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Signals controlling rest and reactivation of T helper memory lymphocytes in bone marrow

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Abstract Established views on the maintenance of immunological memory have been challenged recently by the description of memory plasma cells and memory T helper (Th) lymphocytes residing in the bone marrow (BM) in dedicated survival niches, resting in terms of proliferation and migration. While memory plasma cells are no longer reactive to antigen, memory Th lymphocytes are in a state of attentive rest, and can be reactivated fast and efficiently. Here, we discuss the signals controlling these resting states, which the memory lymphocytes receive from their microenvironment.

Keywords T helper memory · Niche · Bone marrow

Abbreviations
BM Bone marrow
Th T helper

Resting immune memory

"Memory" is a key feature of acquired immunity. In T cell-dependent immune responses, clonally expanded, antigen-specific lymphocytes with extended lifespan maintain information on the antigen for long time periods, even in the apparent absence of the antigen. However, nature and lifestyle of memory lymphocytes have largely been enigmatic. In recent years, it has been shown for plasma cells and for T helper (Th) lymphocytes that, after immune reactions, a dedicated subpopulation of competent memory precursor cells migrates into the bone marrow (BM) and there differentiates into memory cells, which rest in terms of proliferation and migration. These memory cells survive in specialized microenvironments, also called "niches". Memory plasma cells are maintained on CXCL12-expressing stromal cells of the BM [1–3]. These stroma cells may apparently also recruit eosinophils [3], basophils [4], and megakaryocytes [5] to provide accessory signals for the memory plasma cells. Memory Th cells are maintained on IL-7-expressing stromal cells of BM [6], while naive T cells are maintained on IL-7-expressing stromal cells of secondary lymphoid organs, called fibroblastic reticular cells [7].

Microenvironments of resting memory cells

We have previously reviewed the organization by stromal cells of memory lymphocytes [8, 9]. IL-7 and CXCL12 of stromal cells are established survival factors of memory Th cells and memory plasma cells, respectively. Memory plasma cells in addition require other factors for survival such as a proliferation-inducing ligand (APRIL), which is mainly provided to them by eosinophils and megakaryocytes, though not so much by stromal cells [3, 10]. These findings support the concept of dedicated niches for memory cells organized by stromal cells, but also requiring accessory cells. This could also explain why not every CXCL12-expressing stromal cell in murine BM hosts a plasma cell. Some 1–2 % of BM cells are CXCL12-expressing stromal

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cells, while only 0.1 % of murine BM cells are memory plasma cells [2]. This disparity of the frequencies could indicate the complexity of the niches, although at present an alternative explanation cannot be ruled out, namely those laboratory mice simply have too few infections to fill their memory plasma cell pool, as compared to humans, who have about 1 % of BM plasma cells. A further problem so far not resolved is how short-lived eosinophils and megakaryocytes can support long-lived plasma cells.

Murine memory Th cells are at most 0.1 % of all murine BM cells, while IL-7-expressing stromal cells make up 2–4 %. Does this indicate the requirement for additional, restricting accessory factors, other than IL-7, cooperatively controlling the survival of memory Th cells?

Memory plasma cells, memory Th cells, and memory CD8 T cells highly express Bcl-2 [11–13]. Vikstrom and colleagues [14] have shown that Mcl-2 is essential for memory B cell formation but not for their persistence. Expression of Bcl-2 in germinal center memory B cells apparently promotes their survival [15, 16]. Bcl-2 could be the anti-apoptotic molecule required and sufficient for survival of all memory lymphocytes. The expression of Bcl-2 in resting memory Th cells is probably induced by IL-7, because memory Th cells upregulate Bcl-2 in response to IL-7 during a 6-day culture period [17]. We have compared the transcriptional expression of Bcl-2 between splenic and BM CD44^{hi} CD4 T cells [6]. Of the anti-apoptotic Bcl-2 family proteins, i.e., Bcl-2, A1, Bcl-w, Bcl-xL, and Mcl-1, only Bcl-2 is highly expressed by BM CD44^{hi} CD4 T cells, which in the BM contact IL-7-expressing stromal cells (Fig. 1; edited [6]). Memory plasma cells obtain survival factors from CXCL12-expressing stromal cells [2, 18], APRIL- and IL-6-expressing megakaryocytes [5], and eosinophils [3]. IL-6 is known to induce Bcl-2 via STAT3-dependent signaling [19], and deficiency of B cell activating factor (BAFF), a simile of APRIL, both addressing

