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Single-cell dynamics of T-cell priming

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The recent application of *in vivo* imaging to characterize the dynamics of T-cell activation by dendritic cells (DCs) has reshaped long-held beliefs of how adaptive immune responses are initiated. However, to improve our fundamental understanding, these new observations must be synthesized with the diverse theories and paradigms in the field, many of which were established before the advent of the cutting-edge techniques in a modern immunologist's toolbox. A number of factors have been investigated that combine to determine the ability of the DC to activate a naïve T cell: the rules that govern the ability of a T cell to find antigen-bearing DCs; the parameters that define the dose and quality of the antigenic signal; and the mechanisms used by the T cell to interpret a given antigenic signal. Considering T-cell activation to be determined by the sum of interdependent factors might allow us to integrate seemingly disparate observations and hypotheses and to formulate testable predictions for further experimentation.

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Current Opinion in Immunology 2007, **19**:249–258

This review comes from a themed issue on
Lymphocyte activation
Edited by Ulrich von Andrian and Federica Sallusto

Available online 12th April 2007

0952-7915/$ – see front matter

DOI 10.1016/j.coi.2007.04.013

Introduction

Naïve T cells are activated by interactions with antigen-presenting cells (APCs), usually dendritic cells (DCs), in secondary lymphoid organs (SLOs) [1]. To give rise to effector cells, T cells must be exposed to more than one activation signal [2], comprised of a peptide–MHC (pMHC) complex (the antigen) that stimulates the T-cell receptor (TCR) [3] as well as costimulation by B7 family members that bind to CD28 expressed on T cells [4]. T cells are also influenced by cytokine signals from the environment [5]. In this article, we review our current understanding of how interactions with DCs provide these prerequisite signals to T cells, how these interactions change over time, and how they might be regulated. We will specifically focus on the parameters that determine how information between T cells and DCs is exchanged. What controls the ability of T cells to find and contact DCs? How are antigenic signals communicated to T cells by DCs? What determines how T cells interpret such antigenic signals?

At the outset, it is important to note that the requirements for effective activation are distinct for different T-cell subsets, including CD4 versus CD8 T cells. The latter can commit to a program of proliferation and effector/memory differentiation after only short-term stimulation in the presence of appropriate cytokines [6]. By contrast, CD4 T cells need constant antigenic stimulation for sustained proliferation [7], although shorter stimulation might suffice in some settings [8]. In addition, the kinetics and consequences of T-cell activation are critically determined by initial assay conditions, such as the nature of the activating signal (e.g. chemicals such as phorbol esters and/or calcium ionophores, antibodies, mitogens, superantigens, pMHC complexes or proteins presented by professional or non-professional APCs), the prior history of the T cells (e.g. naïve versus resting memory versus proliferating, primary effector versus cell line) and the environment (e.g. immobilized ligands on two-dimensional surfaces or in three-dimensional gels, explanted tissues or whole animals, SLOs or non-lymphoid tissues). Unfortunately, there are no uniformly standardized conditions; this adds considerable complexity to any attempt to make sense of the current literature.

Beyond the issue of experimental conditions, there is also great diversity in the selection of assays used to measure T-cell activation. Commonly acquired parameters include: intracellular calcium flux; the (sometimes transient) induction of activation markers; T-cell proliferation (measured by various techniques); cytokine production; cytotoxicity; and memory cell differentiation and/or survival. All these approaches have merit, in that each measures a viable consequence of T-cell activation, but different activating stimuli can vary substantially with regard to the magnitude and kinetics at which they modulate individual activation parameters. With these caveats in mind, this review will attempt to bring together a broad sampling of the current literature. We will primarily concentrate on our understanding of the events that occur during activation of naïve CD8 T cells by DCs that present cognate pMHC.

