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Making the T Cell Receptor Go the Distance: A Topological View of T Cell Activation

Review

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T cell activation presents a paradigm for both signal transduction and the orchestration of extracellular interactions that lead to the incredible sensitivity and specificity of antigen recognition. While great progress has been made in our understanding of the molecular machinery of T cell receptor (TCR)-initiated signal transduction, the basic vocabulary for understanding events at the cell surface and how these events regulate the signaling machinery is in a nascent stage. Recently, several new concepts have been introduced into the literature. These concepts, particularly those of two-dimensional affinity, serial TCR triggering, and kinetic proofreading, are likely to transform the way in which we think about T cell activation (Dustin et al., 1996a; McKeithan, 1995; Valitutti et al., 1995). Understanding protein interactions in membranes is a new frontier in biology and is a general area in which the T cell activation system is poised to take the lead.

Here, we will discuss recent work on this area, define some key questions, and present a topological model of T cell activation. A key feature of this model is the idea that engagement of the TCR by antigen does not directly result in T cell activation. Rather, it is the rearrangement of membrane proteins in the area of contact between the T cell and the antigen-presenting cell (APC) that is the critical event. Topological features of the T cell surface determine which proteins are present at the cell contact and their specific arrangement. This rearrangement is critical for signaling because it induces the apposition of protein kinases with their substrates at the cell contact. It also results in the exclusion of negative regulators such as tyrosine phosphatases from the contact. Thus, the TCR functions primarily as a choreographer of adhesion molecules, coordinating the stable contact between the T cell and the APC, which in turn drives a specific rearrangement of proteins at the contact cap.

The Basic Problem

T cell activation occurs when the TCR is engaged by its ligand, a specific peptide bound to a major histocompatibility complex (MHC) molecule (Babbitt et al., 1985). This is thought to occur when a T cell interacts with an APC, a simple enough process (Unanue, 1984). On closer examination, however, it is apparent that several large obstacles stand in the way of this simple interaction.

The Problem of Affinity

First, the TCR has a relatively low affinity toward its ligand. Recently, the ability to purify soluble TCRs and peptide-bound MHC molecules has allowed the affinity of the TCR–MHC interaction to be measured. These studies demonstrate that the receptor binds its ligand with dissociation constants ranging between 10^{-4} to 10^{-7} M (Alam et al., 1996; Corr et al., 1994; Lyons et al., 1996; Matsui et al., 1994). This is relatively weak binding when compared with immunoglobulins, for example, which frequently have dissociation constants of $<10^{-9}$ M (Mason and Williams, 1980). However, TCRs and antibodies function in completely different arenas. While antibody must be able to neutralize toxins and bind antigen in the blood and tissue fluids, the TCR works in the confines of a cell–cell contact. What are the characteristics of the cell–cell contact that allow low affinity interactions to drive the activation machinery?

The Problem of Ligand Concentration

Second, the number of specific ligands for a particular TCR on an APC is very small (Demotz et al., 1990; Harding and Unanue, 1990). Low concentration of ligand further complicates the problem of low affinity. The function of the APC is to process and display proteins contained in both the intracellular and extracellular environment for display on the cell surface (Cresswell, 1994). T cells monitor these peptides for the presence of a ''foreign'' peptide. Given the large spectrum of proteins in the cellular environment, the MHC, at any given time, is bound to a tremendous number of different peptides (Falk et al., 1991). Therefore, the number of MHC molecules containing the exact same peptide is likely to be very low. In addition, as these specific ligands are probably randomly distributed on surface of the APC, only a small fraction of a specific antigenic ligand is likely to be initially present in the area of cell contact between the T cell and the APC. Are there specific mechanisms that exist to facilitate the detection of low concentrations of specific antigen by the TCR?

The Problem of TCR Size

Finally, as noted by others, the TCR (and the MHC) is a relatively small molecule compared to the plasma membrane glycocalyx (van der Merwe and Barclay, 1994; Figure 1). The dimensions of the TCR–MHC complex can be directly measured from the recently solved crystal structures of a TCR–MHC complex (Garboczi et al., 1996; Garcia et al., 1996a). These structures demonstrate that the complex is compact and that the distance spanned by the TCR–MHC complex is approximately 15 nm. By definition, therefore, the distance between the T cell and APC plasma membranes in the area of contact between the TCR and MHC must be approximately 15 nm.

The problem is that most membrane proteins on the surface of the T cell are significantly taller than the TCR. Buried under these taller proteins, the TCR is sterically hindered from engaging its ligand, peptide–MHC. For example, the two most abundant proteins on the surface of the T cell, comprising approximately 30% of the surface area, are the glycoproteins CD45 and CD43 (Cyster et al., 1991). Sized by electron microscopy, the extracellular domains of CD43 and CD45 extend from the surface of the T cell by at least 45 nm, at least six times the height of the TCR. Significant reorganization of proteins at the contact between the T cell and the APC must therefore occur to allow TCR engagement of MHC. What are the forces that drive this reorganization?

