

REVIEW

T-lymphocyte interaction with stromal, bone and hematopoietic cells in the bone marrow

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Mature T cells in the bone marrow (BM) are in constant exchange with the blood pool. Within the BM, T-cell recognition of antigen presented by dendritic cell (DC) can occur, nevertheless it is thought that BM T cells mostly receive non-antigenic signals by either stimulatory, for example, interleukin (IL)-7, IL-15, tumor necrosis factor family members, or inhibitory molecules, for example, transforming growth factor- β . The net balance is in favor of T-cell proliferation. Indeed, the percentage of proliferating T cells is higher in the BM than in spleen and lymph nodes, both within CD4 and CD8 T cells. High numbers of memory T cells proliferate in the BM, as they preferentially home to the BM and have an increased turnover as compared with naive T cells. I propose here that the BM plays an essential role in maintaining normal peripheral T-lymphocyte numbers and antigen-specific memory for both CD4 and CD8 T cells. I also discuss BM T-cell contribution to the homeostasis of bone metabolism as well as of hematopoiesis. It emerges that BM T cells play unexpected roles in several diseases, for example AIDS and osteoporosis. A better knowledge on BM T cells has implications for currently used clinical interventions, for example, vaccination, BM transplantation, mesenchymal stem cell-based therapies.

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Mature T cells circulating in the periphery enter primary lymphoid organs through the blood route. In the thymus, most single positive CD4 and CD8 T cells represent recently generated naive T cells, which have completed their development in the organ and are ready to egress. Nevertheless, a small proportion of thymic mature T cells are immigrants from the periphery, as shown by studies in rodents, lambs and pigs.^{1–3} For a greater discussion on immigrant thymic T cells, the reader can refer to other articles in this issue.^{4,5} In the bone marrow (BM), mature T cells represent about 3–8% of total nucleated cells.⁶ BM T cells arrive from the blood route and, after homing to the BM, can move back to the blood and then migrate to other lymphoid organs⁶ (Figure 1). At present, the functions of either immigrant thymic or BM T cells are not completely defined, nor it is known whether the two populations of T cells share similar functions. Another unsolved question is whether the T cells that home to one of the two primary lymphoid organs—thymus or BM—migrate along a recirculating pathway involving both organs.

Recently, we have proposed that the BM is a crucial organ in mature T-cell traffic and contributes greatly to long-term cytotoxic memory as well as to homeostatic regulation of peripheral CD8 T cell numbers^{6–10} (Figure 2a). We and others have shown not only that the BM can prime naive T cells and recruit effector T cells, but also that it serves as a site of preferential proliferation for CD4 and CD8 T cells.^{7–14} In addition to its well-known hematopoietic function, it is recognized

that the BM plays a role in several physiological and pathological processes, including B-cell memory, long-term antibody production, inflammatory response, bone metabolism, tissue repair.^{15–20} In this review, I will discuss the role played by the BM in T-cell responses. Moreover, I will provide some examples of how T cells can modulate the function of other cells in the BM environment, such as mesenchymal stromal cells, osteoclasts, osteoblasts and hematopoietic precursors. By looking at the two sides of the same coin, that is, the effects of the BM on T cells and vice versa, I will propose an integrated view of the functional interactions between immigrant T cells and resident cells in the BM.

RECIRCULATING T CELLS GET IN AND OUT OF THE BM

By performing adoptive transfer experiments in mice, we and others observed that memory T cells preferentially accumulate in the BM, in comparison with naive T cells.^{12,24} Elegant intravital microscopy studies of mouse BM by von Andrian and co-workers showed that CD8 T cell rolling in BM microvessels occurs through L-, P- and E-selectins, whereas sticking is mostly mediated by the interaction between the lymphocyte integrin $\alpha 4\beta 1$ and the endothelial adhesion molecule VCAM-1.¹² As regards chemokines, SDF-1 (CXCL12) is involved in $\alpha 4\beta 1$ integrin activation.¹² This is in agreement with the finding that high levels of CXCL12—a master regulator of hematopoietic stem cell recruitment into the BM—are present in normal BM

sinusoidal endothelium.^{25,26} T-cell expression of the CXCL12 receptor CXCR4 is modulated by antigen, interleukin (IL)-2 family cytokines and tumor necrosis factor (TNF) family members.²⁷ Memory T cells show a higher responsiveness to CXCL12 than naive T cells, as observed in transendothelial migration assays under shear flow.²⁸

The pattern of chemokine receptor expression has been investigated in human BM T cells, either in healthy individuals,¹² or in patients

with cancer, osteoarthritis, viral infections or autoimmune diseases.^{29–32} The concept emerging from these studies is that human BM T cells have a unique chemokine receptor phenotype, characterized by high expression of CXCR4, CCR5, CXCR6, CX3CR1, but not of CXCR3.^{29–32} Thus, in addition to CXCL12, human BM T cells respond to a distinct set of inflammatory chemokines, that is, CCL3, CCL4, CCL5, CXCL16, CX3CL1. Interestingly, the migration pattern to the BM is altered in the case of T cells from KLF2 (Kruppel-like factor 2)-deficient mice.³³ In the absence of KLF2, naive CD4 T cells express a set of inflammatory chemokine receptors normally expressed by either activated/memory T cells or non-T cells, including CCR5 and CCR3. In association with such deregulated expression, KLF2-deficient naive CD4 T cells show an increased migration to the BM, as well as to non-lymphoid peripheral organs, such as colon, liver, kidney, skeletal muscle and brain, whereas their number is reduced in the spleen and lymph nodes.³³

As regards CCR7, a lymph node homing receptor, normal human BM CD8 T cells include similar proportions of CCR7⁺ and CCR7⁻ cells.¹² Two subsets of CD45RA⁻ memory T cells have been defined based on CCR7 expression: CCR7⁺ central memory and CCR7⁻ effector memory cells.³⁴ Both subsets are found in human BM, although their percentages are dissimilar in different studies, a discrepancy possibly related to donor characteristics and/or experimental procedures.^{12,30,32} Furthermore, it has been reported that virus-specific BM CD8 T cells are mostly central memory in cytomegalovirus infection³⁵ and effector memory in HCV infection.³⁶ A thorough analysis of cytomegalovirus-, Epstein-Barr virus (EBV)- and Flu-specific CD8 T cells in matched samples from blood and BM showed that the CD8 T-cell homing profile varies with the type of infection—acute versus persistent—as well as with the viral epitope—latent versus lytic—suggesting a link between disease pathogenesis and

