13 Artificial Methods for T Cell Activation: Critical Tools in T Cell Biology and T Cell Immunotherapy

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13.1 Introduction

T lymphocytes play a conductor role in the immune system by orchestrating complex interactions between innate and adaptive immune cells. Protection from various infections as well as cancers is provided in an antigen-specific manner with immunological memory as a result of the functions of healthy T lymphocytes.

The special capability of T cells – to recognize antigens from various pathogens or endogenous mutations in a very specific, sensitive, and selective manner - is conferred by a clonally restricted receptor molecule, the T cell receptor (TCR). Therefore, any discussion regarding biomaterials that are designed for the engineering of T cell functions cannot be made without a proper discussion of the structure and the function of TCR and its related molecules. The TCR is membrane bound, and specifically interacts with not a soluble antigen by itself but an antigenic peptide combined with a molecule encoded by the major histocompatibility complex (pMHC) which is expressed on a membrane of an antigen presenting cell (APC) or a target cell. Thus, the cellular interface between the T cell and the counterpart cell is very important in determining T cell functions, and has been described as the immunological synapse (IS).

We will first briefly review the biology of TCR and IS. And we will focus our discussion to the biomimetic designs that have been developed to study and/or engineer T cell functions through recapitulating or modifying TCR-pMHC interactions and the spatiotemporal molecular dynamics within the IS.

13.2 T Cell Receptor (TCR)

The TCR is a heterodimer of either *alpha and beta* chains or *gamma and delta* chains, and the T cell that expresses the corresponding pairs is called αβ T cell or γδ T cell, respectively (Davis and Bjorkman 1988). The αβ TCR demonstrates a high degree of antigen specificity and thus becomes a hallmark molecule of the adaptive immune system along with the antibody of the B cell. By contrast, γδ T cells recognize classes of antigens present on ranges of pathogens, whose functions more belong to innate immunity. In this chapter, we will focus our discussion on αβ T cells.

The α and β TCR chains are classified as members of the immunoglobulin superfamily. Each chain contains one variable and one constant domain which are structurally homologous to the variable and constant domains of immunoglobulins. The TCR variable domain contains three complementary determining regions (CDR1-3) that are hypervariable. The

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interaction between αβ TCRs and antigen is determined by the combined affinities 1) between CDR1 or 2 loops and conserved regions of the MHC molecule and 2) between CDR3 and antigenic pMHC (Garcia et al. 1996).

In order to provide immune protection from a wide range of pathogens with antigen specificity, healthy human individuals might possess 10^7~10^8 different TCR specificities (clonotypes) (Arstila et al. 1999). In fact, the theoretically possible TCR diversity (number of different pairs of α and β TCR chains that can be produced) far exceeds the number of TCR repertoire present at a given point in time (Nikolich-Zugich et al. 2004). This amazing diversity of immune receptors (TCR and immunoglobulins) originates from their unique genetic features. Unlike most other genes that contain single complete genes in a given locus, the genes encoding TCR chains (along with the genes encoding immunoglobulin heavy and κ or λ light chains) are composed of families of gene segments that should be rearranged before they can be transcribed. For humans, TCR α and β chain genes are located on chromosomes 14 and 7, respectively. Each TCR chain is composed of gene segments that encodes the leader peptide, the variable, and the constant regions. The germline DNAs for variable region in chromosome 14 (TCR α chain) consist of many alternative variable (V) and joining (J) segments. In chromosome 7 (TCR β chain), an additional set of segments called diversity (D) gene segments are located between V segments and J segments. In the recombination process, a mechanistic rule guarantees that each TCR chain is precisely formed by joining of only one of each V and J segments (TCR α chain), or V, D, and J segments (TCR β chain). This is achieved by recombination that is restricted to only occur between the two different types of recombination signal sequences (RSSs) flanking each V, D, and J genes. This random combinatorial way of forming TCR α and β chains using alternative gene segments significantly contributes to the diversity of TCR antigen specificities. Another major mechanism to diversify the TCR repertoire is junctional diversity. As part of the recombination process, the junctions between the V and J segments or between V, D, and J segments are additionally diversified with palindromic (P) sequences of cleaved sites (by action of recombination activating gene proteins (RAG1 and RAG2) as well as randomly added new sequences (N-nucleotides) by action of terminal deoxynucleotidyl transferase (TdT). As CDR3 is encoded at the junctions between the V and J segments or between V, D, and J segments, this mechanism to create junctional diversity heavily contributes to the increase of functional diversity in TCR repertoire.

Even if there are exceptions (Padovan et al. 1993; Davodeau et al. 1995; Heath et al. 1995; Cochran et al. 2001), allelic exclusion of genes for the TCR α and β chains occurs in most developing T cells in the thymus (thymocytes). So a given immature T cell would likely express one kind of functional αβ TCR. Among the enormous TCR repertoire that are randomly generated by the above-mentioned mechanisms (order of 10^{15} for the αβ receptor) (Nikolich-Zugich et al. 2004), i.e. the repertoire of thymocytes, only a small fraction of thymocytes survive after the two following selection processes. First, only the thymocytes bearing receptors capable of binding self-MHC molecules can survive (positive selection). Second, thymocytes bearing high-affinity receptors for self-MHC molecules or self-peptide presented by self-MHC are eliminated (negative selection). The surviving thymocytes without apoptosis after both selection processes become mature T cells bearing receptors that are "self-restricted" as well as "self-tolerant". It is worth mentioning that some of the gene rearrangement (particularly TCR α-chain) occur simultaneously involving RAG-1, RAG-2, and TdT activities during the positive selection.

Both TCR α and β chains have a positively-charged transmembrane domain and a short cytoplasmic tail at its C-terminus (Blumberg et al. 1990). The cytoplasmic region of αβ TCR is not long enough to transduce the signal initiated by interaction with antigenic pMHC. The accessory molecules that are critical for TCR signal transduction and even for the successful expression of TCR are designated as the CD3 complex. The CD3 complex is composed of three dimers (γε,

δε, and ζζ or ζη) formed by five invariant polypeptide chains (γ, δ, ε, η, and ζ). A negatively-charged residue within the transmembrane regions of all CD3 chains interacts with positively charged amino acid residues in the transmembrane region of each TCR chain, which helps the association between CD3 and TCR polypeptide chains (Call and Wucherpfennig 2004). The cytoplasmic tails of the CD3 chains contain a common sequence called the immunoreceptor tyrosine-based activation motif (ITAM), which interacts with tyrosine kinases to play a major role in signal transduction. The CD3 ζ and η chains each contain three ITAMs, whereas the γ, δ, and ε chains each contain only a single copy of ITAM.

