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Investigative and Clinical Applications of Synthetic Immune Synapses

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Abstract

The immune synapse has emerged as a compelling model of cell-cell communication. This interface between a T cell and antigen presenting cell serves as a key point in coordinating the immune response. A distinguishing feature of this interface is that juxtacrine signaling molecules form complex patterns that are defined at micrometer and sub-micrometer scales. Moreover, these patterns are highly dynamic. While cellular and molecular approaches have provided insight into the influence of these patterns on cell-cell signaling, replacing the antigen-presenting cell with a synthetic, micro-/nano-engineered surface promises a new level of sophistication to these studies. Micropatterning of multiple ligands onto a surface, for example, allowed the direct demonstration that T cells can sense and respond to microscale geometry of the immune synapse. Supported lipid bilayers have captured the lateral mobility of natural ligands, allowing insight into this complex property of the cell-cell interface in model systems. Finally, engineered surfaces have allowed the study of forces and mechanosensing in T cell activation, an emerging area of immune cell research. In addition to providing new insight into biophysical principles, investigations into immune synapse function may allow control over *ex vivo* T cell expansion. Bioreactors based on these concepts may find immediate application in enhancing cellular-based immunotherapy.

Introduction

Cell-cell communication is a powerful organizational principle in living systems. The ability of cells to properly recognize and respond to each other is critical for a range of tissue- and organ-level functions including development, homeostasis, and repair. Conversely, disruption or imbalance in these interactions is the basis of several diseases. Juxtacrine signaling, in which two cells communicate through direct contact and engagement of membrane proteins, is particularly important as it provides the micrometer-scale, cell-by-cell spatial resolution needed to create complex, multicellular structures such as epithelial sheets and stem cell niches. Furthermore, it is increasingly recognized that cell-cell interfaces involved in juxtacrine signaling show complexity at smaller dimensions, exhibiting intricate structures that are defined at sub-micro- and nano-scale levels; such structures were first described in neuron-neuron synapses, but have more recently been identified in the context of cells of the immune system¹. As a result, there is currently much interest in studying cell-cell communication in controllable, *ex vivo* models. Contemporary micro- and nano-scale fabrication techniques can be used to replace one cell with an artificial counterpart to understand how the other, target cell responds to specific aspects of the extracellular environment. This approach has been extremely successful in the context of cell interaction with the extracellular matrix (ECM); patterning of fibronectin, collagen, and other matrix proteins onto a variety of surfaces, from rigid glass to soft elastomers, has revealed a wealth

of information about how cells spatially integrate ECM signaling to drive overall cell response^{2–4}. Such systems have also been useful in creating microscale patterns of cell-cell adhesion molecules, particularly cadherins^{4–6}. However, immobilization of these proteins onto a surface fails to capture a fundamental property; cadherins and other membrane-associated proteins often exhibit long-range lateral mobility. This and other properties of real cell surfaces pose key challenges in modeling cell-cell communication in a reductionist, *in vitro* system.

This review describes recent advances in capturing cell-cell interfaces in the specific context of T lymphocytes, key regulators of the adaptive immune response. Activation and subsequent function of these cells has wide implications in the body's response to an invading pathogen as well as development of treatments to a range of diseases, including cancer and autoimmunity^{7, 8}. T cell activation occurs in large part through direct contact with an Antigen-Presenting Cell (APC) within a micrometer-scale cell-cell interface region termed the "immune synapse" (IS) owing to its asymmetrical structure and similarity to the neuron-neuron synapse. This interaction plays a major role in determining the subsequent proliferative and lineage fate of the T cell^{9, 10}; although a mature cell type, T cells possess the capacity for significant self-renewal as well as the potential to differentiate toward different fates such as short-lived effector cells, long-lived memory cells and regulatory T cells. These fates are critical to the orchestration, balance, and control of a protective immune response. Knowledge regarding the functioning of the IS may yield important insights into T cell activation and differentiation processes that could advance future vaccine development. Adoptive immunotherapy using T lymphocytes cultured *ex vivo* on artificial cell-like scaffolds has also moved into the clinic with some remarkable results, especially in cancer¹¹. Applications of this technology to the treatment of cancer, infectious diseases like HIV infection, and autoimmunity are imminently on the horizon.

The immune synapse as a dynamic material interface

The archetypal T cell-mediated immune response begins with the display of a peptide, derived from a pathogen or other biological structure and loaded in a Major Histocompatibility Complex (MHC, creating a pMHC), on the surface of an APC (Fig. 1A). This pMHC complex is recognized by a T cell presenting a T Cell Receptor (TCR) complex with appropriate affinity, initiating a cascade of intracellular signaling. The response of the T cell, which includes short-term behavior such as activation and longer-term modulation of proliferation and differentiation, depends in large part on the engagement of additional receptor-ligand complexes between the two cells. It is this small ($\sim 75 \mu\text{m}^2$) region of cell-cell contact that is termed the immune synapse (Fig. 1B,C); while this review focuses on synapses formed by T cells, this concept has broadened to include interfaces formed by other lymphocytes. The range of receptor-ligand pairs that have been observed within the IS is strikingly extensive and dependent on many factors¹², including the type of T cell and APC that are participating in this interaction and the presence of soluble factors in the environment. However, two key signaling systems in addition to TCR-pMHC have been the major focus in hybrid cell-surface interfaces (Fig. 1B). The first of these is CD28, a member of the immunoglobulin superfamily of proteins that is presented on the T cell surface. Upon ligation with either CD80 or CD86, CD28 provides a costimulatory signal that augments TCR-based signaling and is essential for full activation of naïve T cells. The second system is LFA-1, a member of the integrin family of receptors, which binds to ICAM-1 on the APC and provides cell-cell adhesion as well as a costimulatory function. Concurrent engagement of TCR with LFA-1 and/or CD28 provides activation of many types of T cells, and these three receptors form a core set of signals that is well-suited for *in vitro*, reductionist studies of T cell function.

