How and Why Does the Immunological Synapse Form? Physical Chemistry Meets Cell Biology

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Introduction

T lymphocytes (T cells) play an important role in orchestrating an adaptive immune response to foreign pathogens. A key event for T cell activation is an appropriate interaction between the T cell antigen receptor (TCR) and a limited number of foreign major histocompatibility complex (MHC)-peptide (MHCp) complexes displayed on the surface of the antigen-presenting cell (APC) (*1*, *2*). Other costimulatory and adhesion molecules also bind in the cell-cell junction. Much attention has been devoted to these extracellular binding events (*3*, *4*), as well as to the intracellular steps involved in signal transduction pathways (*5*) implicated in T cell activation. Most of these individual events are fast compared to the time scale over which full T cell commitment is achieved. An important recent discovery is that T cell recognition of antigen is accompanied by the formation of a specialized cell-cell junction that has been labeled the immunological synapse (*6*-*9*). The mature synapse is characterized by a specific pattern of segregated cell surface molecules in the T cell-APC junction (*7*, *8*). Striking images obtained with video microscopy (*7*, *8*) demonstrate that the mature synapse is several microns in diameter, evolves over many minutes (~30 min), and is sustained for well over an hour. Investigating these slow processes may provide mechanistic insights that have not been easily accessible by studying the fast events noted earlier.

Although data on synapse formation and the underlying molecular events are emerging at a rapid rate, several questions concerning the genesis of the synapse and its biological function remain unresolved. These questions are motivating a plethora of experiments. The resulting wealth of data must be understood in terms of a mechanistic framework if it is to be useful for controlling the immune response. In this regard, quantitative models can be a useful complement to experiments, because they can help develop mechanistic hypotheses that are experimentally testable.

Here, attention is focused on a proposed quantitative model for immunological synapse formation (*10*). Results from this model suggest that early signaling events provide a dynamic cellular environment that enables self-organization processes, which lead to the large-scale redistribution of cell surface receptors observed during synapse formation. I describe how comparing model predictions for conditions amenable to robust synapse assembly with experimental data leads to two intriguing and experimentally testable hypotheses. One concerns the role that certain null peptides can play in synapse formation. The other proposes different functions for the synapse in immune responses that involve gene transcription in the nucleus from those that do not.

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Large-Scale Self-Organization Follows Early Signaling Events to Form the Synapse

In vitro experiments cannot address many issues pertinent to in vivo interactions between T cells migrating in chemokine gradients and APCs. However, in vitro experiments (*7*-*9*, *11*) have provided data on the redistribution of membrane proteins in the junction between T cells and APCs or between T cells and supported bilayers that mimic an APC. For example, Grakoui *et al*. (*8*) used a bilayer containing fluorescently labeled MHCp (a TCR ligand) and intercellular adhesion molecule (ICAM) (a ligand for the integrin LFA1 on the T cell) as an APC mimic. Video microscopy was used to monitor membrane protein redistribution during the interaction of live T cells with the bilayer. Shortly (less than a minute) after the T cell ceases to crawl, a central region of integrins (LFA1 and ICAM) surrounded by a peripheral ring of TCRs and MHCp's is observed. The T cell and the bilayer are in closest apposition at the periphery. Over many minutes, this pattern inverts (Fig. 1, upper panels), and a mature synapse with TCR-MHCp in the center [called the central supramolecular activation cluster (cSMAC)] and a peripheral ring of integrins [called the peripheral supramolecular activation cluster (pSMAC)] emerges. Now, the T cell and the bilayer are in closest apposition at the center of the junction (Fig. 1, panel A). Figure 2 is a schematic illustration of the basic phenomenology that is observed. Synapses with ICAM at the periphery and MHCp clustered in the middle have been observed in experiments with living APCs as well (*7*, *9*, *11*).

What are the processes that underlie the large-scale assembly of synaptic patterns? A mathematical model has recently been proposed for interactions between flexible membranes containing complementary sets of receptors and ligands, which will be referred to as the synapse assembly model (*10*). The model aims to describe experiments such as those by Grakoui *et al*. (*8*). The basic processes considered are receptor-ligand binding and dissociation kinetics, intramembrane receptor protein mobility, shape changes of the cell membrane attached to a cytoskeleton, and TCR down-regulation (*12*). The synapse assembly model proposes specific ways in which these individual processes influence each other (*10*). For example, the binding and distribution of receptors is strongly influenced by the free energy cost of bending the cell membrane to accommodate receptor-ligand pairs with different topographic sizes (the TCR-MHCp pair is ~15 nm, and the LFA1-ICAM pair is ~42 nm).