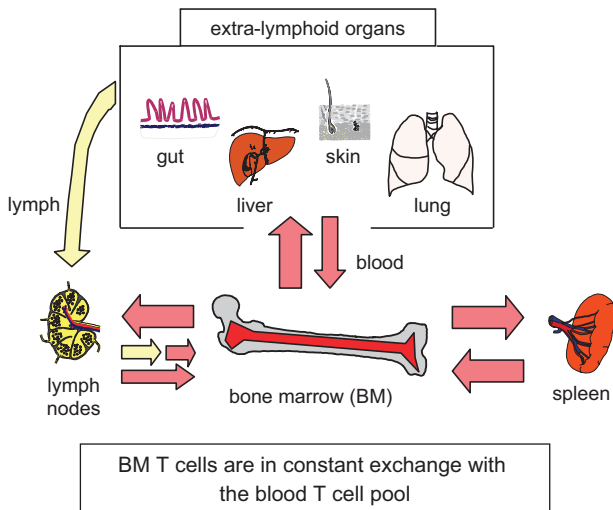


Figure 1 Bone marrow (BM) T-cell recirculatory pathways. Mature T cells get in and out of the BM through the blood route. The figure shows the T-cell recirculatory pathways in which BM is integrated. Very little is known about the kinetics of T-cell recirculation in different organs.

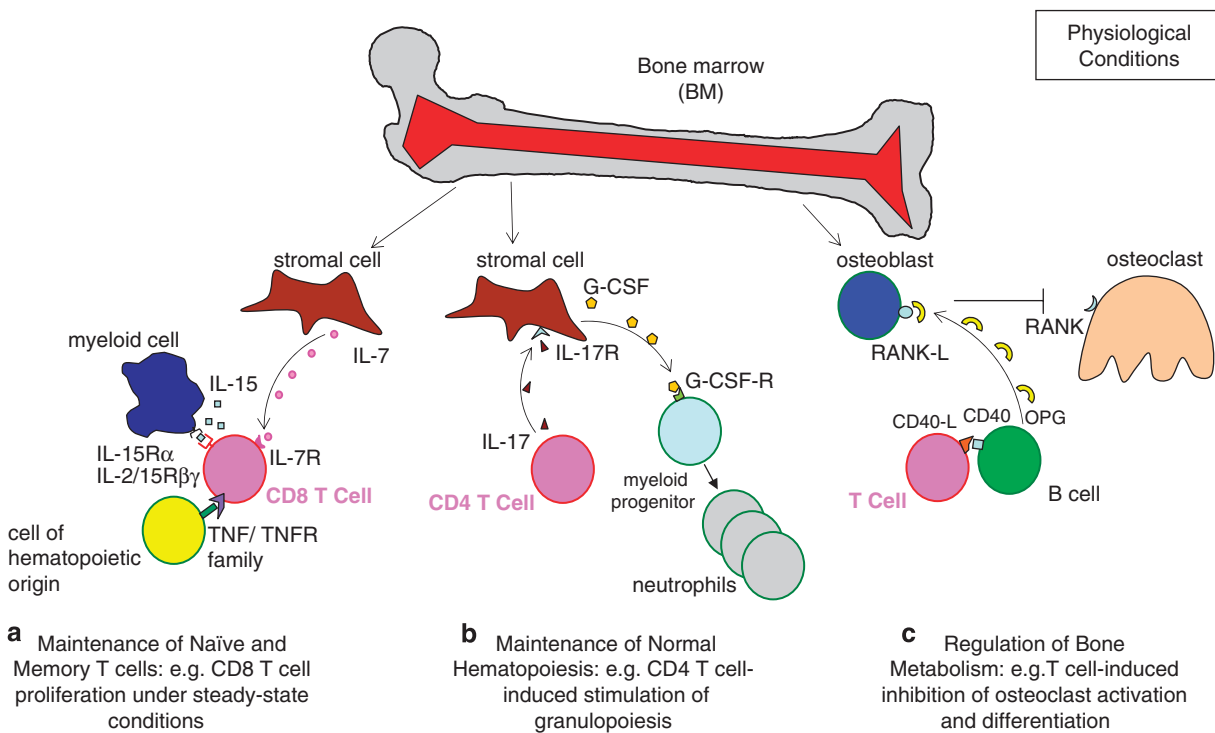


Figure 2 Bone marrow (BM) T cells contribute to the homeostasis of the immune system as well as of different cell types present in the BM environment. The figure represents some examples of BM T-cell involvement in the physiological regulation of immune system⁹ (a), hematopoiesis^{21,22} (b), bone metabolism (c).²³

CD8 T-cell traffic to the BM.³¹ However, CD8 T-cell accumulation in the BM cannot be simply explained by cell recruitment to the sites of antigen persistence. For example, in EBV-chronically infected individuals, CD8 T cells specific for the EBV-lytic epitopes were increased by 3–5 times in the BM as compared with the blood, but EBV load was not increased and lytic antigens were not expressed in the BM.³¹

T cells can egress from the BM only through the blood route, as there are no lymphatic vessels that drain this organ.⁶ Indeed, the BM is the only lymphoid organ that is not integrated in the lymphatic system. Seminal experiments of *in situ* labeling of BM cells in pigs and sheep showed that T cells continuously leave the BM through blood and recirculate to other lymphoid organs.^{37,38} At present, the nature of the molecules regulating T-cell egress from the BM and how this process can be interfered with are still unsolved questions. In agreement with its effects on hematopoietic precursor cells, granulocyte colony-stimulating factor (G-CSF) has been shown to mobilize regulatory T cells from the BM.³⁹ Further studies are required to define the effects on BM T-cell exit of the S1P-analog FTY720, a blocking agent for T-cell egress from thymus and lymph nodes.^{40,41}