13.3 T Cell Activation

The molecular details of signaling cascades following T cell activation and the subsequent alteration of numerous gene expressions is beyond the scope of this chapter. Instead, we will review the most critical molecular components and cellular behaviors that should primarily be considered when developing the biomimetic design of biomaterials that engineer T cell functions. The initial interest lies on the molecular components and their functions within the interface between a T cell and an antigen presenting cell (APC).

Due to the unique role of T cells which respond to pathogens in an antigen-specific manner, the interaction between TCR and pMHC determine its fate and function in almost every stage of a T cell's life span from its early development in the thymus to the fully activated and terminally differentiated effector stage in the periphery. As mentioned earlier, within the thymus, interactions between TCR and self-MHC molecules or self-peptide presented by self-MHC molecules and the consequent intracellular signals determine the fate of developing T cells. The T cells that have completed their development stages exit the thymus and circulate the bloodstream and the lymph through secondary lymphoid organs such as lymph nodes and the spleen in surveillance of foreign antigens. These "non-activated" T cells that have not encountered foreign antigens are called "naïve".

In addition to TCR, there are two kinds of co-receptors designated as CD4 and CD8 on the T cell membrane, and an individual T cell is committed to express only one kind of co-receptor before it is fully developed and exit the thymus. Reflecting their respective effector functions and roles, CD4+ and CD8+ T cells are called "helper" T cells and "cytotoxic" T cells, respectively. TCRs on CD4+ T cells only interact with exogenous peptides presented by class II MHC molecules which are only expressed by phagocytic professional ACPs such as dendritic cells (DCs), macrophages, and B cells. Meanwhile, TCRs on CD8+ T cells only interact with self- or virus-originated peptides presented by class I MHC molecules which are expressed by most cells including professional APCs. Despite this critical difference between CD4+ and CD8+ T cells, the naïve population of both types are commonly required to be properly activated before they can function as effector cells.

As described earlier, the interaction between TCR and the foreign peptide presented by either class I or class II MHC molecules expressed on the plasma membrane of professional APCs becomes a primary signal for T cell activation and dictates the initial activation of naïve T cell. However, a mere TCR-pMHC interaction is not sufficient to fully activate a naïve T cell. The additional requirement is called co-stimulation, and there are multiple molecular interactions between a T cell and an APC that provide co-stimulatory signals. The most important one is the engagement of CD28 on the T cell by CD80 (B7-1) or CD86 (B7-2) on the APC. It is shown that the TCR engaged by pMHC without co-stimulation signals induce the T cell to an "anergic" state (Appleman and Boussiotis 2003). Once the T cell becomes anergic, it maintains this inactivated state even when the T cell is exposed to APC with both antigenic pMHC and costimulatory molecules. The third component that completes the activation of the T cell is various cytokines. Some of these soluble cytokines are secreted either by the T cell itself or by neighboring APC or other cells regulated by

feedback loops. The cytokines secreted by the T cell itself can work as both autocrine and paracrine. As an example, interleukin-2 (IL-2) secreted by T cell is important for the clonal expansion of antigen-specific T cell populations and their differentiation into effector cells.

13.4 Spatiotemporal Molecular Reorganization at the Immunological Synapse

As a result of the interaction between the TCR and its cognate pMHC, the T cell and APC or a target cell form a tightly apposed cellular interface, which is called the immunological synapse (IS). Over the past couple of decades, the spatiotemporal dynamics of molecules within the IS has been extensively studied (Grakoui et al. 1999; Bunnell et al. 2002; Huppa and Davis 2003). The name of IS was initially coined after the initial observation of concentric rings (so called bull's-eye pattern) of membrane receptors and adhesion molecules on T cell membrane upon T cell-APC interaction. Subsequent studies discovered that TCR and CD28 molecules are concentrated within the innermost circle (central supramolecular activation cluster, cSMAC), which are surrounded by peripheral SMAC (pSMAC) of integrins (e.g. LFA-1). Lastly, the outermost ring or distal SMAC (dSMAC) is occupied by proteins that have relatively longer ectodomains (e.g. CD43, CD45). Since then, many studies have been attempted to define the true functions of the IS with the bull's-eye pattern. And continuous TCR signaling is required for maintenance the SMAC structure of IS and extended effector functions (Huppa et al. 2003). Later it was discovered that the lasting TCR signaling is maintained by the microclusters that are continuously and newly generated in the peripheral or distal regions of IS (Yokosuka et al. 2005). It is now well accepted that the microcluster of TCR and other signaling molecules (kinases and adaptors) are the activation signaling cluster. These newly generated TCR microclusters in the distal region are translocated into the central region (cSMAC), where the TCR signaling ceases while TCR molecules break apart from other signaling components. Therefore, cSMAC might function toward the regulation of TCR signaling and molecular recycling. Nevertheless, it has been suggested that the selective and polarized secretion of lytic granules are another function of the cSMAC and the bull's-eye pattern of IS between cytotoxic T cells and target cells (Davis and Dustin 2004).

Furthermore, the bull's-eye pattern is not the unique molecular pattern of IS. For example, a helper T cell forms a multifocal IS in its interaction with a dendritic cell that present the cognate antigens (pMHCs) (Brossard et al. 2005; Dustin et al. 2006). The double positive (CD4 + CD8+) thymocytes also form a decentralized multifocal pattern of TCR accumulation surrounded by ICAM-1 molecules when examined on a lipid bilayer (Hailman et al. 2002).

More recently, owing to the progress in super-resolution microscopy techniques, the spatiotemporal dynamics of TCR and other molecules have been studied in a molecular length scale. And it has been shown that these critical signaling components including TCR complex (e.g. CD3 ζ) (Lillemeier et al. 2010), co-receptor (e.g. CD4) (Roh et al. 2015), adaptor (e.g. Lat) (Lillemeier et al. 2010; Sherman et al. 2011; Williamson et al. 2011), and kinases (e.g. p53lck) (Rossy et al. 2013) are all residing within nano-sized clusters even before the activation of T cells, and these nanoclusters coalesce to form bigger clusters (microclusters) upon engagement of TCR molecules with the cognate pMHCs.

Many steps of these intricate spatial reorganizations within IS and their downstream signaling events are intricately related to cytoskeletal dynamics (e.g. actin rearrangements)

(Miletic et al. 2003; Tskvitaria-Fuller et al. 2003) and integrin adhesion (e.g. LFA-1 to ICAM-1) (Burbach et al. 2007).