How to study T-cell priming *in vivo*

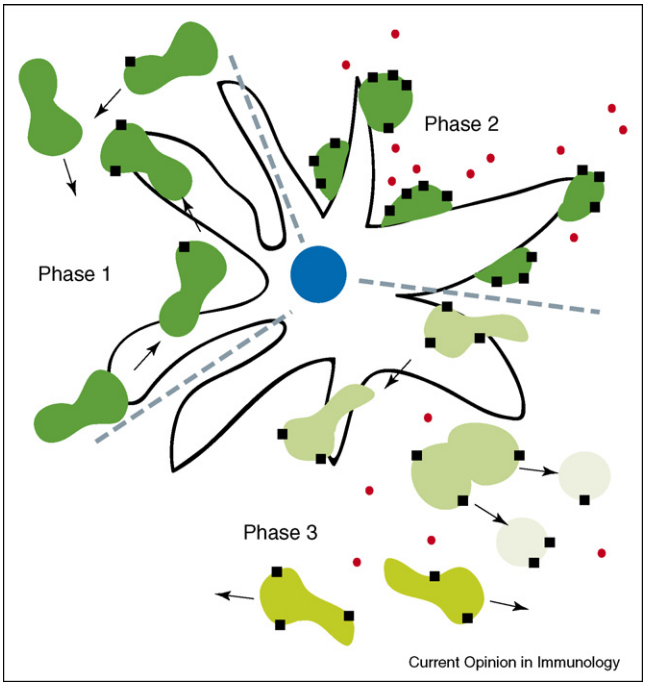
As only few naïve T cells can recognize any given epitope ($\sim$1 in 10^6–10^7), each antigen-bearing DC must interact with many T cells in the hope of being found by a T cell that recognizes a cognate antigen on its surface. The rules

that govern this mutual search are fundamental to understanding how immune responses are initiated — an issue that has been studied for decades.

Early investigations were conducted *in vitro*, including analyses of T cell–APC interactions as well as more recent imaging studies of T cells interacting with reconstituted lipid bilayers [9] or with DCs embedded in collagen gels [10]. These studies showed that T cell–APC interactions induce changes in T-cell morphology, motility and signaling [9,11]. These changes were found to occur during contacts that lasted from less than one hour to more than ten hours [10,12–15]. However, *in vitro* studies have limitations because they lack the highly complex tissue environment of SLOs. In addition, many early studies used T-cell lines and artificial or engineered APCs other than DCs — a scenario that might be more similar to effector cell interactions with their targets. Histology-based *ex vivo* examinations of lymphoid tissues are also limited because they can only provide static snapshots of a highly dynamic process. Thus, during approximately the past five years, immunologists began to realize that an accurate and dynamic view of T-cell priming requires direct *in vivo* observations of T cells and DCs in SLOs.

Since 2002, several groups have demonstrated the application of multiphoton microscopy (MPM) and confocal microscopy to study T cells in intact murine lymphoid tissues, both with and without antigen stimulation. Vigorous T-cell motility was detected in lymph nodes (LNs) at baseline, and this motility was greatly reduced after antigen stimulation [16–18]. One of these groups also noted a transition to high T-cell motility at later time points [17]. Using intravital MPM in mouse popliteal LNs, a small LN in the back of the mouse knee, our group has investigated the kinetics of CD8 T-cell priming by transferring fluorescently tagged antigen-pulsed DCs and naïve TCR transgenic CD8 T cells to recipient mice. We found that priming occurred in three distinct phases in this experimental setting (Figure 1) [19,20]. The first phase was characterized by short interactions between DCs and T cells and lasted for approximately eight hours. The frequency and duration of T cell–APC contacts during this first phase was similar to the brief random collisions that occur even in the absence of antigen. However, T cells were clearly activated during phase one, as evidenced by enhanced expression of the activation markers CD44 and CD69 during this period. Phase two was characterized by a transition to long-lasting ($\geq$1 h) DC–T cell interactions, as well as full activation of the T cells (measured by activation markers) and initiation of cytokine production. This phase lasted until the end of the first day after initial antigen encounter (i.e. approximately twelve hours). Finally, in the third phase, T cells left their DC partners, proliferated and returned to high motility [19]. Eventually, activated cells must leave the LN to exert their effector functions at peripheral sites

Figure 1



Phases of T-cell priming. Three phases of CD8 T cell priming have been demonstrated using intravital and *ex vivo* MPM. Phase one, lasting for the first several hours after a T cell has entered an SLO, is characterized by short interactions between T cells and DCs, with the upregulation of activation markers (symbolized by black squares) starting by the end of that phase. Phase two, lasting for the rest of the first day, is characterized by long-term DC–T cell interactions and the initiation of cytokine secretion (symbolized by red circles). Phase three is characterized by a return to short-term T cell–DC interactions and T-cell proliferation (symbolized by the variable dilution of the cytoplasmic green stain in that period).

of antigen challenge. The rules that govern the transitions from one interactive phase to the next are not yet understood, but we hypothesize that the initial phase of brief serial contacts represents a period during which T cells receive and integrate antigenic and costimulatory signals from DCs; the transiently interacting T cells appear to transition to the second phase only after they have exceeded a certain signal threshold.