A striking feature of the immune synapse is that receptor-ligand pairings within this interface are organized into distinctive patterns of micrometer-scale and smaller. The original descriptions by Monks *et al.*¹³ and Grakoui *et al.*¹⁴ identified what came to be the defining “bullseye” pattern of molecules, consisting of a micrometer-scale central Supramolecular Activation Cluster (cSMAC) region surrounded by a peripheral Supramolecular Activation Cluster (pSMAC) corresponding to zones of concentrated TCR-pMHC and LFA-1-ICAM-1 binding, respectively. Subsequent studies showed intriguing variations on this basic theme (Fig. 1C), which correlate with different T cell – APC interactions^{15–19, 20}. For example, Th2 cells, a class of differentiated CD4+ T cells involved in defense against extracellular parasites, form multifocal mature synapses²¹, while regulatory T cells form highly stable synapses, showing an additional, temporal variation on IS structure²². These different T cell – APC interactions produce distinct outcomes, suggesting that the micro- and nano-scale organization of the immune synapse conveys part of the language of communication between T cells and APCs. Importantly, these structures are observed in the “mature” immune synapse, a stable configuration maintained between a lymphocyte and APC during the course of their interaction. In contrast, the organization of TCR and other ligands in the developing synapse is highly dynamic over the initial few minutes of interaction. In particular, the classic bullseye pattern begins with formation of LFA-1-ICAM-1 pairs in the center of the interface and TCR-pMHC microclusters originating in the periphery¹⁴; this is inverted relative to the mature IS. Understanding the full impact that IS structure and dynamics have on T cell function, as well as the underlying mechanisms, remains a contemporary challenge in immune cell biology.

A fundamental issue in these studies is that signaling downstream of TCR, CD28, and LFA-1 is associated predominantly with phosphorylation cascades, involving intermediates that are soluble in the cell cytosol. Such molecules typically exhibit mobility that is high (diffusion coefficients on the order of $\mu\text{m}^2/\text{msec}$) relative to the rates at which protein activity can be regulated; it is difficult to obtain graded concentrations of activity of these molecules over the micrometer-scale distances that define the immune synapse²³. However, TCR/CD28/LFA-1 signaling also involves intermediates that are associated with the cell membrane, either stably via a transmembrane domain or transiently through post-translational attachment of lipids or binding to specific membrane components, and thus typically exhibit diffusion coefficients on the order of $\mu\text{m}^2/\text{sec}$, values that make spatially resolved cell signaling at these scales plausible. For example, activation of CD28 results in binding of PI3K, which phosphorylates PIP2 lipids into their biologically active counterpart PIP3, leading to membrane association and activation of molecules that are sensitive to these lipids, including Akt and PDK1. Akt translocates back to the cell cytosol following this membrane-associated activation, leading to cytokine production and secretion, a high-level response that is discussed later in this review. Similarly, a major product of antigen-specific binding of TCR is phosphorylation of CD3 ζ , which then allows binding of Zap70. This local protein-protein interaction results in modulation of membrane-associated intermediates that can separate from the TCR complex, leading to transcriptional regulation and modulation of cell function; these pathways share the same potential for spatial regulation, in this case of TCR signaling. TCR signaling also involves activation of pathways that modulate the assembly, disassembly, and activity of the cytoskeleton, allowing for spatial regulation of signaling at scale of micrometers and smaller. The importance of spatial considerations in IS signaling is also seen in cell-ECM communication (represented by current understanding of focal adhesion) as well as cadherin-based cell-cell communication.

Engineered surfaces capturing specific aspects of the extracellular environment play a central role in research of all of these systems. However, the IS illustrates major challenges in this approach when applied to cell-cell communication. For example, elastomers and hydrogels of controlled elasticity have been used very successfully in the context of

understanding how stromal cells sense the rigidity of the extracellular matrix; elastic modulus is an appropriate representation of a key aspect of this matrix. In contrast, cells, including APCs, exhibit additional properties of mechanical response that have proven difficult to capture in engineered systems. The proteins involved in cell-cell binding are associated with the cell membrane, imparting both long-range and short-range mobility that is more akin to viscosity than elasticity. Cells also have an underlying cytoskeletal network that interacts with membrane proteins in numerous ways and is defined over multiple distance scales ranging from micrometers to tens of nanometers. The following sections describe recent advances in capturing these properties in the specific context of the IS.

Microscale organization and dynamics in synthetic immune synapses

The adaptation of microfabrication techniques to biological systems has been transformative to cellular physiology research. Early studies in this direction used ultraviolet ablation to pattern slides precoated with ECM matrices to guide neuron growth^{24, 25}. Later, Kleinfeld *et al.* demonstrated a more robust, photolithography-based method adapted from the microelectronics industry to create patterns of cell adhesive and non-adhesive surface chemistries, introducing the idea of patterning neurons and other cells with arbitrary geometries on a cell culture surface²⁶. However, the equipment needed to carry out this work was difficult to access by the typical cell culture laboratory at that time. A series of key studies from the Whitesides group made micropatterning, particularly of biological molecules, accessible to a wider range of researchers with limited microfabrication access^{27, 28}. These advances led to the explosion in research showing that microscale geometries are important to cellular physiology. Since that time, clean room technologies have become more accessible, as have techniques for patterning at finer resolutions. Today, a wide range of methods for patterning proteins, lipids, nucleic acids, and other biomolecules at resolutions reaching into several nanometers are available to researchers in the life sciences.