13.5 Biomimetic and Artificial Methods to Induce T Cell Activation

To artificially activate T cells, biomimetic design is intrinsically involved. First, biochemical reagents that can effectively engage with the TCR should be selected. Depending on the antigen-specificity requirement, the reagents will be varied, for example, an anti-CD3 antibody for the activation of general T cell population vs. a specific pMHC or a collection of different pMHCs for the activation of antigen-specific T cells. Second, the use of accessory ligands (e.g. for co-stimulatory signals or for effective adhesion) should be considered. Third, the format of presentation (e.g. soluble, plate-bound, membrane-bound, etc.) for such ligands to T cells needs to be determined. All of these design components are deterministic factors for molecular affinity between receptor and ligand and their binding kinetics within immunological kinapses (transient contacts with pro-migratory junctions) and immunological synapses (contacts for a prolonged period with stable junctions) (Fooksman et al. 2010).

Although the exact mechanism of how the very initial TCR triggering is induced is unknown, a number of leading models (van der Merwe and Dushek 2011), namely aggregation model, conformational change model, segregation or redistribution model, serial triggering model (Valitutti et al. 1995), and kinetic proofreading model (McKeithan 1995; Rabinowitz et al. 1996), have been suggested to explain its mechanisms. Historically, various artificial methods of inducing T cell activation have been developed to test these models and hypotheses. In addition, many surface-associated methods were developed as biomimetic tools to study the spatiotemporal dynamics of IS under the microscope. Nevertheless, these various methods can induce varying degrees of T cell activation (Table 13.1) and thus might be useful for particular biomedical applications.

13.5.1 Soluble Reagents

Typically the affinity between TCR and its cognate agonist pMHC has been measured by the use of surface plasmon resonance (SPR). In SPR measurement, one is bound to the surface, and the other binding partner is in the solution, which might well reflect the interactions between soluble reagents and the TCRs on T cell membranes. And from SPR measurements, TCRs have relatively low affinities (K_D values in the range of 1–100 μM) against their cognate agonist pMHCs (Stone et al. 2009), which are combined effects of slow association rates and intermediate dissociation rates. These affinity values are similar to those of antibodies against their antigen in their primary responses, *i.e.* before affinity maturation.

Reflecting these low solution affinity values between TCRs and pMHCs, agonistic pMHC monomers in solution are incapable of inducing T cell activation (Cochran et al. 2000). In fact, the soluble form of high-affinity anti-TCR Fab is incapable of activating T cell either (Yoon et al. 1994). In stark comparison, anti-CD3 antibody in solution can induce T cell activation even if it is partial (Bekoff et al. 1986). Activation can be more robust by using anti-CD28 antibody simultaneously or employing some accessory cells. Thus it is clear that the crosslinking of TCR-CD3 complex is critical for T cell activation. This notion has been further confirmed by well-designed soluble reagents (Fig. 13.1). Cochran et al. (2000, 2001) first introduced maleimide functional groups to the amine side chain of lysine residues within oligopeptides with precisely controlled distance between the lysine residues. By introducing free thiol (cysteine side group) at either α or β chain of recombinant pMHC molecule, 2, 3, or 4 pMHC monomers could be attached to a single peptide chain. All dimer, trimer, and tetramer agonist pMHC could fully activate the T cells (Cochran et al. 2000) and the pMHC dimers coupled through shorter intermolecular distances were

Table 13.1 Comparison of TCR activation platforms and responses

Ligand and presentation platform	Ligand: TCR[a]	TCR activation	Comments	Reference
Soluble				
Agonist pMHC monomers	1:1	No activation	No activation despite binding of monomers to TCR	Cochran et al. (2000)
Agonist pMHC dimers	2:2	Full and sustained	Intermolecular distance is critical	Cochran et al. (2000, 2001)
Agonist/ endogenous pMHC heterodimers	2:2	Full and sustained	CD4 involvement is critical	Krogsgaard et al. (2005)
Anti-TCR fab	1:1	No activation	Low affinity (μM K_d), might not be stable	Yoon et al. (1994)
Anti-CD3 antibody	2:2	Partial	Signaling not blocked by actin depolymerization	Bekoff et al. (1986), Wolff et al. (1993)
Crosslinked anti-CD3	2:2+	Full and transient	CD69 upregulation	Bekoff et al. (1986)
Immobilized surface anchored				
Anti-CD3 antibody	2:2+	Full and sustained	Stable bivalent ligand induces clustering, may generate force for mechanotransduction	Bunnell et al. (2002)
Fluid membrane anchored				
Agonist pMHC monomers	1:1+	Full and sustained if >0.2 pMHC/ μm^2	Requires F-actin, myosin IIA and adhesion to ICAM-1	Grakoui et al. (1999), Varma et al. (2006), Ma et al. (2008), Ilani et al. (2009)

Adapted and modified from Reference Fooksman et al. (2010)
[a]Stoichiometry of engagement
+ Higher-order crosslinking may be present

consistently more potent than those coupled through longer intermolecular distances (Cochran et al. 2001). Furthermore, the same crosslinking strategy was employed by Krogsgaard et al. to construct a heterodimer of agonist pMHC coupled with endogenous pMHC molecules (Krogsgaard et al. 2005). And these heterodimers were capable of inducing T cell activation in a CD4 dependent manner (Krogsgaard et al. 2005). Altogether, T cell activation can be effectively induced by designing soluble reagents that can crosslink the TCR-CD3 complexes. Even if it is still unclear whether and how exactly the strength, duration, and signaling outputs on T cell activations using different soluble reagents are different, the implications on various applications will be further discussed in latter sections.

