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European Journal of Immunology

HIGHLIGHTS

REVIEW

Maintenance of quiescent immune memory in the bone marrow

50th anniversary REVIEW series

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The adaptive immune system has the important ability to generate and maintain a memory for antigens once encountered. Recent progress in understanding the organization of immunological memory has challenged the established paradigm of maintenance of memory by restless, circulating, and "homeostatically" proliferating lymphocytes. Among other tissues, the bone marrow has emerged as a preferred resting place for memory lymphocytes providing both local and systemic long-term protection. Why the bone marrow? There, mesenchymal stromal cells provide a privileged environment for quiescent memory B and T lymphocytes, the protagonists of secondary immune reactions, and for memory plasma cells providing persistent humoral immunity. In this review, we discuss the dedicated role of the bone marrow for the maintenance of memory lymphocytes and its implications for immunological memory.

Keywords: bone marrow · tissue-resident lymphocytes · immune memory · quiescence · memory lymphocytes

Introduction

The observation that humans surviving an infection can acquire immunity to reinfection has already been documented over 2000 years ago by Thucydides in his account of the Peloponnesian Wars [1]. We thus adapt to the infectious challenges of our environment. Probably as old are the attempts to generate immunity intentionally, by variolation [2] and later by vaccination [3]. An understanding of how immunity works had to wait until the foundation of cell biology in the mid-19th century [4, 5] and the discoveries of infectious pathogens, spearheaded by Louis Pasteur and Robert Koch. A wave of seminal discoveries followed, identifying fungi, bacteria, and viruses as pathogens, macrophages, lymphocytes, and antibodies as essential elements of innate and adaptive, cellular, and humoral immunity (reviewed in [6]). About 60 years ago, T and B lymphocytes were identified as essential elements of the adaptive immune system, and B lymphocytes as the precursors of antibody-secreting plasma cells [7, 8]. Since then, we are trying to put the puzzle together and understand how these cells memorize antigenic encounters and provide specific immunity over long time periods. We will use the term "memory" here for antigen-specific imprinting of the immune system and maintenance of information in the absence of the original antigenic stimulus. We will discuss evidence regarding the compartmentalization, maintenance, and lifestyle of memory lymphocytes, and highlight the role of the bone marrow in maintaining quiescent memory cells.

The cells of adaptive immunological memory

At about the same time when T and B lymphocytes were discovered, it became clear, by drainage of the thoracic duct of rats, that lymphocytes circulating through blood and lymph are required to mount primary immune reactions, but are dispensable for secondary immune reactions, three weeks after primary immunization [9]. This was the first formal demonstration that recall immune responses do not require circulating lymphocytes but

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[Correction added on 1 September 2021, after first online publication: Peer review history statement has been added.]

instead are performed by tissue-resident lymphocytes. Since then, antigen-experienced lymphocytes circulating through the blood have been extensively studied and, for a while, have been considered entirely representative for adaptive immunological memory in humans [10, 11], while mouse lymphocytes of the spleen and lymph nodes have been considered tantamount to mouse immune memory. Yet, it is remarkable that despite this intense research over many years, circulating antigen-experienced lymphocytes remain unknown. We neither understand their lifestyle, nor do we know when and how they enter circulation and when they leave again. We assume that circulating antigen-experienced lymphocytes are autonomous and maintained independent of contact to other cells, while in blood or lymph. They have been associated with systemic immunological memory, scanning the body for their cognate antigen and engaging in secondary immune reactions in the spleen and lymph nodes when confronted with their antigen there. Memory B and T lymphocytes have been classified primarily in two dimensions: first, with respect to whether they represent more or less pluripotent stages of lymphocyte development (e.g., for memory T lymphocytes in a line progressing from "memory stem cells" [12, 13], to "central memory cells", "effector memory cells," [14] and finally "terminal effector memory cells" [15]), and second, with respect to functional imprinting (reviewed in [16]), originally based on expression of distinct cytokines, later certain transcription factors, defining T helper type 1, Th2, Th17, T follicular helper, and regulatory lineages, today better recognized as a "landscape" of functional T cell diversity [17]. The taxonomy for memory B lymphocytes is less developed, with memory B cells still expressing IgM antibodies and those expressing antibodies of switched isotype, mostly analyzed for the mouse spleen [18, 19], only anecdotally for the bone marrow [20].