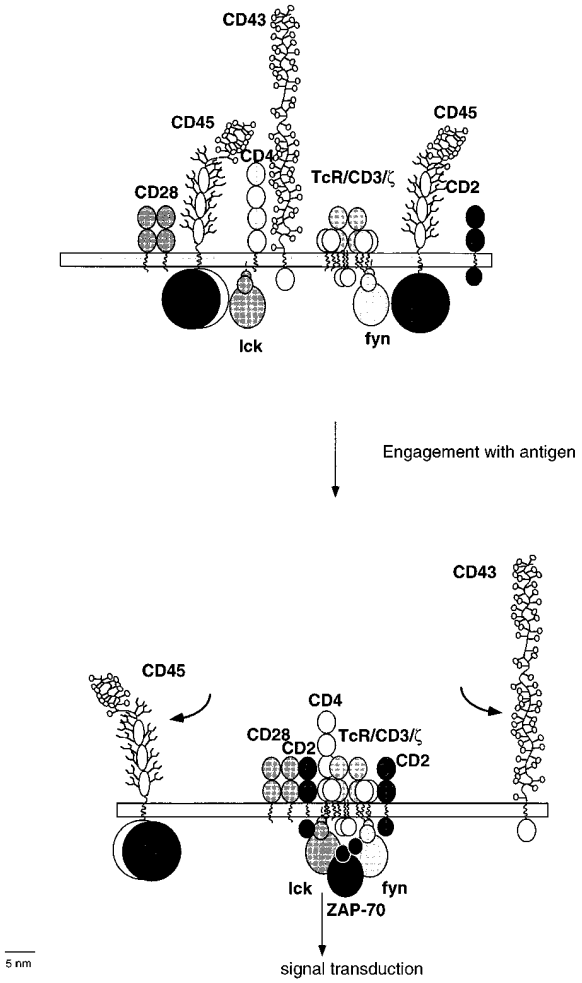

Figure 1. Schematic Depiction of Different Sizes of T Cell Membrane Proteins and Their Arrangement before and after Engagement with Antigen

In the resting T cells, large and small proteins, kinases, and phosphatases are randomly distributed. In this figure, the smaller, low molecular isoform of CD45 is depicted. Antigen engagement (lower panel) results in the segregation of large and small proteins, as well as kinases (ovals) and phosphatases (circles). Small balls represent immunoglobulin folds. Tree-like stick figures represent carbohydrate modifications.

Affinities and Two-Dimensional Kds

Solution measurements, as stated above, suggest that the affinity of the TCR–MHC complex is relatively weak (Alam et al., 1996; Corr et al., 1994; Lyons et al., 1996; Matsui et al., 1994). However the concentration of membrane proteins cannot be expressed as a solution concentration, making it difficult to interpret directly the physiological significance of a solution affinity measurement.

The concentration of a membrane protein can, however, be expressed as a surface density. Thus, it has been proposed that the most relevant affinity parameter for the TCR interaction with MHC molecules is the two-dimensional affinity (2D Kd; Bell et al., 1984; Dustin et al., 1996a). In essence, this affinity measurement can be thought of as the density of receptors in the membrane required to achieve 50% binding. Expressed in this way, the question becomes: what is the receptor density on the surface that is required to drive the interaction between the TCR and the MHC?

While the 2D Kd for interaction of TCR and MHC–peptide molecules has not yet been measured directly, the 2D Kd for the interaction between the adhesion molecules CD2 and CD58 was recently determined (Dustin et al., 1996a). Because solution affinity measurements of CD2–CD58 complexes are kinetically similar to TCR binding to peptide–MHC (van der Merwe et al., 1993, 1994), measurements of the 2D affinity constants for CD2–CD58 may be applicable to interactions such as the TCR binding to peptide–MHC. The message of these studies is loud and clear: an interaction that has a poor biological affinity in solution can give rise to a good biological affinity in a contact area! The 2D Kd measured for CD2–CD58, 21 molecules/μm^2, is biologically relevant and favored in vivo given the resting density of CD2 of about 200–300 molecules/μm^2 (Dustin et al., 1996a).

It is important to realize that 2D affinities are not absolute but will be affected by the topology of the contact area. This is because a perfect 2D affinity constant assumes that the interacting membrane proteins are in the same plane and can only move in two dimensions. In reality, however, the contact area between the two cells is not perfect. Membrane flexibility and the elasticity of membrane anchoring allow membrane proteins to move in three dimensions. What this means is that the observed 2D affinity will vary as the rigidity and the distance between the two membranes change (Figure 2). Conditions that bring membranes closer together or increase membrane rigidity (or both) will reduce the third dimensional component, resulting in an increased observed 2D affinity. Therefore, the 2D affinity is dynamic; conditions that bring membranes closer together and that enhance membrane rigidity will dramatically enhance 2D affinity.