13.5.2 Surface-Bound Ligands

The physiological TCR and pMHC interactions essentially occur between two cell membrane surfaces. Therefore, simply anchoring the soluble ligands onto a surface might bring a change in the nature of interactions. Indeed, monomeric agonistic pMHCs on either fixed plastic surfaces or fluid lipid bilayers in extremely dilute surface density (< 10 per cell) can effectively trigger the activation of cognate T cells (Ma et al. 2008), while the solution counterparts cannot. This surface-anchorage effect could be interpreted in multiple ways, but it is at least clear that it is dependent on T cell adhesion to the surface and intact cytoskeletal function (Ma et al. 2008; Ilani et al. 2009). Additionally, the above-mentioned SPR-based affinity measurements may not represent the whole picture of how these affinity values and parameters are related to the T cell activation. In

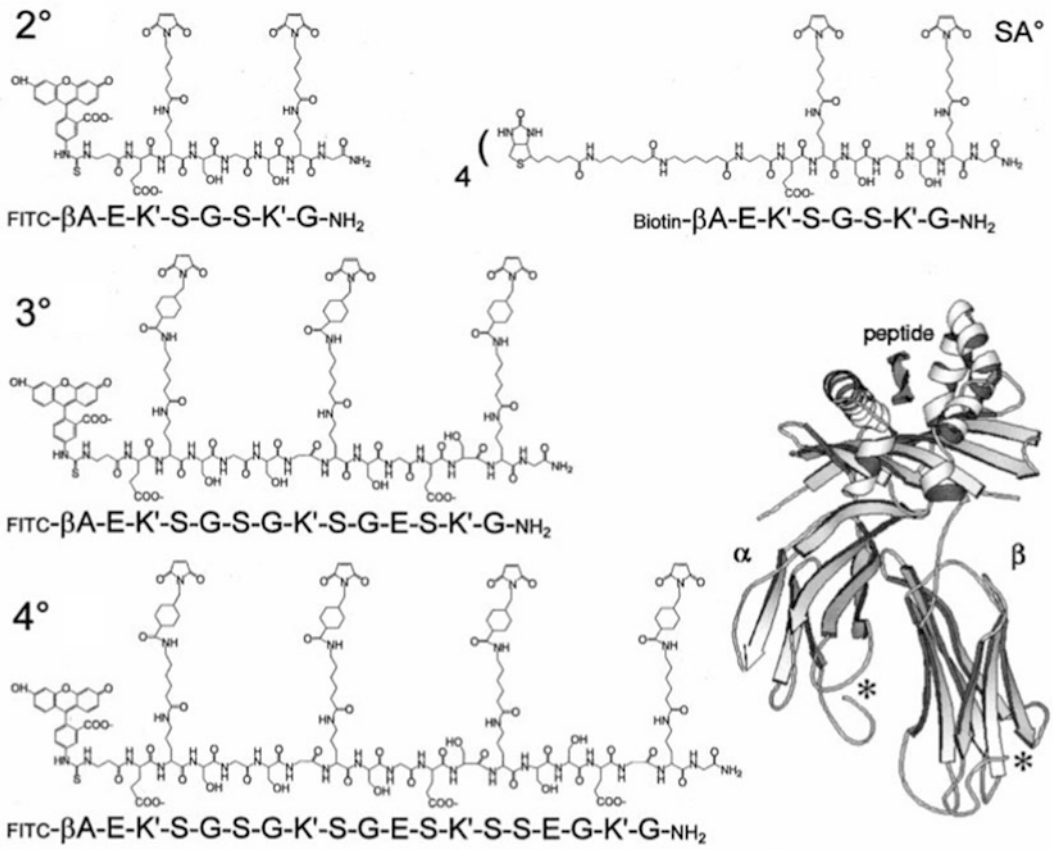

Fig. 13.1 Design and chemical structures of soluble pMHC oligomers: All crosslinkers carry maleimide groups for coupling to cysteine resides introduced into HLA-DR1. Left, fluorescein-labeled, peptide-based crosslinkers for production of MHC dimers (2°), trimers (3°), and tetramers (4°). Upper right, biotin-labeled dimeric crosslinker for production of higher order streptavidin-linked oligomers (SA^o). Lower right, ribbon diagram of the HLA-DR1-peptide complex (Stern et al. 1994), showing the position of cysteine residues (positions marked with asterisks) introduced at the end of either the α or β subunit connecting peptide region. The HLA-DR1 model is shown at approximately 80% of the scale of the chemical diagrams of the cross-linking reagents. Adapted from reference (Cochran et al. 2000). Copyright© 2000, Cell Press

fact, on a plasma membrane, membrane-associated molecules are constricted in 2D diffusion, while the molecules in solution phase are freely movable in 3D. The loss in kinetic degrees of freedom due to the newly formed molecular association between any pair of molecules is considered to be 6 (x, y, and z translation and x, y, and z rotation), 3 (x, y translation and z rotation), or 0 for molecules in soluble (3D), anchored in 2D fluidic membrane, or fixed in immobilizing hard surface, respectively (Fig. 13.2). Therefore, it is expected that the affinity values are intrinsically increased by restricting the soluble activating ligands (pMHCs and other co-stimulatory and integrin binding molecules) onto 2D surfaces (faster on rate and slower off rate). It is indeed commonly observed that more robust T cell activation is induced by surface-bound pMHC or anti-CD3 antibody than by the soluble counterpart.

However, measuring 2D binding properties of TCR and pMHC in practice turned out to be more complicated. Recently, the 2D binding kinetics of TCR-pMHC have been explored using two

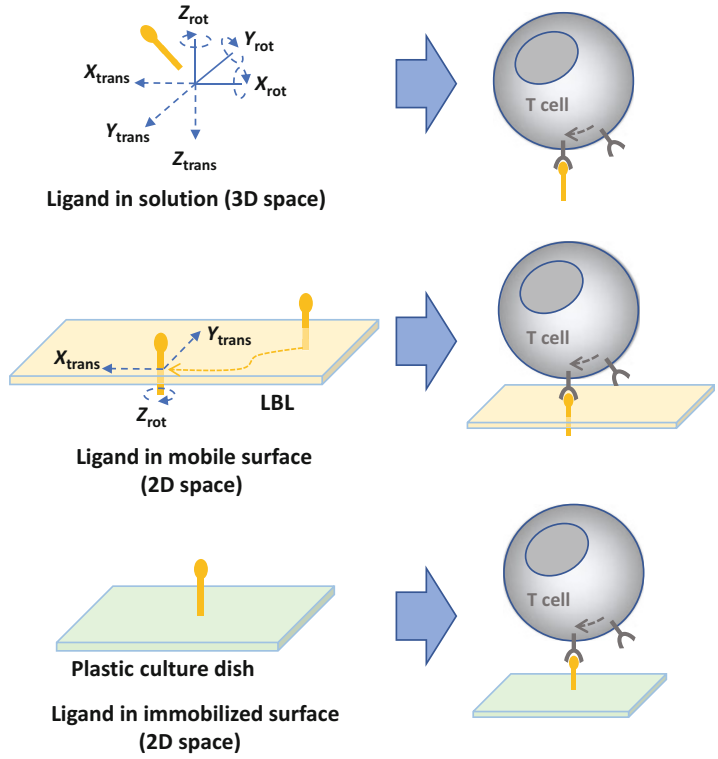

Fig. 13.2 Receptor binding to ligands in a solution (3D), in a lipid bilayer (mobile 2D), and in a solid surface (immobilized 2D). The degrees of freedom before the receptor-ligand binding are 6, 3, and 0, for solution, mobile 2D, and immobilized 2D, respectively. The physiological immune receptor-ligand interactions typically occur at the interface between two opposing cell membranes (immunological synapse)