Each individual phenomenon (such as binding or receptor mobility) is determined by molecular and microscopic processes that occur on short length scales. The synapse assembly model does not resolve details of processes that occur on short (molecular) length scales, nor do the imaging experiments (*7*-*9*). Rather, it describes how these processes are inextricably coupled to each other and how cooperativity among these phenomena leads to receptor protein redistribution on large (nanometers to micrometers) length scales.

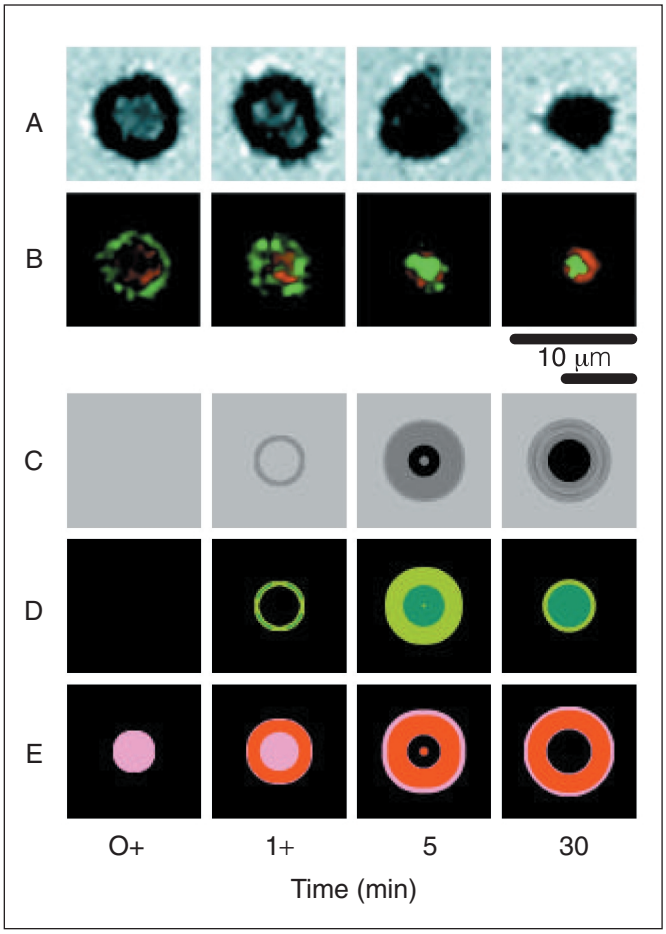

Fig. 1. (**A** and **B**) Results from experiments done by Grakoui *et al.* (*8*), in which a T cell is interacting with a supported bilayer mimic of the APC. These images were taken looking down from above the T cell. (A) Time evolution of the shape of the T cell during synapse formation. The darker the color, the closer the apposition between the T cell membrane and the supported bilayer. (B) Overlay of MHCp (green) and ICAM (red) concentrations in the intercellular junction. Movies that make these observations of the spatiotemporal evolution of protein patterns and cell shape vivid can be seen at (http://www.sciencemag.org/feature/data/1040037.shl). (**C** through **E**) Results of calculations done by Qi *et al.* (*10*) using the synapse assembly model. (C) The evolution of cell shape. Again, the darker the color, the closer the apposition between the two membranes. (D) The evolution of MHCp concentration (green). (E) The evolution of ICAM concentration (red). The different shades of color in (D) and (E) reflect different levels of concentration, with darker colors corresponding to higher concentrations. The similarity between the results of the synapse assembly model (*10*) and experimental observations shown in (A) and (B) is striking.

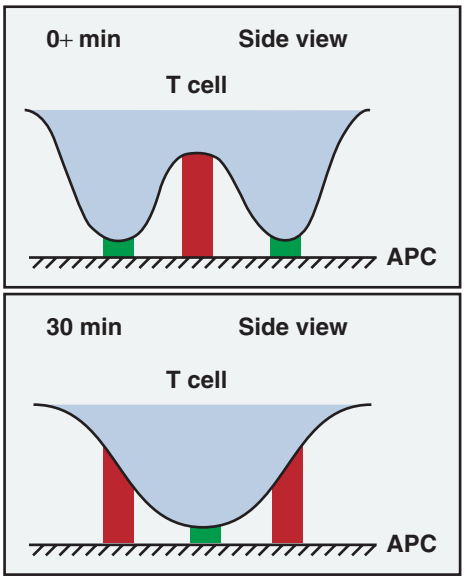

Fig. 2. Schematic illustration (side view) of the results shown in Fig. 1. MHCp (green), ICAM (red).