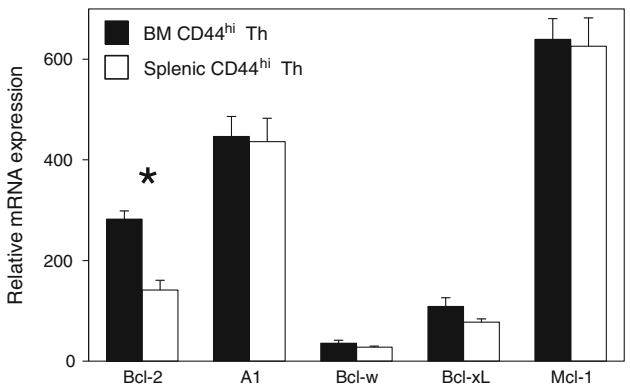

Fig. 1 Expression of anti-apoptotic factors in BM or splenic CD44^{hi} CD4 T cells. In accordance with the gene expression data [6], of the anti-apoptotic factors only expression of Bcl-2 is upregulated in BM memory CD4 T cells

B cell maturation antigen (BCMA) of memory plasma cells, could be compensated for by overexpression of Bcl-2 in B cells, suggesting that BCMA signals also enhance Bcl-2 expression [20]. Finally, it has been shown that the survival-promoting effects of CXCL12 on thymic dendritic cells are mediated by the up-regulation of Bcl-2 [21], i.e., CXCL12 can also enhance Bcl-2 expression in memory plasma cells. All of this is in line with the notion that resting memory plasma cells and memory Th lymphocytes use Bcl-2 to survive.

It is believed that IL-7 is essential for the maintenance of Th cell memory. But molecular details are not clear for the resting Th cells of BM. IL-7 has been reported to be required for survival of long-lived peripheral Th cells, in the absence of their proliferation [17]. Also, it has been claimed that IL-7 mediates the transition from effector to effector-memory Th cells [22]. In both investigations, antigen-specific or effector-memory Th cells from spleen or lymph nodes were transferred into IL-7-deficient mice, and their location and survival in the host was analyzed. In the spleen, naive Th cells contact IL-7-expressing follicular reticular stromal cells of periarteriolar lymphoid sheaths (PALS) area [23], while resident antigen-specific splenic effector-memory Th cells reside in the extrafollicular area and do not contact IL-7-expressing stromal cells (Tokoyoda, unpublished data). Resting memory Th cells of BM, however, are docked onto IL-7-expressing stromal cells [6]. At this time, therefore, it remains controversial whether IL-7 is involved in the differentiation of effector Th cells into resting memory Th cells of the BM and/or their maintenance as long-lived cells. In case of the former, the timing of translocation of effector Th cells to IL-7-expressing stromal cells of the BM would be an important facet of their competence to become resting memory Th cells. This competence would be determined by expression of genes coding for molecules associated with migration and adhesion. The effector Th cells would have to egress from IL-7-expressing stromal cells in the PALS area of secondary lymphoid organs, home to the BM via blood flow, and in the BM migrate towards IL-7-expressing stromal cells. CD49b (integrin $\alpha 2$) is regarded an activation marker of naive Th cells and is expressed on effector Th cells [24]. CD49b^{+} CD4 T cells strongly express IFNγ after stimulation with anti-CD3 antibodies in vitro [25]. Splenic effector Th cells, when incubated with the Fab fragment of an anti-CD49b antibody, do not migrate into the BM upon adoptive transfer [6]. CD49b and CD29, also known as integrin $\beta 1$, form VLA2, which binds to collagen. CD29-deficient antigen-specific effector-memory Th cells cannot develop into resting BM memory cells [26, 27]. Likewise, DiLillo and colleagues have shown that memory plasma cells can be quantitatively eliminated from BM by antibodies to VLA4 [28]. Other adhesion molecules are also linked to survival of memory cells, like CD44. Cassese and