While antigen-experienced B and T lymphocytes are abundant in spleen, lymph, and blood, plasma cells, the antibody-secreting progeny of B lymphocytes, are not. This observation had led to the assumption that plasma cells are short-lived and have to be replenished constantly from (memory) B lymphocytes activated by residual antigen [21, 22]. This view began to change with a line of experiments showing that (a) plasma cells from a particular immune reaction, though disappearing from secondary lymphoid organs after a few days, are persisting in the bone marrow for long time periods [23], (b) those plasma cells are not constantly generated de novo, but persist as non-dividing cells for a lifetime [24–26], and (c) they even persist when (activated) B cells are depleted either by irradiation [25] or CD20 antibodies [27, 28], i.e., when their precursors are ablated. IgG-secreting long-lived plasma cells also persist in the absence of their antigen, e.g., after adoptive transfer [25, 29], since they lack surface expression of the B cell receptor [30]. In a true sense, they are "memory" plasma cells.

Compartmentalization of immune memory

While the experiments of Gowans and McGregor had demonstrated that secondary immune reactions would not depend on circulating lymphocytes [9], it remained unclear whether memory lymphocytes still circulate through blood, lymph, and tissues, with extended residency periods in the tissues at any time point [31]. This would imply that (a) circulating lymphocytes represent the entirety of memory, and (b) that memory lymphocytes would scan the body for antigen, in case of B lymphocytes, or antigen-presenting cells, in case of T lymphocytes. An alternative scenario would be that circulating memory lymphocytes represent a distinct compartment of immunological memory, supposedly conferring memory for ubiquitous, systemic antigens and for persisting antigens, in contrast to other compartments of memory cells, which are confined to and resident in distinct tissues. Such tissue-resident memory cells could confer memory for pathogens addressing these tissues preferentially, but also for systemic antigens, in particular, if the resident memory cells were located in secondary lymphoid organs. The difference between a memory conferred by a ubiquitous and circulating memory, scavenging the body for recurrent antigens and pathogens, and a compartmentalized tissue-resident memory, waiting to be reactivated by antigens presented to them in those tissues, is fundamental and raises many questions. How are memory cells routed into different compartments and how are they kept and maintained there? How are they reactivated and what is the contribution of tissue-resident memory cells to systemic immune responses? What is the division of labor between different compartments? How are secondary immune reactions in the non-lymphoid and lymphoid tissues organized, in particular for those antigens for which memory is confined to non-lymphoid tissues?

The evidence for compartmentalization of immunological memory can be summarized in compliance with essentially three postulates:

First, resident memory cells do not leave their tissue. The physical residency of memory cells is evident by a lack of significant emigration of donor-derived resident memory cells from transplanted tissues into the recipient, or vice versa, from the recipient into the transplanted tissues [32, 33]. In mice, this has also been analyzed in parabiosis experiments where migration of resident memory cells from one parabiont into the other one has not been observed [34, 35], despite severe traumatic events involved in both transplantation and parabiosis which one could have expected to induce activation and migration of resident cells. A similar line of evidence is the reverse experiment, injecting antibodies into the blood to selectively label or ablate circulating cells. Thus, in patients with cutaneous T cell lymphoma treated with CD52 antibodies (alemtuzumab), $CCR7^+$ but not $CCR7^-$ memory T lymphocytes were ablated from the skin, defining the latter ones as tissue-resident [36]. Similar results were observed for CD20 antibodies (rituximab) which efficiently depleted circulating but not lymphoid tissue-resident B cells [37,38].

Second, resident memory cells express distinct genes. Transcriptional signatures of tissue-resident memory T lymphocytes have been described [39–41], and discussed with emphasis on the expression of CD69 as a "tissue-retention" marker of tissue-resident memory T cells [42–45]. $CD69^+$ memory T lymphocytes, both $CD4^+$ and $CD8^+$, isolated from a variety of tissues, also

express the transcription factor HOBIT (homolog of Blimp in T cells) [39, 41], which is discussed as key to tissue-residency, and they even may display a discrete pattern of epigenetic imprinting of their DNA [46]. Apart from a common transcriptional signature of tissue-resident versus circulating memory T cells, there are also clear differences, in particular when it comes to the expression of homing receptors attracting those cells or their precursors to their tissue of residency. CD103 (αE integrin) and CLA (cutaneous lymphocyte antigen) are expressed by resident memory T cells of the skin and other epithelial tissues, but not those of the bone marrow. Bone marrow-resident memory T cells express VLA2, which apparently is required for their establishment in the bone marrow [43, 47].