Successive phases of T-cell priming have also been observed by other groups using both CD4 and CD8 T cells [21,22]. A detailed analysis of CD4 T-cell activation uncovered five stages, whereby the first two stages were dominated by brief dynamic interactions similar to the phase one-like interactions of CD8 T cells, while the third stage was reminiscent of phase two. Stage four involved swarming of T cells around DCs before detachment and proliferation (stage five), akin to what we have termed phase three [22]. Two recent studies indicate that this interactive program might be subject to modification by antigen context. When antigen was presented to

T cells by tolerogenic DCs [21] or in the presence of the negative costimulatory molecule cytotoxic T lymphocyte antigen 4 (CTLA-4) [23], T cells did not transition to phase two-like long-lived contacts with DCs, but continued to engage in phase one-like interactions.

Although phased T-cell activation dynamics have been observed by most groups, T-cell priming *in vivo* depends on a complex set of parameters that might modulate the kinetics of T cell–DC communication. Thus, apparently conflicting results have been obtained in another experimental system [24•]. The mode of antigen introduction might be particularly important in this context. For example, our study was performed after subcutaneous injection of peptide-pulsed DCs [19]; others have injected antigenic protein mixed with cytokines and a fluorescent dye to label and deliver antigen to skin-resident DCs [22]. In both cases, the frequency of antigen-presenting DCs that migrated from the injection site to draining LNs was relatively low, and both approaches resulted in successive phases of T cell–DC interactions. However, antigen can also be delivered directly to endogenous LN-resident DCs by coupling a model antigen (such as ovalbumin) to antibodies that bind the DC surface molecule DEC-205 [25]. Using this technique, one group reported that T cells engaged in long-lasting interactions with DCs virtually immediately [24•]. Upon subcutaneous injection, the anti-DEC205–antigen complex can reach the vast majority of endogenous DCs in the draining LN, presumably resulting in a high and ubiquitous antigenic load in this environment. This might have exposed T cells to sufficiently strong levels of antigenic signal to substantially shorten or even abrogate phase one interactions.

Integrating imaging with insights from other immunological investigations

Despite lingering uncertainties and remaining open questions, the plethora of recent MPM studies has allowed unprecedented *in vivo* examinations of T-cell priming at the single-cell level. Ultimately, the results from intravital MPM studies must be compared and reconciled with data and mechanistic insights derived from other experimental approaches. To this end, we will concentrate here on recent conceptual progress in T-cell activation and on how these insights might inform intravital imaging studies and vice versa. The questions we discuss will focus on three key elements: the factors that enable T cells to find DCs and engage in contacts with them; the means by which antigenic signals are delivered to the T cell; and how T cells interpret that antigenic signal.

How T cells find DCs in SLOs

Bringing T cells and DCs together is the first hurdle of T-cell activation. Several factors determine the frequency, localization and duration of T cell–DC encounters. These include trafficking molecules, such as adhesion receptors and chemokines, and microanatomical features, including specialized microvessels and interstitial guidance structures formed by fibroblastic reticular cells (FRCs).

To maximize the number of encounters with DCs, T cells constantly recirculate between the blood and tissues. The physiological half-life of naïve T cells in the bloodstream is short (~30 min), because circulating lymphocytes are continuously recruited into SLOs, where they remain for about one day before returning to the blood [26]. Most SLOs (except the spleen) contain high endothelial venules (HEVs), specialized microvessels that serve as highly efficient ports of entry for lymphocytes [27]. As soon as the recirculating T cells have gained access to a SLO, they begin their relentless search for antigen by crawling in an amoeboid fashion displaying large-scale random walk-like behavior [28]. *In vivo* MPM studies have shown that naïve T cells migrate with a mean velocity of ~12 μm/min [20,28]. Thus, at an intranodal dwell-time of 23 h/day, the cumulative distance traveled by a single naïve T cell exceeds 6 meters per year, nearly one-million times their own diameter (6–7 μm at rest). By comparison, an adult person would have to walk more than 1000 miles per year to accomplish this feat.

