Linking immunity and hematopoiesis by bone marrow T cell activity

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Abstract

Two different levels of control for bone marrow hematopoiesis are believed to exist. On the one hand, normal blood cell distribution is believed to be maintained in healthy subjects by an “innate” hematopoietic activity, i.e., a basal intrinsic bone marrow activity. On the other hand, an “adaptive” hematopoietic state develops in response to stress-induced stimulation. This adaptive hematopoiesis targets specific lineage amplification depending on the nature of the stimuli. Unexpectedly, recent data have shown that what we call “normal hematopoiesis” is a stress-induced state maintained by activated bone marrow CD4+ T cells. This T cell population includes a large number of recently stimulated cells in normal mice whose priming requires the presence of the cognate antigens. In the absence of CD4+ T cells or their cognate antigens, hematopoiesis is maintained at low levels. In this review, we summarize current knowledge on T cell biology, which could explain how CD4+ T cells can help hematopoiesis, how they are primed in mice that were not intentionally immunized, and what maintains them activated in the bone marrow.

Introduction

Hematopoiesis control, i.e., the signals that govern lineage commitment and hematopoietic stem cell maintenance in adult bone marrow, has been extensively studied in the last decades. A large part of this control depends on the bone marrow microenvironment, where a complex network including multiple cell types such as fibroblasts, osteocytes, endothelial cells, and all bone marrow-derived cells regulates hematopoiesis through cytokine secretion and cellular interactions. In addition, hematopoiesis can be “endocrinally” regulated through cytokines produced outside the bone marrow. While bone marrow-derived stimuli have been viewed as important to maintain basal blood cell production, peripheral stimuli have been related to hematopoiesis amplification during stress situations such as infection, inflammation, irradiation, or hypoxia. Thus, modulation of hematopoiesis has been viewed as a systemically controlled phenomenon (1-4).

We have recently revisited this concept...
by showing that blood cell counts in normal mice are not the result of basal “innate” hematopoietic activity (5). On the contrary, they reflect the antigenic stimulation of bone marrow CD4+ T cell. In other words, “normal hematopoiesis” is not an innate state, but an adaptive state, maintained by antigen stimulation. In view of these novel findings and considering the potential role of CD4+ T cells as a source of hematopoietins, we proposed these cells to be key regulators of what we currently understand as “normal” hematopoiesis (5).

Here, we review the data that indirectly relate T cell and hematopoiesis and discuss the recent findings which redefine hematopoiesis as a T cell-dependent phenomenon. We also summarize the current knowledge on T cell biology to explain how T cells can help hematopoiesis, how they are primed in untreated (not intentionally immunized) mice, and how they are maintained in an activated-state in the bone marrow.

**T cells and hematopoiesis - the old history**

The role of thymus-derived cells in hematopoiesis was first suggested 30 years ago. Using the neonatal thymectomy model, several investigators described that 1-day thymectomized mice were anemic, showed arrested erythroid maturation and reduction in the number of spleen colony-forming units in the bone marrow and spleen (6,7). In addition, intravenous injection of live thymocytes accelerated hematopoiesis reconstitution in sublethally irradiated mice (8). The specificity of the T cell population that causes GVHD and the population that helps hematopoiesis is still an unsolved question. Different T cell subpopulations have been reported to be able to promote allogeneic bone marrow engraftment without causing GVHD (12,17). More specifically, the role of bone marrow T cells in the pathogen-
esis of GVHD seems to be very minor, if any (17). Only recently have these T cells been studied. The total bone marrow T cell population comprises 2-3% of all bone marrow cells. About one third of them are CD3+CD4-CD8- or αβTCR+NK1.1+ cells (18). The role of these unusual T cells in the bone marrow, specifically regarding hematopoiesis, has not been evaluated. The remaining two thirds are conventional αβTCR+ T cells enriched in activated and memory CD4+ and CD8+ T cells (5,17,19,20). The potential of these cells as hematopoietin providers or their cytokine profile and the stimuli promoting their activation have never been investigated.