completely different methods, (i) optical microscopy based on fluorescent resonance energy transfer (FRET) (Huppa et al. 2010) and (ii) mechanical assay based on observation of bonding and deformation (Huang et al. 2010). Intriguingly, in both studies, 2D k_{off} was measured to be significantly faster than the solution (3D) k_{off} values. Again in both studies, the 2D k_{on} values varied in large dynamic ranges (Huang et al. 2010; Huppa et al. 2010) while the 3D counterparts did not (Huang et al. 2010). Furthermore, both the dramatically varying 2D k_{on} rates and fast 2D k_{off} correlated with activation potency, while 3D k_{off} values showed inverse correlation (Huang et al. 2010). All of these interesting data indicated that the TCR-pMHC interaction is subject to mechanical forces, mainly by the actin cytoskeleton, and that the local environment of the direct vicinity of TCR-CD3 complex (e.g. surface density, molecular orientation) dynamically changes upon TCR-pMHC engagement.

Even if measuring 2D binding properties is a very complicated task for the above-mentioned reasons, at least it is clear that localizing TCR triggering signals (pMHC and other ligands) in 2D with biomimetic biomaterial-based design has tremendous implications in T cell activation and functional programing.

13.5.2.1 Immobilized Ligands on Surfaces

As the simplest method of immobilization, ligands (proteins) can be non-specifically adsorbed to 2D surfaces. For in-vitro activation of T cells and resulting T-cell expansions, non-specifically coating the culture dish with anti-CD3 antibodies along with anti-CD28 antibodies has long been used (Lewis et al. 2015). Some examples of antibody clones that are widely employed for this purpose include clone 145-2C11 (anti-mouse CD3ε), OKT3 or HIT3a (anti-human CD3), 37.51 (anti-mouse CD28), and CD28.2 (anti-human CD28).

These antibodies and/or pMHC and recombinant B7 molecules were also immobilized on the surface of synthetic microbeads, which is often called artificial antigen presenting cells (aAPCs) even though the particles are incapable of processing the antigens per se (Curtsinger and Khazaeli 1997; Trickett and Kwan 2003; Walter et al. 2003; Kim et al. 2004; Schilbach et al. 2005; Durai et al. 2009). Both polystyrene beads of 5–6 μm in diameter (so called latex beads) (Curtsinger and Khazaeli 1997; Walter et al. 2003; Schilbach et al. 2005) and commercially available magnetic beads (e.g. Dynabeads®) (Trickett and Kwan 2003; Durai et al. 2009) have been extensively employed as aAPC platforms to expand antigen-nonspecific (with anti-CD3 antibody) or antigen-specific (with pMHC molecule) T cell populations.

Recombinant membrane proteins (e.g. pMHC) or soluble antibodies were successfully incorporated onto the surface of polystyrene beads by non-specific adsorption with or without the aid of co-incubating surfactants (Curtsinger and Khazaeli 1997). For better control over surface density and potentially better molecular orientation, more specific interactions, such as streptavidin to biotin (Walter et al. 2003; Schilbach et al. 2005), anti-Fc antibody or protein A/G to Fc portion of antibody (Trickett and Kwan 2003) or Fc-containing recombinant proteins (Durai et al. 2009), have been employed for the conjugation of T cell activating reagents to the surface of aAPC beads. It is worth mentioning that magnetic Dynabeads® presenting anti-CD3 and anti-CD28 antibodies have been employed as a gold standard for the expansion of CD8+ cytotoxic T cells in recent T cell adoptive therapy trials, due to their easy purification steps using magnets.

In order to observe spatiotemporal dynamics of TCRs and other signaling molecules under microscope, pMHC and B7.1 molecules have also been immobilized on thin glass slides (Lillemeier et al. 2010; Roh et al. 2015). By incubating positively charged poly(L-lysine) (PLL) chains that are conjugated with biotin residues on top of negatively charged glass slides, the surface of the glass slide could be effectively modified with biotin residues. After subsequent incubation with streptavidin, biotinylated pMHC and B7.1 molecules could be immobilized on glass slides. The observation of the T cell membrane molecules interacting with these immobilized activating ligands under the microscope revealed that the TCRs form microclusters, as a result of actin-dependent clustering (concatenation) of smaller nanoclusters, but they could not develop into mature SMACs, potentially due to the spatially fixed activating ligands (Lillemeier et al. 2010).

This observation clearly demonstrates the potential limitations of the surface-immobilized antibodies or natural ligands, which is associated with their incapability of generating dynamic remodeling of physiological IS between T cells and APCs or target cells. This leads us to the discussion of development of artificial platforms with which ligands are presented in a mobile layer.

13.5.2.2 Mobile Ligands Attached to Plasma Membrane-Mimetic Fluidic Surfaces

The molecules within the IS dynamically reorganize during T cell activation as mentioned above. Therefore, the importance of providing lateral mobility of molecular ligands to T cells has long been recognized. The supported lipid bilayer (LBL) has been developed as an effective biomimetic platform to study cell-cell interactions between immune cells (McConnell et al. 1986; Sackmann 1996). The initial observation of the bull's eye pattern of IS was made on top of glass-slide-supported lipid bilayer presenting agonistic pMHC and ICAM-1 (Grakoui et al. 1999). For the preparation of this artificial membrane layer, they first expressed glycosylphosphatidylinositol (GPI)-modified pMHC and ICAM-1 in CHO and BHK cells, respectively, and successfully incorporated them into liposomes containing 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC). Finally, these liposomes were incubated on a clean glass slide. Similarly designed mobile LBLs have been employed to study the spatiotemporal dynamics of IS under the microscope (Varma et al. 2006).

Instead of GPI-anchored proteins, others expressed recombinant proteins (pMHC, B7.1, and ICAM-1) with multi-histidine tag (His-tag) for specific association onto glass-slide supported lipid bilayer composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1,2-dioleoyl-sn-glycero-3-[(N-(5-amino-1-carboxypentyl)iminodiacetic acid) succinyl] (nickel salt) (DGS-NTA(Ni)) (Huppa et al. 2010; Roh et al. 2015).

