COMMENTARY

Tissue resident T cell memory or how the magnificent seven are chilling in the bone

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Following infection, tissue-resident memory T cells (Trm) are thought to be left behind at sites of antigen encounter to protect affected tissues against subsequent reinfection. In this issue of the European Journal of Immunology, however, Pascutti et al. demonstrate that both murine and human CD8+ Trm specific to seven different pathogens, including systemic, skin, and lung tissue-localized pathogens, accumulate in the bone marrow (BM). These cells have a CD69+ phenotype, develop independently of local antigen, and require IL-15, Blimp-1, and Hobit for their differentiation and maintenance. Following restimulation, these cells expand and rapidly produce cytokines. While some of these responses may protect the BM from infection, the consideration that some of these pathogens or their antigens might never reach the BM suggests additional functional roles of BM Trm, possibly in supporting hematopoietic functions via cytokine production following infection. It will be further interesting to determine whether BM Trm contribute to the circulating effector pool following reinfection with tissue-localized or systemic pathogens and whether these cells can be elicited by vaccination.

Keywords: Blimp-1 · bone marrow · Hobit · IL-15 · tissue resident memory T cells

See accompanying article by Pascutti et al.

Until 10 years ago, memory T cells were thought to primarily circulate through the body and either home to secondary lymphoid tissues for reactivation (central memory) or to sites of inflammation (effector memory) [1]. It was noted, however, that infection could also induce local depots of memory T cells that remained localized at the sites of pathogen encounter [2], sometimes for years [3]. These non-recirculating cell populations have subsequently been termed tissue-resident memory (Trm) T cells. Both mouse and human CD4+ and CD8+ Trm are characterized by the loss of receptors that allow them to migrate back into the blood stream, including the sphingosine-1-phosphate receptor that is downregulated in a Krüppel-like factor 2-dependent manner due to constitutive expression of CD69 by Trm [4, 5]. In addition, Trm generally express integrins that anchor them in epithelia or collagen within the tissues, including CD103 and CD49a, respectively [6]. The establishment of Trm likely depends on expression of certain chemokine receptors, such as CXCR3 and CXCR6 [7], allowing their initial homing to peripheral sites. In addition, various transcriptional pathways, including NOTCH1/RBPJ, Hobit, Blimp1, and Runx3, have been described for both Trm establishment and maintenance [3, 8]. Depletion of circulating T cell memory cells could demonstrate that murine Trm are sufficient to restrict a variety of infectious disease agents, including viruses, bacteria, and fungi [9]. Depletion models were a vital evidence to show that lung Trm provide optimal protection against RSV and influenza A virus [10, 11]. Furthermore, CD4+ Trm, which seem to even persist longer in lungs and require less replenishment from the peripheral blood compared to CD8+ Trm [11–13], protect...
from *Bordella pertussis* and *Mycobacterium tuberculosis* infection in the lung [14, 15]. This protection does not necessarily result from superior effector functions of Trm cells, in which they often resemble effector memory T cells with respect to proliferation, cytokine production, and cytotoxicity [16–18]. Instead they seem to be at the right time at the right place to rapidly counter cytokine production, and cytotoxicity [16–18]. Instead they seem to be at the right time at the right place to rapidly counter re-infections.

In the study by Pascutti et al. of the current issue of the *European Journal of Immunology*, the authors investigate tissue residency of CD8\(^+\) T cells in seven viral and bacterial infections: the herpesviruses HSV, human and murine CMV, and EBV, influenza A virus, lymphocytic choriomeningitis virus, and the bacterial infection *Listeria monocytogenes* [19]. Despite distinct routes of infection (intranasal, intraperitoneal, intravenous, or skin-scarification) and distinct patterns of pathogen localization (systemic, lung-localized, skin-localized) as well as tropism, the authors describe that, in all cases, a pool of pathogen-specific CD8\(^+\) Trm is established in the BM (Fig. 1). Even when only infection primed CD8\(^+\) T cells are transferred to naïve recipient mice, a proportion of these home to the BM and subsequently express phenotypic and transcriptional hallmarks of Trm, including CD69, CD49a, CD101, and Hobit along with low levels of sphingosine-1-phosphate receptor and Krüppel-like factor 2. These murine BM CD8\(^+\) Trm were shown to efficiently produce cytokines like IFN-γ, even without restimulation, and to degranulate despite expressing the perforin-mediated cytotoxic machinery only at low levels. These findings provide evidence for the interesting possibility that Trm persist not only at the site of primary infection, but also as a depot in the BM, even despite the absence of cognate antigen or inflammatory cues in the local tissue environment.