Processes that occur on molecular length scales determine specific measurable quantities. For example, ligand binding and dissociation kinetics and the size of the receptor-ligand complexes are determined by the specific molecular identity of the receptors. The rates at which receptors move laterally in the membrane and mechanical characteristics of the cell membrane are determined by cytoskeletal processes (for example, those involving the molecules talin, myosin, and moesin) that control the microscopic environment of the cell membrane. The value of the magnitude of forces due to each specific short-length-scale process that occurs in the cellular environment is manifest in the synapse assembly model as a parameter whose value must be supplied in order to solve the partial differential equations.

Early signaling events most likely determine the specific values of these measured parameters. TCR-associated phosphorylation and intracellular calcium concentrations peak within a minute of cell-cell contact (*13*-*20*). The associated signaling events may regulate the magnitudes of the forces arising from the short-length-scale processes. Given that such cellular events set the values of parameters describing short-length-scale processes in the proposed synapse assembly model (*10*), one can ask the following question: Can the large-scale redistribution of proteins that characterizes synapse formation result from self-organization processes determined by the way in which short-length-scale processes influence each other in the proposed model? Using values of parameters that correspond to the experiments done by Grakoui *et al.* (*8*), the model (*10*) predicts spontaneously emergent large-scale patterns that closely resemble the spatiotemporal evolution of protein patterns and cell shape observed in the experiments (Fig. 1, lower panels).

There have been debates about whether synapse formation is an "active" or "passive" process (*7*, *21*, *22*). The synapse assembly model suggests an alternative way to think about synapse formation. Rather than classifying processes as active or passive, it is instructive to think in terms of processes that occur on short and large length scales. Many cellular processes that occur during T cell recognition of antigen, some of which are regulated by early signaling events, occur on short length scales. These short-length-scale processes influence each other because they involve receptors and ligands embedded in a common cell membrane linked to a cytoskeletal complex. Based on physical concepts, the synapse assembly model proposes specific ways

in which these processes are coupled. Results from the model show that the individual short-length-scale processes act in concert to enable large-scale redistribution of membrane proteins leading to synapse formation.

The integrins LFA1 and ICAM bind first in the middle of the cell-cell junction, simply because they are longer than the TCR-MHCp complex. This discourages binding of TCR-MHCp in the central region, because accommodating two pairs of receptor-ligand complexes with disparate sizes in the same region necessitates bending the cell membrane into highly curved shapes. The finite bending energy of the cell membrane makes the free energy of such curved shapes high and thus unfavorable. The topographical size differences of the two types of receptor-ligand pairs, and the fact that they are embedded in a cell membrane that is connected to the relatively stiff cytoskeleton, also drives segregation of MHCp and ICAM1 in the mature synapse. Two segregated receptor protein patterns are possible: MHCp inside and ICAM outside, and the reverse. The former is favored, because whereas the energy gained because of receptor-ligand binding can be the same in both instances, the free energy of bending the membrane is higher when the shorter molecules are at the periphery.

Mathematical analyses make the preceding arguments quantitative. They also make clear that formation of large-scale synaptic patterns requires a mechanism for receptor protein mobility [either diffusion or convection due to cytoskeletal flow (*22*)].

The synapse assembly model can help in the study of how changes in key molecular and microscopic features can disrupt synapse formation by changing the values of the parameters and predicting the effect on synapse formation. As noted earlier, these parameters reflect processes that occur on short length scales in the cellular environment and are determined by early signaling events.

Can quantitative models for the large-scale redistribution of cell membrane proteins during synapse formation shed light on the role of the synapse in facilitating antigen recognition and T cell effector functions? Clearly, any definitive predictions require incorporating a downstream signaling model into a synapse assembly model. However, comparisons of experimental data with the current form of the synapse assembly model can be useful. T cell effector functions depend on the identity of the peptide bound to the MHC. The kinetics characterizing the binding of a specific TCR with MHCp depend on the identity of the peptide. Do differences in TCR-MHCp binding kinetics affect synapse formation? Are fast-binding self or null peptides spectators or participants during synapse formation? If they are spectators, how do T cells recognize a few antigenic peptides from a vast pool of others? Is robust synapse assembly correlated with T cell effector functions? The juxtaposition of experimental data with model predictions for how robust synapse assembly depends on TCR-MHCp binding kinetics has suggested two intriguing, experimentally testable hypotheses pertinent to these questions (*23*). The genesis of these hypotheses is now described.

