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Nanoengineering of Immune Cell Function

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Abstract

T lymphocytes are a key regulatory component of the adaptive immune system. Understanding how the micro- and nano-scale details of the extracellular environment influence T cell activation may have wide impact on the use of T cells for therapeutic purposes. In this article, we examine how the micro- and nano-scale presentation of ligands to cell surface receptors, including microscale organization and nanoscale mobility, influences the activation of T cells. We extend these studies to include the role of cell-generated forces, and the rigidity of the microenvironment, on T cell activation. These approaches enable delivery of defined signals to T cells, a step toward understanding the cell-cell communication in the immune system, and developing micro/nano- and material- engineered systems for tailoring immune responses for adoptive T cell therapies.

INTRODUCTION

The immune system protects against pathogens and other agents. The adaptive immune system, a recently evolved part of the vertebrate immune system, forms a strong line of defense against biological challenges through its ability to recognize new antigens, develop an appropriate response, and rapidly recall this action on subsequent re-exposure. T lymphocytes are a key regulatory component of this system, coordinating the activity of other cells and directly carrying out specific immune functions. Given these roles, T cell manipulation has been proposed as a therapeutic treatment for many diseases, most notably cancer [1–5]. A key step in many of these treatments, collectively referred to as adoptive immunotherapy, is the *ex vivo* stimulation and expansion of T cells, with subsequent re-introduction of these cells into the patient. This process is most often carried out by presenting T cells with ligands to specific receptors present on the T cell surface. The T Cell Receptor (TCR) complex which provides the primary antigenic signal conferring specificity and the CD28, and LFA-1 receptors which provide costimulatory and adhesive signals have been the most intensively used. Additional co-receptors assist TCR signaling in responding to different types of major histocompatibility complex (MHC) antigen presenting molecules. CD4 assists with recognition of extracellular peptides on MHC class II and CD8 assists with intracellular peptides presented on MHC class I. Mature T cells express CD4 or CD8 and both types of cells are valued adoptive immunotherapy. *In vivo*, these ligands are presented to T cells on the surface of specialized Antigen Presenting Cells (APCs), and this association with the plasma membrane is required for optimal effect. While ideal for use in stimulating T cells *in vivo*, culture and growth of native APCs is difficult and inconsistent. As such, the growth of T cells for contemporary adoptive immunotherapy is often initiated by ligands presenting on engineered beads or artificial APCs [6–9] (Fig. 1B). These approaches allow production of sufficient quantities of T cells for clinical use, but have severe limitations including lack of control over the phenotype or performance of the resultant cells [4].

We propose that the process of T cell expansion can be improved by designing biomaterials to better capture the native T cell/APC interface, a small (70 μm^2) region termed the immunological synapse (IS), which serves as a major point of communication. The binding of TCR to peptide-loaded MHCs (pMHC), CD28 by B7, and LFA-1 by ICAM-1, three major receptor-ligand complexes, occurs in this interface, directing a range of subsequent cell functions including polarization, cytokine secretion, signal integration, and asymmetric cell division [10–13]. An emerging picture is that the micro- and nano-scale details of this structure play important roles in coordinating the function of these cells. This report describes recent progress towards understanding these aspects, which may lead to new design rules for biomaterials that when used in T cell expansion will improve adoptive immunotherapy.

1. MICRO-/NANO-SCALE PATTERNING

A distinguishing characteristic of the immune synapse is the presence of highly complex, micrometer-scale organizations of signaling complexes within this structure. The mature synapse was initially described as a microscale, annular disc of LFA-1/ICAM-1 supramolecular activation clusters (SMACs) surrounding centralized clusters of TCR/pMHC [14]. However, additional patterns of these and other receptor pairs, including CD28/B7, have been identified (Figure 2A) [15–18], raising the possibility that the different motifs may convey specific instructions to the T cells, and conversely be used to control cellular activation.

Recent years have seen the application of micro- and nano-patterning methods to understanding the immune synapse [19–22]. Most notably, Doh and Irvine [19] introduced a lithographic approach to create complementary patterns of ICAM-1 and an activating antibody to CD3 (a key signaling component of the TCR) on material surfaces, and demonstrated that preactivated T cells recognize different microscale patterns of these ligands. Our group built upon this concept [21], introducing techniques to combine higher numbers of patterns on a single surface (Figure 2B and C). By applying multiple rounds of microcontact printing to a single surface, we created independently-defined patterns of activating antibodies to CD3 and CD28, surrounded and separated by ICAM-1. These immobilized proteins were highly effective in patterning the engagement of T cell receptors (Figure 2C).