Assuming that the 2D affinity of the TCR–antigen is similar to that of CD2–CD58, about 20 molecules/μm^2, and assuming a density of TCRs in the membrane of about 200 molecules/μm^2, interactions of the TCR with its ligand would be favored and might be predicted to be sufficient to generate a cell contact. In most cases, however, any avidity that could be generated would be compromised by the extremely limited numbers of specific peptide–MHC complexes present on the APC. At the lower end of the spectrum (about 100 peptide–MHC complexes per APC), the density of specific peptide–MHC complexes is estimated to be only 0.1–1 molecules/μm^2. Thus, the T cell must utilize other mechanisms to generate the attractive force to overcome physical limitations to TCR contact.

Serial Triggering of TCRs

The idea of serial engagement of the TCR was first proposed by Lanzavecchia and colleagues to explain how T cells could be activated by 100 specific peptide–MHC complexes on an APC (Valitutti et al., 1995). They propose that a single MHC–peptide complex can trigger up to 100 TCRs, thus explaining how a minimal number of peptide–MHC complexes could result in activation of

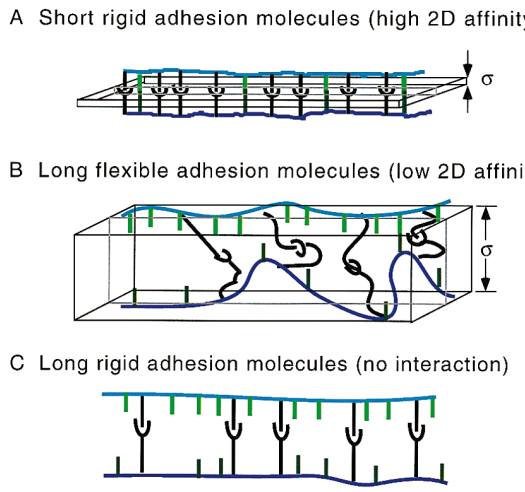

Figure 2. What Is ''Two-Dimensional'' Affinity?

Thinking about the concept of 2D affinity illuminates key issues for T cell activation. Membrane protein concentrations can be quantitated as 2D densities. In reality, however, adhesion molecules are three-dimensional (3D) structures and can have a variable height from the cell surface. Furthermore, the contact area between the two cells is not ideal. Membrane flexibility, cytoskeletal dynamics, and molecular motion add a third dimensional component to interactions between molecules attached to cell surfaces. What this means is that the 2D affinity is not absolute, but depends on the distance between the two membranes and their rigidity. The 2D affinity is, therefore, related to the 3D affinity by the equation

$$3D\ Kd\ (molecules/\mu m^3) = 2D\ Kd\ (molecules/\mu m^2) \div \sigma,$$

where σ represents the constrained third dimensional distance (Bell et al., 1984). Thus, as σ gets smaller, the 2D affinity increases. The figure illustrates how size similarity between adhesion molecules (black lines) and TCRs (green lines) is critical for achieving a high 2D affinity. The boxes are drawn to suggest the 3D volume in the cell–cell interface.

An optimal 2D affinity will be favored by small, topologically similar or identical adhesion molecules that hold membranes rigidly at a uniform distance (small σ in [A]). Inclusion of large adhesion molecules in the contact area produces a large variance in the distance between membranes (large σ) elevating the 2D Kd (B). Finally, aggregation of large, rigid adhesion molecules into the contact is predicted to inhibit TCR engagement (C). Because the sizes of CD2–CD58 and CD28–CD80 are very similar to the size of the TCR–MHC complex, these adhesion molecules play a unique and important role in T cell activation. They enhance the sensitivity of T cells to antigen because they can hold the two membranes at the perfect distance to maximize the 2D affinity of the TCR. Lateral interactions may also promote efficient packing of TCRs and accessory molecules to achieve high local protein density and increased membrane rigidity.

a T cell (Valitutti et al., 1995). Assuming that receptor engagement results in internalization, they measured the number of surface TCRs after stimulation and found a correlation between the internalization of 8000 receptors and T cell activation (Viola and Lanzavecchia, 1996). Using T cells containing two different TCRs, they showed that internalization is specific, as it requires specific antigen recognition.

Because of differences in half-lives, it is not surprising that a single MHC–peptide complex can trigger multiple TCRs. MHC–peptide complexes can have half-lives on the order of hours (Nelson et al., 1994), while the association of the TCR with MHC–peptide has a half-life of seconds (Matsui et al., 1994). Thus, assuming that unliganded TCRs can be recruited and held together in the contact area, the high chemical off rate of the TCR–MHC complex will result in TCR–MHC partner swapping. Although partner swapping has not been directly demonstrated for the TCR–MHC interaction, it has been demonstrated for the CD2–CD58 interaction (Dustin et al., 1996a). Thus, the potency of any particular peptide–MHC complex will be related to both its overall affinity and its off rate. The affinity (2D Kd) of the TCR for the peptide–MHC complex will determine the minimal number of receptors that can be engaged at equilibrium. The off rate will determine how many additional TCRs can be engaged by a single peptide–MHC complex.

