Because activated B cells express Fas and can die in a Bim-dependent manner, it is tempting to suggest that both Bim and Fas play crucial roles in regulating B cell numbers via cell autonomous processes. B cell-specific deletion of Fas led to accumlation of lymphocytes (to the same extent as seen in T cell- plus B cell-specific deletion—although in the B cell-specific case, without expansion of the $B220^+CD3^+$ T cells), suggesting that Fas-mediated B cell death plays a role in the regulation of B cell numbers. The relative contribution of T cells or B cells lacking Fas signaling to disease is difficult to assess for the reasons mentioned above, however, because the defective T cells show enhanced expression of FasL.

To fully understand the complex interplay between these modes of cell death in the control of lymphocyte numbers, what we need, it seems, is a way to specifically block Fas signaling in a specific cell type while eliminating the effects of Bim in the same or different cell. But there is a problem; Fas signals via an adaptor molecule, FADD, and an initiator caspase, caspase-8, and elimination of either of these impacts the ability of the T cell to activate and/or proliferate (reviewed in Pellegrini et al., 2005). How this occurs, and whether we can interfere with the death pathway while sustaining the activation pathway mediated by these molecules, is not yet known (but there are hints that the answer is yes, we can). But perhaps the important message is that this pathway plays two roles, positive and negative, in the control of lymphocyte number. A T cell that loses Fas upregulates FasL to kill other cells (and dies as survival factors become limiting). If instead it loses Fas signaling (FADD, or caspase-8) it does not effectively expand. Loss of Bim allows the cell to survive under limiting conditions, but these cells are nevertheless killed by Fas signaling (or are at least limited by a Fas-dependent process, such as killing of dendritic cells).

One autoreactive T lymphocyte, with unchecked capability for expansion, can kill the organism. That's why the game of T cell survival is so hard to win.

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The Importance of Prolonged Binding to Antigen-Presenting Cells for T Cell Fate Decisions

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How critical is it for T cells to stably arrest on antigen-presenting cells? In this issue of *Immunity*, Scholer et al. (2008) demonstrate profound effector and memory defects for $CD8^+$ T cells encountering "nonsticky" antigen-presenting cells lacking intercellular adhesion molecule-1.

It has become apparent that the stable interaction of T cells with antigen-presenting cells (APCs) is not simply dictated by the presence of peptide-MHC (pMHC) complexes on the stimulating APCs. In vivo, T cells often circle for many hours, loosely engaging in transient interactions with APCs before arresting to form stable contacts (Mempel et al., 2004; Miller et al., 2004). But what changes occur that permit this stable interaction?

Scholer et al. and others started to address this with the observation that dendritic cells (DCs) from immunized animals exhibit a modest upregulation of intercellular adhesion molecule-1 (ICAM-1) that peaks synchronously with the period of time when T cells stably arrest on the DCs in vivo. In vitro, they subsequently demonstrated the expected result that ICAM-1-deficient DCs are defective in mediating prolonged T cell engagements. Such a result is, in effect, a modern version of lymphocyte-function-associated