Besides conventional T cells, an unusual type of “T cells”, the “facilitating cells”, was described in bone marrow by Ildstad’s group (21). These cells comprise 0.4% of total bone marrow cells and are CD8+CD3+CD45 RB-Thy1+class II+cells. Curiously, these “T cells” do not express any known form of T cell antigen receptor (TCR). They are devoid of allospecific activity and can help hematopoiesis. Therefore, although functional data on the activity of these cells are abundant (21-23), their existence remains extremely controversial.

In addition to the BMT model, the role of T cells in hematopoiesis was also investigated in infectious disease models (24-26). It appears that hematopoiesis amplification, required to clear pathogens, is deficient in athymic mice. This is true for the diverse blood cell types, although it is more important within the granulocytic compartment. In these situations, T cells contribute secretory cytokines, including GM-CSF, IL-3, IL-4, IL-5, IL-6, IL-13, and oncostatin M, which all contribute to amplifying granulocyte generation in bone marrow.

Introducing the recent findings

T cells have been shown to be involved in hematopoiesis in different stress situations such as reconstitution after sublethal irradiation, bone marrow engraftment after BMT, and amplification of hematopoiesis during infection, as cited above. However, it is not clear if T cells contribute to hematopoiesis in the absence of such stresses. In other words, do T cells play any major role in the maintenance of normal hematopoiesis? Can T cells contribute to normal hematopoietic activity upon interaction with syngeneic bone marrow cells? And if so, what is the stimulus?

The closest replies to these questions were obtained in the experiments reported by Lord and Schofield (8) and by Bonomo et al. (27). The former showed that injection of syngeneic thymocytes accelerates bone marrow reconstitution after sublethal irradiation. The latter, using an in vitro model, showed that syngeneic T cells stimulate the growth of hematopoietic progenitors.

With the above findings in mind, we devoted our efforts to the study of normal hematopoiesis in athymic nude mice since these animals lack all conventional T cells (5). Our findings were surprising: nude mice have a severe reduction in the number of granulocytes in peripheral blood, despite the high frequency of granulo-monocytic progenitors in the bone marrow. Nude mice reconstitution with fetal thymus or purified CD4+ T cells not only restores the normal granulocyte counts in peripheral blood, but also reduces the frequency of progenitors in bone marrow. In contrast, purified CD8+ T cells are completely inefficient in restoring normal hematopoiesis. The progenitors that accumulate in the nude bone marrow are SCA1+CD11b+ cells, representing committed myeloid progenitors. These cells are not intrinsically unable to differentiate, since exogenous stimulation with growth factors promotes their differentiation in vitro. Therefore, they do not differentiate in vivo because nude mice lack the T cell-derived stimulus, probably inside the bone marrow, which is necessary for that process to occur. In other words, T cells are important to
promote terminal differentiation of these committed progenitors. These findings are not exclusive for athymic mice, since they have been confirmed in other T cell-deficient mouse lineages such as SCID and RAG−/−.

We also found a strong correlation between the number of CD4+ T cells in bone marrow and restoration of granulocyte counts in the peripheral blood of T cell-reconstituted mice. The same was true for reduction in the number of bone marrow GM precursors (5). These data strongly suggest that bone marrow CD4+ T cells were responsible for the effect on hematopoiesis. Thus, since to help hematopoiesis T cells must be activated, we looked at the activation status of these cells in the bone marrow of normal mice. In agreement with previous descriptions (17,19) we found an increased proportion of activated CD4+ T cells in mouse bone marrow - most of them showing an early activated phenotype (5).

