CHAPTER 9

Serial Triggering Model

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Abstract

T-cells recognize a foreign antigen when presented on antigen-presenting cells (APCs) in the context of a peptide bound to major histocompatibility complex (MHC). The recognition of an antigen takes place at the T-cell:APC contact site where an "immune synapse" is formed and the multichain T-cell antigen receptor (TCR) is triggered. This initiates a signal transduction cascade that involves activation of tyrosine kinases, which in turn activate downstream events that elicit a diverse array of effector functions. T-cell activation requires a sustained signal that lasts for several hours. However, TCR affinity to its antigen is low and activation of TCR induces only a brief spike of intracellular signals. The serial triggering model resolves these seemingly paradoxical requirements for T-cell activation. The model states that sustained signaling is accomplished by the concerted action of multiple T-cell receptors that are sequentially engaged with and triggered by the peptide:MHC complex. In this chapter, we review the serial triggering model and two other models that expand this model. These models describe kinetic aspects of T-cell activation such as the pivotal question of how the T-cell "counts" the number of serially triggered receptors over time and how it determines that a threshold level has been reached for the activation of T-cell response.

T-Cell Receptor Signaling Cascade

A great deal of progress has been made in the past few years towards elucidating the cascade of signaling events and molecular mechanisms involved in the control of T-cell activation. T-cell activation is initiated in secondary lymphoid organs where T-cells encounter antigen-presenting cell (APC). T-cell activation leading to a productive response (i.e., cytokine secretion and proliferation) requires interactions of T-cell antigen receptors (TCRs) and peptides presented by major histocompatibility complex (MHC) molecules in an adhesive junction between the T-cell and APC. The main signaling pathways elicited by binding the TCR and some of the coreceptors are briefly described below.

The interaction of TCR with peptide:MHC complex (pMHC) leads to the phosphorylation of tyrosines of immunoreceptor tyrosine-based activation motifs (ITAMs) presented on the ζ and CD3 signaling subunits of the multichain TCR complex. Phosphorylation of these tyrosines by the Src family protein tyrosine kinase, Lck, promotes recruitment and activation of the nonreceptor tyrosine kinase zeta-associated protein 70 (ZAP-70) which in turn activates several target proteins including the adaptors, linker for activation of T-cells (LAT) and SH2 domain-containing leukocyte protein of 76 kDa (SLP-76). Phosphorylation of LAT and SLP-76 serves as a docking site, among others, for the SH2 domain of the phospholipase Cγ1 (PLCγ1). PLCγ1 catalyzes the formation of the second messengers, inositol 1, 4, 5-triphosphate and diacylglycerol, which trigger calcium flux and contribute to protein kinase C (PKC) and Ras activation, respectively.

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Multichain Immune Recognition Receptor Signaling: From Spatiotemporal Organization to Human Disease, edited by Alexander B. Sigalov.