Naïve CD4+ T cells isolated from mice were able to recognize different patterns of activating antibodies to CD3 and CD28, as measured by secretion of IL-2, a key cytokine involved in T cell activation. Figure 2D compares IL-2 secretion across a select set of patterns indicated along the bottom axis of that graph, revealing a set of design rules: 1) activation of naïve CD4+ T cells is enhanced by engaging CD28 in the periphery of the T cell-substrate interface, as opposed to the center, 2) a similar change in CD3 engagement had a minor influence on IL-2 secretion, and 3) segregation/colocalization of the CD3 and CD28 signaling had a very small impact on T cell function.

Together, these studies point to a powerful role of spatial organization in directing T cell activation and, potentially, subsequent differentiation and function. Incorporation of these patterning concepts into biomaterial design may provide a similar, new level of control over T cell expansion.

2. MODULATING T CELL ACTIVATION THROUGH SUBSTRATE RIGIDITY

The mechanical rigidity of the extracellular environment is increasingly recognized as a modulator of numerous cell functions, including formation/disassembly of adhesion complexes (i.e., focal adhesions), cell spreading, gene transcription, and cell fate [23–25].

This rigidity-sensing is coupled with force generation by the acto-myosin cytoskeleton in the cell, which may alter the phosphorylation of key molecules and switch integrins between resting and active states [26,27].

In T cells, formation of an IS also involves a high level of acto-myosin activity, which is initiated by TCR microclusters [28] and driven by continuous centripetal transportation of these microclusters [28–31]. Inhibiting actin polymerization abrogates the formation of TCR microclusters [28] and the activation processes [32,33]. Myosin IIA was found to be the motor protein for the centripetal actin flow and microcluster movement [34]. Actin polymerization and myosin II-induced retraction at the periphery of the IS result in "contractile oscillation" [35], which may help stabilize integrin-mediated adhesion through force-mediated signaling [36]. Inhibition of myosin IIA activity significantly reduced phosphorylation of Zap70 and LAT, two pivotal signaling events in T cell activation [34], implying that the mechanical force generated inside T cell may play a role in elevating activation signals.

We tested this hypothesis by intervening with the mechanical contraction process using antibody-coated substrates of varied rigidities. The bulk rigidity of planar polydimethylsiloxane (PDMS, Sylgard 184) substrates, which were coated with activating antibodies to CD3 and CD28 (Figure 3A) was controlled by varying the ratio of elastomer base to curing agent, resulting in substrates ranging in Young's modulus from tens of kiloPascals to several MegaPascals (Figure 3B) [37]. PDMS was prepared as a thin (hundreds of micrometers) layer backed by glass. Cells can probe the first few micrometers of elastomer depth [23], so these preparations appear as a half-infinite slab to the cells, yet are thin enough to allow microscopy and handling. The amount of antibody adsorbed to each surface was measured using an ELISA-based approach, and was found to be similar across this range of compositions/rigidities ($P < 0.005$), varying by approximately 5%.

Figure 3B compares IL-2 secretion by naïve mouse CD4+ T cells as a function of PDMS formulation; the Box plots in this figure illustrate one representative experiment of three repetitions. T cell costimulation was sensitive to the rigidity of the underlying substrate, where cells on the stiffer substrate had higher IL-2 secretion. All samples were statistically different from each other by Kruskal-Wallis and ANOVA tests ($\alpha = 0.05$, $n > 2000$ cells per surface).

Control over the mechanical properties of an activating surface may similarly modulate long-term T cell function, including division, proliferation, and differentiation, providing a new level of control over *ex vivo* T cell expansion.

3. LATERAL MOBILITY OF SIGNALING CLUSTERS

Unlike extracellular matrix proteins, ligands to proteins involved in cell-cell communication, such as those presented by an APC, exhibit lateral mobility across the cell surface, owing to its association with the plasma membrane. Supported lipid bilayers (SLBs) presenting membrane proteins capture the lateral mobility of the natural cell surface, and have emerged as a powerful model for investigating cell signaling [38–46]. Using this system, Chan et al. [47] demonstrated that laterally mobile, GPI-tethered CD58 was much more potent than an immobile counterpart in promoting cell interaction. Subsequent implementations of this model provide a rare look into the organization of the immunological synapse [39,40]. T cells cluster and reorganize various components of antigen-presenting cells that have been isolated and tethered to planar supported lipid bilayer, a phenomenon not possible if the proteins were immobilized to the experimental surface.