The large number of activated T cells in the bone marrow of normal mice creates another problem: since these mice were not intentionally immunized, how did their bone marrow T cells become primed? Possibly, T cells were being primed by their specific antigens (the cognate antigen) following the conventional pathway of T cell activation. This priming could be in the bone marrow or at the periphery, from where the antigen-primed cells would migrate to the bone marrow. However, since our data showed that bone marrow T cells were activated while lymph node cells were not, it seemed possible that bone marrow T cells had their own rules of activation. In this case, T cells would be primed by endogenous weak ligands that could work in synergy with other bone marrow-specific stimuli for T cell activation.

In order to answer this question, we designed an experiment that dissociated these two hypotheses. We used RAG−/− TCR transgenic mice whose T cells all express TCR specific for an exogenous antigen, ovalbumin (OVA), presented by the I-Aβ major histocompatibility complex (MHC) molecule (DO11.10) (28). If bone marrow T cells were activated in DO11.10 mice and hematopoiesis was normal, this would mean that bone marrow T cells are primed by endogenous ligands in a distinct fashion. However, if bone marrow T cells were not activated, and hematopoiesis was abnormal, this would mean that bone marrow T cells must recognize the cognate antigen to become primed and help hematopoiesis. Through hematopoiesis and bone marrow T cell analyses, we found that the second hypothesis proved to be correct. In fact, DO11.10 RAG−/− mice had the same hematopoietic profile as nude mice, showing that bone marrow T cells must recognize their specific antigens in order to help hematopoiesis (5).

The conclusion was that, since hematopoiesis in normal mice is maintained by antigenic stimulation of bone marrow T cells, it does not represent a basal state! On the contrary, it is already an induced state (Figure 1). However, current literature defines

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Figure 1. Model of “adaptive” T cell-instructed hematopoiesis. T cells are primed by the cognate antigen and reach the bone marrow, where they can help hematopoiesis by i) direct cytokine production; ii) inducing cytokine production by the bone marrow stromal cells (through cytokines or cellular interaction). These cytokines act by promoting terminal differentiation of myeloid progenitors. BM = bone marrow; APC = antigen-presenting cell; HSC = hematopoietic stem cell.
normal hematopoiesis as “a basal state that maintains normal blood cell counts in the absence of hematopoietic stresses” (1-4). Can we consider antigenic stimulation a stress? We believe that the answer is yes. The source of antigen for bone marrow T cell activation is unknown. It may be a harmless environmental antigen, a pathogen-derived antigen, or even a self antigen. We will discuss this question in more detail in the next sections. The fact is that normal hematopoiesis does not represent a basal state, and our finding shows that the old view of a two layer-controlled hematopoiesis (normal x stress-induced) must be reviewed. Our suggestion would be to revisit the concept of “normal”, and replace it with innate hematopoiesis, as the basal thymic-independent bone marrow activity based only on the constituents of innate immunity.

The first problem: how T cells can help hematopoiesis

It seems obvious to think that CD4+ T cells regulate hematopoiesis through cytokine secretion. Indeed, CD4+ T cells are wonderful cytokine producers, not only quantitatively, but also qualitatively, including the major T cell cytokines that control the immune responses such as IFN-γ, IL-4, IL-5, IL-13, IL-10 and TGF-β, and the “minor” cytokines, whose effects on adaptive immune responses are less prominent (29). This group includes GM-CSF, IL-3, IL-6, IL-17, and oncostatin M - all involved in the regulation of hematopoiesis (30-37). The major problem is that most of these cytokines are not T cell exclusive. Therefore, it is very difficult to determine if the lack of T cells significantly affects the role of these cytokines.

In addition to having a direct activity as a source of hematopoietins, T cells can also stimulate cytokine production by bone marrow stromal cells (Figure 1). It has been shown that oncostatin M and IL-17 produced by T cells can stimulate IL-6 production by endothelial cells and osteocytes (32,36). IL-6 has an important effect on neutrophil production. IL-6−/− mice are neutropenic. Also, in the absence of IL-6, residual neutrophils found in G-CSFR−/− mice are eliminated (34).