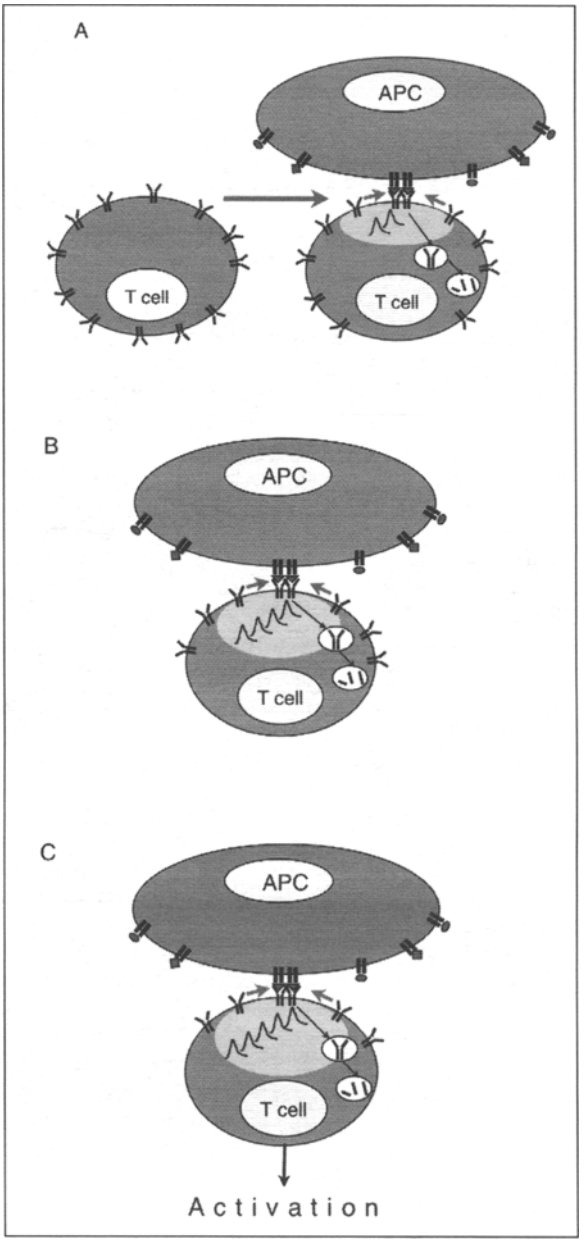

Figure 1. Naïve T-cell encounters a specific antigen on APC that presents on its surface multiple peptide antigens in association with MHC molecules (A). Following TCR triggering, signaling is initiated (red) and the triggered TCRs are internalized and degraded. New TCRs are moving into the T-cell:APC contact site where they are serially triggered by the pMHC complexes (A and B). Transient signal spike elicited by an individual TCR is represented by a curve with each spike adding to the falling phase of the one before. Eventually successive TCR-mediated transient signals are integrated up to the threshold of activation (C). Curves represent individual signal spikes.

PKC and Ras activation results in the activation of several members of the mitogen-activated protein kinase (MAPK) superfamily. The MAPKs are serine/threonine kinases that activate signaling cascades, resulting in the activation of the transcription factor NF-κB and AP-1. The transcription of several key genes involved in the T-cell response require both of these factors. Hence, the tyrosine phosphorylation cascade ultimately activates several transcription factors, such as NF-AT and AP-1, that in turn direct the transcription of new genes needed for T-cell response.

Vav1 protein expressed exclusively in the hematopoietic system is a signaling molecule that couples TCR to its effector function. Studies of Vav-deficient mice indicated that Vav is required for signaling through the TCR.[1,2] The block in $Vav^{-/-}$ T-cells appears to be in a proximal component of the TCR signaling pathway since the defect could be rescued by treatment with phorbol ester and ionomycin, pharmacological agents that mimic the downstream events induced by TCR stimulation.[1-4] Studies using Vav-deficient T-cells have indicated that Vav is the regulator of TCR-induced actin polymerization.[1,3] Defects in TCR signaling in Vav-deficient T-cells can be mimicked by treating T-cells with cytochalasin D (CD) that blocks actin polymerization. CD inhibits interleukin 2 (IL-2) production, cell proliferation and TCR capping[3,5,6] without impairing the initiation of early receptor events such as activation of tyrosine kinase, MAPK and JNK. The Wiskott-Aldrich syndrome protein (WASP) also plays an important role in the cascade linking TCR stimulation and actin polymerization. Upon TCR stimulation, both human[7,8] and mouse[4] WASP-deficient T-cells share defects in actin polymerization and T-cell proliferation with Vav-deficient cells. Thus, both Vav and WASP proteins appear to be crucial in linking the TCR signaling to cytoskeleton reorganization.