Third, resident memory cells have a discrete antigen-receptor repertoire. If the antigen–receptor repertoire of memory cells of one tissue differs from the antigen-receptor repertoire of their sister cells in other tissues, and in particular, from that of circulating memory cells, this is clearly demonstrating their residency. For example, confinement of memory T cells with particular specificities to the bone marrow has been shown originally for mice [43], later also for humans, with a preponderance of bone marrow memory $CD4^+$ T lymphocytes for the maintenance of long-term memory to systemic pathogens, i.e., childhood pathogens like measles [48]. Likewise, memory T cells specific for pathogens infecting epithelial tissues are largely found in epithelial tissues [49–51], while they are less abundant in the bone marrow [48]. While a discrete antigen-receptor repertoire and restriction of distinct specificities to a particular tissue is sufficient to define a memory compartment, and probably is the strongest argument for compartmentalization, it is not a necessary condition. In principle, compartments could also be linked in ontogeny, i.e., memory cells or their precursors of a given immune reaction could have been delegated to and could have become residents of different compartments.

According to those criteria for compartmentalization, populations of resident memory T lymphocytes, both $CD4^+$ and $CD8^+$, have been described for epithelial and mucosal tissues, (in particular skin, lung, intestine), as well as for bone marrow, but also for secondary lymphoid tissue, like the spleen [39,45,48]. These are mainly $CD69^+$ memory T lymphocytes, while for $CD69^-$ memory lymphocytes the discussion continues as to what extent they are tissue-resident [45].

A new level of understanding of the heterogeneity and compartmentalization of immunological memory may be reached by defining transcriptomes and receptor repertoires on the level of individual cells. Until recently, memory B lymphocytes had been regarded a more or less homogeneous population prominent in the spleen with some cells circulating, and mostly divided into memory B cells expressing IgM antibodies and those expressing IgG or IgA, and memory B cells participating in secondary immune reactions in germinal centers versus those directly differentiating into antibody-secreting plasma cells outside of germinal centers [18, 19, 26]. IgM^+ memory B cells have been described for the bone marrow [20]. Resident IgG^+ memory B cells of the lung had been described [52, 53], but the true extent of heterogeneity of memory B cells remained unclear. When we recently determined the absolute numbers of memory B cells expressing IgG antibodies in various mouse tissues, it became evident that most of them were residing in either spleen or bone marrow, at about equal numbers, in particular in wild mice [54]. Single-cell transcriptomes coupled with BCR repertoire sequencing revealed an unforeseen heterogeneity of six memory B cell populations, one of them found exclusively in the spleen and one found exclusively in the bone marrow, and only one population, comprising about 20% of all IgG^+ memory B cells, qualifying as circulating cells. It may take some time to put this heterogeneity into a functional context, but for now, it is evident that IgG^+ memory B cells are mostly resident, in spleen and bone marrow, and circulating memory B cells are not representative of B cell memory as such.

So far, when it comes to the compartmentalization of immunological memory, the field has largely focused on resident memory T lymphocytes of epithelial and mucosal tissues, regarding them as cells protecting their tissue against recurrent local challenges, and considering circulating memory T lymphocytes as a correlate of protection against systemic challenges, i.e. the second line of immune defense. Why then do we find so prominent and exclusive compartments of memory plasma cells, memory T cells and memory B cells in the bone marrow?

Why bone marrow?

Throughout life, the bone marrow is the primordial source of hematopoietic cells, including lymphocytes. Bone marrow is not connected to the lymphatic vessel system, but intimately connected to the blood, providing rapid access to systemically disseminated antigen. Bone marrow volume is proportional to the size of the body, making it a perfect candidate to define the size of hematopoietic compartments. Its parenchyma is organized by a network of reticular mesenchymal stromal cells, which make up only a few percent of all bone marrow cells. The stromal cells of this network are not proliferating, they do not express *Mki67* [55], a marker of proliferating cells, nor do they incorporate EdU, which would be indicative of DNA replication [56]. As such, the network of stromal cells is stable over time, though it is flexible in space [57]. Although stromal cells are quite heterogeneous according to their transcriptomes, clusters of stromal cells can be defined by their expression of genes relevant with respect to their communication with hematopoietic cells [55]. It is obvious that lifelong hematopoiesis requires a delicate balance of maintaining quiescent stem and progenitor cells and controlling proliferation and differentiation of their offspring, in response to activating signals. These requirements can be extrapolated to the maintenance of immunological memories for a lifetime: in the apparent absence of their antigen, quiescent memory B and T lymphocytes are poised to be reactivated, and when reactivated by antigen, will proliferate and differentiate [58]. Memory plasma cells are quiescent in terms of proliferation, too, and maintained by stromal cells [59], as presumably terminally differentiated antibody-secreting "factories".