The authors further demonstrate that murine BM Trm could be significantly expanded following antigen encounter or infection and, additionally, that sequential infections add to the total Trm pool, rather than “new” Trm replacing the “old” Trm from preceding infections. These findings are both particularly surprising. First, because it has been suggested that the BM mainly maintains resting memory cells [20] and second, because this niche was thought to limit the total memory T cell pool due to the requirement for direct interactions with limited VCAM1 and IL-7-expressing stromal cells [21, 22]. The BM is known to be enriched for memory CD4\(^+\) T cells, which accumulate at this site following initial immune responses [21] and can be subsequently mobilized during secondary pathogen encounter to support antibody responses more efficiently than other CD4\(^+\) T cell sources [21]. The current study by Pascutti et al, not only suggests that CD4\(^+\) memory and CD8\(^+\) Trm occupy the same niche within the BM [22], possibly with CD4\(^+\) T cells preferentially utilizing IL-7 and CD8\(^+\) T cells preferentially utilizing IL-15 as their respective survival cytokines [23], but further, that, unlike CD4\(^+\) T cell memory, CD8\(^+\) Trm can expand during secondary immune responses at this site without any obvious limitations and may not be mobilized into the periphery at high numbers like CD4\(^+\) cells.

What then, is the role for these pathogen-specific BM CD8\(^+\) Trm in host protection? The most straightforward possibility is that they restrict subsequent infections at this site. Protection from EBV infection in the BM represents an interesting example. The authors of this study found that EBV specific human CD8\(^+\) T cells, despite being present at half the frequency of human CMV specific CD8\(^+\) T cells in the BM, were enriched in CD69\(^+\) Trm cells. Previously it had been demonstrated that lytic EBV antigen (BZLF1)-specific CD8\(^+\) T cells were preferentially enriched in human BM, while latent EBV antigen (EBNA3A)-specific CD8\(^+\) T cells were difficult to detect [24]. Lytic EBV replication can be primarily observed in plasma cells in healthy EBV carriers [25].
Additionally, co-infection with the Kaposi sarcoma associated herpesvirus, which stimulates plasma cell differentiation in EBV infected B cells, increases lytic EBV replication [26]. It is known that, following infection, the BM is enriched for plasma cells that are maintained for years due to the survival-promoting tissue niche, providing VCAM-1, APRIL, and BAFF signaling [27]. Thus, immune restriction of lytic EBV infection in BM-localized plasma cells could benefit from a Trm compartment that recognizes early lytic EBV proteins. Furthermore, CD8+ T cell responses against such early lytic EBV antigens have indeed been shown to diminish lytic EBV replication in an in vivo model of EBV infection [28]. Thus, a Trm depot could specifically protect the important hematopoietic compartment from re-infections or chronic infection reactivation in the BM.

Alternatively, the BM may act as a reservoir for CD8+ Trm specific for pathogens, which preferentially infect other tissue sites. In this case, following reinfection, these BM Trm might be mobilized and migrate to the affected tissues to provide protective functions, as has been previously demonstrated for CD4+ memory T cells with a tissue residency profile [21]. Such a scenario would also be interesting from the perspective of vaccination. As vaccines are typically administrated at one site (i.e. the deltoid muscle) to protect from infection at another site (i.e. the respiratory tract), the generation of a BM Trm intermediate that could act as a source for protective memory T cells would be an intriguing possibility.

Pascutti et al., however, raise an interesting additional possible role for these BM-localized CD8+ Trm, namely that the preferential cytokine producing function of these cells, either with or without restimulation, could support steady-state or emergency hematopoiesis [29]. Along these lines it was already shown that IFN-γ by CD8+ T cells promote myelopoiesis either by stimulating hematopoietic stem cells directly into proliferation [30] or myeloid progenitor cells while retaining hematopoietic stem cell quiescence [31, 32]. These functions were suggested to result either from a direct effect of IFN-γ on these hematopoietic stem and progenitor cell populations [30, 31] or via IFN-γ stimulated IL-6 production by BM mesenchymal stem cells [33]. Therefore, infections could elicit a CD8+ Trm cell population in the BM which would support hematopoiesis and stimulate myelopoiesis that would, in turn, support tissue repair following infections. Such a positive feed-back loop for improved hematopoiesis after local and systemic infections via Trm cells in the BM is an attractive theory that would need to be further substantiated in future studies. Nevertheless, the accumulation of CD8+ Trm in the BM after infection, independent of the presence of the causative viruses or bacterium in this tissue, provides a new perspective on the role and functions of Trm in tissue residency.

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Abbreviation: Trm: tissue-resident memory

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