In order to mimic the preclustered membrane microdomains on the APC surface, Albani and colleagues introduced neutravidin to the supported LBL. Biotinlyated cholera toxin B (CTB) along with biotinylated antibodies and pMHC complexes were first bound to neutravidin. By utilizing the affinity between CTB and the monosialoganglioside (GM1), the whole protein complex with neutravidin as a scaffolding unit was attached to supported LBL composed of phosphatidylcholine, cholesterol and GM1.

LBL was also formed on glass substrates displaying various patterns of chromium lines (100 nm wide and 5 nm high) fabricated using electron-beam lithography (Mossman et al. 2005). These nanoscale chromium lines became barriers for the lateral movement of pMHC-TCR complex, which created IS with artificial geometric molecular patterns. Within these repatterned molecular structures in IS, the TCR microclusters mechanically trapped in the peripheral regions showed prolonged signaling compared to the central counterparts (Mossman et al. 2005), which reinforced the idea that cSMAC may function as a signal termination site.

The aAPCs with laterally mobile activating ligands have also been constructed in a non-planar geometry, i.e. spherical shape. Liposomes of 60–90 nm in size were constructed with cholesterol and phosphatidylcholine to effectively present agonistic pMHC molecules (60–160 per aAPC) to detect and activate the cognate T cell population (Prakken et al. 2000). Similar to the above-mentioned planar LBL, the affinity between CTB and GM1 has been employed to construct the liposome aAPCs decorated with neutravidin-scaffolded anti-CD3, anti-CD28, and anti-LFA1 monoclonal antibodies (mAbs) (Zappasodi et al. 2008). These lipid aAPCs were similarly effective to the commercially available magnetic bead (anti-CD3, anti-CD28 mAbs) counterpart in activation and expansion of T cells (Zappasodi et al. 2008). The viscous oil droplets (liquid colloids) created by shear-induced rupturing in viscoelastic complex fluids have been surface grafted with anti-CD3 and anti-CD28 mAbs as aAPCs that are more similar to the physiological cells in size (a few microns) (Bourouina et al. 2012). Upon interacting with these aAPCs, T cells were activated to induce intracellular signaling and developed cytoskeleton-dependent dynamics at the interfaces (Bourouina et al. 2012).

In addition to these artificially constructed aAPCs, virus-like particles (VLP) were produced by mammalian cells (HEK293 cells) infected with Moloney murine leukemia virus (Derdak et al. 2006). In order to effectively decorate the VLPs with TCR ligands (either scFv of anti-CD3 antibody or pMHC) as well as the costimulatory ligand (CD80) and the intracellular adhesion molecule (ICAM-1), the recombinant constructs of these molecules were linked to GPI anchor sequences. These molecules were specifically localized into lipid rafts on HEK293 cells, which were heavily transferred to the membranes of budding VLPs. Using this VLP-based aAPC, T cells were successfully stimulated either in antigen-specific or non-specific manner (Derdak et al. 2006). Exosomes (Thery et al. 2002) are another important category of cell-derived vesicles that can act as aAPC. These small (30–150 nm) extracellular vesicles secreted from cellular endosomes of dendritic cells could present pMHC molecules as well as costimulatory and adhesion molecules to CD4+ or CD8+ T cells (Zitvogel et al. 1998). Exosomes derived directly from tumor cells have also been employed to boost the antigen-specific T-cell immune responses in vivo (Wolfers et al. 2001). These anti-tumor activities might be a result of combined effects of direct interaction between exosomes and T cells as well as indirect priming of T cells through exosome targeted DCs (Syn et al. 2017).

13.6 Conclusions

At the basis of the central roles of T cells in the adaptive immune system, there is the specific molecular interaction between TCR and pMHC molecular complex. The antigen recognition in a very selective, sensitive, and specific manner induces the activation of T cells with intricate signaling pathways. As the T cell activation continues, the interface between the T cell and the APC or the target cell develop into mature IS with spatially reorganized molecular structures in various sizes (from molecular nanostructures to cellular microstructures).

A variety of biobimetic artificial methods to induce T cell activation has been pursued. Actually many of these methods were developed as a tool to study the molecular and cellular mechanisms of T cell activations. Here we briefly reviewed these methods in three different categories, i.e. i) soluble, ii) immobilized-surface-bound, and iii) mobile-surface-bound forms.

As adoptive T cell therapy continuously creates tremendous success cases in pre-clinical and clinical studies, the need for effective and clinically-relevant methods of T cell activation and expansion is also ever-growing. Various aAPCs summarized above have tremendous potential, while genetically-modified cell-based aAPCs (Maus et al. 2002) also may have ample room to contribute with their unique attributes (Kim et al. 2004).

Here, we have discussed the physicochemical implications of presenting ligands in different formats (e.g. 3D vs. 2D, immobilized vs. fluidic). These differences may affect the strength, duration, and/or the nature of T cell activations, which in turn may yield different phenotypes and functions of final T cell populations (Li and Kurlander 2010). Therefore, the platform of activation should be carefully selected for each intended purpose, and further studies are warranted to better understand the relationship between the final fate of the T cell and the methods of artificial T cell activation varied by above-mentioned engineering parameters as well as other parameters such as mechanical properties of the substrate (O'Connor et al. 2012).

References

Appleman LJ, Boussiotis VA (2003) T cell anergy and costimulation. Immunol Rev 192:161–180

Arstila TP, Casrouge A, Baron V, Even J, Kanellopoulos J, Kourilsky P (1999) A direct estimate of the human alphabeta T cell receptor diversity. Science 286(5441):958–961

Bekoff M, Kubo R, Grey HM (1986) Activation requirements for normal T cells: accessory cell-dependent and -independent stimulation by anti-receptor antibodies. J Immunol 137(5):1411–1419

Blumberg RS, Alarcon B, Sancho J, McDermott FV, Lopez P, Breitmeyer J, Terhorst C (1990) Assembly and function of the T cell antigen receptor. Requirement of either the lysine or arginine residues in the transmembrane region of the alpha chain. J Biol Chem 265(23):14036–14043

Bourouina N, Husson J, Hivroz C, Henry N (2012) Biomimetic droplets for artificial engagement of living cell surface receptors: the specific case of the T-cell. Langmuir 28(14):6106–6113