The microscale organization of proteins observed in the immune synapse made this structure a compelling target for micro- and nano-scale fabrication techniques. Traditional cellular and molecular approaches had provided initial insight into how IS organization influences function; for example, Tseng *et al.*²⁹, focusing on junctions between primary mouse T cells and artificial APCs, suggested that segregation of CD28 and TCR within the immune synapse enhances T cell activation. However, the comparison between segregated and colocalized CD28 and TCR was accomplished by truncating the cytosolic domain of CD80 in the APC. This manipulation may have unanticipated consequences on activity of the protein itself and not simply localization, a limitation associated with many manipulations based on cellular and molecular techniques. The use of a micropatterned surface to dictate the layout of the immune synapse (Fig. 2A) circumvents these problems. In this direction, Doh and Irvine³⁰ first described the use of a novel, water-soluble, biotinylated photoresist³¹ in creating micropatterned surfaces that presented patterns of ligands to TCR and LFA-1. TCR activation was provided using an antibody to CD3 ϵ , a component of the TCR that when properly engaged bypasses the antigenic recognition requirement and initiates downstream signaling processes. The ability of specific antibodies to activate receptors on the T cell surface is well established, and given the versatility of antibodies, this approach has found widespread use in synthetic systems. Activation of LFA-1 was accomplished by immobilizing onto the surfaces a fusion protein containing the extracellular domain of ICAM-1, the natural ligand to LFA-1, fused with the Fc region of human IgG. By avoiding the transmembrane and cytosolic regions of the full-length protein, this approach simplifies patterning of ICAM-1 while the Fc domain provides versatile tethering to material surfaces. Using this system, Doh and Irvine presented to preactivated CD4⁺ T cells artificial synapses based on a range of anti-CD3 geometries, including a single central feature, a cluster of

smaller dots, and an annulus, surrounded and separated by ICAM-1. The anti-CD3 features were effective in halting migration of T cells and initiating activation. Moreover, secretion of key cytokines indicative of T cells function (specifically, IL-2 and IFN- γ) was sensitive to pattern geometry (Fig. 2B), providing the first direct demonstration that IS layout can modulate activation by TCR and ICAM-1 signaling. A later study by Shen *et al.*³² used microcontact printing to study activation of T cells to the spatial organization of three ligands – TCR, the costimulatory CD28 receptor, and ICAM-1. Sequential rounds of microcontact printing provided the ability to create patterns of anti-CD3 and anti-CD28 (which, like anti-CD3, activated its respective receptor upon binding) that were independent of each other in layout; these patterns were then backfilled with ICAM-1. Using this system, Shen demonstrated that costimulation of naïve CD4⁺ T cells from mouse were sensitive to the layout of CD28 (Fig. 2C). However, in contrast to the observations of Tseng *et al.*²⁹, the separation of CD3 and CD28 was shown to have only a minimal effect on T cell activation; presentation of anti-CD28 as a cluster of small features in the periphery of the IS, as opposed to a single, centralized feature had a larger effect than the relative position to TCR signaling. By allowing the comparison of different IS layouts using the same molecular ligands, as well as inclusion of patterns not observed in natural cell-cell interfaces, these two studies provided a key, direct demonstration that the microscale layout of the IS, as a model of other cell-cell junctions, can influence higher-level cellular functions. Subsequent studies in these directions use micropatterned surfaces to explore the kinetics of specific receptor-ligand interactions associated with the immune synapse and the directed interaction of T cells and B cells on multicomponent arrays^{33, 34}. Cellular immunology has thus served as a strategic, and perhaps unexpected, context in which the power of multicomponent, micropatterned surfaces to understand cell signaling has been demonstrated³⁵. Such approaches will undoubtedly find application in a wide range of cellular systems.

However, a fundamental limitation in studies using substrate-tethered ligands is that such systems do not capture the natural mobility of the proteins along the APCs surface, which is reflected in the reorganization of the immune synapse. This immobilization influences signaling events; for example, in T cells interacting with immobilized anti-CD3, SLP-76, a intracellular signaling adapter protein, sheds from static clusters of Zap70, a process attributed to the immobilization of TCR³⁶. As SLP-76 is a target of Zap70, this separation is not likely to happen when TCR-pMHC is mobile, and the influence of this dissociation is not known. The lateral mobility of ligands on the APC surface can be captured in a different system, the supported lipid bilayer. As reviewed in detail elsewhere^{37–39}, this system consists of a self-assembled, planar bilayer structure of phospholipids, mimicking cellular membranes. A combination of hydration, van der Waals, and other forces maintains the bilayer in close proximity to an appropriate substrate, most commonly glass. A thin (sub-nanometer) layer of water separates the bilayer and support, imparting lateral mobility of the phospholipids and other membrane components, including tethered proteins, in the plane of the bilayer. This system has found extensive use as a mimic of the APC surface, although additional applications in other cellular systems are being developed^{40–42}. Indeed, the system used by Grakoui *et al.* in early explorations of this interface was based on supported lipid bilayers presenting pMHC and ICAM-1 proteins tethered to the membrane by a GPI moiety⁴³, where the lateral mobility imparted by this structure allowed reorganization and inversion of microclusters into the cSMAC and pSMAC structures of the bullseye pattern (Fig. 2D). However, in its basic construction, membrane lipids and tethered proteins exhibit free Brownian diffusion coefficients on the order of a few $\mu\text{m}^2/\text{sec}$, corresponding to a mobility that is too high to support attachment and spreading of cells and thus presents little resistance to motion in the plane of the bilayer as applied by the T cells. Notably, this diffusion coefficient is in keeping with that of a homogenous, fluid-phase lipid bilayer, but proteins on real cell surfaces often exhibit diffusion coefficients that are several orders of magnitude lower. Pioneering work by the Kusumi group using single molecule tracking

indicated that membrane protein diffusion is in fact not purely Brownian, but hindered over distances of tens to hundreds of nanometers^{44, 45}. The resultant model of hop-diffusion suggests that this complex diffusive behavior results from interactions between the cell cytoskeleton and membrane proteins.