Besides cytokines, cell-cell interaction seems to be important for the contribution of T cells to hematopoiesis. T cells can interact with bone marrow stroma through the CD40-CD40L (CD154) pathway (Figure 1). In fact, hematologic recovery after syngeneic BMT is accelerated by treatment with soluble CD40L in mice (38). This protocol increases colony-forming units-GM frequency in mouse bone marrow and spleen, and stimulates platelet and leukocyte recovery in peripheral blood. CD40L appears to directly stimulate the production of Flt3 by several cell types and of thrombopoietin by bone marrow stromal cells (39). This shows that, although T cells are not indispensable for megakaryopoiesis in normal mice, they can contribute to platelet generation under damage-induced conditions, i.e., after irradiation.

Further evidence for cellular interaction in T cell contribution to hematopoiesis came from mixed chimera experiments. Since MHC compatibility between T cells and hematopoietic progenitors is required for bone marrow engraftment, it is clear that interaction between the two cell types, in an MHC-restricted way, is obligatory (16). How T cells are activated and what activates them is discussed in the next sections.

The second problem: how bone marrow T cells become antigen-primed cells

We and others have found an increased number of activated T cells in mouse bone marrow (5,17,19,20). This is true for both CD4+ and CD8+ T cells. However, normal mice are not intentionally immunized: how
can they have such a high number of activated T cells in bone marrow? We have found that only the cognate antigen can activate bone marrow T cells (5). Which are the cognate antigens in “normal” mice? We envision two possible explanations: the stimulation by environmental antigens or by cognate self antigens. In fact, mice - like humans - are exposed to antigens present in the environment. These environmental antigens probably prime some T cell clones, although their study has not attracted much interest lately.

The second explanation suggests that bone marrow T cells are primed by specific autoantigens present only in bone marrow. At present there are no data supporting or excluding this hypothesis, except for the fact that the CD4 T cell population of bone marrow is composed of lymphocytes preferentially expressing VB families, especially VB3 and VB7, suggesting that bone marrow T cells have some preferences in terms of antigen recognition (19). It is very difficult to choose between these two hypotheses. Our experimental model uses the DO11.10 RAG-/- mice in which all T cell clones respond to OVA, excluding the possibility of studying both T cell priming by environmental antigens and cognate self-antigens (5). Experiments with DO11.10 in animals with a non-RAG-/- background have revealed that their bone marrow T cells are activated, showing that T cells expressing endogenously rearranged TCR α-chains can be primed by other antigens. However, we could not distinguish if these TCRs recognize self or environmental antigens.

Another important issue is the priming site for bone marrow T cells (Figure 2). Although we know that T cells must be present in bone marrow to help hematopoiesis, we do not know if they were primed in bone marrow or in the periphery. Activation of virgin T cells must include TCR recognition of cognate antigens. Then, T cells can only be primed in the bone marrow if the antigens reach it. Feuerer et al. (40) showed that this is possible for blood-borne antigens. They transferred transgenic OT-I CD8+ T cells (which recognize pOVA + H-2Kb) to normal B6 mice, and immunized these mice.
with OVA intravenously. They found that 50% of bone marrow CD8+ T cells displayed the early-activation marker CD69 6 h after immunization, while in the spleen and lymph nodes the frequency of activated cells was 25 and 5%, respectively. The authors concluded that priming of bone marrow T cells predominantly occurred in bone marrow. However, they did not show the total number of CD8+ T cells in each organ. Probably spleen and lymph nodes have a much higher number of activated CD8+ T cells than bone marrow. In addition, Di Rosa and Santoni (41) showed that antigen-primed T cells have an increased capacity to migrate to bone marrow when compared to naive cells. Thus, it is possible that CD69+ T cells in the bone marrow represent peripherally primed T cells that migrate to bone marrow after antigenic stimulation (Figure 2).