It is increasingly recognized that the plasma membrane exhibits considerable micro- and nano-scale order. Sub-cellular patterning of a single type of SLB has been studied using techniques of e-beam lithography [41,48], micro-contact printing [49], photolithography and parylene peel-off [50]. The ability to pattern multiple bilayers of different composition on a single surface is important for bridging between studies using uniform SLBs and micro-patterned immobilized proteins [19,22]. However, patterning at sub-cellular resolution has been elusive, owing to the limitations of non-mixing laminar flow [51–54]. More recently, researchers have patterned SLBs with sub-micrometer precision and multiple compositions using AFM-related techniques [55–57], but these are not well-suited for covering the relatively large areas intended for cell-based experiments (millimeters to centimeters on a side).

As shown in Figure 4A, we introduced a SLB patterning strategy that takes advantage of diffusive transport in supported membranes after formation on a substrate [58]. A bilayer-compatible substrate is divided into multiple regions, consisting of two large open regions separated by a middle region which contains a continuous barrier that divides the entire surface into two topologically distinct regions. A three-flow chamber system is used to form three different types of lipid bilayer on this surface; bilayers containing two different tethered proteins are deposited on the outer regions, while a plain bilayer is formed on the middle zone. Over time, tethered biomolecules will diffuse from the outer regions into the interdigitated middle region, leading to an interlacing of regions each separately containing different components (Figure 4B). Importantly, the spatial resolution of the resultant bilayers is determined by the barrier, providing finer resolution than that provided by laminar flow, reaching potentially into the realm of tens of nanometers [59]. With this approach, multiple ligands can be presented to cells, each confined to separate regions of the cell-surface interface while retaining the mobility required for effective membrane protein function. Figure 4C shows the use of this platform in presenting spatially segregated, micropatterned ligands to the T cell surface proteins TCR and LFA-1.

The supported lipid bilayer model has been extremely useful for contemporary investigations into the impact of membrane protein mobility on cell signaling. Adaptation of these design rules into new biomaterials, however, poses several challenges, including the fragility and limited lifetime of supported lipid bilayer. Continued advances in artificial amphiphilic molecules capturing key properties of lipids may yield to more effective techniques for including lateral mobility into material systems. Conversely continued design of polymer structure may allow the capture of the nanoscale behavior of natural lipids into these systems.

CONCLUSIONS

This article reviews recent advances in understanding the role of micro- and nano-scale organization and cellular biomechanics in directing T cell function. Together, these principles may lead to biomaterials that provide significantly enhanced control over T cell expansion, leading to improved implementation of adoptive immunotherapy.

Acknowledgments

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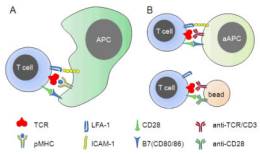

Figure 1. T cell activation

(**A**) Activation of T cells *in vivo* is mediated in large part by contact-mediated communication with Antigen Presenting Cells (APCs). (**B**) For therapeutic *ex vivo* expansion, activation is commonly carried out by replacing the APC with either engineered cells or beads that engage the same receptors involved in T cell/APC interaction.

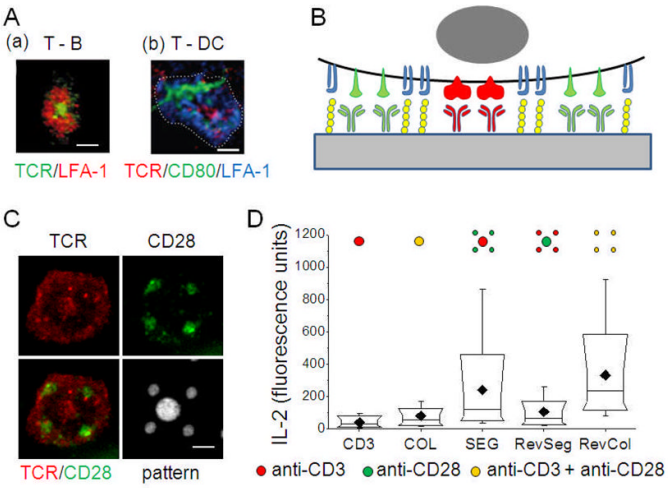

Figure 2. Micropatterned activation of T cells

(**A**) Signaling complexes for distinct patterns of organization within the immune synapse. Adapted from [14] and [18]. (**B**) Micropattering of activating ligands to cell surface receptors allows the study of how cells respond to specific organizations of signaling complexes. (**C**) Demonstration of the ability to control receptor organization using activating antibodies to CD3 (central 2 μm dot) and CD28 (satellite 1 μm features). (**D**) Comparison of IL-2 secretion by mouse naïve CD4+ T cells on specific microscale patterns of CD3 and CD28. Scale bars: 2 μm.

