indicating effective stimulation on PA.

Conclusions: We demonstrate rigidity dependent activation of naïve CD4+ T cells the dependence of IL-2 on PDMS. These results are being extended onto polyacrylamide gels to explore more physiologically relevant ranges of elastic modulus. These results demonstrate the role of rigidity in an emerging field of cell signaling, and by modulating T cell activation may provide new tools for improving adoptive immunotherapy.

References:

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[4]Shen K. PNAS. 2008; 105: 7791-7796.