T-cell homing to the BM is somehow linked to the egress of other rival T cells. Our studies suggested that T-cell colonization of the BM is a competitive process between the incoming T cells and the resident T cells, which already inhabit the same saturable niche.²⁴ Indeed, adoptively transferred CD44^{high} memory T cells easily displaced T cells from the BM of young mice, in which the lymphoid periphery mostly contained CD44^{int/low} naive T cells, but not of old thymectomized mice, which had plenty of CD44^{high} memory T cells in their lymphoid organs.²⁴ Other studies showed that, a few days after the blood circulation of two immune congenic mice had been connected by parabiosis, the BM of each mouse contained equal numbers of the two types of congenic antigen-specific memory CD8 T cells, implying that most memory CD8 T cells resided in the BM for a short time and then were displaced by incoming rival cells from the blood.⁴² Similar results were found in the spleen, lung and liver, but not in the brain, peritoneal cavity and intestinal lamina propria, where memory CD8 T-cell entry from the blood was delayed.⁴² In mice with chronic colitis, both adoptive transfer and parabiosis experiments suggested that pathogenic CD4 T cells continuously recirculate among colonic lamina propria, mesenteric lymph nodes, thoracic duct, peripheral blood, spleen and BM.^{43,44} Taken together, these results suggest that, either in healthy or disease conditions, BM memory T cells are in equilibrium with a blood-borne cell pool, which has the capacity to seed multiple organs (Figure 1).

BM T CELLS AND SYSTEMIC IMMUNITY

Recently, our studies, as well as those of others, have suggested that the contribution of BM T cells to systemic immunity is greater than thought previously.^{6–8,11–13} Although the percentage of CD4 and CD8 T cells within BM nucleated cells is only around 5%, as the whole BM is a relatively large organ, the absolute number of BM T cells and that of spleen T cells are in the same range: we estimated that in a healthy young adult human 25×10^9 T cells are present in the BM and 31×10^9 in the spleen.⁶ The BM TCR⁺ cells comprise both $\alpha\beta$ -T cells and $\gamma\delta$ -T cells, as well as natural killer T cells, which are 'innate-like' lymphocytes expressing the $\alpha\beta$ -T-cell antigen receptor in conjunction with natural killer cell markers. Moreover, the BM contains a subset of CD8 α^+ TCR⁻ unconventional cells, which contributes to the facilitation of allogeneic hematopoietic stem cell engraftment upon BM transplantation.⁴⁵ Canonical CD4 and CD8 T cells are found in the BM in a ratio of roughly 1:2, which is quite the reverse of the ratio

observed in the blood, in which CD4 are more abundant than CD8 T cells.

It has long been known that T cell–B cell cooperation in the BM can lead to antibody formation.¹⁵ In the last decade, it has been shown in mice that the BM can function as a site of T-cell priming, under conditions in which antigen-loaded dendritic cell (DC) are found in the BM, either because the antigen is blood-borne and local BM DC capture it, or because circulating DC carrying the antigen migrate to the BM.^{11,46,47} Antigen-loaded DC in the BM can also restimulate memory T cells.^{11,48} In several human diseases, antigen-specific T cells have been found in the BM.^{29,31,36,49–53} In some instances, BM clearly represented a target organ of effector T cells (Figures 3a and b), for example, in the hematological malignancies acute myelocytic leukemia and multiple myeloma,^{49,50} and in idiopathic thrombocytopenic purpura, an autoimmune disease.^{32,54} Nevertheless, this was not the case in other instances, such as in the following examples. The BM was enriched in Flu-specific CD8 T cells after resolution of acute influenza virus infection.³¹ Similarly, EBV-chronically infected individuals had a higher frequency of CD8 T cells specific for EBV-lytic epitopes in the BM as compared with the blood, but this finding was not correlated with an increased viral load.³¹ The case of patients with solid tumors is a difficult one, because the presence of rare tumor cells in the BM cannot be completely ruled out. Still, in several cancer patients, high frequencies of tumor-specific T cells were found in the BM, in the absence of apparent metastases in this organ.^{52,55} We observed in mice that antigen-specific CD8 T cells were present in the BM after immunization with different antigens introduced through various routes, making it unlikely that antigen localization in the BM was responsible for preferential homing to this site.^{7,8} Taken together, these findings suggest that the BM is enriched in antigen-experienced T cells, both in the presence and in the absence of local antigen.

We and others documented that both in mice and humans BM T cells have a different activation state compared to corresponding peripheral cells.^{7,9,30,31,21} When tested for antigen-specific response several months after immunization, CD8 T cells from mouse BM secreted interferon- γ after a 40-h stimulation, whereas those from the spleen required a 6-day stimulation.⁷ In untreated mice, both CD44^{high} and CD44^{int/low} CD8 T cells from the BM showed a higher Forward Scatter as well as a reduced IL-7R α (CD127) membrane expression than their splenic counterparts, suggesting that the BM was enriched in activated cells.^{7,9} Unlike CD8 T cells responding to chronic antigenic stimulation, which show reduced membrane expression of both CD127 and IL-2/IL-15R β (CD122),⁵⁸ CD8 T cells in the BM comprised a lower percentage of CD127⁺ cells, but a higher percentage of CD122^{high} cells, than corresponding cells in spleen and lymph nodes⁹ (E Parretta and F Di Rosa (2008), unpublished data). After stimulation with anti-CD3 mAb, effector memory CD8 T cells from human BM had a higher killing activity as compared with corresponding cells from peripheral blood.³⁰ EBV-specific CD8 T cells from the BM showed a higher antigen-specific interferon- γ response than corresponding peripheral blood cells.³¹ This is in contrast with the lower response showed by cytomegalovirus-specific CD8 T cells, a discrepancy possibly due to the pathogen, as cytomegalovirus persists in hematopoietic progenitors and may thus affect BM T-cell responsiveness.³¹ As compared with their lymph node counterparts, BM CD4 T cells from untreated mice contained a higher proportion of activated/memory cells, as assessed by both their expression profile of CD62L, CD45RB, CD69 and their increased production of IL-4 and interferon- γ .²¹ Overall, these results suggest that memory CD8 and CD4 T cells are both preferentially recruited to the BM and activated in the organ environment by non-antigenic stimuli.

