immunity induced by those receptors. TRIM21 is expressed by most cells, but high TRIM21 expression has been observed in myeloid cells such as macrophages and dendritic cells (DCs)[9]. McEwan *et al.* find that infection of mouse macrophages by antibody-coated adenovirus leads to activation of NF-κB and expression of proinflammatory cytokines, such as interleukin 12 (ref. 2). The innate signaling by TRIM21 might therefore also be important in DC-induced adaptive immunity. Future studies will need to investigate how the sensing of intracellular antibodies by TRIM21 in DCs affects the polarization of helper T cells and antigen presentation. Also, as DCs and macrophages are professional antigen-presenting cells with quite elaborate uptake and intracellular transport mechanisms, it will be interesting to determine the efficiency of the transfer of antibodies into the cytosol and activation of TRIM21 in these cells. Cross-presentation pathways allow antigens to escape from the endocytic pathway; this might also orchestrate the entry of antibody-antigen complexes into the cytosol, which can lead to activation of TRIM21.

The identification of signaling from TRIM21 to intracellular antibody-pathogen complexes might have important implications for the development of vaccination strategies. For example, the adenovirus type 5 vector was used in the STEP HIV vaccine trial in which preexisting antibodies to the vector resulted in a twofold greater incidence of HIV acquisition among vaccinated recipients than among recipients of placebo, presumably due to more activation of DCs–T cells by antibody-vector immune complexes[10]. Better understanding of the signaling pathways triggered by TRIM21, crosstalk with other innate signaling networks and the 'flavor' of adaptive immune responses will be very important not only in understanding the relevance of this mechanism to defense against infections but also in using this mechanism to improve vaccination strategies.

COMPETING FINANCIAL INTERESTS
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T cell priming goes through a new phase

David A Blair & Michael L Dustin

The priming of naive T cells requires antigen presentation by activated dendritic cells, yet the optimal generation of effector and memory CD8$^+$ T cells requires subsequent T cell–T cell interactions during the critical differentiation period.

A naive T cell encountering antigen for the first time has a wide range of options open to it, and its primary instructor in making these 'choices' is considered to be the dendritic cell (DC). In this issue of *Nature Immunology*, Gérard *et al.* provide compelling evidence for secondary synapse circuits between aggregating T cells, after the interaction with DCs, as a previously unknown phase in T cell priming[1] (**Fig. 1**). They undertake extensive pharmacological and genetic analysis that supports the proposal of an important role for the intercellular adhesion molecule ICAM-1 on CD8$^+$ T cells in the generation of effector and memory cells after the DC has done its job.

ICAM-1 was identified as an adhesion molecule on the basis of the ability of a monoclonal antibody to ICAM-1 to inhibit the self-aggregation of activated lymphocytes (either B cells or T cells) dependent on the integrin LFA-1 ($\alpha_L\beta_2$)[2]. ICAM-1 also stood out in early screens for activation antigens on lymphocytes[3] and stromal cells, including endothelial cells. Subsequently, ICAM-1 has attracted attention as a critical molecule for the establishment of asymmetric T cell division that leads to the development of effector and memory T cells[4,5]. Those studies assumed that the critical ICAM-1 molecules are on the surface of non-T cells, but the results of Gérard *et al.* point to an important and previously unknown role for ICAM-1 induced on T cells by activation and provide evidence that ICAM-1 functions in the formation of T cell–T cell synapses in aggregates[1], a concept that has been put forward before by this group[6].