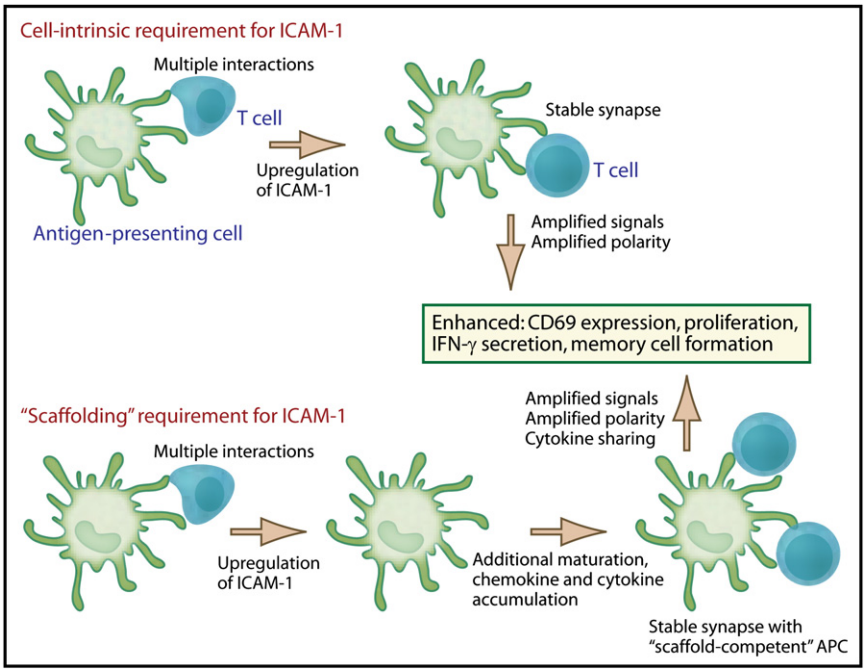

Figure 1. Requirements for ICAM-1 on APCs Mapped Directly or Indirectly to T Cell-APC Interactions

The top shows the cell-intrinsic requirement for ICAM-1. ICAM-1 is upregulated on APCs after short interactions with T cells, thereby leading to enhanced adhesion and a stable synapse. The stable synapse improves activation and amplifies TCR signals and polarity. The bottom shows the scaffolding requirement for ICAM-1. Dynamic T cell interactions cause upregulation of ICAM-1 on APCs in addition to increasing expression of other costimulatory molecules. These factors increase adhesion of both the original T cell and other bystander cells. The aggregation of these cells leads to amplification of cytokine and chemokine signaling. The APC then matures into a scaffold-competent cell, able to form stable synapses with antigen-specific T cells. Signaling and polarization are amplified, and T cell clusters increase local concentrations of cytokines. In both cases, ICAM-1 expression is necessary for stable T cell-APC synapses that enhance CD69 expression, proliferation, IFN-γ secretion, and memory cell formation.

antigen-1 (LFA-1) blockade experiments performed with some of the first monoclonal antibodies in mixed-leukocyte reactions (MLRs); such assays showed LFA-1-ICAM interactions were critical for T cell activation. In another modern turn, they subsequently show a profound reduction in cell arrest on APCs in vivo as well.

But what is the mechanism of the ICAM-1 contribution and how does this relate to cell arrest? Previously, it has been argued that ICAM-1 "costimulates" T cell activation through LFA-1 signaling in much the same way that B7 costimulates T cells through CD28. However, as shown here again, LFA-1-ICAM engagement appears to function quite distinctly from CD28 costimulation. Notably, the absence of ICAM-1 from the entire host had limited effects on early measures of TCR signaling such as CD69 upregulation or upon proliferation over the first 3 days.

In contrast, activation of T cells in vivo under conditions in which ICAM-1 is lacking resulted in shorter T-APC contact times. Shorter contact correlated with specific defects in CD8+ T cell priming for gamma-interferon (γIFN) production as well as substantial defects in memory formation. Furthermore, cells stimulated under such conditions exhibit a proliferative profile resembling ones generated by other "tolerizing" stimuli such as direct targeting of antigen to DEC-205$^+$ DCs in the absence of CD40 engagement. Although cells stimulated in the absence of ICAM-1 resemble such tolerance, it is not yet proven that they are identically "tolerant." It should also be noted that the majority of tolerance-related results in this study also derive from experiments in which ICAM-1 was lacking in the entire host and not just on the APC. The possible effect of ICAM-1 on other aspects besides T cell-APC contacts (for example, in effective homing and trafficking) should not be discounted.

The implications here are that prolonged APC interactions strengthen the T cell response, in part by regulating the ability of T cells to stably assemble. Another possibility is that short interactions are inherently tolerogenic and inhibitory for memory or effector cell formation. A single long-lived interaction might overcome this. Whereas this promotes the concept that a stable interaction in vivo generates a full activation program, the complete components of such a program remain a mystery.