Kinetic Models of T Cell Activation

Recently, McKeithan and McConnell separately proposed models of T cell activation that they call ''kinetic proofreading'' or ''kinetic editing'' models, respectively (McKeithan, 1995; Rabinowitz et al., 1996). These models were proposed to explain how a low affinity receptor, the TCR, could distinguish between small differences in antigen. The TCR, for example, which makes contact with only a few amino acid side chains of the antigenic peptide, can easily distinguish single, conservative amino acid changes in the peptide (Evavold et al., 1993).

These models propose that small differences in receptor affinity correlate with differences in the duration of receptor engagement. Because T cell activation requires the assembly of multiple layers of proteins to the phosphorylated TCR (Chan and Shaw, 1996), a certain amount of time is required to assemble the correct signaling complex. Thus, the formation of the complete complex will require stimuli that exceed a certain threshold of strength and duration. Altered peptide ligands (APL), which are slightly lower affinity ligands for the TCR (Lyons et al., 1996), stimulate the TCR but are unable to sustain signaling long enough to generate the complete signaling complex. Thus, the system allows the TCR to respond in an off or on fashion to antigenic stimuli within a very narrow affinity range. These models are a breakthrough in our understanding of TCR signaling.

A key feature of these kinetic models is the importance of signal duration. But these models do not address exactly how signal duration is achieved, assuming simply that signal duration is directly related to receptor affinity. Because of issues of TCR size and affinity, it seems likely that signal duration will be closely related to the stability of the cell–cell contact formed. Extracellular binding events, which are largely dependent on 2D affinities, will therefore determine the duration of TCR signaling.

Can we integrate these concepts of 2D Kd, serial triggering, and kinetic proofreading into a model of TCR recognition and activation?

Initiating Interactions between T Cells and APC

Cells adhering to other cells must overcome their tendency to repel each other owing to their net negative surface charge (Springer et al., 1987). The attractive force necessary to overcome this barrier is mediated

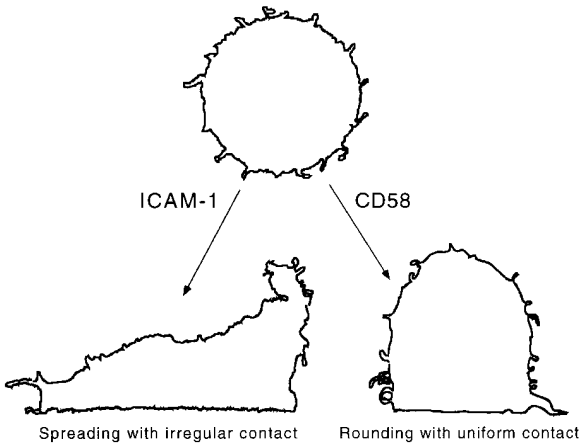

Figure 3. Lymphocytes Plated on ICAM-1 or CD58 Exhibit Distinct Behaviors

A round lymphoblast displays dramatically different behavior when it lands on a substrate coated with ICAM-1 (CD54) or CD58. ICAM-1 is the ligand for LFA-1 and mediates cells spreading with a highly irregular cell–substrate separation. In contrast, CD58 is the ligand for CD2 and maintains a rounded cell morphology with a regular cell–substrate spacing. These profiles were traced from electron micrographs of human T lymphoblasts on planar bilayers containing the respective ligands (Dustin and Springer, 1988). Original electron micrographs are courtesy of Dr. J. Caulfield.

largely by the action of adhesion molecules such as CD2 and LFA-1.

CD2 and LFA-1 represent two different ways to provide adhesion, differing in topology, affinity, and cellular responses. CD2 is a small molecule that is buried in the glycocalyx, while LFA-1 is relatively tall and extends above the glycocalyx. CD2 mediates adhesion through many low affinity bonds (Dustin et al., 1996a; Figure 3, left). In contrast, LFA-1 mediates adhesion with a few high affinity bonds (Lollo et al., 1993; Figure 3, right). Most important for our discussion, they exhibit distinct cellular responses: T cells plated on a CD2 ligand generate a small, circular contact area, while T cells plated on ICAM-1, an LFA-1 ligand, generate a broad contact area (Dustin and Springer, 1988; Figure 3). What is the significance of these distinct properties?

Clustering allows CD2 to be recruited into the TCR contact, while spreading allows LFA-1 to be pushed away from the TCR contact (Dustin et al., 1996b). CD2 clustering produces a high local density of CD2–CD58 bonds (Dustin et al., 1996a; Moingeon et al., 1991). This allows CD2 to generate an attractive force sufficient to bring together the membrane of the T cell and the APC to $\sim$15 nm. On the other hand, LFA-1 spreading strengthens cell–cell adhesion by broadening the cell contact, enhancing CD2 and TCR engagement, and reducing bond strain on the potentially more fragile TCR–MHC and CD2–CD58 interactions. In addition, the few LFA-1 bonds can be readily displaced from the center of the contact because they are sparsely distributed over a large area. Thus, LFA-1 is a general adhesion molecule helping cells make contact, while CD2 appears to be more specifically adapted to helping the TCR make contact.