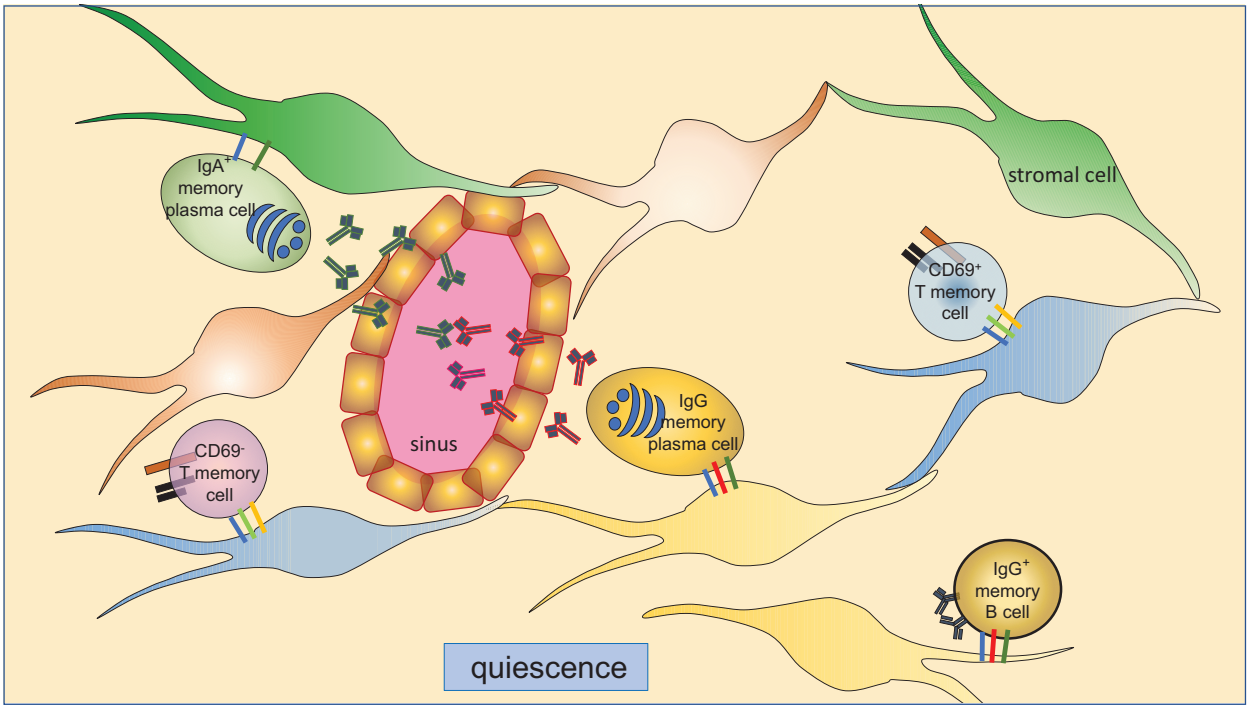

Figure 1. Organization of the resting memory lymphocytes in the bone marrow. Following the successful resolution of an immune reaction, antibody-secreting memory plasma cells and memory B and T cells persist as quiescent cells (non-proliferative, non-migratory) in dedicated survival niches organized by bone marrow stromal cells. The immune memory cells dock individually onto dedicated stromal cells, which control their maintenance. The numbers of dedicated stromal cells available define the size of the memory compartments.