Guidance by the stromal network

The T-cell area of LNs is permeated by a network of branching collagen fibers that are ensheathed by specialized stromal cells known as FRCs [29–32]. The microscopic channels formed by the FRC sleeve are accessible to small molecules in the subcapsular sinus, thus establishing a conduit by which lymph-borne antigens and bioactive molecules are transported to the vicinity of HEVs in the cortex [29]. Recent work has shown that the migrating T cells crawl along the outer surface of the FRC scaffold, which serves as a foothold and guidance structure [33••]. This allows the traveling T cells to scan LN-resident DCs that form a dense network in the paracortex [34]. Some of these endogenous DCs are tightly associated with the stromal network and collect lymph-borne antigens from FRC conduits [32]. By contrast, peripheral tissue DCs carrying antigen to draining LNs by way of the lymph are not associated with FRC conduits. These DCs penetrate the paracortex and congregate around HEVs [35]. Thus, T and B cells that home to LNs by way of HEVs are first exposed to those DCs that have most recently departed from peripheral sites and carry the most up-to-date information from these regions [20,36]. Interestingly, upon subcutaneous injection of antigen, the consequences of T-cell activation by DCs that carried the antigen from the skin were not equivalent to those induced by resident DCs that acquired the antigen while in the LNs; only priming by migratory DCs promoted delayed-type hypersensitivity [37]. Whether this difference in outcome is reflected at the level of cell–cell interactions during priming is not yet known.

Guidance by chemoattractants

The mechanisms that guide and/or retain DCs and T cells at particular locations within LNs are still incompletely understood. However, both the homing of T cells in HEVs and the entry of DCs from peripheral tissues into the draining lymphatics as well as their migration from the lymph into the LN cortex depends on the chemokine receptor CCR7 and its ligands — CCL19 and CCL21 [38]. LN stromal cells can produce several chemokines, including CCL19 and CCL21 [39]. This is relevant to interstitial T-cell migration because T-cell motility in LNs is markedly attenuated in CCR7-deficient T cells and in *plt/plt* mice, which lack the genes for CCL19 and LN-expressed CCL21 [40,41]. Indeed, *in vitro* studies have shown that the presence of homeostatic chemokines, including CCL21, promotes T-cell chemokinesis (i.e. motility in the absence of a concentration gradient) and accelerates T-cell encounters with and activation by DCs [42]. By contrast, CCR7-deficient T cells can still mount antiviral responses, albeit at a reduced level [43], indicating that CCR7-dependent T-cell migration and tissue organization are not an absolute prerequisite for antiviral cytotoxic T lymphocyte activation and effector differentiation, at least in certain settings.

The T cell–APC dialogue: chemokines chiming in

Chemokines can enhance and modulate T-cell activation by DCs in more than one fashion. For example, DC presentation of cognate antigen to CD4 T cells in LNs induces the local production of CCR5 ligands, as well as a change in cytokine milieu within the LN that induces CCR5 expression on naïve CD8 T cells [44••]. The $CCR5^+$ CD8 T cells are then preferentially attracted to nearby DC–CD4 pairs. This CCR5-dependent recruitment is necessary for CD8 T cells to receive optimal 'help', as evidenced by the finding that inhibition of CCR5 ligands compromises long-term CD8 memory generation [44••].

Several recent observations further indicate that chemokines can modulate immune responses by mechanisms independent of their chemoattractant activities [45]. For example, DCs can bind chemokines, including CCL21, on their surface and use them to induce T-cell adhesion [46]. This occurs before immune synapse formation, and might lower the activation threshold of the tethered T cells. Other chemokine-induced mechanisms include effects on DC maturation, the polarization of effector activities, and the communication between T cells and APCs [45]. Early studies of the latter effect had suggested that TCR activation constitutes an instant 'stop' signal that overrides pro-migratory chemoattractant signals [9]. However, *in vivo* and *in vitro* observations of brief serial encounters between T cells and APCs have called this concept into question [10,19,22]. Indeed, certain chemokine gradients have the potential to suppress T-cell activation by preventing formation of the immunological synapse (see below) [47]. More recent findings indicate that DC-derived chemokines and the surface distribution of T cell expressed chemokine receptors can control the duration of T cell–APC contacts, at least *in vitro* [48••]. In these experiments, G_i-coupled chemokine receptors, such as CCR7, promoted the migratory dispersal of T cells, but T-cell activation induced a redistribution of other chemokine receptors, particularly CCR5 and CXCR4 that signaled through G_q and/or G_{11}. These chemokine receptors became sequestered in the immunological synapse and were stimulated by APC-derived ligands. Therefore, the T cells became more resistant to distractions from other chemokine sources, T cell–APC conjugates were stabilized, and T cell activation was enhanced [48••]. These *in vitro* observations are intriguing in the context of the three-phase T-cell activation process described *in vivo* [19]. For example, one might speculate that the phase one–two transition is at least partially regulated at the level of chemokine receptor mobility on T cells and/or the induction of chemokine secretion by engaged DCs. It will be important to explore these questions in future MPM experiments.