Gérard *et al.* examine the role of LFA-1–ICAM-1 interactions at various times during the primary CD8$^+$ T cell response through the use of blocking antibodies or T cells from genetically manipulated mice to inhibit such interactions, and particularly focus on the role of ICAM-1 induced on the T cells[1]. The paradigm at present states that T cell priming in the lymph nodes occurs in three distinct phases[7]. In phase I, T cells make brief contacts with DCs for 2–8 hours, which leads to upregulation of expression of the early activation marker CD69. In phase II, T cells arrest motility with DCs and initiate cytokine production. In phase III, the motility of the T cells increases and the cells begin to proliferate. Gérard *et al.* propose a splitting of phase II into an early DC- and antigen-dependent T cell–DC synapse and a late DC- and antigen-independent phase, which they call the 'critical differentiation period' (CDP), essential for protective memory CD8$^+$ T cell responses[1] (**Fig. 1**). They find that inhibiting LFA-1 interactions within 2 hours of immunization results in less activation and diminished effector function of CD8$^+$ T cells. This result is not surprising, because it has already been established that LFA-1–ICAM-1 interactions contribute to the sensitivity of T cells to complexes of peptide and major histocompatibility complexes presented on DCs and are critical for stable T cell–DC interactions in phase II. Gérard *et al.* show that when LFA-1 interactions are inhibited at 24 hours after immunization (at the beginning of the CDP), there is no effect on CD69 or the ability to initiate proliferation measured at 3 days; however, the later acquisition of effector function is abrogated[1]. Inhibition of LFA-1 at 60 hours after immunization (at the end of the CDP) has no effect on activation or the acquisition of effector function. Inhibition of LFA-1 during the CDP also results in fewer antigen-specific CD8$^+$ T cells in the effector population

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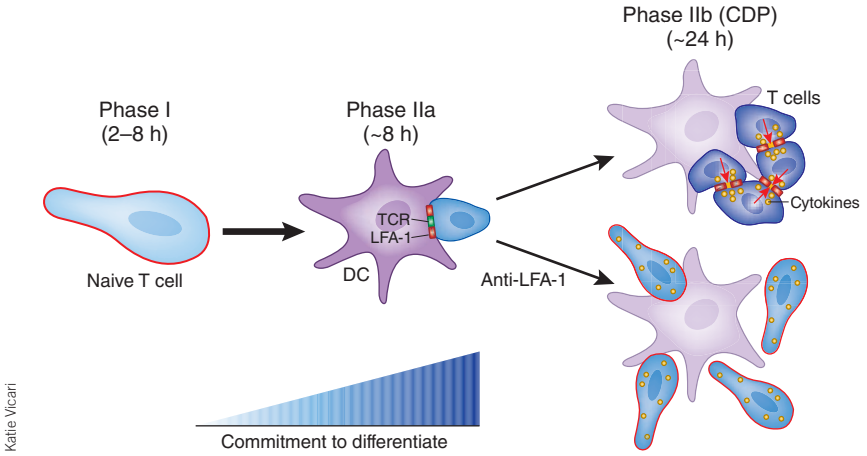

Figure 1 Model for CDP, a new phase in T cell priming. In phase I, T cells are highly polarized and scan DC networks even as antigen recognition is initiated. LFA-1 remains nonpolarized. In phase II, the T cells decelerate and form stable interactions with antigen-bearing DCs in which LFA-1 (red) and TCR (green) are polarized to the interface. Gérard *et al.* have defined the CDP[1], which seems equivalent to a later part of phase II. Activated T cells expressing ICAM-1 are now able to form antigen-independent T cell–T cell synapses, which allows efficient sharing of cytokines by directed secretion (yellow). This process is needed to complete commitment for differentiation into effector and memory cells. TCR, T cell antigen receptor; anti-LFA-1, antibody to LFA-1.

at day 8. Whether this is due to the survival of cells entering the response or a cessation of proliferation after LFA-1 inhibition remains to be determined. Indeed, continued antigen availability during the proliferative phase of the response drives maximum population expansion of T cells[8,9]. Nonetheless, stable LFA-1 interactions throughout the CDP are required for the generation of effector cells.