Surface-immobilized and membrane-tethered ligands to receptors on the T cell surface thus represent two experimental extremes, with the real APC membrane being somewhere between these states, owing to the micro- and nano-scale details of cellular structure. Several groups have begun to capture this spatial complexity by modifying the basic supported lipid bilayer structure to capture the presence of barriers to free diffusion that the cytoskeleton, or other nanoscale structure within the immune synapse, would present. In these methods, a silicon oxide surface, which is one of only a handful of materials that supports bilayer formation, is patterned with nano-scale barriers of another material that does not support bilayer formation, such as metal or plastic (Fig. 2E). Mossman *et al.*⁴⁶ used this approach to partition a supported lipid bilayer into independent patches using continuous barriers of such materials, providing short-range mobility to pMHC and ICAM-1 ligands while restricting long-range diffusion. These surfaces demonstrated that restricting the central-targeting motion of TCR clusters enhances TCR signaling. Subsequent studies by Tsai *et al.*⁴⁷ used periodic, nanoscale gaps in these otherwise perfect barriers to capture the semi-permeable behavior of cytoskeleton in the hop-diffusion model. The impact of this more physiological model of cell membranes remains to be elucidated. However, these systems illustrate the difficulties in capturing the complete mechanical properties of the cell membrane in synthetic systems.

Mechanobiology and synthetic immune synapses

Mechanobiology represents an emerging area of research in immune cell function. The early studies of IS geometry by Grakoui *et al.* suggested the importance of an active actin cytoskeleton underlying the cell membrane; contraction of this structure is critical for the observed inversion and reorganization of TCR and LFA-1 observed in the immune synapse. Moreover, the edge of the IS is highly active, exhibiting periodic ruffling and contraction reminiscent of stromal cells⁴⁸, further suggesting a role of mechanical forces in T cell signaling. The ability of mechanical forces in TCR signaling was finally demonstrated by Kim *et al.*⁴⁹, in which both shear and normal forces produced were able to initiate TCR signaling. Furthermore, Husson *et al.*⁵⁰ demonstrated that TCR engagement induces actin polymerization that can lead to the application of significant forces to the extracellular environment. Most recently, Judokusumo *et al.*⁵¹, using polyacrylamide gels presenting antibodies to CD3 and CD28, demonstrated quantitative modulation of T cell activation based on Young's modulus over a range of tens to hundreds of kiloPascals (Fig. 3A,B). Together, these studies reveal a complex, self-reinforcing configuration in which TCR signaling may be modulated in response to the mechanical rigidity of the extracellular environment. The mechanisms for this response remain to be explored (Fig. 2B & C). Such an effect is now well established in the context of stromal cells, including stem cells⁵², interacting with ECM proteins. The parallel ability of T cells to sense rigidity, independent of integrin-based signaling, represents a new frontier of mechanobiology that is rapidly emerging.

In the context of the immune synapse, these results suggest that the mechanical rigidity of the APC, defined at some relevant distance scale, may function to modulate T cell activation and subsequent function. This emerging story is proving to be highly complex and surprising; for example, human CD4+ T cells are also sensitive to substrate rigidity, but show stronger expansion on softer surfaces⁵³, a behavior that would not be expected from the short-term results observed with mouse cells. It is also noted that the mechanical

properties of the APC, like other cells, may not be completely described in terms of rigidity owing particularly to the mobility of proteins along the plasma membrane, even in the context of membrane-cytoskeleton interaction noted above. Current studies seek to understand how these more complex aspects of cell surfaces, particularly viscosity and nanoscale mobility associated with hop-diffusion, may influence T cell activation. Finally, we recognize that the interface between a T cell and APC is not necessarily a planar structure. A recent report from the Davis laboratory revealed the complex topology between these cells, with processes from the T cell indenting and protruding into, but not breaking the plasma membrane of, a partner APC⁵⁴. The impact of this topology on T cell – APC communication remains an important area of current research.

Clinical Applications

The adaptive immune response has remarkable potency and specificity for clearing pathogens. Immunotherapeutic strategies are being aggressively explored in the realm of cancer, with recent successes highlighting the efficacy of this general approach⁵⁵. Injected antibodies such as rituximab for B-cell malignancy provide improved response rates and survival for targeted cancers compared to chemotherapy alone, and represent clinically accepted applications of immunotherapy⁵⁶. In comparison, cellular-based immunotherapy has the potential to improve tumor control by providing a persistent source of responsive cells and the complete machinery to target the disease. Of the approaches currently under consideration, adoptive transfer is likely to be one of the most robust forms of cellular immunotherapy⁸. For example, adoptive immunotherapy with allogeneic donor leukocytes, in which cells isolated from a donor are transfused into a patient, generates potent anti-leukemic effects through graft vs. leukemic (GVL) effects; however, the benefit is confined largely to patients with myeloid leukemia⁵⁷. Autologous immunotherapy, in which the cells come from the patient, avoids graft-vs.-host disease and thus promises more targeted therapy with less off-target tissue toxicity associated with allogenic approaches.

A major challenge in this approach is the limited repertoire of clinically usable expansion systems for both CD4+ and CD8+ T cells. *Ex vivo* expansion, in which cells from the patient are stimulated and allowed to proliferate outside the body before reintroduction, is a central step in these therapies. While dendritic cells and other APCs can be used to initiate this expansion, the ability to manipulate the composition and state of differentiation of these cells to direct T cell expansion is challenging at best. This is critical because the nature of transferred T cells (e.g. T_H1, T_H2, memory or effector) is likely one important factor for long-term persistence and efficacy of adoptively transferred T cells⁵⁸. As a result, *ex vivo* expansion is currently carried out using cell-sized magnetic microbeads coated with anti-CD3 and anti-CD28 agonist antibodies, which have made possible large scale and relatively cheap culture of T cells for many early phase clinical trials^{59–63}. However, synthetic platforms that mimic the natural T cell:APC interface may offer additional advantages in control of T cell activating signals over these bead-based systems.