colleagues have shown that ligands of CD44, like specific antibodies or hyaluronic acid, can prolong the survival of memory plasma cells isolated from the BM [29]. All these observations underpin the crucial relevance of correct adhesion for the persistence of memory cells in their BM niches and their survival. CD44, VLA2, and VLA4 seem to be of more general relevance for immune memory.

A central point in the discussion of memory cells of BM is whether they are resting in terms of antigenic stimulation. Both for memory plasma cells and for Th cells, this can clearly be denied. For plasma cells, it has been shown that their persistence in the BM is not influenced by antigen or antigen–antibody complexes [30, 31], although once dislocated from their niches, BM plasma cell apoptosis is speeded up by FcγIIb signals, i.e., immunocomplexes [32]. For Th lymphocytes, it has been shown that antigen-specific effector-memory Th cells can persist in major histocompatibility complex (MHC) class II-deficient mice, i.e., in the absence of cognate antigen recognition [33] and resting memory Th cells of the BM do not contact MHC class II-expressing cells, but nevertheless are stably maintained over extended time periods [6]. On the other hand, tonic T cell-receptor (TCR) stimulation induces homeostatic proliferation of effector-memory Th cells, a mechanism discussed as contributing to the maintenance of the long-lived peripheral effector-memory Th cell pool [34].

Microenvironments for reactive immune memory

Naive Th cells, when activated by antigen-presenting cells, proliferate and produce cytokines. They differentiate into effector Th cells and some may become long-lived memory cells. Both effector and memory Th cells can help other immune cells. CD154 (CD40 ligand) is an essential co-stimulatory molecule of Th cells to control B cell maturation. Upon TCR stimulation, CD154, but also cytokines, are quickly re-expressed on in vitro-differentiated Th1 "effector" cells and BM CD44^{hi} "memory" Th cells (Fig. 2; edited [6]). At least, in humans, BM-resident memory Th cells are also polyfunctional, i.e., individual cells express a variety of effector cytokines in a coordinated fashion [35]. So, while resting in terms of proliferation, migration, and overall gene expression [6], memory Th cells of BM are highly attentive to antigen and react to it faster and more efficiently than peripheral effector-memory cells. How is this resting, attentive state organized on the molecular level? Currently, this is completely unclear. First of all, it is unclear how the precursors of BM memory Th cells are induced to enter this state and become attentive resting memory cells. Is it an imprinting or a tonic signal? Did the cell receive it during its original antigenic stimulation or later, once it had entered the memory niche of BM?

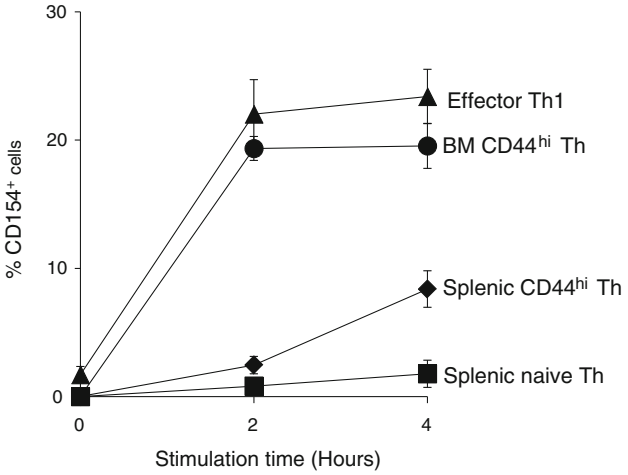

Fig. 2 BM memory CD4 T cells express CD154 quickly. Splenic CD44^{lo} T, CD44^{hi} T, BM CD44^{hi} T or in vitro-differentiated Th1 cells were stimulated with plate-bound anti-CD3 in the presence of Brefeldin A and, at the time points indicated, were fixed and stained with anti-CD154