Co-Agonist Peptides Promote Synapse Formation

Values of the association constant (k_{on}) and dissociation constant (k_{off}) characterizing TCR-MHCp binding kinetics depend on the specific TCR expressed and the MHCp complex that binds across the cell-cell junction (*3*, *24-30*). Lee *et al*. (*23*) used the synapse assembly model (*10*) to compute the range of values of k_{on} and k_{off} that result in a mature synapse with the classic pattern of ICAM outside and MHCp inside. In performing these calculations, they assume that all other processes that occur on short length scales remain unchanged from those in earlier experiments. More explicitly, they assume that early signaling events that set the magnitudes of parameters describing processes, such as receptor mobility and membrane mechanics, remain unchanged when analyzing different TCR-MHCp pairs. The veracity of this assumption cannot be determined at the present time.

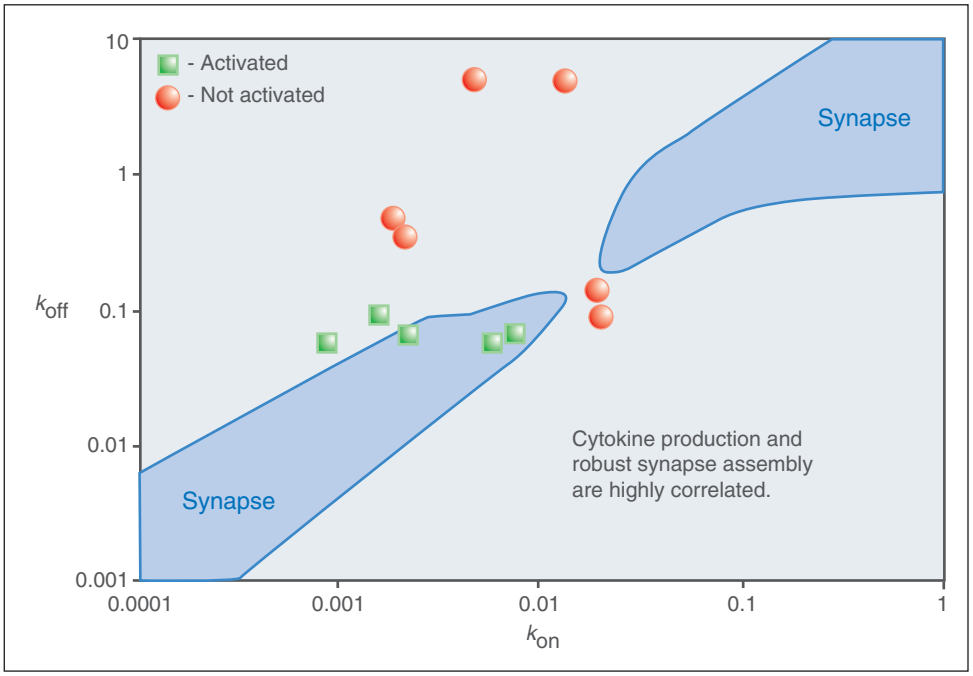

Fig. 3. Predictions of the synapse assembly model (*23*) for the dependence of robust synapse assembly on TCR-MHCp binding kinetics compared to measurements of cytokine production by specific TCR-MHCp pairs. The two regions enclosed by curves are predicted to lead to the formation of a mature synapse. The green data points correspond to TCR-MHCp pairs that lead to efficient cytokine production, and the red data points correspond to TCR-MHCp pairs that do not activate cytokine production under the conditions studied. The experimental data are taken from (*8*, *27*, *40-42*). The units of k_{off} are s^{-1} and those of k_{on} are $\mu m^2\ mol^{-1}\ s^{-1}$. The two-dimensional units used for k_{on} can be converted to three-dimensional units according to methods described in (*8*).

A mature synapse with a classic cSMAC and pSMAC pattern formed only over a narrow range of values for k_{on} and k_{off} (*23*) (Fig. 3). An interesting prediction of the model was that

certain peptides with fast binding kinetics characteristic of null or self peptides (peptides that, by themselves, do not activate T cells) can form a synapse. However, this was predicted to be true only if early signaling effects maintained the values of parameters characterizing other processes to be those corresponding to experiments with the agonist peptide MCC88-103 (the initial parameters were determined from studies with this peptide). Binding of this MHCp complex to TCR derived from the 2B4 T cell line enables synapse formation and T cell activation.