Due to the changes in the cytoskeleton, T-cells interacting with APCs undergo rearrangement of cytoskeletal elements that in turn drives the reorganization of specialized lipid microdomains and surface receptors to face the zone of contact with the APC. This active accumulation of signaling complexes and TCRs at the T-cell: APC interface results in formation of a supramolecular structure, termed "the immune synapse", that serves to increase the amplitude and duration of TCR signaling (reviewed by ref. 9). This process requires the engagement of costimulatory receptors, such as CD28.[10,11] Interestingly, in T-cells, the molecular adaptor Cbl-b has been shown to selectively inhibit TCR-mediated Vav activation, receptor clustering and raft aggregation, whereas CD28 costimulation has been found to be necessary to overcome this inhibitory effect.[12-14]

Hence, T-cell activation involves a series of signal transduction events, cytoskeleton polarization and dynamic accumulation of accessory molecules at APC: T-cell contact sites. The overall integration of these events determines the functional outcome of TCR engagement.

Serial Triggering Model

Despite knowing the major players in the signaling pathway triggered by TCR recognition of pMHC, full understanding of T-cell activation should take into account the time scale and kinetic aspects of the process.

Blocking the pMHC:TCR interaction, actin polymerization[15] or inhibiting Lck kinase activity[10] aborts TCR signaling as well as T-cell effector function. Hence, activation of TCR induces a very transient increase in tyrosine-phosphorylated intermediates and a brief spike in intracellular calcium concentration that is rapidly lost. However, a sustained elevation of signaling intermediates and intracellular calcium is an indispensable step for a productive T-cell activation.[15,16] In fact, elevated intracellular calcium levels need to be maintained for several hours to permit downstream signaling events such as NF-AT translocation to the nucleus.[17] Therefore, prolonged TCR occupancy is required to maintain sustained signaling essential for full T-cell activation.

While prolonged TCR occupancy is required for full T-cell activation, a single APC can simultaneously present multiple MHC-bound antigens, many of which are autologous peptides. Therefore, on the APC surface, the fraction of MHC molecules occupied with a specific antigen recognized by an individual T-cell is very low. It is estimated that as few as 100 pMHC complexes displayed on the APCs are sufficient to trigger T-cell activation.[18,19] This high sensitivity of TCR recognition for the low-frequency pMHC molecules and the requirement for prolonged TCR

occupancy seems to be in sharp contrast to the low affinity and the high off rate of TCR:pMHC interaction, the half-life of which is estimated to be in the range of seconds to a few minutes.[20-24] Any molecular model explaining activation by triggered receptors must reconcile the two opposing kinetic and seemingly paradoxical features of T-cell activation. On the one hand, the signal emanating from each triggered TCR is short-lived and decays in the absence of a continuous TCR triggering.[15,25-27] Yet on the other hand, T-cell activation requires intracellular signaling prolonged over several hours.

The serial triggering model proposed by Lanzavecchia and coworkers[29] reconciles these apparently paradoxical findings and explains how so few pMHC complexes can over time engage enough TCRs to transduce an activation signal. In fact, they have shown that it is the low affinity of the TCR:pMHC interaction that allows for the high sensitivity of T-cells scanning for the rare antigen bound to the MHC molecule. According to the serial triggering model, a single pMHC complex can engage and trigger up to 200 TCRs one after the other and thus enables a small number of pMHC complexes to achieve a high and prolonged TCR occupancy. This process of TCR serial triggering takes place in the immune synapse where a pMHC complex presented on the APC surface triggers a TCR on the T-cell surface. The latter dissociates shortly after its activation, due to its low affinity and high off-rate and gets internalized and degraded (Fig. 1A). The pMHC complex is now available to engage with a new TCR that has transited to the APC:T-cell contact site where TCR triggering transpires (Fig. 1B). The continuous supply of new TCRs to replace those dissociated and consumed is derived by the cytoskeleton rearrangement. These dynamic changes in surface molecular topology and immune synapse organization are important in sustaining TCR signaling. Hence, the low affinity binding of the TCR to pMHC complex plays an important part in achieving serial triggering as it allows optimal turnover of the TCR:pMHC interaction (reviewed by refs. 30,31).