Epigenetic memory

Although molecular details are scarce, it has long been noted that genes for cytokines and other effector functions are imprinted epigenetically in effector-memory cells, by demethylation of DNA, and modification of histones by acetylation and methylation [36, 37]. Th1 cells but not Th2 cells increase histone acetylation at the IFNγ locus [38], while the IL-4 gene is demethylated in Th2 cells but not Th1 cells [39]. Most notably, it has been shown that key transcription factors of Th lineage differentiation, like GATA3, directly control demethylation of functional genes of this lineage, i.e., IL-4, and that this demethylation determines the fast, TCR-dependent but STAT4-independent reexpression of the IL-4 gene, i.e., cytokine memory [40]. It has also been shown that the lineage-specific epigenetic imprinting of cytokine genes does not only require additional critical and timed costimulators [41] but also requires progression of the activated naive Th cells into the S-phase of cell cycle [42].

Of particular interest is CD154, a critical costimulator for B cells and conditio sine qua non for B cell and plasma cell memory [43]. Naive Th cells and splenic effector-memory Th cells express it slowly. BM memory-phenotype Th cells and in vitro-differentiated effector Th1 cells express CD154 very quickly (Fig. 2). The molecular reason for this is not known, although there is indirect evidence that again this regulation may occur epigenetically: in Th

cells of female patients with systemic lupus erythematosus (SLE), the gene for CD154 is demethylated on both X chromosomes, while it is not on the inactivated X chromosomes of healthy females [44].

Metabolism and resting memory

While memory plasma cells are resting in terms of proliferation and mobility, they do synthesize proteins at a high rate requiring an extremely active metabolism. For resting memory Th lymphocytes of BM, the situation may be different. Activated T lymphocytes maximize their energy metabolism by glycolysis; they express the glucose transporter Glut1 [45]. Regulation of Glut1 surface expression is associated with PI3K/AKT activation following cytokine stimulation or stimulation via TCR and CD28 [46]. In contrast, resting T cells of the periphery probably mostly generate their energy by oxidative phosphorylation. It has been suggested that the metabolic changes from effector to effector-memory cells are regulated by the mammalian target of Rapamycin (mTOR) [47, 48]. Recent studies have shown that rapamycin, an immunosuppressive drug, promotes the generation of effector-memory CD8 T cells [49–51]. mTOR-deficient or rapamycin-treated Th cells fail to differentiate into Th1, Th2, or Th17 cells, and instead differentiate into Foxp3$^+$ regulatory T cells [52, 53]. At this time, however, the role of mTOR for metabolic regulation of resting, attentive memory Th cells from BM remains unknown, although it should be noted that their location in the sinusoid region of BM privileges them with respect to oxygen consumption.

Concluding remarks

The signals that control the development and the maintenance of memory lymphocytes are poorly understood. For memory plasma cells, a molecular code emerges for their maintenance in the BM, in dedicated survival niches organized by CXCL12-expressing stromal cells. This code includes CXCL12 itself, IL-6, and ligands of BCMA, VLA4, and CD44. For Th lymphocytes, the confusion is enhanced by the current debate on the relative contributions to memory of peripheral, mobile, and BM-resident, resting long-lived cell populations. For the attentive, resting Th memory cells of BM, we can say that they have access to signals for the IL-7 receptor, VLA2, CD44, and oxygen from their microenvironment, while signals for the antigen-receptor are apparently absent, unless the immune system encounters the cognate antigen again. Comparing the survival signals for memory plasma cells and memory Th cells, it is obvious that the survival codes share elements like CD44, but differ in others. It will be of high scientific and translational interest to decipher the memory code completely, and also for memory B cells and cytotoxic memory T cells. Knowing the code will allow the manipulation of immunological memory in an unprecedented fashion strengthening good memories and erasing bad ones.

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