The antigenic signal

To understand the many interdependent forces that alter the antigenic signal delivered to the T cell by the DC, as well as their implication for tolerance versus programming of effector responses, we must consider the subcellular arrangement of interacting molecules at the T cell–APC surface as well as the factors that determine antigenic strength.

A moving target: the immunological synapse

The immunological synapse (IS) is a highly orchestrated arrangement of interacting macromolecules at the interface between T cells and APCs, and has been well-covered in an excellent recent review [49]. Its role in the initiation or potentiation of signaling events in T cells is a topic of ongoing debate. Following stable IS formation, microclusters of TCRs and phosphorylated signaling molecules form peripherally and then migrate toward the center of the IS where they may be degraded [50,51•]. Blockade of actin cytoskeleton remodeling was able to block microcluster formation, which also seemed to prevent TCR–pMHC interactions in the contact region [50].

These *in vitro* results have not yet been confirmed *in vivo* — in fact, the formation of an IS during T cell–DC interactions remains to be carefully characterized in intact tissues, although initial observations have been made [17]. The resolution needed to accomplish this feat (e.g. by intravital MPM) is pushing current technological limits because investigators must employ several spectrally distinct fluorescent tags that delineate molecular components of the IS without interfering with their

normal function or distribution. The development of such tools will be crucial to determine how T-cell behavior and signaling are linked.

Antigen signal

What can be considered the 'dose' of antigen received by a T cell can more accurately be thought of as the sum of many variables, including the density of antigen-bearing DCs, the level of antigen per DC, the affinity of that antigen for the TCR, and the duration of antigen availability. (An additional essential determinant is the availability of costimulatory molecules, which we assume to be available at optimal levels for this discussion.)

Considering dose as a sum of these variables allows us to dissect the antigen signal into its many parts. If we start by considering the role of the density of cognate antigen-bearing DCs, the APC-to-T cell ratio correlates positively with T-cell activation up to a certain range, beyond which larger APC numbers do not have a major impact [52,53]. Similarly, increasing density of cognate antigen per APC leads to more efficient T-cell activation and effector differentiation [54–56]. However, a long-standing concept in T-cell biology states that antigen levels beyond an upper bound might yield *de facto* tolerance [57] caused by termination of effector responses triggered by activation-induced cell death [58]. In addition, there is an inverse relationship between antigen dose and TCR affinity: low-affinity TCRs can be activated by high-dose antigen, and vice versa [59]. Finally, to study the effect of limited exposure to antigen, different models have been used to stimulate TCR-transgenic CD8 T cells [60,61]. A short pulse of antigen was shown to trigger a differentiation program that resulted in complete effector differentiation and the formation of long-lived memory cells. Most recently, an elegant system was used to deliver antigen for a controlled period of time *in vivo*. This study made use of diphtheria toxin receptor transgenic DCs that could be acutely deleted by administration of diphtheria toxin [62]. The duration of the antigen pulse correlated with the degree of the primary expansion. Seven hours of antigen exposure was sufficient for development of full effector function and memory cell differentiation, but longer exposure to antigen was required for full primary T-cell expansion. Whether and to what extent this reflects on the role of phase one versus phase two observed by intravital MPM studies [19] remains to be determined.