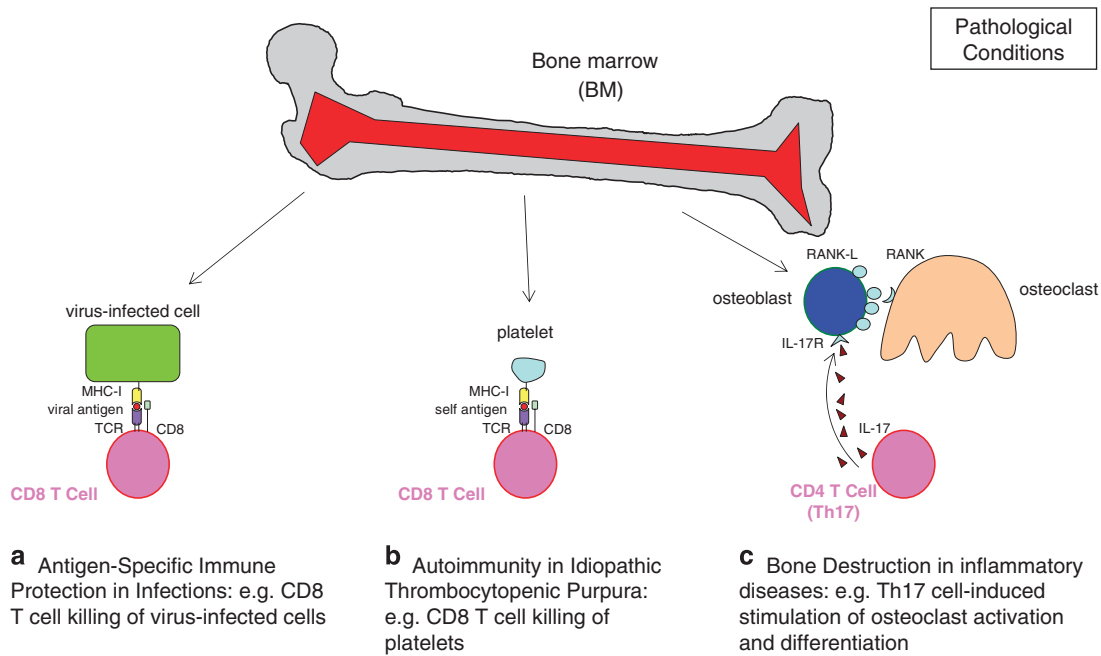


Figure 3 Under pathological conditions, bone marrow (BM) T cells contribute to either protection or damage of different cell types present in the BM environment. The figure represents some examples of BM T-cell involvement in the pathogenesis of viral infections⁵⁶ (a), autoimmune diseases⁵⁴ (b), inflammatory diseases (c).⁵⁷

By performing both BrdU incorporation experiments and *ex vivo* cell cycle analysis in mice, we and others showed that the percentage of proliferating cells within antigen-specific memory CD8 T cells is higher in the BM than in spleen, lymph nodes, liver or lung.^{8,13} Similar results were found in the case of CD44^{high} memory^{8,13} and CD44^{int/low} naive CD8 T cells,⁹ suggesting that the BM is a crucial organ for the proliferation of peripheral cytotoxic T cells. The BM contribution to the turnover of mature recirculating CD8 T cells was even more evident when the absolute numbers of proliferating CD8 T cells were taken into account.^{8,10} Indeed, 6–10 weeks after immunization, the number of proliferating antigen-specific memory CD8 T cells in the BM largely exceeded that in spleen, lymph nodes and liver taken all together.⁸ Although we detected proliferating antigen-specific memory CD8 T cells in the thymus, their exceedingly low number suggests that the thymus does not play a relevant role in antigen-specific memory CD8 T-cell division (E Parretta *et al.* (2004), unpublished data). As regards naive CD8 T cells, they had a lower turnover than memory CD8 T cells,⁵⁹ mostly attributable to a decreased proliferation rate.¹⁰ Still, the percentage of proliferating CD8 T cells was higher in the BM than in either spleen or lymph nodes; taking into account cell numbers, the BM contained about 20% of the total proliferating CD44^{int/low} CD8 T cells present in the spleen, lymph nodes and BM taken all together.⁹ In non-human primates, Silvestri and co-workers recently showed that the BM is a key organ for the homeostatic proliferation not only of CD8 T cells, but also of CD4 T cells.¹⁴ In two different species of non-human primates, the percentage of cells expressing the proliferation marker Ki-67 was higher in the BM as compared with lymph nodes and peripheral blood, within both CD4 and CD8 T-cell subsets.¹⁴ Interestingly, upon infection with SIV, the percentage of Ki-67⁺ CD4 T cells was higher in the BM than in peripheral blood in sooty mangabeys, in which infection is non-pathogenic and normal CD4 T-cell counts are preserved despite high levels of SIV replication, but not in rhesus

macaques, which progress to AIDS. These results indicate a role for the BM in counteracting SIV-induced CD4 T-cell loss in sooty mangabeys, with important implications for the pathogenesis of AIDS in human immunodeficiency virus-infected humans.¹⁴ On the basis of all these findings and expanding upon our previous hypothesis on CD8 T cells,⁷ I propose that the BM plays an essential role in the maintenance of antigen-specific T-cell memory and greatly contributes to the homeostasis of naive T cells, for both CD4 and CD8 cell subsets.^{6,8–10,13,14}

I speculate that homing to the BM represents a ‘default’ pathway, which guarantees that recirculating memory T cells receive the appropriate signals for their maintenance, in the absence of either ongoing strong immune responses or inflammations in other sites. In certain disease conditions, for instance acute viral infections, recirculating memory T cells would proliferate not only in the BM, but also in the sites of vigorous immune reactions, in response to either crossreactive antigens⁶⁰ or high levels of stimulatory cytokines.⁶¹ Interestingly, in a mouse model of T-cell-mediated gut inflammation in which IL-7 is essential to maintain disease, recirculating pathogenic CD4 T cells do not require intestinal IL-7 to give persistent colitis, as long as they have access to IL-7 in the BM, and possibly the spleen.⁴⁴ In this context, it is intriguing that T-cell homing to the BM and T-cell migration to inflamed extra-lymphoid organs share a group of regulatory molecules, that is, $\alpha 4\beta 1$ -integrin and a defined set of inflammatory chemokine receptors, as mentioned above.^{12,29–32}