These distinct behaviors are not passive, but are specifically orchestrated by the T cell cytoskeleton. Consistent with this, cytoskeletal inhibitors can potently inhibit T cell activation (Valitutti et al., 1995). These processes reflect the need of the T cell to rapidly reorganize and concentrate accessory molecules and receptors at the contact membrane when engaged by an APC. This mechanism of rapid protein aggregation at one pole of the cell is reminiscent of the phenomenon of immunological capping, first described over 25 years ago, in which cross-linking of membrane proteins results in clustering and aggregation of proteins at one pole of the cell (Unanue et al., 1972). Selective capping, by increasing surface density of topologically similar molecules, would be a simple and rapid way to increase the 2D affinity of a membrane receptor.

The formation of the contact cap may also be stabilized by lateral interactions. Recently, it was shown that the presence of CD8 increases the avidity of the TCR by slowing the off rate (Garcia et al., 1996b). Lateral interactions might allow these molecules to pack together in organized protein lattices. The involvement of many different proteins in the contact suggests that a specific packing arrangement of the TCR with accessory molecules might be important for efficient engagement of MHC–peptide. It was recently proposed that the CD4 might play such an important organizing role (Sakihama et al., 1995).

The formation of these lateral arrays would have important effects on TCR engagement. First, concentrating the TCR and accessory molecules would enhance their local density. Second, these concentrated areas of similarly sized proteins would then generate a low or ''bald'' area facilitating engagement of the TCR by peptide–MHC. Third, tight clustering of molecules in the contact cap would increase the rigidity of the membrane, enhancing the 2D affinity of interacting membrane proteins. Finally, high concentrations of TCR may promote serial engagement by forcing MHC–peptide complexes to percolate through a dense field of TCRs.

Protein Size and Threshold Formation

Based on the short height of the TCR–MHC complex, receptor engagement dictates that not just LFA-1 but larger proteins in general be excluded from cell–cell contacts. A threshold level of force is therefore required to drive these proteins from the contact. Many factors can contribute to determining how much force is required to exclude these larger proteins from the contact, including the density, the size, and the charge of these larger proteins.

The surface of the plasma membrane is covered by a negatively charged glycocalyx, contributed mainly by sialic acid linked to N- and O-linked oligosaccharides of glycoproteins and glycolipids (Despont et al., 1975; Springer et al., 1987). This forms a cloud of negative charge averaging about 20 nm deep on the surface of lymphocytes. The presence of CD45 and CD43 can extend this surface even higher (Cyster et al., 1991). When the T cell and APC initiate contact, the two $>$20 nm high glycocalices, totaling $>$40 nm, must be compressed to approximately 15 nm to allow TCR engagement to occur.

The glycocalyx responds to this compression by extruding tall glycoproteins that are not specifically held in the contact.

In addition to vertical/compressive forces, lateral forces are probably also important. One physical principle is that the cell tries to maintain an optimal average spacing of like (predominantly negative) charges. Crowding/clustering of the TCR, CD2, and CD28, which are held in the contact cap via ligand binding, should result in a redistribution of unliganded, highly charged molecules such as CD45 and CD43 outside the area of crowding. Thus, the contact cap is organized under two major principles: exclusion of large, charged unliganded molecules, and the clustering of receptors based upon interactions with ligands.

The function of tall, highly glycosylated proteins such as CD43 and CD45 seems likely, therefore, mainly to set activation thresholds; they provide a repulsive force that the cell must overcome before TCR engagement can occur (van der Merwe and Barclay, 1994). Activation thresholds could be regulated by varying levels of expression of these proteins as well as their size, charge, and initial proximity to the TCR. For example, if large forms of CD45 interact laterally with the TCR in some cells, this would increase the activation threshold by forcing the avidity of the initial contact to drive away CD45. In support of this, expression levels of both CD43 and CD45 vary during hematopoiesis, and both molecules exhibit developmentally regulated changes in size and charge density (Ellies et al., 1996; Thomas, 1989). The regulation of these physical characteristics is likely to be critical for T cell function.

For example, memory T cells are characterized by a low threshold of T cell activation and by the expression of a distinctive marker, CD45RO. CD45RO, generated by mRNA splicing, is the smallest, least glycosylated form of CD45 (Thomas, 1989). This suggests that the low thresholds of T cell activation in memory T cells are directly related to the expression of smaller isoforms of CD45. Similarly, positive selection of thymocytes requires a 10-fold lower level of TCR–MHC affinity compared with activation of mature T cells (Alam et al., 1996). It is interesting to speculate that this difference in threshold may be related to the 5-fold lower level of sialic acid and reduced CD43 on the surface of thymocytes (Despont et al., 1975; Ellies et al., 1996). Experiments using transgenic and knockout animals support the idea that CD43 also sets T cell thresholds. T cells from CD43 knockout animals have increased adhesion and sensitivity to T cell activation (Manjunath et al., 1995). The role of these proteins in setting thresholds potentially explains the regulated expression of these proteins in T cell development and can explain why these proteins are alternatively spliced.