In the bone marrow, memory plasma cells and memory B and T lymphocytes are dispersed throughout the parenchyma, at frequencies of up to 1%. As individual cells they dock onto stromal cells, apparently one at a time (Fig. 1). The reason for this restriction is currently not clear, but it may be fair to speculate that a critical element of the synapse between memory cells and stromal cells is confined to the synapse, making a particular stromal cell invisible for other memory cells or incapable of hosting them. $CD4^+$ and $CD8^+$ mouse memory T lymphocytes seem to be competing for the same niche, since the average distances between any two $CD4^+$ or $CD8^+$ memory cells are the same as that between a $CD4^+$ and a $CD8^+$ memory cell [60]. For both, it has been described that they contact stromal cells expressing IL-7, or to be more precise: expressing a gene encoding green fluorescent protein, introduced into the *Il7* gene locus [43, 60]. Likewise, IgG-expressing mouse memory B cells and IgG-secreting memory plasma cells are hosted by stromal cells expressing laminin [54, 61].

The synapse between stromal cell and immune memory cell is complex, and its molecular nature has not yet been fully elucidated. For memory plasma cells, VCAM-1 and ICAM-1 of the stromal cell, binding to VLA-4 and LFA-1 of the plasma cell apparently are essential, since antibodies to VLA-4 and LFA-1 eliminate plasma cells from mouse bone marrow [28], and laminin expressed by stromal cells seems to be essential for IgG-secreting memory plasma cells of the bone marrow, although its receptor on plasma cells still is enigmatic [61]. While CXCL12 binding to CXCR4 of plasma cells supports the survival of plasma cells in vitro [62], it is unclear whether it is essential for their persistence in vivo [63]. However, it is essential for homing of activated B cells and plasmablasts to the bone marrow [63, 64]. For memory T lymphocytes, VLA-4 and VLA-2, linking them to VCAM-1 and to collagens 2 and 11 [47], respectively, have been identified as part of their synapse with stromal cells. Collagen 11 may be of particular relevance, since it is nearly exclusively expressed by distinct stromal cells of the bone marrow [47]. For memory T lymphocytes, which also express CXCR4, there is indication that the latter is also relevant for their attraction to the bone marrow [65]. In addition, their homing to the bone marrow is dependent on their expression of CD69. Blocking CD69, or genetically ablating it, blocks their entry into the bone marrow [42], probably by a mechanism involving binding of CD69 to its ligands myosin light chains 9 and 12 of endothelial lining cells of the bone marrow sinusoids [66]. Whether or not CD69 is also retaining established memory T cells in the bone marrow, by blocking expression of sphingosine-1-phosphate receptors, and thus blocking attraction of the cells into the blood [67], remains to be shown.

Maintaining immune memory cells: (homeostatic) proliferation versus stromal cell contact-induced quiescence

It had been demonstrated that long-lived (memory) plasma cells persist as non-proliferating cells, not incorporating BrdU over extended time periods [24] and refractory to treatments killing

proliferating cells [25, 68]. Instead their survival could be linked experimentally to two essential signals from their environment, (a) a redundant signal provided by either BAFF or APRIL, ligands of the cytokine receptors TACI and BCMA of bone marrow plasma cells [59, 69, 70], and (b) cell contact to stromal cells via VLA-4 and LFA-1 [28, 59].

APRIL is not produced by stromal cells, but by several other cell types of the bone marrow, in particular eosinophilic granulocytes, which are abundant in the vicinity of plasma cells of the bone marrow [56, 71]. It has been postulated that eosinophilic granulocytes may be essential for plasma cell persistence, based on the analysis of mice deficient for eosinophils [71, 72]. More recently, however, alternative explanations have been provided for this observation [73–76], and a subpopulation of bone marrow stromal cells expressing BAFF has been identified [55], suggesting that BAFF and/or APRIL can be provided by several cell types, including eosinophilic granulocytes, but obviously in a redundant fashion [56].

The signals provided by cell contact to stromal cells, and by APRIL or BAFF, are necessary for the persistence of bone marrow plasma cells [28, 69, 70], and they are sufficient to keep them alive in cell culture, securing them from apoptosis [59]. While APRIL/BAFF induced NF-κB signaling in bone marrow plasma cells prevents activation of caspase 12, as induced by protein synthesis–related stress of the endoplasmic reticulum, stromal cell contact-induced phosphoinositide 3-kinase (PI3K) signaling prevents activation of caspases 3 and 7, counteracting mitochondrial stress. Thus, both survival signals synergize in providing resilience of memory plasma cells to environmental and endogenous stress. Whether or not NF-κB signaling is also required to maintain memory B and T lymphocytes alive, as quiescent cells with very little protein synthesis in steady state, remains to be shown. The direct contact of memory T and B cells to stromal cells in the bone marrow, however, suggests that stromal cell-induced PI3K signaling may be critical for their maintenance as it is for memory plasma cells.

