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Maintenance of memory T cells in the bone marrow: survival or homeostatic proliferation?

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Six years ago, Radbruch and colleagues discussed in *Nature Reviews Immunology* (Organization of immunological memory by bone marrow stroma. *Nat. Rev. Immunol.* **10**, 193–200 (2010))[1] how distinct stromal cell subsets in the bone marrow can support the lifelong persistence of plasma cells and memory T cells. These authors proposed that the bone marrow might serve as a depot for resting non-circulating memory T cells. Furthermore, they discussed how memory T cells might be maintained in the bone marrow by survival factors, such as interleukin-7 (IL-7), as opposed to by proliferative factors, such as IL-15. This view was in contrast with the largely accepted notion at the time that recirculating memory T cells are maintained by a homeostatic equilibrium between proliferation and death long after antigen clearance[2]. Furthermore, it did not accommodate previous data concerning the proliferation[3,4] and recirculation[5,6] of memory $CD8^+$ T cells in the bone marrow.

Recently, the idea that was originally proposed in *Nature Reviews Immunology*[1] was revived by the identification of quiescent, non-migratory tissue-resident memory T (T_{RM}) cells in the skin, gut and other organs[7]. Indeed, Radbruch *et al.* hypothesized that bone marrow memory T cells might share several features with T_{RM} cells, and they suggested that their previous and newly generated findings supported this concept[8,9]. However, it might be misleading to chiefly consider bone marrow memory T cells as non-circulating, non-dividing cells.

Experiments using Ki67 staining in mice and humans have shown that, at any given time-point, 95–98% of memory $CD8^+$ T cells in the bone marrow are in the G0 phase of the cell cycle[8,9]. Of the remaining cells, some are in the G1 interval, and a few (that is, 0.2–1.7%) are actively proliferating in S/G2/M[3,8,9]. However, this still means that the proportion of memory $CD8^+$ T cells proliferating in the bone marrow is reproducibly two- to fourfold higher than the proportions (that is, 0.05–0.80%) proliferating in the spleen, lymph nodes or blood[3,8,9]. This is true also when cell division is measured for one or more days. For example, in a 3 day-bromodeoxyuridine (BrdU)-labelling analysis, the average frequency of dividing antigen-specific memory $CD8^+$ T cells was 4% in the bone marrow and 2% in the spleen[4]. Moreover, when carboxyfluorescein succinimidyl ester (CFSE)-labelled antigen-specific memory $CD8^+$ T cells were transferred into non-immunized animals, they showed higher rates of proliferation in the bone marrow than in the spleen and lymph nodes[3,10]. In general, the data concerning memory $CD8^+$ T cell cycling in the bone marrow are all in agreement. However, memory $CD4^+$ T cell proliferation requires further investigation, as antigen-specific cells have not been examined in the bone marrow[11].

Despite the apparent consistency of the data concerning memory $CD8^+$ T cells, their interpretations differ. Radbruch's group proposes that these data suggest similarity between bone marrow memory $CD8^+$ T cells and peripheral T_{RM} cells, reinforcing the concept that a resting non-proliferative state following antigen clearance is the hallmark of memory $CD8^+$ T cells[8,9]. These authors suggest that the number of memory $CD8^+$ T cells proliferating in the bone marrow is negligible and may have been overestimated owing to a BrdU-related artefact[9]. They also showed by RNA microarray analysis that bone marrow memory $CD8^+$ T cells resembled their spleen counterparts and that both had overtly different transcriptomes from memory $CD8^+$ T cells stimulated *in vitro*[9]. Moreover, they have suggested that bone marrow memory $CD8^+$ T cells are sessile, as up to 60% of them express CD69, a molecule that in $CD4^+$ T cells is essential for retention in the bone marrow[8,9]. Finally, they suggest that the colocalization of bone marrow memory $CD8^+$ T cells with IL-7-producing stromal cells supports the idea of IL-7-driven survival of memory $CD8^+$ T cells in the absence of proliferation[9].

However, it could be argued that although the frequency of memory $CD8^+$ T cells that are dividing in the bone marrow is low, the absolute numbers of proliferating memory $CD8^+$ T cells is much higher in the bone marrow than in the spleen and lymph nodes[3,4]. As regards BrdU-related artefacts, they may occur at high BrdU doses[9] but seem uncommon at the standard BrdU dose that was used in bone marrow T cell studies[3,4,12]. Notably, recent adoptive transfer experiments in genetically modified mice have shown that IL-15 in the bone marrow promotes proliferation and inhibits interleukin-7 receptor subunit-α (IL-7Rα) expression in memory $CD8^+$ T cells, independently of antigen co-transfer or treatment with innate receptor agonists[13]. In respect to molecular data[9], it is perhaps not surprising that transcription profiles were highly diverse when comparing *ex vivo*-isolated and *in vitro*-stimulated cells; besides, some differences between freshly obtained spleen and bone marrow $CD44^{hi}CD8^+CD3^+$ T cells might have been missed owing to the cell sorting strategy. For instance, IL-$7R\alpha^{hi}$, but not IL-$7R\alpha^{low}$, T cells were selected for analysis, and yet IL-$7R\alpha^{low}$ T cells are enriched in the bone marrow[8,13], reflecting *in vivo* exposure to IL-15 (REF. 13). Moreover, global transcription data, CD69 expression profiles and colocalization in tissue sections do not address *in vivo* T cell migration. In fact, *in situ*-labelling studies and parabiosis experiments have shown that memory T cells do recirculate to and from the bone marrow[5,6].

In conclusion, the available evidence supports the view that the bone marrow is a 'stopping point' where recirculating memory $CD8^+$ T cells are stimulated to proliferate before continuing to move around the body[3,4,12,13]. Notably, lodging into the bone marrow is a competitive process among memory T cells[14], which is an element to be considered especially in interpretation of adoptive transfer data[11,14]. Furthermore, reported diversities in the repertoire of antigen specificity between human bone marrow and blood memory $CD4^+$ T cells after *in vitro* restimulation[8] might reflect several features, including: differences in T cell recruitment into the bone marrow, undetected ongoing responses against common pathogens (for example, *Candida albicans* and *Cytomegalovirus*) and differences between *in vitro* and *in vivo* responses. Finally, recent parabiosis experiments demonstrated that a small percentage (up to 5%) of memory $CD8^+$ T cells in the spleen and lymph nodes are non-migratory[15]. Therefore, it is possible that a minor population of memory $CD8^+$ T cells in the bone marrow (as opposed to the majority) might be sessile, but direct evidence for this is lacking at the moment.

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