Flexible and Hierarchical T-Cell Activation Thresholds

Commitment of T-cells to cytokine production and proliferation requires TCR signaling sustained for as long as several hours, achieved through serial engagements. Upon productive interaction with the pMHC molecule, the triggered TCR initiates a signaling cascade and is subsequently internalized and degraded.[32] Experimentally, the extent of TCR downmodulation from the T-cell surface enables researchers to measure the number of receptors triggered before the onset of activation.[33] It has been shown that T-cell activation is elicited when a defined number of TCRs are triggered and internalized. However, it was further shown that different responses are elicited at different activation thresholds, as measured by the number of triggered and internalized TCRs. Cytotoxic T-cells (CTLs) can kill a target cell displaying as few as a single antigenic pMHC complex that triggers only a very small number of TCRs.[34] On the other hand, cytokine response requires significantly higher levels of antigen concentrations and TCR occupancy.[29] Single-cell analysis established the existence of a hierarchy in TCR signaling threshold for induction of distinct cytokine responses.[35] For example, IL-2 production requires higher TCR occupancy than interferon-gamma (IFN-γ). These single-cell studies further demonstrate that the hierarchy in activation thresholds observed in cell populations reflects the hierarchy that takes place at the individual cell level. As a result of this mechanism, antigen dosage may dictate not only quantitative but also qualitative aspects of the immune response due to changes in the relative levels of cytokine produced. Interestingly, the induction of IL-4, a Th2-like cytokine, has been reported to require a lower antigen dose and receptor ligation as compared to IFN-γ, showing that antigen dose and the extent of TCR triggering may effect the development of distinct T helper cell phenotypes.[36,37]

It is important to note that the threshold number is flexible. Costimulation can significantly lower the number of TCRs required for individual responses[29] without affecting TCR occupancy or triggering as evident by TCR down-regulation.[10] Studies of CD28-mediated costimulation suggest that the stability of the TCR-induced phosphorylation signal is flexible and can be regulated.[10] The changes in the stability of TCR-evoked signaling mediated by CD28 result in a decreased threshold number of triggered TCRs required for activation. Taken together these

findings suggest that the stability of phosphorylation induced by an individual TCR is critical for setting the threshold number of TCRs required for activation. The effect of costimulation on TCR signal duration may result from costimulator-promoted reorganization of membrane microdomains and immune synapse maturation that stabilizes signal spikes.[10,11,38,39] On the other hand, the pregnancy-associated immunoregulatory protein, placental protein 14 (PP14; also known as glycodelin), was shown to decrease the stability of TCR-induced phosphorylated proteins[40] and to elevate the T-cell activation threshold, thereby rendering T-cells less sensitive to a given level of TCR stimulation.[41]

Temporal Summation as a Mechanism for Signal Integration

As indicated above, to fully activate T-cells, antigen-stimulated TCR signaling needs to be sustained for as long as several hours, thus requiring ongoing TCR triggering. The magnitude of the T-cell response correlates with the level of TCR occupancy,[25,26] thus pegging distinct cytokine and other functional responses to a different level of TCR occupancy at the single cell level. Furthermore, the threshold number of triggered TCRs required for T-cell activation is flexible and costimulation can significantly lower the number of TCRs required for individual responses. Given the transient nature of the TCR response, it is not clear how a T-cell integrates signaling events from multiple triggered TCRs over several hours. Thus, pivotal questions arise as to: (1) how serially triggered receptors that are promptly internalized (reviewed by ref. 32) can be "counted" by the T-cell and (2) how their transient signaling events are accumulated over time and integrated to yield a threshold level sufficient to induce a corresponding biological response.

