CD69 guides CD4$^+$ T cells to the seat of memory

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Immunologists' views on the cellular basis of immune memory have undergone substantial modifications over the last decade, with new findings advancing the field from mostly descriptive studies of their presumptive phenotypes to those that allow the features distinguishing memory cells from short-lived effectors to be known and understood (1). Although such studies have helped to define the functional qualities of memory cells, more recent attention has been paid to the "where" of immune memory through comparison of the number and quality of memory T and B cells in various anatomical sites. These studies have produced the rather surprising finding that, rather than residing in the secondary lymphoid organs where most immune responses are initiated, many types of memory cells have been shown to preferentially accumulate in the bone marrow (BM), a site more often associated with hematopoiesis (2). As expected, such observations immediately lead back to the question of "how" this process occurs in terms of homing behavior and retention within specialized niches. In PNAS, Shinoda et al. provide a compelling answer for CD4$^+$ memory T cells by showing that expression of the activation marker CD69 is critical for the persistence of CD4$^+$ T cell memory in the bone marrow environment (3).

Memory Niches

CD4$^+$ memory T cells are critical for the generation of high-affinity memory B cells, long-lived plasma cells, and memory CD8$^+$ T cells, a fact that makes them essential to maintaining protective immunity to many classes of infectious pathogens (4–6). Despite this important immunoregulatory role, there is still much to discover about the differentiation, diversity, and maintenance of CD4$^+$ memory T cells in the body. Following immunization, CD4$^+$ T-cell responses involve an initial clonal expansion that produces the various specialized effector cells needed to deal with the specific antigenic challenge that is followed by a contraction phase in which the majority of these effectors die off. Whereas it is generally accepted that the remaining memory T cells undergo slow homeostatic proliferation to maintain their numbers and can mount a more rapid response to antigen reencounter than their naive precursors, the intrinsic and extrinsic factors that govern their survival are less well understood. An additional layer of complexity is added by the fact that CD4$^+$ T cells can be divided into different functional subsets such as effector memory (T_{EM}), central memory (T_{CM}), and follicular helper (T_{FH}), each of which possesses distinct functional capacities and sites of action and may also display different homeostatic and localization characteristics as memory cells (7, 8). Despite this complexity, a common theme connecting these subsets may be their need for cytokine environments that ensure their survival. In the absence of antigen, memory T cells are maintained by survival signals and by homeostatic cytokines, with IL-7 serving as the predominant homeostatic cytokine for CD4$^+$ memory T cells, although some contribution from IL-15 has also been observed (9). As such, it is surmised that memory CD4$^+$ T cells would seek an environment where these memory-maintaining cytokines are readily available. Although such environments can be found within secondary lymphoid organs, the bone marrow has emerged as a preferential site of residence for many types of memory cells including CD4$^+$ and CD8$^+$ T lymphocytes and long-lived plasma cells, which constitute an important component of humoral memory (2, 4–6, 9, 10). These observations have led directly to the question of what features of the bone marrow environment attract and retain memory lymphocytes there and what features the cells must possess to traffic there.

Expression of the activation marker CD69 is critical for the persistence of CD4$^+$ T cell memory in the bone marrow environment.

CD69 Shows the Way

Clues regarding the homing behavior of CD4$^+$ T_{MEM} cells began to emerge from analysis of the surface markers they express. Adoptive transfer experiments that allow longitudinal tracking of antigen-specific CD4$^+$ T cells have revealed that following the primary expansion and contraction phases of the response to immunization, they relocated to the bone marrow where they associated with IL-7$^+$ stromal cells (10). The BM-resident CD4$^+$ T cells displayed a distinct pattern of surface molecule expression that included the integrin α2, which mediated homing to BM, as well as the differentiation antigen Ly6C and the activation marker CD69. Constitutive expression of CD69 in the BM CD4$^+$ T cells was notable, as it is normally associated with acute activation of T cells, whereas the CD69$^+$ BM-resident memory CD4$^+$ T cells appeared to have entered a resting state, with greatly reduced levels of gene expression and DNA synthesis compared with the antigen-experienced CD4$^+$ T cells in the spleen. This resting state could be rapidly reversed, however, as the BM-resident CD4$^+$ T cells were found to quickly provide help for B-cell responses upon challenge with the appropriate antigen. Although neither its precise role in immunity nor its ligand are known, CD69 is not required for the development of CD4$^+$ T cells per se, as mice lacking this gene are still able to generate CD4$^+$ T-cell and CD4$^+$ T-cell–dependent immune responses (11, 12). A prominent role for CD69 has been described for regulating lymphocyte egress from secondary lymphoid organs through interaction with the sphingosine 1-phosphate receptor-1 (S1P1), qualifying it as being directly involved in the localization of T cells (13). Armed with this knowledge, Shinoda et al. use an adoptive transfer system to study the relevance of CD69 expression for CD4$^+$ T-cell memory. Using an adoptive transfer system (figure 1 in ref. 3) that allows wild-type and CD69$^{-/-}$ antigen-specific transgenic CD4$^+$ T cells to be tracked in different lymphoid compartments, they find that both populations can be readily detectable in comparable numbers at early time points after immunization. In the memory phase, however, the CD69-deficient cells fail to accumulate in the BM whereas their wild-type counterparts do so efficiently. This outcome does not appear to result from reduced egress from lymphoid organs, as the number of wild-type vs. CD69$^{-/-}$ antigen-specific CD4$^+$ T cells in the blood is comparable at every phase of the immune response. In addition to their reduced numbers, the CD69-deficient CD4$^+$ T cells are also functionally compromised, as they fail to provide efficient help for B cells in the form of high-