What is the nature of the "complete" activation program that is set into play when LFA-1-ICAM interactions are permitted to occur? In the simplest scenario (Figure 1), longer contacts are likely to allow greater recruitment of TCRs and associated signaling molecules to the site of pMHC engagement, the immunological synapse (IS). A higher density of receptors correlates with a higher signal output (Grakoui et al., 1999), and so a simplistic model would argue that longer duration times and associated accumulations at the IS would provide improved activation via a T cell intrinsic effect. An extension of this idea was recently provided by Chang et al., who proposed that more stable interactions are better at inducing a stable cell polarization program within T cells and thus in promoting differentiation through a process of asymmetric cell division (Chang et al., 2007). This is also a cell-intrinsic mechanism in which longer and more stable interactions may facilitate a greater degree of commitment to a hyperpolarized polarity state within the T cell. The in vitro data of Scholer et al. (2008) support this sort of restricted requirement for ICAM-1 in mediating the arrest phase because in vitro-matured bone-marrow-derived dendritic cells lacking ICAM-1 were specifically defective in stopping. Nonetheless, the situation in vivo may prove more complicated.

Sticky APCs may also serve as a communal meeting ground for multiple T cell interactions over time. In such a model (Figure 1), the ICAM-1 is also a broad prerequisite for a DC to act as a scaffold for a great many different cell types. Loss of ICAM-1 would then represent a defect in amplification capability; each T cell interaction is unable to improve the stimulatory quality of the APC in multiple respects, including expression of other

costimulatory molecules such as B7 and CD40, not just with respect to its ICAM-1 expression.

Notably, over time and multiple encounters, a qualified APC might effectively become a repository for a collective history of multiple T cell responses. One way of storing this information is probably in the form of bound inflammatory chemokines. DCs can be shown to effectively present the T zone chemokine SLC on their surface, thereby altering synapse dynamics (Friedman et al., 2006). Two studies from the past two years support the existence of a CCR5-ligand "tagging" method whereby T cells that are weakly activated can be recruited to dendritic cells that are or were actively engaging other T cells (Castellino et al., 2006; Hugues et al., 2007). This mechanism appears to take advantage of chemokines made during early rounds of T-DC interactions, to recruit T cells to sites where other T cells have begun to assemble. Because chemokine-mediated recruitment typically uses LFA-1-ICAM-1 interactions in lymphocytes, this would be a program that may also be cut short by loss of ICAM-1 on DCs.

In either case, these results bear a curious relationship to recent studies of protein kinase Cθ (PKCθ)-deficient T cells, which have the dual phenotype of poor IS stability and, at least in some circumstances, increased IL-2 production (Sims et al., 2007). In that study, it was argued that continued scanning, an effect normally promoted by PKCθ, provided a positive benefit to T cells because they effectively could accumulate activating signals from many APCs. Subsequently, they may preferentially arrest on the "best." Such a result is not in conflict with the Scholer result. Indeed, transient-engagements may allow T cells to collectively select the best APCs in the lymph node and ultimately give them the best chance of collectively aggregating on ICAM-1-positive (or upregulated) DCs when enough short engagements have been made with that APC. It is then informative that PKCθ deletion, although being somewhat of a positive benefit for T cell IL-2 production in an antigen-rich environment (e.g., on bilayers where each move away from one IS means that the cell can get more signal), is rather detrimental for activation and effector development of T cells in vivo (Marsland et al., 2004), in which being unable to stop means being unable to commit when the best APC is finally found.

It is interesting to further speculate on the possibilities for adhesion to play a more generalized role in regulating systemic functions in immunity. A sticky surface could come to contain multiple activating T cells—indeed, such large clusters have been observed in vivo. This physical proximity would serve to increase local concentrations and availability of cytokines within the scaffold. In addition, it may provide a mechanism by which a collection of initially weak TCR signals may, through bulk action, evade the spoiling effects of regulatory T cells that might also compete for APC occupancy (Tang et al., 2006). In the coming years, it will be interesting then to differentiate purely T cell intrinsic effects of stopping for a synapse as compared to the role that stopping has in promoting larger system-wide properties in which T cells and DCs participate.

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