Phosphatase Exclusion and Signal Initiation: The Special Case of CD45

Based on its size, the removal of CD45 should have a positive effect on T cell activation, but it does not; T cells lacking CD45 cannot be activated (Pingel and Thomas, 1989). As CD45 is a tyrosine phosphatase, this suggests that its enzymatic activity and its size are critical for its function. The current dogma suggests that CD45 is required for T cell activation because it is required for activation of $p56^{lck}$ (Ostergaard et al., 1989). $p56^{lck}$ is regulated by phosphorylation of tyrosines at positions 394 (Tyr-394) and 505 (Tyr-505). Phosphorylation of Tyr-394 is required for full activity of $p56^{lck}$, and phosphorylation of Tyr-505 inhibits $p56^{lck}$ kinase activity. In resting T cells expressing CD45, most (>80%) of the $p56^{lck}$ molecules are dephosphorylated at both Tyr-505 and Tyr-394. Therefore, most of the $p56^{lck}$ molecules are in a partially active state (Cooper and MacAuley, 1988). Given the presence of active $p56^{lck}$, why are there so few tyrosine phosphorylated proteins in resting T cells?

One possibility is that although CD45 activates $p56^{lck}$ by dephosphorylating it, CD45 keeps $p56^{lck}$ impotent by rapidly dephosphorylating any substrate phosphorylated by $p56^{lck}$ or by keeping the critical activating tyrosine, Tyr-394, unphosphorylated. Consistent with this idea, it has been reported that CD45 is associated with $p56^{lck}$ in resting T cells (Guttinger et al., 1992). Thus CD45 has a dual function: to activate kinases such as $p56^{lck}$ and also to maintain the resting state of dephosphorylation. For $p56^{lck}$ to phosphorylate a substrate stably, CD45 must be physically separated from $p56^{lck}$ or its substrate. The function of CD45 to promote a general state of dephosphorylation in the resting cell is supported both by the abundance of CD45 (about 10% of the surface) and also by its K_{cat} toward tyrosine-phosphorylated substrates (about 3 logs higher than the K_{cat} of tyrosine kinases toward their substrates; Fischer et al., 1992).

Based on its size, it has been proposed that CD45 is actively excluded from cell contacts (Davis and van der Merwe, 1996). In contrast, the tyrosine kinases $p56^{lck}$ and $p59^{fyn}$ are held in the contact via their association with CD4 and CD3, respectively. Thus, removal of CD45 from the proximity of these enzymes promotes kinase activity and subsequent phosphorylation of substrates localized in the contact cap such as the TCR. Because CD45 is required for src kinase function, T cell activation cannot proceed in the absence of CD45. Thus, the decreased threshold gained by loss of CD45 is nullified by the inability of src kinases to be activated.

This model is significant because it suggests that receptor clustering is insufficient by itself to initiate TCR signaling. Rather, it is phosphatase exclusion that initiates T cell activation. Receptor activation, in this model, is not directly linked to ligand engagement, but to contact cap formation. The contact generates an area that is relatively free of phosphatase activity. Any receptor that is present in the cap is then activated because of the presence of active kinases associated with the TCR and coreceptors. A key variable is the concentration of TCRs in the cap. This will partially depend on the affinity of the TCR toward its antigen. However, the kinetics of this interaction are also important. For example, a high affinity interaction with a slow off rate would limit the the number of recruited TCRs to the same number of specific MHC–peptide complexes. But a high affinity combined with a fast off rate will allow the same affinity to recruit many more TCRs to the contact cap via the process of serial engagement. Once engaged, TCRs may be held in the contact area by lateral interactions

with coreceptors and accessory molecules and cytoskeletal interactions (Rozdzial et al., 1995; Symons et al., 1996).

Serial Triggering: A Counter or a Timer?

The serial triggering model proposes that single hit engagement by ligand induces internalization of the activated receptor. When approximately 8000 receptors have been internalized, T cell activation ensues (Valitutti et al., 1995; Viola and Lanzavecchia, 1996). Thus, the model infers that the cell ''counts'' how many receptors have been internalized. However, the threshold number is flexible. For example, the participation of costimulation (CD28) significantly lowers the number of receptors required for activation (Viola and Lanzavecchia, 1996).

But does the cell really internalize each TCR immediately after receptor engagement? It seems equally plausible that engaged TCRs are held in the contact cap for signaling and then shed (Dustin et al., 1996b) or internalized en bloc after disengagement from the APC. This distinction is important for our understanding of TCR signaling kinetics. Receptor occupancy of the TCR for at least 2–4 hr is required before activation events such as interleukin-2 (IL-2) secretion can occur (Kumagai et al., 1987; Weiss et al., 1987). However, receptor internalization occurs within 30–60 min of receptor engagement with antibodies (Krangel, 1987). Thus, immediate internalization of engaged receptors might not be able to sustain signaling long enough for T cell activation, particularly if the stimulus is strong, in which case the TCR would be rapidly depleted from the surface. For these reasons, successful T cell activation will likely involve a mechanism that prevents, rather than promotes, internalization. Packing of proteins into the contact cap might be one way internalization is inhibited. When the protein density in the contact cap is high, the TCR is interacting laterally with other surface molecules bound to ligands on the other cell. It is difficult to envision how the cell could isolate a lone activated receptor and internalize it under these conditions. Furthermore, if, as we argue, T cell activation is triggered mainly by phosphatase exclusion, receptor activation is not directly linked to receptor engagement. Rather, receptors are activated because they are located in zones that are free of phosphatases in the contact cap.