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affinity antibodies in vivo compared with their wild-type counterparts. This outcome is subsequently shown to involve a failure of antibody-secreting plasmablasts to home to BM, suggesting that BM-resident memory CD4+ T cells control either the establishment or the maintenance of plasma cells within that environment. A significant part of the help provided by $CD4^+$ T cells for the generation of high-affinity antibodies and plasmablasts involves transmission of signals via CD154 (CD40L). CD69-deficient $CD4^+$ T cells were found to express lower levels of CD154 than wild-type controls, suggesting this deficiency may contribute to their defective helper function. A range of other functionally relevant parameters are found to be normal in $CD69^{-/-}$ $CD4^+$ T cells, however, including the ability to generate phenotypically normal T_{FH} cells and germinal center (GC) B cells. The picture emerging from these observations is that CD69 regulates the entry and/or maintenance of $CD4^+$ T cells within the BM environment. As BM is accessed from the bloodstream, Shinoda et al. monitor the migration of differentially labeled wild-type and $CD69^{-/-}$ activated $CD4^+$ T cells following i.v. transfer to recipient mice and find that although both types of cells equilibrate in the spleen to similar levels, $CD69^{-/-}$ T cells are conspicuously decreased in the BM at both early and late time points. That CD69 inhibits BM entry is further supported by the demonstration that blocking antibodies to CD69 substantially inhibit wild-type B cells from relocating to the BM without affecting their ability to differentiate into functional helper cells.

The report by Shinoda et al. assigns a critical role to CD69 in mediating the relocation of $CD4^+$ T cells from blood to BM, one that likely accompanies their effector to memory transition. This observation raises the question, Which features of the BM environment are able to attract and retain these cells, and might CD69 play a role in this? The authors shed light on this question by using a powerful GFP reporter gene system to demonstrate that the majority of CD69-expressing cells in the BM associate with laminin$^+$ stromal and epithelial cells, compared with the minor fraction of their GFP-negative counterparts. This finding suggests that CD69 expression directs the memory $CD4^+$ T cells to specialized niches where they might receive the necessary cytokine and survival signals involved in their transition to the resting yet reactive state that is observed in the study by Shinoda et al. Although the nature of these signals is incompletely understood, it appears that IL-7 plays a role as the majority of antigen-experienced $CD4^+$ memory T cells in the BM are associated with VCAM-1^+ IL-7-expressing stromal cells (10). Curiously, memory maintenance does not appear to involve signals transmitted via the T-cell receptor, as no class II-expressing cells are found in the immediate vicinity of the BM-resident memory $CD4^+$ T cells. The existence of specialized BM niches for memory cell maintenance has been described for plasma cells that home to $CXCL12^+$ VCAM-1^+ stromal cells that make up only 1% of total BM cells (14). A slightly different situation exists for $CD8^+$ T cells that appear to migrate in significant numbers to BM but remain motile and actively proliferate (15, 16). The report by Shinoda et al. sheds important light on how $CD4^+$ T cells may find their way into the specific BM niche that provides their necessary survival cues and may even allow unique approaches to identifying the long-sought ligand of CD69. The most proximal clues to the mechanism underlying the observations that Shinoda et al. (3) make may come from the effect of CD69 on S1P1 function, as a receptor pathway involved in lymphocyte egress from one specialized environment may be involved in facilitating entrance to another. In any event, our understanding of the cellular mechanism through which T-cell memory is orchestrated and maintained is made clearer and thereby more compelling by Shinoda et al. (3), and the field looks forward to the new studies ref. 3 will undoubtedly influence.

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