Although it is clear that bone marrow dendritic cells can present antigens in an immunogenic manner, this is restricted to antigens that reach the bone marrow. Probably, this will not be the case for environmental antigens, which enter the body predominantly through mucosal and subcutaneous routes. If environmental antigens are the responsible for the activation of the majority of bone marrow T cells in normal mice, probably these cells were primed at the periphery and then migrated to the bone marrow. As described above, antigen-primed T cells, both CD4+ and CD8+, have a high capacity to migrate to the bone marrow (41). Lack of ICAM1 and CD18 molecules - which are important to direct T cells to lymph nodes - does not affect T cell migration to the bone marrow. Interestingly enough, adoptive transfer of activated T cells to 1-year-old mice thymectomized at 4 weeks of age precludes the access of activated cells to the bone marrow due to the presence of a pre-existing memory population in bone marrow (41).

The existence of a memory T cell pool in bone marrow was described by several groups (17,19,20). The CD8+ compartment of memory T cells in bone marrow is particularly interesting. Long-lived memory CD8+ T cells can be found in mouse bone marrow 4-7 months after immunization (20). However, it is not clear if the bone marrow memory compartment is permanent or constantly renewed. Parabiosis experiments favor the second hypothesis since, following parabiosis, memory CD8+ T cells rapidly equilibrate into the lymphoid organs of each parabiont, including bone marrow (42). Recently, Di Rosa’s group showed that bone marrow is an important location for T cell memory maintenance (Di Rosa F, personal communication). They showed that memory bone marrow CD8+ T cells are in a highly proliferative state and contribute to a large fraction of total CD8+ memory T cells in normal mice. Regarding bone marrow CD4+ T cells, there is no definitive report showing that they are memory cells. However, if this possibility proves to be correct, it is possible that maintenance of hematopoiesis at an optimal level in normal mice (and humans) is regulated by memory T cells.

The third problem: how the bone marrow maintains T cells activated

Bone marrow T cells are primed in bone marrow or at the periphery and then migrate to bone marrow. However, what happens after priming? Are T cells maintained in an activated state in the bone marrow? Or, on the contrary, are bone marrow T cells a pool of activated cells in constant interchange with the periphery? We favor the first hypothesis for two reasons. The first is that the presence of an activated T cell population is a very highly reproducible result described in several mouse lineages (5,17,19,20). The second is that adoptive transfer of activated T cells to mice thymectomized during adult age impairs the migration of activated cells to the bone marrow (41). In other words, there is a competition for bone marrow seeding and the pre-existing bone marrow T cell
These findings show that these T cells are, in fact, antigen-primed, but also suggest that they are being restimulated in the bone marrow. The remaining question concerns the restimulation of these cells (Figure 3). Is it a cognate antigen/TCR stimulus? Is there a role for self-ligands and cytokines? Although the T cell population of bone marrow is composed of clones bearing a restricted Vß repertoire, the population is polyclonal (19). Thus, it is unlikely that all the antigens required to activate a polyclonal T cell population will reach the bone marrow (Figure 3A). In other words, if bone marrow T cells are a resident population that is constantly restimulated, probably the source of this stimulation is not the cognate antigen, or at least not only the cognate antigen.