Brossard C, Feuillet V, Schmitt A, Randriamampita C, Romao M, Raposo G, Trautmann A (2005) Multifocal structure of the T cell - dendritic cell synapse. Eur J Immunol 35(6):1741–1753

Bunnell SC, Hong DI, Kardon JR, Yamazaki T, McGlade CJ, Barr VA, Samelson LE (2002) T cell receptor ligation induces the formation of dynamically regulated signaling assemblies. J Cell Biol 158 (7):1263–1275

Burbach BJ, Medeiros RB, Mueller KL, Shimizu Y (2007) T-cell receptor signaling to integrins. Immunol Rev 218:65–81

Call ME, Wucherpfennig KW (2004) Molecular mechanisms for the assembly of the T cell receptor-CD3 complex. Mol Immunol 40(18):1295–1305

Cochran JR, Cameron TO, Stern LJ (2000) The relationship of MHC-peptide binding and T cell activation probed using chemically defined MHC class II oligomers. Immunity 12(3):241–250

Cochran JR, Cameron TO, Stone JD, Lubetsky JB, Stern LJ (2001) Receptor proximity, not intermolecular orientation, is critical for triggering T-cell activation. J Biol Chem 276(30):28068–28074

Curtsinger JW, Khazaeli A (1997) A reconsideration of stress experiments and population heterogeneity. Exp Gerontol 32(6):727–729

Davis MM, Bjorkman PJ (1988) T-cell antigen receptor genes and T-cell recognition. Nature 334 (6181):395–402

Davis DM, Dustin ML (2004) What is the importance of the immunological synapse? Trends Immunol 25 (6):323–327

Davodeau F, Peyrat MA, Romagne F, Necker A, Hallet MM, Vie H, Bonneville M (1995) Dual T cell receptor beta chain expression on human T lymphocytes. J Exp Med 181(4):1391–1398

Derdak SV, Kueng HJ, Leb VM, Neunkirchner A, Schmetterer KG, Bielek E, Majdic O, Knapp W, Seed B, Pickl WF (2006) Direct stimulation of T lymphocytes by immunosomes: virus-like particles decorated with T cell receptor/CD3 ligands plus costimulatory molecules. Proc Natl Acad Sci USA 103(35):13144–13149

Durai M, Krueger C, Ye Z, Cheng L, Mackensen A, Oelke M, Schneck JP (2009) In vivo functional efficacy of tumor-specific T cells expanded using HLA-Ig based artificial antigen presenting cells (aAPC). Cancer Immunol Immunother 58(2):209–220

Dustin ML, Tseng SY, Varma R, Campi G (2006) T cell-dendritic cell immunological synapses. Curr Opin Immunol 18(4):512–516

Fooksman DR, Vardhana S, Vasiliver-Shamis G, Liese J, Blair DA, Waite J, Sacristan C, Victora GD, Zanin-Zhorov A, Dustin ML (2010) Functional anatomy of T cell activation and synapse formation. Annu Rev Immunol 28:79–105

Garcia KC, Degano M, Stanfield RL, Brunmark A, Jackson MR, Peterson PA, Teyton L, Wilson IA (1996) An alphabeta T cell receptor structure at 2.5 A and its orientation in the TCR-MHC complex. Science 274(5285):209–219

Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML (1999) The immunological synapse: a molecular machine controlling T cell activation. Science 285(5425):221–227

Hailman E, Burack WR, Shaw AS, Dustin ML, Allen PM (2002) Immature CD4(+)CD8(+) thymocytes form a multifocal immunological synapse with sustained tyrosine phosphorylation. Immunity 16(6):839–848

Heath WR, Carbone FR, Bertolino P, Kelly J, Cose S, Miller JF (1995) Expression of two T cell receptor alpha chains on the surface of normal murine T cells. Eur J Immunol 25(6):1617–1623

Huang J, Zarnitsyna VI, Liu B, Edwards LJ, Jiang N, Evavold BD, Zhu C (2010) The kinetics of two-dimensional TCR and pMHC interactions determine T-cell responsiveness. Nature 464 (7290):932–936

Huppa JB, Davis MM (2003) T-cell-antigen recognition and the immunological synapse. Nat Rev Immunol 3 (12):973–983

Huppa JB, Gleimer M, Sumen C, Davis MM (2003) Continuous T cell receptor signaling required for synapse maintenance and full effector potential. Nat Immunol 4 (8):749–755

Huppa JB, Axmann M, Mortelmaier MA, Lillemeier BF, Newell EW, Brameshuber M, Klein LO, Schutz GJ, Davis MM (2010) TCR-peptide-MHC interactions in situ show accelerated kinetics and increased affinity. Nature 463(7283):963–967

Ilani T, Vasiliver-Shamis G, Vardhana S, Bretscher A, Dustin ML (2009) T cell antigen receptor signaling and immunological synapse stability require myosin IIA. Nat Immunol 10(5):531–539

Kim JV, Latouche JB, Riviere I, Sadelain M (2004) The ABCs of artificial antigen presentation. Nat Biotechnol 22(4):403–410

Krogsgaard M, Li QJ, Sumen C, Huppa JB, Huse M, Davis MM (2005) Agonist/endogenous peptide-MHC heterodimers drive T cell activation and sensitivity. Nature 434(7030):238–243

Lewis MD, de Leenheer E, Fishman S, Siew LK, Gross G, Wong FS (2015) A reproducible method for the expansion of mouse CD8+ T lymphocytes. J Immunol Methods 417:134–138

Li Y, Kurlander RJ (2010) Comparison of anti-CD3 and anti-CD28-coated beads with soluble anti-CD3 for expanding human T cells: differing impact on CD8 T cell phenotype and responsiveness to restimulation. J Transl Med 8:104

Lillemeier BF, Mortelmaier MA, Forstner MB, Huppa JB, Groves JT, Davis MM (2010) TCR and Lat are expressed on separate protein islands on T cell membranes and concatenate during activation. Nat Immunol 11(1):90–96

Ma Z, Sharp KA, Janmey PA, Finkel TH (2008) Surface-anchored monomeric agonist pMHCs alone trigger TCR with high sensitivity. PLoS Biol 6(2):e43

Maus MV, Thomas AK, Leonard DG, Allman D, Addya K, Schlienger K, Riley JL, June CH (2002) Ex vivo expansion of polyclonal and antigen-specific cytotoxic T lymphocytes by artificial APCs expressing ligands for the T-cell receptor, CD28 and 4-1BB. Nat Biotechnol 20(2):143–148