BM T CELLS AND THE LOCAL MICROENVIRONMENT

The anatomical location of the niches where T cells localize in the BM and the cellular components of such niches are current topics of investigation. *In vivo* imaging studies showed that adoptively transferred T cells migrated to specific BM perivascular regions, which were localized parasagittally within the frontal and parietal bones of the mouse skull and were defined by a specialized endothelium highly

positive for both SDF-1 and E-selectin expression.²⁶ Such BM perivascular spaces, organized around sinusoids and venules, were preferentially used by hematopoietic stem/progenitor cells as well as by leukemic cells for BM seeding.²⁶ T cells were found in these regions for at least 2 weeks after transfer.²⁶ Similar perivascular domains were defined by DCs expressing green fluorescent protein in the mouse skull of CX3CR1^{gfp/+} mice.⁶² After intravenous transfer, both B and T lymphocytes were found in these areas, which were called 'BM immune niches'.⁶² The reasons for the anatomical restriction of the 'BM immune niches' to specific areas of BM skull are unclear, nor it is known whether similar niches are present within long bones and, if so, in which location. How BM T-cell number is regulated—whether at the level of cell number in each niche or at the level of total niche number, or both—is still an unsolved issue. Our studies in healthy mice suggested that the space available for T cells in the BM is limited and saturable.²⁴ It is also possible that BM T-cell niches are differently regulated either under steady state conditions or during immune responses, as well as in different disease conditions. Another open question is whether the low oxygen tension of the BM environment affects BM T-cell function and survival, or if their perivascular location protects them from hypoxia.⁶³ A better knowledge of the T-cell niches in the BM will be critical to design new strategies for modulating the function of T cells by targeting their BM microenvironment.

As previously discussed, at any given time a fraction of BM CD8 T cells proliferates within the organ. Nevertheless, after they were taken out from the BM environment, purified mouse CD8 T cells did not show a higher proliferative response to IL-7, IL-15, IL-21 *in vitro*, nor to poly:I:C treatment *in vivo*, as compared with corresponding cells from the spleen.⁹ This suggests that BM T cells are not committed to self-renewal, but rather are stimulated in the organ environment. We characterized some of the molecular events induced in the CD8 T cells within the mouse BM, such as increase of both phosphorylated STAT-5 and p38 MAPK intracellular levels, reduction of IL-7R α membrane expression.⁹ Taken together, our findings suggest that CD8 T cells are activated and proliferate in the BM because they are able to integrate signals received from several molecules, such as IL-7, IL-15, TNF family members, which are abundant in the organ environment^{9,64,65} (Figure 2a). A possible approach to characterize the factors involved in CD8 T-cell stimulation in the BM will be to study the immune response in genetically modified mice, in which putative modulatory molecules acting on BM CD8 T cells are regulated in both organ-specific and inducible manner.

BM T CELLS AND MESENCHYMAL STROMAL CELLS

Within the BM, cells of hematopoietic origin are embedded in a heterogeneous mixture of stromal cells of mesenchymal origin, which includes fibroblasts, reticular cells and adipocytes. The mesenchymal cells of the BM arise from a common ancestor, the mesenchymal stem cell, which has both the capacity to self-renew and the ability to differentiate into osteoblasts, adipocytes, chondrocytes and fibroblasts. It is well recognized that BM stromal cells support hematopoiesis, by establishing appropriate anatomical niches and secreting specific cytokines and growth factors.⁶⁶ BM stromal cells support also the maintenance of mature B lymphocytes, long-lived plasma cells, and possibly naive and memory T cells; lymphocyte stimulatory molecules which are produced at high levels by reticular-like BM stromal cells include IL-7, IL-6 and VCAM-1.^{67–71} However, BM stromal cells can also secrete inhibitory factors for lymphocytes, such as transforming growth factor- β and hepatocyte growth factor.⁷² Considering that preadipocytes and adipocytes are emerging as new modulators of immune responses,⁷³ it would be interesting to know whether BM preadipocytes

and adipocytes are different from those present in other organs and whether the increase of BM adipose tissue which occurs with aging influences BM lymphocyte homeostasis and/or function. The fact that, under physiological conditions, the BM is a primary lymphoid organ and supports mature lymphocyte maintenance suggests that the net balance is usually in favor of positive rather than negative modulation of lymphocyte proliferation and survival. As regards lymphocyte effector responses, the BM environment sustains Ab secretion by long-lived plasma cells and maintains T-cell activation to some degree, nevertheless those types of responses that can lead to immune pathology are normally kept in check. However, in a pathological BM environment, for example in the presence of tumors growing in the BM, stromal cells can contribute to suppress antitumor responses.⁷⁴

The identity of the stromal cells establishing the niches for mature B and T lymphocytes, as discussed above, remains uncertain. One major problem is the high degree of stromal cell heterogeneity. By using CXCL12 GFP knockin mice, Nagasawa and co-workers⁷⁵ showed that BM stromal cells expressing high levels of CXCL12 are different from those producing IL-7. The two types of BM stromal cells were found to be associated with B lymphocytes at different developmental stages, so that pre-pro-B cells and plasma cells adjoined CXCL12-expressing stromal cells, whereas pro-B cells were in contact with IL-7-producing stromal cells.⁷⁵ This study represents an interesting example of how to approach the issue of BM stromal cell identity, by taking advantage of genetically modified mice in combination with confocal microscopy. Another important issue is the bidirectional cross talk between lymphocytes and stromal cells.^{76,77} Indeed, T cells can regulate stromal cells by producing IL-17A, a proinflammatory cytokine that, both *in vitro* and *in vivo*, is a potent growth factor for mesenchymal stem cells.⁷⁶ BM fibroblasts and fibroblast-like cells express the IL17A receptor at very high levels, both in humans and mice.⁷⁶ It has been proposed that the facilitating effect of BM T cells on allogeneic hematopoietic cell engraftment is at least partially mediated by BM stromal cells, which in the presence of T cells show an improved capacity of reconstituting BM microenvironment.⁷⁶

