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The lifestyle of memory CD8$^+$ T cells

Özen Sercan Alp and Andreas Radbruch

We are thankful for an excellent opportunity to expand on our Review (Organization of immunological memory by bone marrow stroma. *Nat. Rev. Immunol.* **10**, 193–200 (2010))[1] that has been provided by the correspondence from Francesca Di Rosa (Maintenance of memory T cells in the bone marrow: survival or homeostatic proliferation? *Nat. Rev. Immunol.* (2016))[2].

The traditional concept on how immunological memory is maintained proposes that memory lymphocytes circulate through the body in a quest for cells presenting them their cognate antigen. Their numbers are determined by a balance between (homeostatic) proliferation and cell death. It has been proposed that this proliferation mainly takes place in the bone marrow and is driven by interleukin-15 (IL-15), with an estimated turnover of the entire population within 2 months[3–6]. Memory CD8$^+$ T cells from the bone marrow are thus considered to be part of the circulating memory T cell population.

Observations made by us, for memory T cells from the bone marrow, and by others, for memory T cells from epithelial tissues, challenge this traditional view and suggest that there might be an alternative scenario for the maintenance of systemic and local immunological memory. This scenario suggests that, apart from the memory provided by circulating memory T cells, immunological memory to systemic and local pathogens is provided by populations of memory T cells residing in the bone marrow and in epithelial tissues, respectively. These memory T cells are resting in terms of proliferation and mobility. We originally showed this for mouse memory CD4$^+$ T cells generated in particular immune responses[7]. These cells were maintained exclusively in the bone marrow and disappeared from secondary lymphoid organs. They were resting in terms of transcriptional activity, proliferation and mobility; the latter was evident from their absence from the periphery. They expressed CD69, and this was essential for their persistence in the bone marrow[8]. More recently, we have shown that human memory CD4$^+$ T cells are also residing in the bone marrow[9]. The comparison of memory CD4$^+$ T cell repertoires from the blood and bone marrow of individual donors revealed that memory T cells specific for systemic childhood pathogens in many donors were only and readily detected in the bone marrow but not in the blood, arguing that both compartments are separate and that these bone marrow memory CD4$^+$ T cells are residents of the bone marrow, resting there for their lifespan. A more detailed analysis of the repertoires of circulating and bone marrow-resident memory CD4$^+$ T cells will show whether memory T cells with specificities that are present in the bone marrow and blood are one or two distinct populations.

For memory CD8$^+$ T cells, the situation is much less clear. We are not aware of comparisons of memory CD8$^+$ T cell repertoires from secondary lymphoid organs, blood and bone marrow. Memory CD8$^+$ T cells from the bone marrow do, however, express CD69 (REFS 9,10). Their phenotype is similar to that of tissue-resident cells, especially with respect to the expression of CD69, sphingosine-1-phosphate receptor 1 (*S1PR1*) and Kruppel-like factor 2 (*KLF2*). In human bone marrow, about 70% of the memory CD8$^+$ T cells express CD69 but not *S1PR1*: that is, they are not attracted to enter the blood circulation[9]. It is true that a parabiosis experiment and another experiment using *in vivo* labelling of bone marrow cells have shown that memory CD8$^+$ T cells can emigrate from bone marrow, spleen and lung, but this does not exclude the existence of a separate resident population in those organs[11,12]. In fact, it has been recently shown that all memory CD69$^+$CD8$^+$ T cells in the lung, spleen and lymph nodes are tissue-resident, non-circulating cells[13,14]. For the bone marrow, the definition and exact quantification of a resident population remains a challenge, but a reasonable working hypothesis is that this might comprise all memory CD69$^+$CD8$^+$ T cells, which represent the major part of the memory CD8$^+$ T cells in the bone marrow.

What are the memory CD4$^+$ and CD8$^+$ T cells doing in the bone marrow? We and others have found them allocated to IL-7-expressing stromal cells[10,15]. According to cell cycle analysis by propidium iodide (PI) staining, more than 99% of the memory CD8$^+$ T cells in human bone marrow are either in the G0 or G1 phase of the cell cycle, and Ki67 staining shows that 98–99.5% of the cells are indeed in G0 (that is, resting in terms of proliferation)[9]. Of mouse memory CD8$^+$ T cells, more than 99% of the cells were in G0 or G1, according to PI staining, with 95% of them being in G0 according to Ki67 staining[10,16]. Both approaches are non-invasive and do not involve the manipulation of cells *in vivo*, unlike approaches based on bromodeoxyuridine (BrdU) or carboxyfluorescein succinimidyl ester (CFSE) labelling[3–6]. Indeed, we could demonstrate that BrdU can induce proliferation of memory T cells when given orally at a dose of 1 mg per ml in drinking water[10]. Based on our analysis of proliferation using cell cycle analysis and Ki67 staining, we feel it is fair to state that the extent of homeostatic proliferation has been largely overestimated previously[3–6]. We would not be too surprised if it turns out that homeostatic proliferation does not have any role at all in the maintenance of memory CD4$^+$ and CD8$^+$ T cells.

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Competing interests statement
The authors declare no competing interests.