Rather than count activated receptors, the cell could easily time responses by using either the initiation of new transcriptional events or the assembly of large, multilayered protein complexes as a clock. The 2–4 hr time requirement for TCR engagement thus could represent the time required to form a specific signaling complex and/or for the synthesis of a specific factor required to allow the cell to cross a ''checkpoint.'' The ability to sustain TCR engagement and signaling is thus of critical importance.

Another Paradigm for the Function of Costimulation?

Most current models support the idea that two independent signals are required for T cell activation. The TCR provides the first signal and a requisite second signal is provided by a costimulator molecule. Of numerous molecules that have been implicated as costimulators, the best candidate is CD28. Antibodies to CD28 or its ligand B7 can potently inhibit T cell activation, suggesting that CD28 ligation is required for T cell activation. The current paradigm is that CD28 engagement activates a specific mitogen-activated protein (MAP) kinase, the jun kinase or JNK, whose activation is required for the induction of cytokine gene transcription. (Su et al., 1994). Surprisingly, however, T cells from CD28 knockout animals can still be activated (Shahinian et al., 1993). This suggests that CD28 signaling is not required for T cell activation.

What is the function of CD28? Does it truly transduce an independent signal required for T cell activation? To date, despite intensive efforts, no convincing CD28 signal has been identified (Shahinian et al., 1993; Su et al., 1994). Rather, CD28 ''signaling'' generally requires coengagement of the TCR. This dependence on the TCR suggests that costimulators function mainly to enhance or modify TCR signaling and do not signal independently by themselves.

One way to think about costimulators is that they enhance the strength or duration (or both) of signaling by the TCR by enhancing antigen presentation or the stability of the contact cap. In fact, studies with the CD28 knockout mice suggest that the presence of CD28 is critical only for antigens that have a short half-life (Kundig et al., 1996). CD28 knockout mice could not mount an immune response to a peptide, which is rapidly cleared from the mouse in hours, but had no difficulty mounting a response to a viral infection, which is present in the mouse for several days. This implies that CD28 functions mainly to enhance the duration or efficiency of antigen presentation. CD28 could function purely as an adhesion molecule that enhances the 2D affinity of the TCR and/or might facilitate efficient packing of the TCR at the cell contact.

The signals mediated by costimulators could be explained if longer duration TCR signaling allowed distinct downstream pathways to be activated. For example, the extracellular signal–regulated protein kinase (ERK) pathway might be activated quickly by TCR engagement, while activation of the JNK pathway might require more sustained TCR signaling. Theoretical models, in fact, suggest that MAP kinase cascades are designed to respond in an all-or-none fashion to stimuli once a particular threshold is reached (Ferrell, 1996). In the case of the TCR, the threshold for JNK activation may be higher than the threshold for ERK activation. This is also another way in which the cell can time a response.

Costimulators may also function to potentiate T cell activation by helping to recruit signaling proteins or by enhancing the activation of tyrosine kinases. For example, the presence of a phosphatidylinositide 3-kinase–binding site in the CD28 cytoplasmic tail suggests that association of CD28 with the the TCR might be one way in which phosphatidylinositide 3-kinase is recruited to the TCR signaling complex. The finding that proline-rich src homology 3 (SH3) ligands are strong activators of src kinases suggests that the recruitment of SH3 ligands may play a major role in the activation of src kinases (Moarefi et al., 1997). In this regard, it is interesting to note that CD28 and CD2 contain two and five potential SH3-binding sequences, respectively.

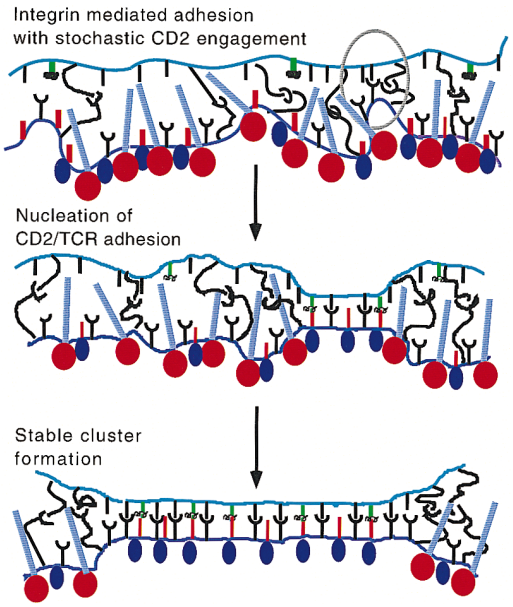

Figure 4. Topological Model for T Cell Activation

(Top) Cell to cell adhesion is initiated by integrins that are not inhibited by charge resulsion. This creates opportunities for smaller adhesion molecules or antigen receptors to engage ligands (circled event in top panel; checkpoint 1).