Mesenchymal stem cells of the BM do not generate only the local stroma. Increasing evidence suggest that, in adult life, mesenchymal stem cells circulate from the BM to injured organs, where they participate in tissue repair.²⁰ Mesenchymal stem cells derived from the BM have been transplanted in patients, for regenerative treatments, mainly in the field of hemato-oncology and musculoskeletal disorders.⁷⁸ A large number of new therapeutical applications are under study. It is therefore very important to better understand the complex interplay between mesenchymal stem cells and the immune system. Mesenchymal stem cells are generally considered immunosuppressive in a non-major histocompatibility complex-restricted manner, although the underlying mechanisms still need to be elucidated; moreover, under appropriate conditions, mesenchymal stem cells can be immunogenic.^{79,80} Mesenchymal stem cells from the BM, irrespectively of the human leukocyte antigen of the donor, have been successfully used for treating patients with steroid-resistant severe acute graft-versus-host disease.^{78,81} Such therapeutic effect might be due to a number of mechanisms, including direct inhibition of T cells, generation of regulatory T cells, induction of tolerogenic DC, increased healing of wounded tissues or reduction of the 'danger' signal.^{80,82,83} *In vitro* studies reported conflicting results on the immunomodulatory properties of BM mesenchymal stem cells. For example, some reports showed that mouse BM mesenchymal stem cells exerted a generalized inhibition on T-cell antigen-specific responses,⁸⁴ whereas other studies documented a negative effect of human mouse BM mesenchymal stem cells on T-cell proliferation but

not on cytotoxicity.⁸⁵ These conflicting results may be due to differences in the species examined as well as in the experimental conditions for generating mesenchymal stem cells and/or for testing lymphocyte responses.⁸⁰ Indeed, a thorough study showed that opposite results were observed when human BM mesenchymal stem cells and T cells were cultured at different stem cell/T-cell ratio; T-cell proliferation in response to IL-2, IL-7, IL-15 was mostly enhanced at low ratios and suppressed at high ratios.⁸⁶ In the light of the potential therapeutic applications, it will be essential to define the rules of stromal cell/T-lymphocyte interaction in the natural BM niche, as well as the immunomodulatory mechanisms triggered by the infusion of BM-derived mesenchymal stem cells.

BM T CELLS AND HEMATOPOIESIS

Within the medullary cavity of bones, hematopoiesis occurs in specialized niches for hematopoietic stem cells. With increasing age, the hematopoietic BM is reduced, being replaced by adipose tissue. Hematopoietic stem cells located in the BM are self-renewing precursors, which give rise to all the lineages of blood cells. Increasing knowledge of the membrane expression of specific set of markers by BM hematopoietic stem cells not only allows for higher grade purifications of these cells and better definition of their functional characteristics, but also opens up the possibility of localizing their position in tissue sections.^{87,88} In the BM, two types of niches for hematopoietic stem cells have been described, one near the endosteum,^{89,90} at the interface between bone and BM, and the other close to blood vessels, associated with sinusoidal endothelium.^{87,91} Candidate BM stromal cells organizing the niches are (i) mouse osteoblasts,^{89,90} (ii) human CD146⁺ reticular cells associated with sinusoidal endothelium⁹¹ and (iii) mouse reticular cells expressing high levels of CXCL12, which are scattered throughout the BM, being associated with hematopoietic stem cells both near the endosteum and around sinusoids.⁹² It is still unclear whether the two types of niches equally contribute to hematopoiesis, or whether they have different functions.^{66,93} Interestingly, the CD146⁺ cells establishing the perivascular niche have the capacity to both self-renew and generate differentiated mesenchymal cells, thus having the characteristics of mesenchymal stem cells.⁹¹ Perivascular niches for hematopoietic stem cells have been described not only in the BM, but also at extramedullary sites, such as the spleen of mice treated with cyclophosphamide/G-CSF, and in the yolk sac, aorta-gonad-mesonephros region and vitelline arteries during the embryonic development, supporting the notion that sinusoidal endothelial and/or periendothelial cells are specialized in organizing the hematopoietic microenvironment.^{87,93} In this context, it is remarkable that T cells in the BM are found perivascularly, and that adoptively transferred hematopoietic stem cells and mature T cells localize to the same subendothelial areas of the BM, as mentioned above.²⁶

Effector T cells in the BM environment can modulate hematopoiesis by producing cytokines and growth factors acting on blood cell progenitors. An example of bridging between T-cell function and hematopoiesis is the regulation of neutrophil production. In normal mice, granulopoiesis was impaired in the absence of activated CD4 T cells in the BM, although hematopoietic stem cells could still give rise to committed myeloid progenitors.²¹ A proposed regulatory circuit involves CD4 T-cell production of IL-17, which stimulates BM stromal cells to secrete G-CSF, which in turn promotes granulopoiesis.^{21,22,94} (Figure 2b). Novel findings in mice link the elimination of apoptotic neutrophils by phagocytic cells to IL-23 and IL-17 production, supporting the following view of homeostatic regulation of granulopoiesis.^{22,95} At low neutrophil number, macrophages and DCs

produce IL-23, which stimulates CD4 T cells, as well as $\gamma\delta$ -T and natural killer T cells, to produce IL-17, which then increases granulopoiesis through G-CSF. At high neutrophil number, apoptotic neutrophils are phagocytosed by macrophages and DCs, with consequent inhibition of IL-23 production by these cells, resulting in a negative feedback on granulopoiesis.^{22,95} Several other factors can regulate G-CSF secretion, further modulating this loop.^{21,95} In agreement with this regulatory circuit, it has been proposed that reduced CD4 T-cell counts might be involved in the granulopenia observed in AIDS patients;^{21,96} however, further studies are required to confirm this hypothesis in humans.