PI3K signaling could also be induced by activation of the antigen-receptors of memory B and T lymphocytes [77, 78], and it has been speculated that persistent antigen may be required to maintain immunological memory [11, 79, 80]. While it is difficult to formally exclude the existence of little amounts of persisting or of cross-reactive antigens, changing the specificity of the antigen-receptors of memory B lymphocytes [81] or deleting it in T lymphocytes [82] by genetic interference, or monitoring the persistence of memory T lymphocytes in a host genetically deficient for antigen-presentation [83], has provided conclusive evidence that a persistent antigen is not required for the maintenance of memory B and T lymphocytes. However, conditional ablation of the antigen-receptor as such in B lymphocytes does impair their survival [84–86], probably a reflection of their internal quality control, and a hint that "tonic" signaling of the BCR may contribute directly to their survival, by inducing PI3K signaling [87]. The relative contributions of "tonic" BCR signaling versus stromal cell-contact induced signaling to the maintenance of memory B lymphocytes remains to be determined, and it could well be different for circulating and resident memory B cells. If antigen is not driving memory persistence, we can refer to true "memory" in the sense that information is maintained in the absence of the original instruction. For resident memory lymphocytes direct, integrin-mediated cell contact to other cells, in case of bone marrow contact to stromal cells, may induce sufficient PI3K signaling to keep them alive as quiescent cells.

For circulating memory lymphocytes that are not continuously contacting other cells while circulating, the situation is less clear. Presumably, their antigen receptors are not engaged, at least those of memory T lymphocytes. Krüppel-like factor 2 (KLF2), whose expression is suppressed by PI3K signaling [88], has been demonstrated to be required for the recirculation of lymphocytes [89]. Transferring mouse splenic memory T lymphocytes, most of them probably representing circulating memory cells, into IL-7 or IL-15 deficient hosts, has shown that these cells require those cytokines to persist in the host, with $CD4^+$ memory T lymphocytes being entirely dependent on IL-7 [90, 91] and $CD8^+$ memory T lymphocytes being dependent on both cytokines [92–94]. Interestingly, transferred memory T lymphocytes homed to various organs of the recipient, including the bone marrow, and proliferated at rates of up to 50% within 14 days [95, 96]. In the apparent absence of antigen, and dependent on the cytokines IL-7 and -15, this has been termed "homeostatic" proliferation, a mechanism for the maintenance of immunological memory by constant replacement of dying memory lymphocytes by newly generated ones. Systemic concentrations of IL-7 and -15 would serve as the rheostat of memory, defining the numbers of memory cells.

For resident memory T and B lymphocytes of the bone marrow, proliferation has not been observed, neither by determining the frequencies of cells in S or G2M phases of cell cycle, nor by determining expression of Ki67 [43, 48, 54, 60, 97], a protein expressed only by cells in cell cycle, not by quiescent cells resting in G0 [98]. In line with this evidence, when administering cyclophosphamide for 14 days to eliminate proliferating memory lymphocytes, the numbers of $CD8^+$ memory T lymphocytes [97] and switched memory B lymphocytes [54] of the bone marrow remain constant. The previous observation that splenic memory T lymphocytes are proliferating [95] is confirmed by cyclophosphamide treatment: 50% of the memory $CD8^+$ lymphocytes of the spleen are depleted by cyclophosphamide within 14 days. Thus, while circulating memory T lymphocytes of the spleen perform homeostatic proliferation, resident memory T lymphocytes of the bone marrow do not. It also remains unclear, whether memory T lymphocytes of the spleen are all proliferating, or just a subpopulation of them, with other cells resembling resident memory T cells of the bone marrow. Memory B cells of the spleen and the bone marrow, most of which are resident, show very little if any proliferative activity [54, 99]. While the numbers of circulating memory lymphocytes may be controlled by systemic cytokine rheostats, the numbers of bone marrow resident memory lymphocytes are controlled by mesenchymal stromal cells providing a niche, that is integrin-mediated cell contact, for an individual memory cell. This may constitute a mechanism adjusting the volume of immunological memory to the volume of the body [100].