Self-recognition is a double-edge sword. If T cells recognize the peripheral self with too much affinity and avidity they initiate a deleterious autoimmune response. However, if T cells are unable to recognize the peripheral self, they cannot survive or their activity is impaired. Then, self-recognition is a quantitative problem, which is necessary for normal T cell activity, but it must be controlled to avoid autoimmunity. The role of self-ligands varies according to the activation status of the T cell. While naive T cells cannot persist in the absence of the thymic positively selecting self-ligands, memory T cells are maintained for long periods of time in the absence of the selecting MHC (43,44). Moreover, self-recognition is required for a normal response of naive T cells to foreign antigens. Dendritic cells can signal T cells in the absence of exogenous antigens, inducing weak proliferation and some signs of activation, i.e., down-regulation of TCR and expression of CD25 and CD69 (45,46). Endogenous peptide/MHC (pMHC) complexes appear to accumulate in the immunological synapse during antigen recognition, and although only agonist pMHC can initiate T cell activation, self pMHC seem to be required for a maximal response (47). Li et al.
suggested that this cooperation between self and agonist ligands is required for optimal CD4-mediated Lck accumulation in the immunological synapse. Finally, Ron Germain’s group (49) showed that self-recognition promotes the foreign antigen sensitivity of naive T cells. These cells deprived of contact with self-ligands show an acute loss of ζ-chain phosphorylation and respond poorly to subsequent cognate antigen stimulation. Likewise, blood naive T cells - which are naturally deprived of contact with self-ligands - also show decreased sensitivity to foreign antigens, confirming the physiological relevance of these findings.

Hematopoiesis control and bone marrow T cells

The role of self-recognition in the behavior of already activated or memory T cells is less understood. Kassiotis et al. (50) showed that memory CD4+ T cells maintained in the absence of any endogenous ligands are less responsive to subsequent antigenic stimulation. In addition, Stefanova et al. (51) suggested that 24-h pre-activated T cells (with the agonist ligand) become hypersensitive and can respond to different pMHC complexes, including some peptides which were antagonists for unprimed T cells. All of these findings strongly suggest that i) self-ligands have a function in T cell activation that is not uniquely related to survival signals, but is also related to maintenance of optimal TCR proximal signaling; ii) activated T cells are less demanding regarding antigen specificity, and can be restimulated by altered ligands which cannot activate the same naive T cells. Therefore, it seems appropriate that self-ligands should contribute to maintaining the activated status of bone marrow T cells (Figure 3B). Further studies are required to confirm this hypothesis.

In addition to self-recognition, some cytokines have remarkable effects on antigen-experienced T cell activation (Figure 3C). While naive T cells must find a cognate antigen to become activated, memory T cells can be activated through cytokines in determined situations. Berg et al. (52) showed that memory CD8+ T cells can secrete IFN-γ when stimulated with IL-12 and IL-18 in the absence of a cognate antigen. Moreover, several investigators have shown that memory CD8+ T cell maintenance - that depends on homeostatic proliferation - is regulated by IL-15 and, to a lesser extent, IL-7 (53,54). In fact, memory CD8+ T cells do not persist in mice lacking IL-15 or IL-15Rα (55,56). In the CD4 compartment, IL-7 is necessary to maintain the memory, but appears to work in association with TCR-derived signals (57). Despite these findings, the existence and extension of cytokine-induced activation of memory in normal mice have not been addressed. However, considering that bone marrow T cells seem to include a large number of memory cells, and that the bone marrow microenvironment is an excellent source of cytokines - especially IL-7 and IL-15 - we cannot exclude a role for cytokines in the maintenance of the activated status of bone marrow T cells.

In summary, it is possible that T cell activity in the bone marrow is under the control of positive activation signals (self-ligands, cytokines, and even accessibility of...
cognate antigen) and negative signals which control the extent of this activation (the suppressive activity of bone marrow stroma). The tuning of these signals enables T cells to contribute to hematopoiesis in the absence of bone marrow damage.

Perspectives

The presence of activated T cells in the bone marrow is required for normal hematopoiesis maintenance. Understanding the mechanisms underlying T cell activity in the bone marrow, can provide new clues not only about immune regulation and immunological memory, but also about clinical problems such as the physiopathology of GVHD, graft failure after BMT and hematologic manifestations in T cell-deficient patients. Finally, T cell activity in the bone marrow shows that immunity and hematopoiesis are intimately connected and that this connection is required to establish the best defense against foreign aggressors, keeping us in a healthy condition. Conceptually, it is important to mention that what we understand as normal hematopoiesis is the response of the basal or “innate” hematopoiesis to the adaptive immune system.

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