Experimental data are consistent with the predicted outcome of the model described above. Video imaging experiments have been performed with T cells interacting with supported bilayers and B lymphoma APC-containing mixtures of a null peptide mutant (MCC K99A) and the agonist peptide MCC88-103 (*8*, *31*). Experiments with 100-fold or 10-fold excess of the MCC K99A mutant showed that a classic synapse pattern forms, with accumulation of null peptides in the cSMAC (*8*, *31*). The binding kinetics of MCC K99A are estimated (*23*, *32*) to correspond to the upper lobe of the two regions in Fig. 3 that are predicted to lead to robust synapse assembly. Thus, the synapse assembly model predicts that this fast-binding peptide mutant can form a synapse. Based on this prediction and the imaging experiments (*8*, *31*), the following testable hypothesis can be proposed: Early signaling events due to the binding of a few agonist MHCp with the TCR set the magnitudes of the various forces involved in large-scale redistribution of receptor proteins to values that are the same as when higher concentrations of agonist peptide are available. Then, large-scale self-organization processes described by the synapse assembly model (*10*) enable the accumulation of certain fast-binding peptides (as MHCp-TCR complexes) in the cSMAC. Fast-binding peptides with binding kinetics corresponding to the upper curve-enclosed lobe in Fig. 3 have been labeled co-agonists by Lee *et al*. (*23*) and may contribute to how T cells can be activated by a few antigenic peptides in the midst of a vast pool of nonactivating self peptides.

Cytokine Production Versus Cytotoxic T Lymphocyte (CTL)-Mediated Killing

Comparisons of how T cell effector functions and the robustness of synapse assembly depend on TCR-MHCp binding kinetics can be useful (*23*). Using the synapse assembly model, a quantitative comparison between robust synapse assembly and cytokine production by T helper ($CD4^+$) cells was performed (Fig. 3). Qualitative comparisons (*23*) with other sets of experimental data (*25*, *26*) have also been performed and support the correlation that TCR-MHCp pairs that result in efficient cytokine production also lead to robust synapse assembly, and vice versa (Fig. 3). However, a similar quantitative comparison between data on CTL-mediated killing by $CD8^+$ cells and robust synapse assembly showed that there was no correlation between CTL-mediated killing and synapse assembly (*23*).

These comparisons suggest a different role for the synapse in $CD4^+$ and $CD8^+$ T cell effector functions. Cytokine production by $CD4^+$ cells requires gene transcription and is thus a postnuclear immune response. Cytokine production occurs over a long period and requires sustained cell-cell engagement (*33*). The TCR-MHCp interaction is insufficiently strong on its own to promote the sustained cell-cell engagement required for cytokine production. The synapse facilitates sustained engagement by confining the TCR and MHCp molecules in a small region with the two membranes in close apposition. This leads to many TCR-MHCp rebinding events that would not occur in a less confined topography (*34*), allowing sustained cell-cell engagement despite the intrinsically transitory nature of TCR-MHCp interactions. Sustained engagement produced by the synapse may facilitate binding of costimulatory molecules, such as CD28 and CD80, necessary for sustained signaling leading to gene transcription and cytokine production (*35*). Sustained engagement may also be necessary for other regulatory processes, such as TCR endocytosis (*13*).

CTL-mediated killing results from the secretion of preformed molecules in the cell and occurs within a few minutes after cell-cell engagement (*36*). CTL-mediated killing is a prenuclear response, which does not require activation of gene transcription and may not require a robust synapse to form through the mechanisms employed by $CD4^+$ cells. This may be why CTL-mediated killing does not correlate with robust synapse assembly when analyzed by the synapse assembly model. Recent experiments imaging $CD8^+$ T cells interacting with a supported bilayer containing no MHCp molecules showed that synapses form that are visually similar to those formed through TCR-MHCp interactions (*37*). In contrast, previous work demonstrated that T helper cells do not form a synapse in the absence of TCR-MHCp binding (*8*). This supports the viewpoint that $CD8^+$ engagement does not result in the formation of synapses through the mechanisms used by T helper cells. What is the role of the synapse in CTL-mediated killing? One speculation is that it may play a role in directing the secretion of preformed toxic molecules to the target cell (*38*, *39*).

Taken together, model calculations and experiments suggest that early signaling events determine the cellular environment necessary for the formation of a self-organized mature synapse. Synapse formation facilitates sustained cell-cell engagement necessary for signal transduction pathways involved in postnuclear immune responses. This hypothesis, and many other unanswered questions regarding how and why synapses form, are currently subjects of intense experimental study. Synergy between these studies and modeling efforts should lead to fundamental new insights into the role of the synapse in T cell function. The results of the experimentation and model prediction tests will lead to correction and improvement of the synapse assembly model (*10*) and hopefully allow it to be applied to other signal transduction paradigms. Such a marriage of physical chemistry and cell biology promises to be exciting.

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