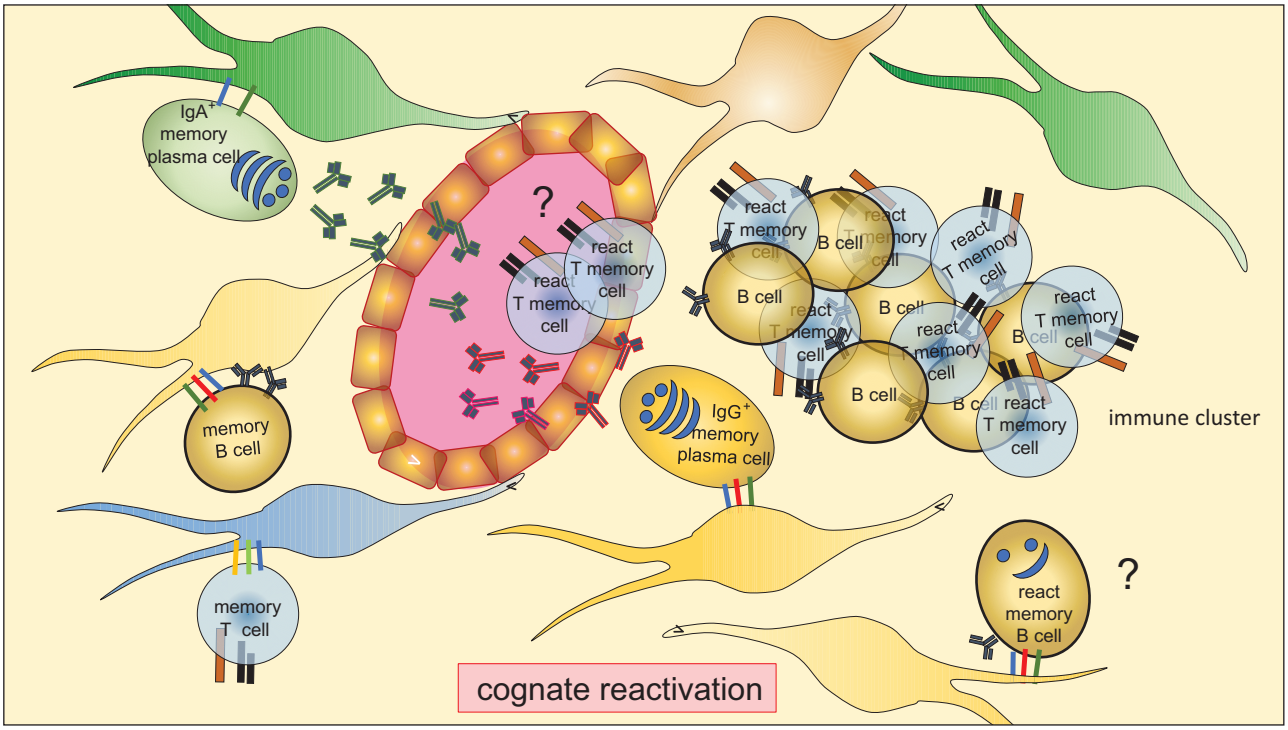

Figure 2. Cognate reactivation of bone marrow memory cells. Upon reencounter with the antigen, which enters directly via the blood into the well-vascularized bone marrow or is transported there by antigen-presenting cells, antigen-specific memory T and B cells are reactivated. Memory T cells proliferate locally, forming immune clusters, and providing local protection. Presumably, others exit the bone marrow and may contribute to secondary immune reactions in spleen and lymph nodes. Memory B cells of bone marrow may directly differentiate into antibody-secreting cells in the bone marrow, providing rapid enhancement of humoral immunity.

Cytokines may yet play a role also for the maintenance of bone marrow resident memory cells, as we and others have shown for memory plasma cells and as was recently for memory B cells requiring APRIL or BAFF [59, 69, 70, 86], but they are not sufficient to maintain the memory cells, and they do not induce proliferation. Resident bone marrow memory cells are maintained as quiescent cells.