The results discussed above demonstrate that capturing the micro- and nano-scale details of the immune synapse in synthetic systems can serve to modulate early activation and function of a range of T cells. In addition, early conceptualization of the immune synapse centered around the role of the APC to direct T cell differentiation⁹, suggesting that this same approach can be used to control longer-term T cell functions that are beneficial to immunotherapy. As an example particularly relevant to cancer, immunologic memory, the ability of an organism to respond rapidly to a repeated challenge with a pathogen, is a fundamental property of the immune system. In a human clinical trial of adoptive immunotherapy for melanoma, persistence of T cells, associated with memory function, appears to predict a positive objective response⁶⁴. T cell memory is thought to result from

expanded, Ag-specific T cells that survive and persist long after a pathogen is cleared. In humans, two populations of memory T cells, central memory (T_{CM}) and effector memory (T_{EM}), have been described based upon their functionality and homing potential (reviewed in ⁶⁵). T_{CM} cells appear to lack significant effector function, but they are capable of rapid response to antigen with subsequent differentiation to effector cells. In contrast, T_{EM} cells are best characterized as “pre-loaded” for effector function. $CD8^+$ T_{EM} cells constitutively express perforin and granzyme B required for cytotoxic T cell lytic function. $CD4^+$ T_{EM} cells are capable of significant cytokine production following antigenic challenge. Evidence derived from studies in mice suggest that T_{CM} cells provide greater long-term persistence and greater efficacy in both infectious and cancer models⁶⁶, although we recognize that the optimal population may be different for different therapies. Regardless, recent studies suggest a model of differentiation in which the immune synapse provides vital cues in directing asymmetric division of $CD8^+$ T cells, generating both memory and effector populations^{67, 68}. Understanding what aspects of the IS direct T cell differentiation and expansion, and applying this knowledge to bioreactor design, may be able to improve control over T cell phenotype during expansion, providing a powerful tool in immunotherapy.

Other strategies for improving T cell expansion are under development. Notably, there are two main strategies for generating large numbers of tumor specific T cells⁷, both of which are typically carried out *ex vivo* using a cell expansion bioreactor system. The first approach is antigen-specific T cell expansion. The advantage of this approach is the selective nature of the expansion and antigen specificity. However, methods generally depend upon the identification of relevant tumor antigens, and they are labor intensive and expensive. Alternatively, polyclonal T cell activation and expansion can generate large numbers of T cells at lower cost, and this approach is generally more rapid and feasible. Nevertheless, it depends upon the presence of tumor-specific T cells within the polyclonal population, and the ability of these cells to find and respond to tumors or tumor antigens presented by antigen presenting cells. Genetic engineering of the T cells during *ex vivo* manipulation using cloned T cell receptors or chimeric antigen receptors to generate desired antigen specificity provides additional tools in the adoptive immunotherapy armamentarium, but the ability to expand cells to clinically relevant quantities (millions to billions of cells for a single treatment) remains a significant challenge. Undoubtedly, future immunotherapy strategies will use a combination of multiple approaches.

Conclusion

Engineering of the immune system is a current area of growth. A variety of approaches, ranging from design of new biomaterials to targeted delivery of therapeutic agents via immune cells show much promise in leveraging the specificity, power, and longevity of the adaptive immune response^{10, 69–75} to treat disease. We focus here on understanding the immune synapse, as evidence suggests that this structure is an important determinant of T cell differentiation. Current understanding of the IS has resulted from traditional cellular and molecular immunology approaches, but techniques for micro- and nano-engineering of substrates, particularly in the directions of surface patterning and the supported lipid bilayer model, have led to important discoveries of IS function. One compelling area of future research is to understand the role of topology on IS-based cell-cell communication; while the studies described in this review have focused on planar surfaces, it is recognized that the actual interface is much more convoluted. An example of this topology is illustrated by the interface between a T cell and K562 APC shown in Fig. 4. In applying the resultant basic discoveries to human health, it is recognized that the immune system is highly complex; T lymphocytes themselves comprise a large collection of different subsets, each with specific functionality and response. In addition, there are key differences between human

immunology and mouse, an important model in research. Future research will continue to be interdisciplinary, and require a broad, collective expertise.

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Abbreviations used in this manuscript

APC	antigen presenting cell
ECM	extracellular matrix
IS	Immune Synaps
MHC/pMHC	major histocompatibility complex/peptide-loaded major histocompatibility complex
cSMAC/pSMAC	central supramolecular activation cluster/peripheral supramolecular activation cluster
T_{CM}/T_{Em}	central memory/effector memory classes of human T cells

References

1. Yamada S, Nelson WJ. Synapses: Sites of Cell Recognition, Adhesion, and Functional Specification. *Ann Rev Biochem.* 2007; 76:267–294. [PubMed: 17506641]
2. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Micropatterned surfaces for control of cell shape, position, and function. *Biotechnol Prog.* 1998; 14:356–363. [PubMed: 9622515]
3. Théry M. Micropatterning as a tool to decipher cell morphogenesis and functions. *J Cell Sci.* 2010; 123:4201–4213. [PubMed: 21123618]
4. Tsai J, Kam L. Rigidity-Dependent Cross Talk between Integrin and Cadherin Signaling. *Biophys J.* 2009; 96:L39–L41. [PubMed: 19289031]
5. Borghi N, Lowndes M, Maruthamuthu V, Gardel ML, Nelson WJ. Regulation of cell motile behavior by crosstalk between cadherin- and integrin-mediated adhesions. *Proc Nat Acad Sci U S A.* 2010; 107:13324–13329.
6. Hunt SJ, Nelson WJ. Fabrication of a dual substrate display to test roles of cell adhesion proteins in vesicle targeting to plasma membrane domains. *FEBS Letters.* 2007; 581:4539–4543. [PubMed: 17803993]
7. June CH. Principles of adoptive T cell cancer therapy. *J Clin Invest.* 2007; 117:1204–1212. [PubMed: 17476350]
8. June CH. Adoptive T cell therapy for cancer in the clinic. *J Clin Invest.* 2007; 117:1466–1476. [PubMed: 17549249]
9. Maldonado RA, Irvine DJ, Schreiber R, Glimcher LH. A role for the immunological synapse in lineage commitment of CD4 lymphocytes. *Nature.* 2004; 431:527–532. [PubMed: 15386021]
10. Maldonado RA, Soriano MA, Perdomo LC, Sigrist K, Irvine DJ, Decker T, Glimcher LH. Control of T helper cell differentiation through cytokine receptor inclusion in the immunological synapse. *J Exper Med.* 2009; 206:877–892. [PubMed: 19349465]
11. Gilham DE, Debets R, Pule M, Hawkins RE, Abken H. CAR-T cells and solid tumors: tuning T cells to challenge an inveterate foe. *Trends Molec Med.* 2012; 18:377–384. [PubMed: 22613370]
12. Vyas YM, Maniar H, Dupont B. Visualization of signaling pathways and cortical cytoskeleton in cytolytic and noncytolytic natural killer cell immune synapses. *Immunol Rev.* 2002; 189:161–178. [PubMed: 12445273]
13. Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature.* 1998; 395:82–86. [PubMed: 9738502]

14. Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML. The immunological synapse: a molecular machine controlling T cell activation. *Science*. 1999; 285:221–227. [PubMed: 10398592]
15. Davis DM, Dustin ML. What is the importance of the immunological synapse? *Trends Immunol*. 2004; 25:323–327. [PubMed: 15145322]
16. Reichardt P, Dornbach B, Gunzer M. APC, T cells, and the immune synapse. *Curr Microbiol Immunol*. 2010; 340:229–249.
17. Singleton KL, Roybal KT, Sun Y, Fu G, Gascoigne NRJ, van Oers NSC, Wulfig C. Spatiotemporal Patterning During T Cell Activation Is Highly Diverse. *Sci Signal*. 2009; 2:ra15. [PubMed: 19351954]
18. Brossard C, Feuillet V, Schmitt A, Randriamampita C, Romao M, Raposo G, Trautmann A. Multifocal structure of the T cell - dendritic cell synapse. *Eur J Immunol*. 2005; 35:1741–1753. [PubMed: 15909310]
19. Purtic B, Pitcher LA, van Oers NSC, Wulfig C. T cell receptor (TCR) clustering in the immunological synapse integrates TCR and costimulatory signaling in selected T cells. *Proc Nat Acad Sci U S A*. 2005; 102:2904–2909.
20. Tseng SY, Waite JC, Liu M, Vardhana S, Dustin ML. T cell-dendritic cell immunological synapses contain TCR-dependent CD28–CD80 clusters that recruit protein kinase C theta. *J Immunol*. 2008; 181:4852–4863. [PubMed: 18802089]
21. Thauland TJ, Koguchi Y, Wetzel SA, Dustin ML, Parker DC. Th1 and Th2 Cells Form Morphologically Distinct Immunological Synapses. *J Immunol*. 2008; 181:393–399. [PubMed: 18566405]
22. Zanin-Zhorov A, Ding Y, Kumari S, Attur M, Hippen KL, Brown M, Blazar BR, Abramson SB, Lafaille JJ, Dustin ML. Protein kinase C-theta mediates negative feedback on regulatory T cell function. *Science*. 2010; 328:372–376. [PubMed: 20339032]
23. Brown GC, Kholodenko BN. Spatial gradients of cellular phospho-proteins. *FEBS letters*. 1999; 457:452–454. [PubMed: 10471827]
24. Hammarback JA, Letourneau PC. Neurite extension across regions of low cell-substratum adhesivity: implications for the guidepost hypothesis of axonal pathfinding. *Dev Biol*. 1986; 117:655–662. [PubMed: 3758485]
25. Hammarback JA, Palm SL, Furcht LT, Letourneau PC. Guidance of neurite outgrowth by pathways of substratum-adsorbed laminin. *J Neurosci Res*. 1985; 13:213–220. [PubMed: 3973933]
26. Kleinfeld D, Kahler KH, Hockberger PE. Controlled outgrowth of dissociated neurons on patterned substrates. *J Neurosci*. 1988; 8:4098–4120. [PubMed: 3054009]
27. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. *Science*. 1997; 276:1425–1428. [PubMed: 9162012]
28. Kumar A, Whitesides GM. Features of Gold Having Micrometer to Centimeter Dimensions can be Formed Through a Combination of Stamping with an Elastomeric Stamp and an Alkanethiol “Ink” Followed by Chemical Etching. *App Phys Lett*. 1993; 63:2002.
29. Tseng S-Y, Liu M, Dustin ML. CD80 Cytoplasmic Domain Controls Localization of CD28, CTLA-4, and Protein Kinase C- θ in the Immunological Synapse. *J Immunol*. 2005; 175:7829–7836. [PubMed: 16339518]
30. Doh J, Irvine DJ. Immunological synapse arrays: Patterned protein surfaces that modulate immunological synapse structure formation in T cells. *Proc Nat Acad Sci U S A*. 2006; 103:5700–5705.
31. Doh J, Irvine DJ. Photogenerated polyelectrolyte bilayers from an aqueous-processible photoresist for multicomponent protein patterning. *J Amer Chem Soc*. 2004; 126:9170–9171. [PubMed: 15281792]
32. Shen K, Thomas VK, Dustin ML, Kam LC. Micropatterning of costimulatory ligands enhances CD4+ T cell function. *Proc Nat Acad Sci U S A*. 2008; 105:7791–7796.
33. Doh J, Krummel MF. Immunological synapses within context: patterns of cell-cell communication and their application in T-T interactions. *Curr Microbiol Immunol*. 2010; 340:25–50.