Interactions during the CDP also influence the generation of memory T cells. Interestingly, Gérard *et al.* find that LFA-1 blockade during the CDP results in alteration of the memory precursor effector cell (MPEC) and short-lived effector cell intermediates measured at day 15 and shifts the population toward an MPEC phenotype[1]. However, although more MPECs develop when LFA-1 interactions are inhibited during the CDP, there is also a concomitant lower frequency of cells with a central memory phenotype measured at 2 weeks, which indicates a defect in the transition from MPEC to central memory cell[10]. Most importantly, blocking LFA-1 interactions during the CDP results in less protection against reinfection with a pathogen. Gérard *et al.* report that when LFA-1 interactions are disrupted during the CDP in mice undergoing vaccination, protection against rechallenge is lost, even when equal numbers of antigen-specific $CD8^+$ T cells are transferred into new mice to eliminate any residual effects of the antibodies[1]. Consistent with the qualitative differences in surviving antigen-specific T cells after vaccination in the absence of ICAM-1, published work has shown that $CD8^+$ memory cells do emerge after viral infection but that these cells, although present in normal numbers, are not protective[11]. In the model used by Gérard *et al.*[1], it is unknown if the pool of memory cells continues to wane over time or if they simply fail to mount a recall response because of intrinsic defects during differentiation of the MPECs. Profiling the molecular signature of the cells during CDP with and without blockade will provide clearer insight into the molecules that regulate downstream differentiation pathways.

Gérard *et al.* use two-photon imaging during the CDP to show that clusters of activated T cells surround DCs in the lymph nodes[1]. This clustering of the T cells is supported by LFA-1-dependent T cell–T cell interactions, as inhibition of LFA-1 with an antibody during the CDP or genetic deletion of ICAM-1, which is induced on T cells during the CDP, disrupts these clusters (**Fig. 1**). Clustering and functional outcomes are weakly dependent on the presence of DCs during the CDP, but the positive effects of the LFA-1–ICAM-1 interaction are largely independent of DCs and antigen in this phase. Histological analysis provides evidence that is consistent with polarized secretion of interferon-γ (IFN-γ) through T cell–T cell synapses. This IFN-γ secretion is important in differentiation, as blockade of LFA-1 or IFN-γ during the CDP results in memory cells with lower expression of IFN-γ after recall. Gérard *et al.* interpret these results in terms of collective 'decision making' by DC-primed T cells that will take place even with the low numbers of antigen-specific T cells in the primary response[1]. It remains to be determined if any effects of LFA-1 blockade or ICAM-1 deficiency are related to reverse signaling by ICAM-1 into T cells during the CDP.

In summary, Gérard *et al.* bring to light a key component of $CD8^+$ T cell differentiation that requires cell-cell interactions beyond the classic T cell–DC interactions in priming[1]. During this CDP, activated T cells engage in secondary synaptic circuits dependent on LFA-1 interactions (**Fig. 1**). It is not clear what signals drive the polarity of cytokine secretion in these synapses, but it is well established that costimulatory signals can drive antigen-independent synapse formation[12]. The work by Gérard *et al.*[1] helps to elucidate such interactions in the context of *in vivo* responses. Published work has also shown that T cell–T cell interactions among proliferating $CD8^+$ T cells activate the Hippo pathway, a conserved developmental pathway important in terminal differentiation[13]. It remains to be determined if other types of non-DC partners can participate in these aggregates to influence T cell differentiation. These secondary synaptic circuits may particularly influence the 'decision tree' of helper T cell responses. Moreover, it remains to be determined if help from $CD4^+$ T cells can involve mixed $CD4^+$ T cell–$CD8^+$ T cell secondary synaptic circuits. Along the same lines, during an immune response to a natural pathogen in which multiple clones of T cells are brought into the response, will the secondary synaptic circuits promote collective 'decision making' across different specificities? Secondary synaptic circuits dependent on LFA-1–ICAM-1 interactions represent an additional phase of T cell priming and will be an exciting target in vaccine development and immunotherapies.

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