In individuals exposed to stress conditions (for example, trauma, burns and so on), BM hematopoiesis undergoes rapid changes due to increased levels of glucocorticosteroids. Indeed, in mice exposed to stress levels of glucocorticosteroids, B lymphopoiesis was inhibited within 24 h and granulopoiesis was enhanced within 36 h.^{97,98} A similar skew of hematopoiesis was found in mice examined 3 days after intraperitoneal injection with either antigen plus adjuvant or adjuvant alone; such skew was related with increased production of the proinflammatory cytokine TNF- α and diminished BM levels of CXCL12 and SCF.¹⁸ The role of glucocorticosteroids was not investigated in this system; nevertheless, they are induced by inflammatory stimuli within a few hours,⁹⁹ although the kinetics and level of production may be different than in stress conditions. In the light of the stimulatory effects of T-cell-derived IL-17 on granulopoiesis, it is time to revisit previous findings on the effects of corticosteroids on mature lymphocyte redistribution. Studies in rodents showed that acute exposure to either pharmacological or natural doses of corticosteroids induced circulating B- and T-lymphocytopenia and T-cell accumulation in the BM.¹⁰⁰⁻¹⁰² A recent study reported that, after *in vitro* treatment with dexamethasone, human T cells showed increased CXCR4-mediated signaling; this might be one of the mechanism underlying corticosteroid-induced T-cell homing to the BM.¹⁰³ Corticosteroid administration in rodents also increased BM cell response to the mitogens PHA, ConA, PWM, suggesting that mature lymphocytes in the BM were fully functional.^{101,104} It is tempting to speculate that IL-17, produced by activated T cells in the BM, at least partially mediates the positive effects of glucocorticosteroids on granulopoiesis.

BM T CELLS AND BONE METABOLISM

Bone marrow is in close contact with bone tissue formed by the organized deposits of type I collagen and hydroxyapatite, a calcium phosphate salt, in which bone cells are dispersed. Rather than being an inert matrix, bone undergoes a continuous turnover: osteoblast activity resulting in bone deposition is counteracted by osteoclast-mediated bone resorption. Osteoblasts are cells of mesenchymal origin, whereas osteoclasts are of hematopoietic origin, belonging to the monocyte/macrophage lineage. Several factors regulating bone homeostasis are also molecular players of the immune response.¹⁹ For example, the TNF family member RANK-L (also called TRANCE, OPGL, ODF), a potent regulator of osteoclast activation and differentiation, is expressed not only by osteoblasts, but also by activated CD4 and CD8 T cells.¹⁹ RANK-L mediates its biological effects by binding to RANK, which is expressed by osteoclast progenitors, mature osteoclasts and DCs.^{105,106} RANK-L can also bind to the soluble protein osteoprotegerin, which acts as an inhibitory decoy receptor; osteoprotegerin is produced by several cell types, including osteoblasts, DCs, B cells and T cells.^{19,23,107,108} By binding to RANK, RANK-L strongly stimulates bone resorption, contributes to lymph node organogenesis, prolongs DC survival and augments DC adjuvant properties.^{19,109,110} Other shared molecules between bone and

immune system include the T-cell-derived cytokines interferon- γ and IL-4, both inhibitors of bone resorption, and IL-17, which in contrast stimulates it.^{19,111}

Bone remodeling is important for both skeletal strength and calcium homeostasis. However, in certain pathological conditions, bone metabolism is unbalanced, so that excessive osteoclast activity, in the absence of increased osteoblast function, leads to bone loss. Several studies have shown a key role for CD4 T cells in mediating bone destruction in immune-mediated inflammatory diseases, including rheumatoid arthritis and periodontitis.^{19,112,113} In these diseases, the pathogenic CD4 T cells belong to the Th17 lineage,^{57,111} a subset of T-helper cells involved in several immune-mediated pathologies.¹¹⁴ Based on experiments in mice, the following mechanism has been proposed: Th17 cells secrete IL-17, that stimulates osteoblasts to upregulate RANK-L, which in turn induces osteoclast activation and differentiation⁵⁷ (Figure 3c). BM T cells may contribute to bone loss also in neoplastic diseases, particularly in multiple myeloma and skeletal metastasis.^{115–117} In multiple myeloma patients, BM T cells express high levels of RANK-L and can thus directly stimulate osteoclasts;¹¹⁵ moreover, increased production of T-cell-derived IL-3 occurring in this disease can inhibit osteoblast generation.¹¹⁶

Increasing evidence supports the notion that T cells are involved in post-menopausal osteoporosis. Experiments in mice showed that, in the absence of estrogens, higher numbers of TNF- α producing T cells were found in the BM; the increased production of TNF- α could both directly stimulate osteoclasts and augment their response to RANK-L.^{118,119} By comparing peripheral blood mononuclear cells from pre- and post-menopausal women, it was observed that (i) estrogen deficiency was associated with an increased production of TNF- α and RANK-L, even without stimulation and (ii) that T cells produced a higher amount of RANK-L as compared with monocytes.¹²⁰ Although further work is necessary to clarify the complex changes leading to post-menopausal osteoporosis in women, a pro-osteoclastogenic contribution of T cells has to be taken into account.

Surprisingly, it was documented that T cells have a protective role on bone turnover under physiological conditions. Hints that this modulation may occur came from *in vitro* studies showing that osteoclastogenesis was inhibited by CD8 T cells.^{108,121} Moreover, after activation with anti-CD3 and anti-CD28 mAb, mouse lymph node CD8 T cells showed a delayed kinetics of RANK-L expression, as compared with corresponding CD4 T cells.¹⁰⁶ Culture of BM cells from CD4 and CD8 T-cell depleted mice showed enhanced osteoclastogenesis in response to 1,25-dihydroxyvitamin D3 stimulation, suggesting that T cells had a suppressive effect in this system.¹²² The protective role of T cells on bone metabolism was clearly documented by *in vivo* studies, showing that both B-cell- and T-cell-deficient mice have decreased bone mineral density.²³ A detailed analysis demonstrated that osteoporosis was prevented by osteoprotegerin produced in the BM by B cells, stimulated by T cells through CD40L/CD40 interactions²³ (Figure 2c). In contrast, IL-17 does not play any relevant role in physiological bone homeostasis, as IL17-deficient mice show normal bone mineral density and skeletal development.⁵⁷ Taken together, these findings support the notion that BM CD4 and CD8 T cells play a protective role in physiological bone homeostasis, using pathways different from those associated with inflammatory bone diseases.