Most kinetic models for T-cell activation do not address specifically the path followed by T-cells from serial engagements to signal strength, assuming simply that prolonged TCR occupancy is the critical event. A more recently proposed model for T-cell activation is based on temporal summation of successive signals from individual TCRs,[42] similar to temporal summation in neural cells, which translate the frequency of presynaptic signals into the size of a postsynaptic potential. Within this model, it is possible to explain how T-cells combine the effects of signals received from successively triggered TCRs. Thus, signaling intermediates produced by serially triggered TCR incrementally and locally build up, with each signaling event adding to the falling phase of the one before (Fig. 1). In this way, small and short signals that are unable to trigger a response by themselves can be summed up over time to reach a threshold level. The magnitude of the signal reflects the rate of TCRs triggering and the life-span of the signal induced by each triggered TCR. Thus, the T-cell "counts" the number of occupied TCRs by summing TCR-induced signals. When threshold amplitude is reached, activation ensues (Fig. 1C). This model suggests that there is more to activating T-cells than simple receptor occupancy. Several phenomena associated with T-cell activation, such as the flexibility in the threshold number of activated receptors required for T-cell activation, are apparent by the model.

According to this model, costimulation extends the duration of individual signals by stabilizing TCR-induced tyrosine phosphorylation,[10] thus extending the time course of an individual signal and driving signal summation. Costimulation not only reduces the level of TCR occupancy required for activation and increases the amplitude of the response, but also decreases the duration of the signaling. In contrast, factors that shorten the duration of the signal, such as PP14,[40,41] will impede signal summation. Furthermore, the temporal summation model posits that summation may occur more effectively if the triggered receptors are in close proximity. Indeed, proximity is an intrinsic characteristic of serial triggering of many receptors by a single pMHC complex that takes place in the centre of the immune synapse.[43] Nevertheless, this model can also explain how a T-cell can integrate and sum up discontinuous signals that are generated over time in a mobile T-cell moving from one APC to another.[44]

An essential feature of the temporal summation model is that signaling events originating from successively triggered TCRs are gradually accumulating over time. Using experimental conditions that provide T-cell-APC or antibody-coated bead contacts and allow for serial TCR triggering at both bulk cell population and at a single T-cell level, several studies have demonstrated that

signaling events originating from serially triggered TCR are not simply sustained but are gradually accumulated and integrated in order to generate a corresponding response.[45-48] The rate and extent of accumulation of signaling intermediates are essentially determined by the level of TCR engagement and augmented by costimulation. As predicted by the temporal summation model, a good correlation exists between the strength of the stimulus and the delay duration for the onset of the response.

Previous studies have shown that the amplitude and duration of calcium signals differentially control transcription factors shuttling to the nucleus. Consequently, the nature of the calcium signal determines the specificity of gene expression.[17,49-52] Hence, the different oscillations patterns and amplitudes of signaling intermediates (such as calcium) generated in the process of serial triggering can be translated to the shuttling of transcription factors into the nucleus at various combinations, consequently producing different biological responses. This may explain how the various threshold levels of TCR occupancy generate different T-cell responses over time.

Summary

Antigen recognition by T-cells is a very sensitive process since only a very small number of peptides presented on MHC molecules and displayed on the surface of APCs are capable of activating T-cells. Receptor engagement leads only to a transient response, while full T-cell activation requires antigen-stimulated TCR signaling to be sustained for as long as several hours. Therefore, activation of T-cells leading to cytokine production and proliferation requires ongoing TCR triggering. This triggering is achieved through serial engagement at the T-cell: APC interface in a structure termed "the immune synapse". However, signaling events originating from serially triggered TCR are not simply sustained but gradually accumulated and integrated over time to reach threshold levels required to elicit a response. Distinct responses have different activation thresholds. Thus, the nature of the antigen, its dose and the presence of costimulatory signals determine T-cell fate and the quantitative and qualitative aspects of the biological outcome. This mechanistic insight into the kinetics of signaling events during TCR serial triggering provides a useful framework to further explore the TCR-triggered intracellular pathways and the impact of TCR serial triggering on T-cell fate in particular and the immune response in general.

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