How T cells evaluate the antigenic signal

Models for individual interactions between TCR and pMHC

Many models have been developed to explain the fine discrimination that TCRs must achieve to differentiate between the abundant self antigen and the rare cognate antigen presented by APCs [63,64]. To begin with, there are models to account for the mechanism by which the analog signal from the pMHC is translated into a digital activation signal for the T cell. The most supported model takes into consideration the dynamic interaction between the pMHC and the TCR and is referred to as kinetic proofreading [65], although there are still issues that are not fully explained by this model [66]. Kinetic proofreading links the intracellular signaling that leads to full TCR activation to the duration of individual pMHC–TCR interactions. These studies often use altered peptide ligands (APLs), which differ from the 'natural' ligand by one or more amino acids, to study the effect of altered interaction with MHC and/or TCR on intracellular signaling [67]. In general, the affinity of a given APL is positively correlated with the duration of its association with a cognate TCR [68,69]. A pair of feedback loops, one rapid and negative (Src homology domain 2 [SH2]-containing tyrosine phosphatase-1; SHP-1) and one delayed and positive (extracelular signal-regulated kinase, ERK, which is also known as mitogen-activated protein kinase, MAPK), has been identified that begins to explain the ability of the TCR to discriminate between cognate antigen and self-antigen [70,71•].

There are also models to explain how pMHC binding to TCR could actually cause 'triggering' of intracellular signaling (and not simply a decision to trigger): kinetic segregation (whereby close contact zones that form upon APC–T cell contacts foster exclusion of large phosphatases and facilitate triggering); heterodimerization (whereby coreceptor and TCR bind to the same pMHC); a model in which the TCR binds to one pMHC and the coreceptor binds to another, forming a lattice of interacting molecules; pseudodimerization (whereby self pMHC and agonist pMHC both engage TCRs); and conformational change (whereby TCR engagement yields structural changes in the CD3 complex) [63,72]. Recent studies have shown evidence for antigen-dependent clustering of TCRs leading to CD3ε conformational change [73,74•]. In addition, the effect of cognate antigen on both the recognition kinetics of TCRs and the conformation of TCR–pMHC interface have been connected using surface plasmon resonance measurements of soluble TCR–pMHC binding, which showed a role for both the half-life of the TCR–pMHC interaction and the change in heat capacity [75].

Serial interactions of molecules and cells

Classic experiments have shown that a relatively small number of pMHC complexes on the surface of an engineered APC can trigger the downregulation of a much larger number of TCRs on responding CD4 T cells [76]. This discovery led to the idea of serial triggering, whereby a few cognate pMHC complexes on a single APC were thought to sequentially stimulate numerous TCRs on a T cell [77]. Imaging studies of DC–T cell contacts in collagen matrices revealed that T cells can engage in a series of short (<10 min) interactions with multiple DCs

(reminiscent of phase one *in vivo*). The sum of these serial encounters was found to yield full T-cell activation [10]. Unlike the original studies that demonstrated serial triggering, cell–cell interactions in these more recent experiments involved single T cells contacting many different DCs. This led to the development of a 'serial encounters' model, which posited that T cells interacting with a series of DCs can sum the signal they receive until full activation is achieved (or not) [78].

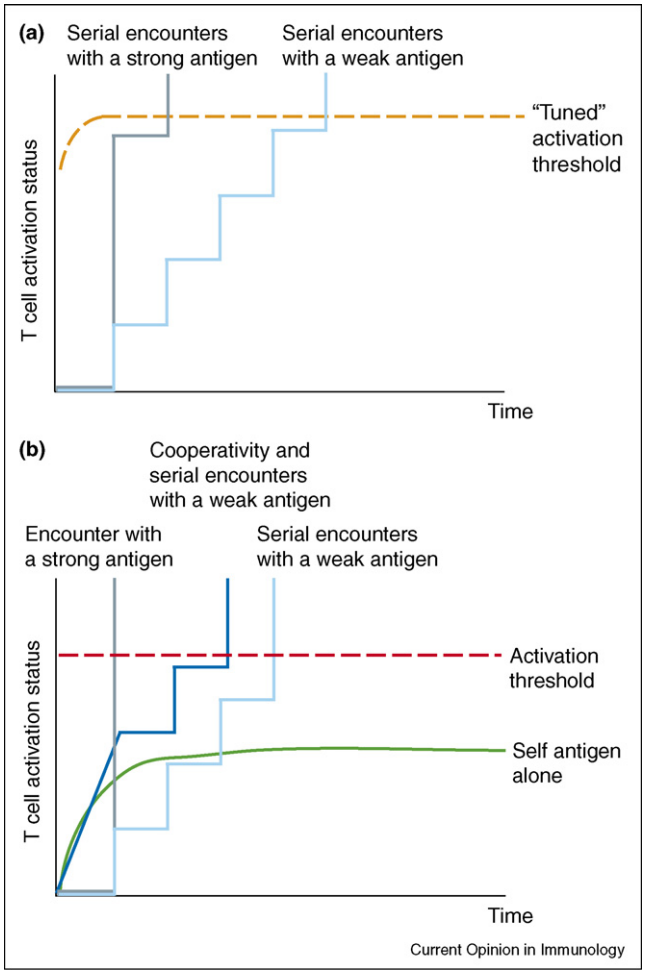
When is enough, enough? Tunable activation thresholds