34. Schwarzenbacher M, Kaltenbrunner M, Brameshuber M, Hesch C, Paster W, Weghuber J, Heise B, Sonnleitner A, Stockinger H, Schutz GJ. Micropatterning for quantitative analysis of protein-protein interactions in living cells. *Nat Meth.* 2008; 5:1053–1060.
35. Jung H-R, Choi J-C, Cho W, Doh J. Microfabricated platforms to modulate and monitor T cell synapse assembly. *WIREs Nanomed Nanobiotechnol.* 2012 in press.
36. Bunnell SC, Hong DI, Kardon JR, Yamazaki T, McGlade CJ, Barr VA, Samelson LE. T cell receptor ligation induces the formation of dynamically regulated signaling assemblies. *J Cell Biol.* 2002; 158:1263–1275. [PubMed: 12356870]
37. Dustin ML, Starr T, Varma R, Thomas VK. Supported planar bilayers for study of the immunological synapse. *Curr Prot Immunol.* 2007; Chapter 18(Unit 18):13.
38. Groves JT, Dustin ML. Supported planar bilayers in studies on immune cell adhesion and communication. *J Immunol Meth.* 2003; 278:19–32.
39. Yu CH, Groves JT. Engineering supported membranes for cell biology. *Med Biol Eng Comp.* 2010; 48:955–963.
40. Kam L, Boxer SG. Cell adhesion to protein-micropatterned-supported lipid bilayer membranes. *J Biomed Mat Res.* 2001; 55:487–495.
41. Perez TD, Nelson WJ, Boxer SG, Kam L. E-Cadherin Tethered to Micropatterned Supported Lipid Bilayers as a Model for Cell Adhesion. *Langmuir.* 2005; 21:11963–11968. [PubMed: 16316139]
42. Yu CH, Law JB, Suryana M, Low HY, Sheetz MP. Early integrin binding to Arg-Gly-Asp peptide activates actin polymerization and contractile movement that stimulates outward translocation. *Proc Nat Acad Sci U S A.* 2011; 108:20585–20590.
43. Groves JT, Wulfig C, Boxer SG. Electrical Manipulation of Glycan-Posphatidyl Inositol-Tethered Proteins in Planar Supported Bilayers. *Biophys J.* 1996; 71:2716–2723. [PubMed: 8913608]
44. Morone N, Fujiwara T, Murase K, Kasai RS, Ike H, Yuasa S, Usukura J, Kusumi A. Three-dimensional reconstruction of the membrane skeleton at the plasma membrane interface by electron tomography. *J Cell Biol.* 2006; 174:851–862. [PubMed: 16954349]
45. Ritchie K, Shan X-Y, Kondo J, Iwasawa K, Fujiwara T, Kusumi A. Detection of non-Brownian diffusion in the cell membrane in single molecule tracking. *Biophys J.* 2005; 88:2266–2277. [PubMed: 15613635]
46. Mossman KD, Campi G, Groves JT, Dustin ML. Altered TCR signaling from geometrically repatterned immunological synapses. *Science.* 2005; 310:1191–1193. [PubMed: 16293763]
47. Tsai J, Sun E, Gao Y, Hone JC, Kam LC. Non-Brownian Diffusion of Membrane Molecules in Nanopatterned Supported Lipid Bilayers. *Nano Lett.* 2008; 8:425–430. [PubMed: 18205424]
48. Sims TN, Soos TJ, Xenias HS, Dubin-Thaler B, Hofman JM, Waite JC, Cameron TO, Thomas VK, Varma R, Wiggins CH, et al. Opposing Effects of PKC θ and WASp on Symmetry Breaking and Relocation of the Immunological Synapse. *Cell.* 2007; 129:773–785. [PubMed: 17512410]
49. Kim ST, Takeuchi K, Sun ZY, Touma M, Castro CE, Fahmy A, Lang MJ, Wagner G, Reinherz EL. The alphabeta T cell receptor is an anisotropic mechanosensor. *J Biol Chem.* 2009; 284:31028–31037. [PubMed: 19755427]
50. Husson J, Chemin K, Bohineust A, Hivroz C, Henry N. Force Generation upon T Cell Receptor Engagement. *PLoS one.* 2011; 6:e19680. [PubMed: 21572959]
51. Judokusumo E, Tabdanov E, Kumari S, Dustin Michael L, Kam Lance C. Mechanosensing in T Lymphocyte Activation. *Biophys J.* 2012; 102:L5–L7. [PubMed: 22339876]
52. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix Elasticity Directs Stem Cell Lineage Specification. *Cell.* 2006; 126:677–689. [PubMed: 16923388]
53. O'Connor RS, Hao X, Shen K, Bashour K, Akimova T, Hancock WW, Kam LC, Milone MC. Substrate Rigidity Regulates Human T Cell Activation and Proliferation. *J Immunol.* 2012 in press.
54. Ueda H, Morpew MK, McIntosh JR, Davis MM. CD4+ T-cell synapses involve multiple distinct stages. *Proc Nat Acad Sci U S A.* 2011; 108:17099–17104.
55. Sharma P, Wagner K, Wolchok JD, Allison JP. Novel cancer immunotherapy agents with survival benefit: recent successes and next steps. *Nat Rev Cancer.* 2011; 11:805–812. [PubMed: 22020206]