Immunological memory – tailored for many challenges

One reason for the intellectual reluctance to appreciate the bone marrow as a prime site for the maintenance of immunological memory [101] may be the unresolved question of how bone marrow resident memory cells participate in secondary immune reactions. Are they reacting in the bone marrow directly, possibly providing local protection to the hematologically most important organ in our body? Do they leave the bone marrow to participate in systemic immune reactions in secondary lymphoid organs? Two options that do not have to be mutually exclusive. While germinal centers have not been observed in bone marrow, upon reactivation, memory T lymphocytes of the bone marrow do leave their niches, proliferate vigorously in “immune clusters” [58] (Fig. 2), and express effector type genes, conferring the potential to protect the bone marrow. Whether some of them also leave the bone marrow and participate in secondary immune reactions of germinal centers in lymph nodes or spleen, is less clear. Upon adoptive transfer, they clearly can home to secondary lymphoid organs and participate in follicular secondary immune reactions [43], as has been shown for tissue-resident memory T cells in general, also by fate mapping and ablation [102], confirming the original report of McGregor and Gowans that secondary immune reactions are dependent on tissue-resident memory lymphocytes [9]. It would be intriguing to speculate that there is a functional division of labor between $CD69^+$ and $CD69^-$ memory T cells of the bone marrow in secondary immune reactions: $CD69^+$ memory T cells as bona fide tissue-(bone marrow-) resident memory cells remaining in the bone marrow upon reactivation and providing local protection of the hematopoietically so important tissue, and $CD69^-$ memory T cells with the ability to leave the bone marrow and participating in the systemic immune responses and germinal center reactions in secondary lymphoid organs. In recall immune responses an initial wave of antigen-reactive memory T cells can be observed already 16–48 h after vaccination [103], suggesting direct mobilization into the blood from their tissue of residence (Fig. 2).

For memory plasma cells the reason for their residence in the bone marrow is clearer, as the bone marrow provides all signals required for persistence and the proximity to blood circulation for continuous dissemination of antibodies via the blood (Fig. 1). Resident memory B lymphocytes of the bone marrow may be direct

precursors of those plasma cells, since they apparently inhabit the same kind of niches [54] (Fig. 2). It has been demonstrated that memory B cell responses are unimpaired in splenectomized patients [104] despite multiple claims that the spleen hosts most memory B cells [104–106]. While the contribution of lymph node-resident memory B cells is still unclear, the bone marrow may represent a major reservoir of memory B cells, which might also, analogous to the memory T cells, segregate functionally: those that directly differentiate into long-lived memory plasma cells, providing immediate protection, and those that migrate out of the bone marrow to participate in germinal center reactions in secondary lymphoid organs.

At this time, the term "resident" refers to the steady-state of memory maintenance, and we know surprisingly little about the behavior of tissue- or bone marrow-resident memory lymphocytes upon reactivation, except that the reactivating antigen has to come to them in the first place, refuting for those cells the old textbook paradigm that memory lymphocytes patrol the body in search of their antigen, or cells presenting it.

While we are still far from understanding immunological memory on a molecular level, we slowly begin to understand its functional and topographic diversity. Bone marrow-resident memory lymphocytes represent a distinct compartment of immunological memory, a compartment that on the level of single cells will most likely display extensive heterogeneity, contributing to both local and systemic protection. The concept of "tissue-residency" has been confined to epithelial tissues for long, neglecting the central role of the bone marrow for the persistence of long-term memory to systemic antigens by dedicated memory T and B lymphocytes, and for humoral immunity as such, as provided by memory plasma cells. We have noticed that we do not have good working hypotheses for "circulating" adaptive memory lymphocytes, their entry into and egress from circulation, nor their whereabouts in between. It remains a challenge to determine the contribution of the various memory lymphocyte populations to secondary local and systemic, extrafollicular, and follicular immune reactions, providing redundant layers of protection, i.e., efficient secondary reactions to recurrent pathogens, both on the level of cellular response and antibody-mediated neutralization.

Acknowledgements: We like to thank Klaus Rajewsky for critical reading of the manuscript and his expert input. This work was supported by the Deutsche Forschungsgemeinschaft (Grant No. 389687267 to A.R.and TRR130 P16 to H.D.C. and A.R.), the European Research Council Advanced Grant IMMEMO (ERC-2010-AdG.20100317 Grant 268987; to A.R.), the Dr. Rolf M. Schwiete Foundation (to H.D.C.) and the Leibniz Association through the Leibniz Science Campus Chronic Inflammation.
Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest: The authors declare no conflict of interest.

Peer review: The peer review history for this article is available at https://publons.com/publon/10.1002/eji.202049012.

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Abbreviation: **PI3K**: phosphoinositide 3-kinase

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Received: 25/3/2021
Accepted article online: 19/5/2021