Migrating T cells in SLOs are constantly exposed to self pMHC complexes that engage TCRs without fully activating them. If this subthreshold stimulation (noise) increases, the threshold of stimulation necessary for full T-cell activation (signal) has been proposed to increase as well [79], although it is important to keep in mind that the availability of costimulation also plays an important role in setting the threshold for activation [80]. It has been proposed that T-cell activation, on a single cell basis, is based on a tunable activation threshold set according to the recent history of TCR stimulation [81,82]. Further studies have considered how this tuning might take place biochemically [59,71•,83]. This model can account for the variable responses of T cells to APLs in varied environments, including T-cell development in the thymus versus priming in SLO [84].

Germain and co-workers [85] have pointed out that the tunable activation threshold model predicts a paradoxical role for self-antigen, which would serve as a selecting agent in the thymus, but would exert a dampening effect in the periphery. In contrast to this prediction, this group reported a synergy between self and foreign antigen [86]. This suggests a feedback regulation model of ligand discrimination whereby self-antigen facilitates mature T-cell responses to cognate antigens by pre-clustering TCRs and pre-docking low levels of both positive and negative signaling molecules. The benefits of the clustering and pre-docked activating molecules outweigh the low-level concurrent pre-docking of negative molecules, thus facilitating full activation by subsequent agonists. This view is consistent with data that suggest a role for 'pseudodimers' of foreign pMHC and self pMHC complexes that can cooperate in T-cell activation [87•,88].

We can apply these recent conceptual advances to ask how dwell time alters the activation threshold of a T cell after it has homed to a SLO from the blood (Figure 2). Elegant work has shown that blood-borne T cells show minimal evidence for TCR stimulation, which was measured by phosphorylation of CD3ζ chains. By contrast, when T cells were isolated from LNs, where T cells are constantly exposed to self-MHC, partial CD3ζ phosphorylation was evident [86]. Therefore, upon entering a LN, T cells begin to integrate self-antigen signals. According to the classic tunable activation threshold concept, T cells would therefore require increasingly strong cognate signal to become activated, whereas cooperativity between self and cognate antigen should progressively lower their threshold for full activation by a cognate antigen. In this model, which yields feedback regulation of ligand discrimination, prolonged serial