56. Cramer P, Hallek M. Hematological cancer in 2011: New therapeutic targets and treatment strategies. *Nat Rev Clin Oncol*. 2012; 9:72–74. [PubMed: 22231754]
57. Loren AW, Porter DL. Donor leukocyte infusions after unrelated donor hematopoietic stem cell transplantation. *Curr Opin Oncol*. 2006; 18:107–114. [PubMed: 16462177]
58. Berger C, Jensen MC, Lansdorp PM, Gough M, Elliott C, Riddell SR. Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. *J Clin Invest*. 2008; 118:294–305. [PubMed: 18060041]
59. Brentjens RJ, Riviere I, Park JH, Davila ML, Wang X, Stefanski J, Taylor C, Yeh R, Bartido S, Borquez-Ojeda O, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. *Blood*. 2011; 118:4817–4828. [PubMed: 21849486]
60. Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, June CH. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci Transl Med*. 2011; 3:95ra73.
61. Levine BL, Bernstein WB, Connors M, Craighead N, Lindsten T, Thompson CB, June CH. Effects of CD28 costimulation on long-term proliferation of CD4+ T cells in the absence of exogenous feeder cells. *J Immunol*. 1997; 159:5921–5930. [PubMed: 9550389]
62. Rapoport AP, Stadtmauer EA, Aqui N, Badros A, Cotte J, Chrisley L, Veloso E, Zheng Z, Westphal S, Mair R, et al. Restoration of immunity in lymphopenic individuals with cancer by vaccination and adoptive T-cell transfer. *Nat Med*. 2005; 11:1230–1237. [PubMed: 16227990]
63. Scholler J, Brady TL, Binder-Scholl G, Hwang WT, Plesa G, Hege KM, Vogel AN, Kalos M, Riley JL, Deeks SG, et al. Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. *Sci Transl Med*. 2012; 4:132ra153.
64. Robbins PF, Dudley ME, Wunderlich J, El-Gamil M, Li YF, Zhou J, Huang J, Powell DJ, Rosenberg SA. Cutting edge: persistence of transferred lymphocyte clonotypes correlates with cancer regression in patients receiving cell transfer therapy. *J Immunol*. 2004; 173:7125–7130. [PubMed: 15585832]
65. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol*. 2004; 22:745–763. [PubMed: 15032595]
66. Klebanoff CA, Gattinoni L, Torabi-Parizi P, Kerstann K, Cardones AR, Finkelstein SE, Palmer DC, Antony PA, Hwang ST, Rosenberg SA, et al. Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells. *Proc Nat Acad Sci U S A*. 102:9571–9576.
67. Chang JT, Palanivel VR, Kinjyo I, Schambach F, Intlekofer AM, Banerjee A, Longworth SA, Vinup KE, Mrass P, Oliaro J, et al. Asymmetric T lymphocyte division in the initiation of adaptive immune responses. *Science*. 2007; 315:1687–1691. [PubMed: 17332376]
68. Reiner SL, Sallusto F, Lanzavecchia A. Division of labor with a workforce of one: challenges in specifying effector and memory T cell fate. *Science*. 2007; 317:622–625. [PubMed: 17673652]
69. Hirosue S, Kourtis IC, van der Vlies AJ, Hubbell JA, Swartz MA. Antigen delivery to dendritic cells by poly(propylene sulfide) nanoparticles with disulfide conjugated peptides: Cross-presentation and T cell activation. *Vaccine*. 2010; 28:7897–7906. [PubMed: 20934457]
70. Hubbell JA, Thomas SN, Swartz MA. Materials engineering for immunomodulation. *Nature*. 2009; 462:449–460. [PubMed: 19940915]
71. Ali OA, Emerich D, Dranoff G, Mooney DJ. In situ regulation of DC subsets and T cells mediates tumor regression in mice. *Sci Trans Med*. 2009; 1:8ra19.
72. Swiston AJ, Gilbert JB, Irvine DJ, Cohen RE, Rubner MF. Freely suspended cellular “backpacks” lead to cell aggregate self-assembly. *Biomacromolecules*. 2010; 11:1826–1832. [PubMed: 20527876]
73. Yoo JW, Irvine DJ, Discher DE, Mitragotri S. Bio-inspired, bioengineered and biomimetic drug delivery carriers. *Nat Rev Drug Disc*. 2011; 10:521–535.
74. Acharya AP, Dolgova NV, Clare-Salzler MJ, Keselowsky BG. Adhesive substrate-modulation of adaptive immune responses. *Biomaterials*. 2008; 29:4736–4750. [PubMed: 18829103]

75. Acharya AP, Dolgova NV, Moore NM, Xia CQ, Clare-Salzler MJ, Becker ML, Gallant ND, Keselowsky BG. The modulation of dendritic cell integrin binding and activation by RGD-peptide density gradient substrates. *Biomaterials*. 2010; 31:7444–7454. [PubMed: 20637504]

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Figure 1. Basic components of an immune synapse

(A) T cell activation begins with engagement of the TCR with a peptide-loaded MHC. (B) TCF-pMHC, CD28-CD86/CD80, LFA-1-ICAM-1 form a core set of molecules defining the immune synapse. (C) Signaling complexes are organized into distinct patterns within the IS, which are dependent on the specific T cell – APC interaction. Red dots represent TCR-containing microclusters, while the blue dots represent LFA-1, which coalesce into the larger blue areas in the stable synapses. Comparisons between the stable synapse structures are made in the main text.

Figure 2. Capturing the spatial complexity of the IS

(A) Micropatterned surfaces allow arbitrary control over the layout of an artificial IS. A variety of these techniques allow patterning of biomolecules onto the substrate. The small red, green, and blue shapes in this and subsequent panels correspond to ligands to TCR, CD28, and LFA-1, respectively, as was set forth in Fig. 1B. (B) Preactivated T cells respond to the geometry of TCR – LFA-1 signaling. Adapted from Doh and Irvine³¹. (C) Naïve T cells respond to the geometry of a tri-component surface containing patterns of TCR and CD28 ligands, separated and surrounded by ICAM-1. Adapted from Shen *et al.*³². The shaded outlines in panels B & C illustrate the size of a typical T cell on these surfaces. (D) The supported lipid bilayer system provides natural mobility to APC proteins presented on a substrate. (E) Micro- and nano-patterning of the bilayer system captures the influence of cytoskeletal structures on membrane protein dynamics.

Figure 3. Mechanical forces in T cell activation

(A) Activation of T cells by anti-CD3 and anti-CD28 attached onto polyacrylamide gels. (B) Activation of naïve mouse CD4⁺ T cells correlates with increasing gel rigidity, E . * $P < 0.05$, ** $P < 0.005$ compared to 200 kPa surface. Data are mean $\pm$ s.d., $n=7$. (C) Localization of active (phosphorylated) signaling proteins Zap70 and pan-SFK as a function of gel rigidity. Cells were fixed and stained for these proteins 30 minutes after initiation of cell-substrate contact. Panels B & C were adapted from Judokusumo *et al.*⁵¹ and are reproduced with permission of Rockefeller University Press.

Figure 4. Topology of the immune synapse

(A) Scanning electron micrograph of a human CD4+ T cell interacting with a K562 antigen presenting cell. (B) These cells separated during processing, allowing clearer visualization of the cell-cell interface. Images provided courtesy of Dr. Manus Biggs, Columbia University.