(Middle) TCR engagement and initiation of phosphatase exclusion (checkpoint 2).

(Bottom) If sufficient TCR ligand is present, the regions of phosphatase exclusion can coalesce to form a contact cap (checkpoint 3). If the contact cap is sustained for several hours, a naive T cell can be activated to produce cytokines and proliferate.

Key: adhesion molecules, black; TCR, red bars; MHC peptide, green bars; tyrosine kinases, red balls; tyrosine phosphatase CD45, blue ovals.

A Model of T Cell Activation

Figure 4 depicts our sequential model of T cell activation. This model requires that the quiescent T cell pass through three thresholds or checkpoints to reach full activation. Time is a key parameter in this model. Integrins are likely to provide the initial cell–cell contact. The first threshold is adhesion that can overcome charge repulsion between cells. This depends on accessory molecules and the 2D Kd of the antigen receptor for the MHC–peptide complex. This takes seconds. The next threshold is formation of zones of phosphatase exclusion that allow the TCR to initiate signal transduction as manifested by events such at tyrosine phosphorylation and cytoplasmic Ca^{2+} increases. This requires active cytoskeletal concentration of accessory molecules and the TCR to drive out large, unligated molecules such as CD45. This takes minutes. The final threshold is the maintenance of a stable contact cap, which is the fundamental signaling unit. This will depend on having enough TCR ligand to maintain the cytoskeleton-driven clustering and to resist phosphatase infiltration and electrostatic repulsion. If successful, this will take several hours.

This is a self-strengthening system because TCR signaling strongly potentiates the stability of the cell contact and vice versa. The amount of TCR signaling required to maintain the contact will depend upon the constituents of the contact cap; Lanzavecchia found that 8000 TCRs were required when CD2 and CD4 were available, while only 2000 TCRs were required when CD28 was added to the mix (Viola, 1995). If the contact cap is the fundamental signaling unit, the addition of CD28 would strengthen the contact cap. Thus, the number of TCRs and the strength of TCR signaling needed to maintain the cap is decreased. CD28 can contribute an adhesive force by binding to its ligand, B7, but may also facilitate the packing arrangement of molecules in the contact cap.

Each of the sequential checkpoints is more demanding of the TCR–MHC interaction. Thus, it is possible for the process to stall out at different points, leading to different nonsignaling and partial signaling phenomena (Evavold et al., 1993). A diversity of outcomes is generated through a series of all-or-none signaling thresholds in which the 2D Kd, serial ligation/contact cap formation, and kinetic editing determine the ability of a specific TCR to reach the thresholds.

What Is the Mechanism of Antibody-Mediated Activation of T Cells?

In this review, we have focused on the role of cell–cell contacts in the initiation of TCR signaling. Provocatively, we have argued that receptor cross-linking is not sufficient by itself to activate the TCR, but rather that it is phosphatase exclusion mediated by cell–cell contact that is critical. If this is true, how do antibodies activate T cells?

One possibility is that antibody cross-linking of the TCR generates membrane protein aggregates that are relatively devoid of CD45. Although antibody cross-linking induces strong signaling, it might not always result in prolonged signal duration because of receptor down-modulation (Krangel, 1987). That is probably why solution cross-linking of the TCR is not as good as solid-support cross-linking of the TCR at inducing IL-2 secretion (Kubo et al., 1989). Antibody-mediated cross-linking of costimulator molecules might function by affecting the rate of receptor down-modulation or by facilitating the efficient packing of TCRs to increase phosphatase exclusion.

A Generalized Paradigm for Adhesion?

The model we have proposed may have general relevance to our understanding of receptor tyrosine phosphatases and the formation of adhesion complexes. Given the ubiquitous and abundant expression of receptor tyrosine phosphatases, the principle of contact caps and phosphatase exclusion may be a general principle important in adhesion formation. Issues of protein density, size, and charge will dictate that membrane protein reorganization will occur when cells make contact with other cells or extracellular matrix. It is interesting that receptor tyrosine phosphatases were recently implicated in the regulation of focal adhesions and adherens junctions (Brady and Tonks, 1995).

In this review, we have tried to integrate recent work based on T cell adhesion with current ideas about TCR signal transduction. Currently, researchers studying the mechanism of T cell activation are basically divided into

two groups: those studying extracellular interactions and those studying intracellular interactions. We have tried to demonstrate how issues of contact cap formation, the role of adhesion molecules, and the kinetics of membrane protein interaction impact on our ability to understand TCR signaling events. It is becoming increasingly clear that the regulation of intracellular signaling events involves extracellular binding events. The challenge for the next 5 years will be to achieve an integration of these two areas.

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