McConnell HM, Watts TH, Weis RM, Brian AA (1986) Supported planar membranes in studies of cell-cell recognition in the immune system. Biochim Biophys Acta 864(1):95–106

McKeithan TW (1995) Kinetic proofreading in T-cell receptor signal transduction. Proc Natl Acad Sci USA 92(11):5042–5046

Miletic AV, Swat M, Fujikawa K, Swat W (2003) Cytoskeletal remodeling in lymphocyte activation. Curr Opin Immunol 15(3):261–268

Mossman KD, Campi G, Groves JT, Dustin ML (2005) Altered TCR signaling from geometrically repatterned immunological synapses. Science 310 (5751):1191–1193

Nikolich-Zugich J, Slifka MK, Messaoudi I (2004) The many important facets of T-cell repertoire diversity. Nat Rev Immunol 4(2):123–132

O'Connor RS, Hao X, Shen K, Bashour K, Akimova T, Hancock WW, Kam LC, Milone MC (2012) Substrate rigidity regulates human T cell activation and proliferation. J Immunol 189(3):1330–1339

Padovan E, Casorati G, Dellabona P, Meyer S, Brockhaus M, Lanzavecchia A (1993) Expression of two T cell receptor alpha chains: dual receptor T cells. Science 262(5132):422–424

Prakken B, Wauben M, Genini D, Samodal R, Barnett J, Mendivil A, Leoni L, Albani S (2000) Artificial antigen-presenting cells as a tool to exploit the immune 'synapse'. Nat Med 6(12):1406–1410

Rabinowitz JD, Beeson C, Lyons DS, Davis MM, McConnell HM (1996) Kinetic discrimination in T-cell activation. Proc Natl Acad Sci USA 93 (4):1401–1405

Roh KH, Lillemeier BF, Wang F, Davis MM (2015) The coreceptor CD4 is expressed in distinct nanoclusters and does not colocalize with T-cell receptor and active protein tyrosine kinase p56lck. Proc Natl Acad Sci USA 112(13):E1604–E1613

Rossy J, Owen DM, Williamson DJ, Yang Z, Gaus K (2013) Conformational states of the kinase Lck regulate clustering in early T cell signaling. Nat Immunol 14(1):82–89

Sackmann E (1996) Supported membranes: scientific and practical applications. Science 271(5245):43–48

Schilbach K, Kerst G, Walter S, Eyrich M, Wernet D, Handgretinger R, Xie W, Rammensee HG, Muller I, Buhring HJ, Niethammer D (2005) Cytotoxic minor histocompatibility antigen HA-1-specific CD8+ effector memory T cells: artificial APCs pave the way for clinical application by potent primary in vitro induction. Blood 106(1):144–149

Sherman E, Barr V, Manley S, Patterson G, Balagopalan L, Akpan I, Regan CK, Merrill RK, Sommers CL, Lippincott-Schwartz J, Samelson LE (2011) Functional nanoscale organization of signaling molecules downstream of the T cell antigen receptor. Immunity 35(5):705–720

Stern LJ, Brown JH, Jardetzky TS, Gorga JC, Urban RG, Strominger JL, Wiley DC (1994) Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. Nature 368 (6468):215–221

Stone JD, Chervin AS, Kranz DM (2009) T-cell receptor binding affinities and kinetics: impact on T-cell activity and specificity. Immunology 126(2):165–176

Syn NL, Wang L, Chow EK, Lim CT, Goh BC (2017) Exosomes in Cancer nanomedicine and immunotherapy: prospects and challenges. Trends Biotechnol 35 (7):665–676

Thery C, Zitvogel L, Amigorena S (2002) Exosomes: composition, biogenesis and function. Nat Rev Immunol 2(8):569–579

Trickett A, Kwan YL (2003) T cell stimulation and expansion using anti-CD3/CD28 beads. J Immunol Methods 275(1–2):251–255

Tskvitaria-Fuller I, Rozelle AL, Yin HL, Wulfing C (2003) Regulation of sustained actin dynamics by the TCR and costimulation as a mechanism of receptor localization. J Immunol 171(5):2287–2295

Valitutti S, Muller S, Cella M, Padovan E, Lanzavecchia A (1995) Serial triggering of many T-cell receptors by a few peptide-MHC complexes. Nature 375 (6527):148–151

van der Merwe PA, Dushek O (2011) Mechanisms for T cell receptor triggering. Nat Rev Immunol 11(1):47–55

Varma R, Campi G, Yokosuka T, Saito T, Dustin ML (2006) T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. Immunity 25 (1):117–127

Walter S, Herrgen L, Schoor O, Jung G, Wernet D, Buhring HJ, Rammensee HG, Stevanovic S (2003) Cutting edge: predetermined avidity of human CD8 T cells expanded on calibrated MHC/anti-CD28-coated microspheres. J Immunol 171(10):4974–4978

Williamson DJ, Owen DM, Rossy J, Magenau A, Wehrmann M, Gooding JJ, Gaus K (2011) Pre-existing clusters of the adaptor Lat do not participate in early T cell signaling events. Nat Immunol 12 (7):655–662

Wolfers J, Lozier A, Raposo G, Regnault A, Thery C, Masurier C, Flament C, Pouzieux S, Faure F, Tursz T, Angevin E, Amigorena S, Zitvogel L (2001) Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. Nat Med 7 (3):297–303

Wolff CH, Hong SC, von Grafenstein H, Janeway CA Jr (1993) TCR-CD4 and TCR-TCR interactions as distinctive mechanisms for the induction of increased intracellular calcium in T-cell signalling. J Immunol 151(3):1337–1345

Yokosuka T, Sakata-Sogawa K, Kobayashi W, Hiroshima M, Hashimoto-Tane A, Tokunaga M, Dustin ML, Saito T (2005) Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76. Nat Immunol 6(12):1253–1262

Yoon ST, Dianzani U, Bottomly K, Janeway CA Jr (1994) Both high and low avidity antibodies to the T cell receptor can have agonist or antagonist activity. Immunity 1(7):563–569

Zappasodi R, Di Nicola M, Carlo-Stella C, Mortarini R, Molla A, Vegetti C, Albani S, Anichini A, Gianni AM (2008) The effect of artificial antigen-presenting cells with preclustered anti-CD28/-CD3/-LFA-1 monoclonal antibodies on the induction of ex vivo expansion of functional human antitumor T cells. Haematologica 93(10):1523–1534

Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, Ricciardi-Castagnoli P, Raposo G, Amigorena S (1998) Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. Nat Med 4(5):594–600