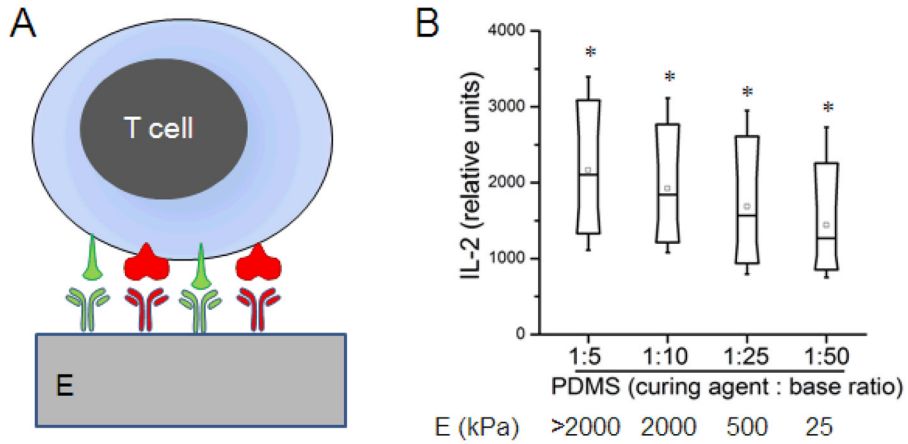

Figure 3. Rigidity sensing by T cells

(**A**) Mouse CD4+ T cells were activated using antibodies to CD3 and CD28, adsorbed onto planar substrates of varying rigidity. (**B**) Box plots comparing IL-2 secretion by cells as a function of substrate rigidity. * Each condition was statistically different from all others, $\alpha = 0.05$, $n > 2000$ cells per surface.

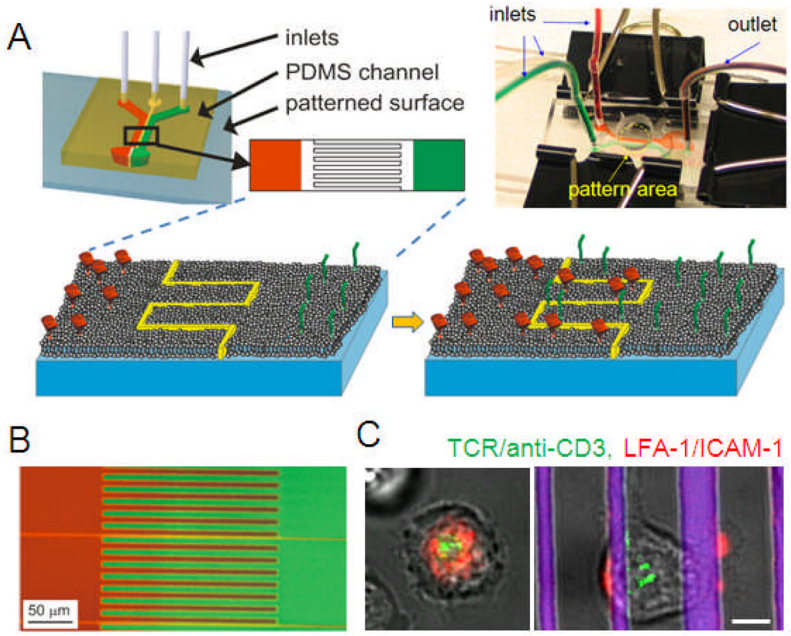

Figure 4. Multicomponent supported lipid bilayers

(**A**) Microfluidic approaches allow patterning of supported lipid bilayers at subcellular levels. (**B**) Example of a two-component lipid bilayer system. (**C**) Comparison of T cell receptor organization on unpatterned (left) and segregated (right) lipid bilayers presenting ligands to TCR and LFA-1; scale bar = 5 μm. Adapted from [58].