Rigidity-dependent Costimulation of Naïve CD4+ T cells

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Statement of Purpose:

Communication between T cells and antigen presenting cells (APCs) forms a key regulatory point of the immune system. This is mediated in large part by cell surface proteins within a small (75 μm^2) cell-cell contact area termed the immunological synapse (IS). An emerging picture of this interface is that dynamic organization of signaling complexes has wide impact on cell communication, driven by the cell cytoskeleton of both the T cell and APC. Previous studies have shown that a disruption of actin cytoskeleton in dendritic cells (DCs) reduces T cell activation [1, 2]. Tseng and colleagues showed that deletion of cytosolic domain of CD80 (the native, APC ligand to the costimulatory molecule CD28) results in a decrease in T cell activation [3]. Our previous study have also proven that localization of CD28 antibody to the periphery of IS, an actin-rich region, enhances T cell activation [4]. Together, these studies suggest that T cells may be sensitive to the mechanical response of the extracellular environment. In this report, we demonstrate that T cell activation, measured by secretion of IL-2, is indeed sensitive to the rigidity of a cell culture substrate, and explore the mechanisms of this regulation.

Methods:

Sylgard 184 PDMS elastomer (Dow Corning) was prepared at a range of curing agent : base ratios between 1:5 and 1:50, yielding elastomers with Young's modulus between 2.1 MPa (1:5) and 25 kPa (1:50). Elastomer slabs were then coated with 1:1 mixture of activating antibodies to CD3 (a key activational component of the TCR) and CD28. Glass coverslips were used as a reference. Naïve CD4+ T cells were isolated from 6~8-week C57BL/6 mice (Taconic) and seeded onto the substrates. IL-2 secretion over the subsequent 6 hours was analyzed using a secretion assay kit (Miltenyi Biotec). In addition, cells were fixed after 30 min in culture and stained for CD28, phospho-p130Cas, and actin. T cell traction forces were mapped using PDMS micro-pillar arrays of 0.75μm in diameter and 5μm to 9μm in height. Pillars were coated with antibodies identically as that for planar surfaces. Data were analyzed with ANOVA and Kruskal-Wallis approaches.

Results:

IL-2 secretion by naïve CD4+ T cells is shown in the representative experiment of Fig. 1. The parts of the box plots indicate the 10, 25, 50, 75, and 90 percentiles, respectively. Surfaces noted by the asterisks (*) were not statistically different from each other (α=0.05); all other comparisons were different (α=0.01). On PDMS, IL-2 secretion correlated with higher rigidity. Surprisingly, IL-2 secretion on glass, the most rigid surface, was similar to that on the softest PDMS surface. However, the concentration of antibodies adsorbed onto glass was ~1/10 of that on PDMS substrates; the concentration on PDMS was independent of elastomer formulation.

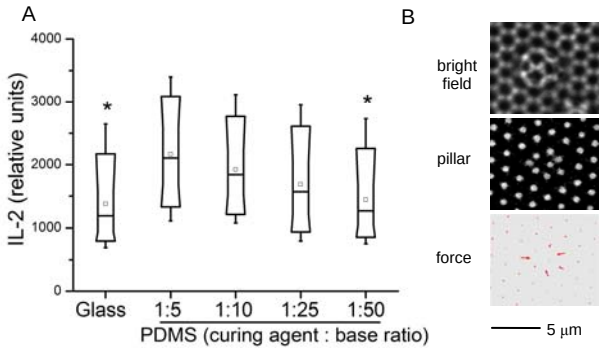

Figure 1 (A) 6 hr. IL-2 secretion by T cells on PDMS correlates with rigitiy (B) force mapping on antibody coated micro-pillar array

To identify potential mechanisms by which these cells can respond to rigidity, we **mapped the traction forces** exerted by cells using elastomer pillar arrays (Fig. 1B). T cells exerted forces on the order of 0.1 nN, providing new evidence into mechanical forces within in this interface. We also compared the distribution of **actin, phospho-p130cas and CD28** (Fig. 2A) between glass and the softest PDMS preparation. Cell spreading and phospho-p130Cas micro-cluster number per cell were significantly different between two substrates (Fig. 2B, *, **, α=0.05).

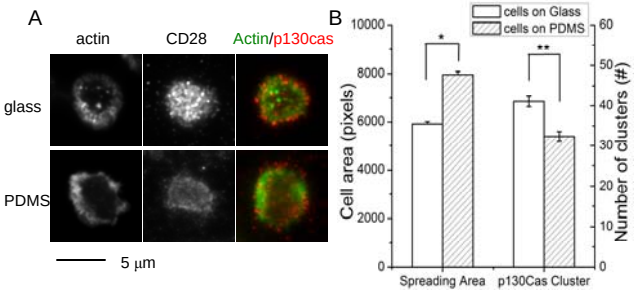

Figure 2 (A) Intracellular staining for actin, CD28 and phospho-p130Cas (B) cell spreading area and phospho-p130cas clusters on glass and PDMS surfaces

Conclusions:

Our results demonstrate a clear modulation of T cell activation as a function of substrate rigidity. Understanding the mechanisms by which rigidity is sensed will be a significant advance in the basic study of immunology as well as the design of new materials and systems to direct *ex vivo* expansion of T cells.

Acknowledgements:

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References:

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