FUTURE PERSPECTIVES: BM T CELLS AS EFFECTOR CELLS FOR TRANSPLANTATION

For their beneficial effects on both tumor treatment and hematopoietic recovery, BM T cells are currently transferred together with

Table 1 Open questions regarding BM T cells

1. <i>Anatomical organization of the BM niche for T cells:</i>	Which are the cellular components of the BM niche for T cells? (candidates include reticular stromal cells, BM DCs) Are T cells localized in the same niche of mature B cells? (it is suggested that B and T cells colocalize together with BM DCs in the so-called 'BM immune niche') Are T cells localized in the same perivascular niche of HSC? Are different T-cell subsets localized in the same BM niche? (for example, CD4 T cells, CD8 T cells, Treg) How is the number of T cells in each BM niche regulated? How is the number of BM niches for T cells regulated? In which anatomical locations are BM niches for T cells localized within bones? (it is suggested parasagittally in the mouse skull; not known for other bones) Does aging affect the BM niches for T cells in terms of distribution among different bones, number of niches, number of T cells, T-cell subset composition?
2. <i>BM T-cell homing and egress:</i>	Which are the chemokines, integrins and other adhesion molecules regulating homing of T cells to the BM? (candidates include the chemokines CXCL12, CCL3, CCL4, CCL5, CXCL16, CX3CL1, the integrin- α 4 β 1) Which are the molecules regulating T-cell egress from the BM? How is the balance between BM T-cell homing and egress regulated?
3. <i>Induction of antigen-specific T-cell response and molecules acting on T cells in the BM:</i>	Under which circumstances does antigen presentation in the BM by BM DCs play a role in either induction or tolerization of T-cell response? Which are the stimulatory molecules acting on BM T cells? (candidates include IL-6, IL-7, IL-15, members of the TNF family such as 4-1BBL) Which are the inhibitory molecules acting on BM T cells? (candidates include TGF- β , HGF) How is the balance between stimulatory and inhibitory molecules regulated?
4. <i>Antigen-specific T-cell effector response and molecules produced by T cells in the BM:</i>	Under which circumstances does antigen-specific T-cell effector function in the BM play a role in either immune protection or immune-mediated pathology? Which are the molecules produced by BM T cells affecting either directly or indirectly bone metabolism? (candidates include IL-17, RANK-L, CD40L) Which are the molecules produced by BM T cells affecting either directly or indirectly hematopoiesis, particularly granulopoiesis? (candidates include IL-17, TNF- α)
5. <i>Alteration of BM T-cell function in the pathogenesis of human diseases:</i>	In which human diseases is BM T cell homing and egress altered? In which human diseases is BM T cell function altered? How do BM T cells contribute to pathological bone resorption in inflammatory diseases? (for example, by producing IL-17, RANK-L) How do BM T cells contribute to the impairment of hematopoiesis in autoimmune disorders targeting blood cells? (for example, by recognition of self-antigen expressed by either progenitors or mature blood cells)
6. <i>Modulation of BM T-cell function for treatment of human diseases:</i>	How can BM T-cell homing and egress be modulated? (for example, by mobilizing treatments) How can BM T-cell function be increased for protection against tumors and viral infections? How can BM T cells be better exploited for GVT and GVL effects upon BM transplantation, without causing GVHD? How can adoptive transfer of BM T cells be exploited against tumors and viral infections?

Abbreviations: BM, bone marrow; DC, dendritic cell; GVHD, graft-versus-host disease; GVL, graft-versus-leukemia; GVT, graft-versus-tumor; HGF, hepatocyte growth factor; IL, interleukin; TNF, tumor necrosis factor; TGF, transforming growth factor.

hematopoietic stem cells in some BM transplantation settings.¹²³ The facilitation of hematopoietic stem cell engraftment by BM T cells might be due to a number of mechanisms such as (i) the elimination of the few hematopoietic host cells left in the BM after the conditioning regimen, (ii) the elimination of host cells which may reject transplanted cells or (iii) the positive effects of T cells on BM stromal cells.¹²³ The antitumor effect, called graft-versus-tumor or graft-versus-leukemia effect in case of hematological malignancies, reduces relapse rates; nevertheless there is certainly a requirement for further improvement. Unfortunately, immune reactions against normal cells of the recipient may occur, leading to graft-versus-host disease. Several approaches are under study to tip the balance in favor of beneficial rather than adverse effects of transferred BM T cells, for example, selective *in vivo* allo-depletion using post-transplantation cyclophosphamide or *ex vivo* pre-transplantation treatment of the graft to induce allo-tolerance.¹²⁴

Further applications of BM T cells might be considered in the near future. Because of their prompt effector response and their increased antigen-responsiveness as compared with corresponding peripheral T cells, BM T cells might be exploited when highly activated effectors are required, such as in the immunotherapy of cancer and in the treatment of severe viral infections.^{7,50} Furthermore, in those cases in which bone homeostasis is impaired, BM T cells might even contribute to re-establish its balance. To effectively design adoptive therapies using BM T cells, one key issue to be considered is the heterogeneous composition of the inoculum. It is well known that the BM contains not only cytotoxic CD8 T cells, but also other T-cell subsets, including regulatory CD4⁺ CD25⁺ T cells.³⁹ A successful example of adoptive T-cell therapy has been shown in a mouse tumor model, showing that tumor-specific effector CD8 T cells from tumor-bearing donors could outcompete regulatory T cells following stem cell transplantation.¹²⁵ Other inhibitory leukocyte populations within the BM include myeloid suppressor cells¹²⁶ and tolerogenic DCs.⁴⁷ A population of strongly inhibitory myeloid cells is induced by G-CSF treatment, as shown by studies in G-CSF-mobilized donors.¹²⁷ Although this is seen as an advantage to reduce the risk of graft-versus-host disease in BM transplantation, caution should be taken because, as observed in experiments in mice, T cells still retain their function after elimination of the inhibitory myeloid cells.^{127,128} Another level of BM T-cell heterogeneity depends on the time elapsed after the last antigenic encounter: it is known that such a lag time influences T-cell responsiveness and activation state,¹²⁹ with different outcomes in the context of either acute or chronic viral infections.³¹

It has been reported that, after T-cell-replete BM transplant, hematological patients with graft-versus-host disease experience extramedullary relapses in the absence of BM recurrences.¹³⁰ This suggests that post-transplant disease is influenced not only by tumor-associated factors and conditioning regimen, but also by adoptively transferred BM T cells, which can effectively control tumor growth within the BM. The future challenge will be to make adoptively transferred BM T cells highly effective in systemic protection (Table 1). A successful outcome is envisioned based on both the functional properties of BM T cells and their enrichment in antigen-experienced T cells. The increasing knowledge of the inflammatory like-homing profile of BM T cells, as well as of their recirculatory pathways, further supports this view.

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