Figure 2



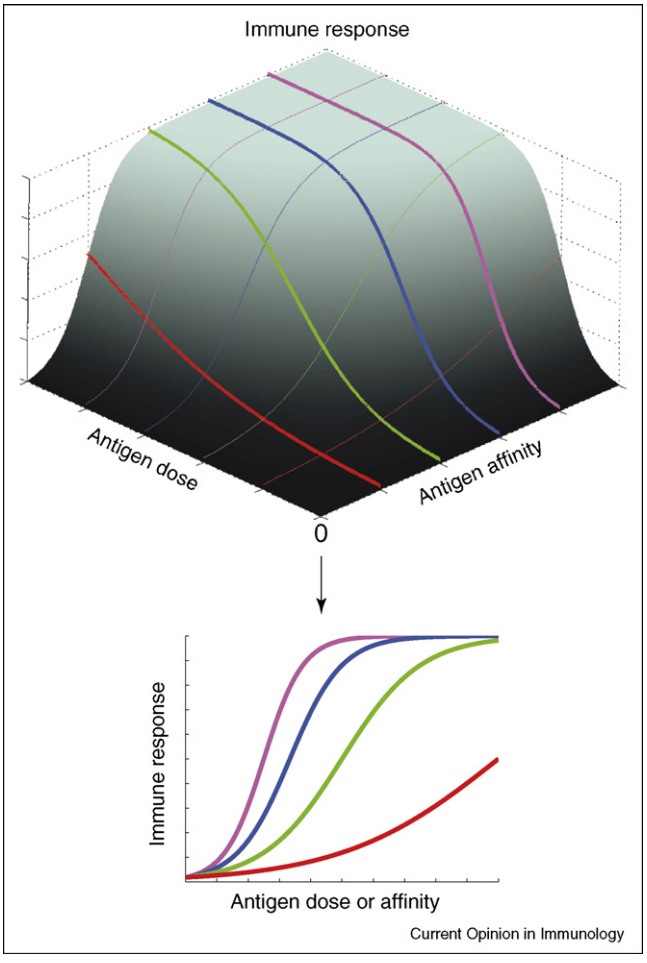
Models of T-cell activation. The diagrams show an arbitrary metric of T-cell activation versus the time that has lapsed since a given T cell has entered an SLO that contains cognate antigen-presenting DCs (which are assumed to be fully mature and presenting optimal costimulation). An arbitrary activation threshold (broken lines) was drawn to mark the level of signal necessary for a full T-cell response. At a very high cognate antigen dose (grey line), T-cell activation can be achieved rapidly. At lower antigen levels, the serial encounters model (in light blue) predicts that small incremental signals are integrated by the T cell over a series of encounters, eventually leading to full activation. **(a)** The tunable activation threshold model (orange broken line) predicts that the presence of self antigen will raise the T-cell activation threshold, increasing the antigen dose necessary for T-cell activation. Therefore, it would take longer for any given cognate dose (light blue or grey) to activate a T cell (if at all). **(b)** With the feedback regulation model of ligand discrimination, T cells interact with self-pMHC molecules that induce partial activation (green). If low antigen levels synergize with self antigen, T-cell activation is facilitated (in dark blue). The activation threshold is marked (red broken line).

engagement of self-MHC after T-cell entry into SLOs would allow a relatively weak antigenic stimulus to intensify T cell–DC interactions beyond a hypothetical activation threshold. By contrast, newly homed T cells that encounter the same stimulus might not reach this threshold. This is an attractive concept to explain, at least in part, why T cells spend some time engaged in phase one before being able to transition to phase two-like interactions.

A cohesive model

The diverse models discussed in this review remain to be reconciled, but most are not mutually exclusive. For example, kinetic proofreading and conformational change could both contribute to how a T cell experiences any given pMHC complex. Although the interactions between TCRs and pMHCs are integrated at the macromolecular level, at the cellular level serial encounters between T cells and DCs in SLOs might allow each T cell to integrate a multitude of self and non-self inputs in its local environment. The decision whether to undergo full-fledged activation could depend on the nature of the antigen and the threshold set for activation. This threshold is probably highly dynamic and adjustable to the environment and experience of each T cell.

Figure 3



Interdependent factors control T-cell priming. The diagram integrates the role of antigen dose and affinity in T-cell priming, as examples of interdependent variables relevant to T-cell priming. Although these parameters are often studied in isolation, these and many other factors are interdependent. The amplitude of the immune response based on altering the level of either antigen dose or antigen affinity is shown in the third dimension. The different color lines indicate different levels of antigen dose or antigen affinity from lowest (red) to highest (purple). For example, a low antigen dose (green line) can still achieve a full immune response if the antigen is high affinity (purple).

Conclusions

Many interdependent factors affect the signals received by T cells; as one variable is modulated, other variables must be adjusted to achieve full activation (Figure 3). For example, an increase in antigen level might reduce the TCR affinity needed to trigger the activation program. Conversely, only T cells with the best TCR 'fit' might be able to respond to sparse antigen levels. A continuing problem in studying these issues is the choice of readout for T-cell activation. Recent advances now allow us to compare the traditional symptoms of T-cell stimulation to observations of the *in vivo* dynamics of T-cell communication with APCs. This strategy holds promise not only for the generation of a more unified theory of T-cell activation but also for practical approaches, for example in vaccine design, cancer therapy and treatment of inflammatory diseases.

Acknowledgements

Thanks to R Germain and A Viola for their comments on the manuscript. Thanks to T Buschman for assistance with figures.

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- • of special interest
- •• of outstanding interest

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