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Inflammatory signals in HSPC development and homeostasis: Too much of a good thing?

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Abstract

Hematopoietic stem cells (HSCs) reside in the bone marrow and are responsible for the life-time maintenance of the blood and bone marrow, achieved through their differentiation into the myriad cellular components and their ability to generate additional stem cells via self-renewal. Identification of intrinsic and extrinsic factors that regulate how the HSC population is maintained over the lifespan of an organism, or those that trigger differentiation into mature hematopoietic cell types, are important goals for regenerative medicine. Recent studies have found that inflammatory signals play a role in the regulation of adult HSC homeostasis and tonic innate immune signals influence HSC development during embryogenesis. Additionally, dysregulation of inflammatory cytokines, and the consequent impact of this on hematopoietic progenitors, may be a contributing factor to the hematopoietic defects that occur during aging and in patients with bone marrow failure syndromes or blood cancers. To update recent findings on this topic, the International Society for Experimental Hematology (ISEH) organized a webinar entitled “The Role of Inflammatory Signals in Embryonic HSC Development and Adult HSC Function,” which we summarize here.

Most effector cells of the hematopoietic system are shortlived, terminally differentiated cells that must be continually replenished throughout life. An estimated 10^{11} cells are produced by the bone marrow every day, and this number increases during stresses such as infection [1]. Hematopoietic stem and progenitor cells (HSPCs) are specialized cells in the bone marrow that maintain blood production for the life span of the organism through their

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extensive capacity for proliferation and differentiation, with their unique self-renewal ability regenerating the HSPC pool throughout life. Thus, understanding the cues that regulate HSPC function is a key to understanding not only blood homeostasis, but also diseases such as aplastic anemia and leukemia. Furthermore, defining the regulation of HSPCs may further reveal ways to use these cells therapeutically to address deficiencies in blood production or for optimized clinical utility in bone marrow transplantation.

In efforts to unravel the molecular mechanisms that regulate HSPC pathways, many groups have uncovered significant roles for inflammatory signals in maintenance of HSPC homeostasis in adult bone marrow. In particular, interferon alpha (IFN α), a type I interferon, induces division of quiescent hematopoietic stem cells (HSCs) [2] via a process that involves either displacement from quiescence-enforcing perivascular niches [3] or cytosolic sequestration of the quiescence-enforcing transcription factor FoxO3a [4]. Interferon gamma (IFN γ) also stimulates division of hematopoietic stem cells, in the context of both microbial infection and exogenous administration [5], which leads to functional impairment of HSPC activity, as assessed by bone marrow transplantation assays. Similarly, the bacterial Toll-like receptor agonist lipopolysaccharide (LPS) and the pro-inflammatory cytokine interleukin (IL)-1 activate HSC division [6–8]. Furthermore, recent studies have revealed a role for immune/inflammatory signals in promotion of HSPC genesis in the developing embryo, in both zebrafish and mouse systems.

Here we highlight these advances by summarizing the ISEH webinar presented on 5 April 2016 by Markus Manz and Trista North (moderated by Katherine King) entitled “The Role of Inflammatory Signals in Embryonic HSC Development and Adult HSC Function.”

Dr. Trista North: The importance of inflammatory signals during HSPC development

The emergence and expansion of HSPCs during embryonic development represent a crucial step for the establishment of a functional hematopoietic system in vertebrates. Accordingly, understanding the molecular pathways that regulate these processes in the embryo will help ultimately allow the production and expansion of HSPCs *ex vivo*. Several research groups have investigated the intrinsic and extrinsic molecules triggering HSC development from aorta/gonad/mesonephros (AGM) regions of midgestation mouse embryos. Recent findings suggest that the innate immune/inflammatory signaling regulates HSC specification and expansion during embryogenesis [9]. In this webinar, Dr. North discussed recent findings from her research group uncovering the complex relationship between the inflammatory signaling network and HSPCs during embryonic development.

During embryogenesis, the emergence of HSPCs from hemogenic endothelium and the subsequent specification of HSCs suggest the presence of sequential waves of stimuli produced by the environmental niche. To identify these conserved modifiers of HSC formation in vertebrates, Dr. North et al. performed a chemical screen by incubating embryos in the presence of compounds with known biological activity. Using the levels of conserved HSC-specific factors *Runx1* and *c-Myb* as readout, they evaluated the effect of each chemical on HSCs by performing *in situ* hybridization on treated embryos [10]. They

found that many compounds affecting HSC development were known to modulate synthesis and signaling of prostaglandin E2 (PGE2), which is a key mediator of inflammatory response in chronic infections and cancer [11]. Accordingly, Dr. North et al. confirmed that PGE2 and other eicosanoid signals, such as cannabinoid receptor 2 (CNR2) and epoxyeicosatrienoic acids (EETs), affect HSC development in the AGM [12,13]. Using transplantation assays of HSCs from murine bone marrow and human cord blood, they also found that PGE2 regulates HSC function and expansion in vivo [12, 14].

Given that embryogenesis occurs in an environment devoid of infection-mediated response, Dr. North et al. investigated how pro-inflammatory signals are stimulated in the embryo and how these extrinsic cues affect HSC development. Previous studies have unveiled the interconnection between nutrients and the hematopoietic system during development [15]. Accordingly, metabolic cues like glucose levels modulate the biological activity of HSCs [16], which can be exacerbated in the context of diabetes and metabolic syndromes. To assess the effect of blood glucose on HSC development in the AGM, Dr. North et al. exposed embryos to higher concentrations of glucose (twofold normal levels) and found that hyperglycemia promotes HSC expansion during embryogenesis, as measured using the HSC markers *Runx1* and *c-Myb* as readout [17]. This effect was dependent on *Hif1a*, a crucial regulator of quiescence and glycolysis in HSPCs, levels of which are modulated by the reactive oxygen species (ROS) produced by energy metabolism. Using VE-cadherin conditional knockout of *Hif1a*, different teams elegantly determined that *Hif1a* is required for HSC induction and expansion in the AGM of vertebrates [18, 19]. These experiments revealed that elevated glucose levels contribute to establishment of a pro-inflammatory environment in the developing embryo, which promotes HSC expansion, and confirmed the previous observation that inflammatory signals affect HSC homeostasis through feedback regulation in adult hematopoiesis.

Although blood glucose levels affect HSC homeostasis during development, other extrinsic cues produced by the environment tightly regulate the baseline HSC regulatory program. Among the different waves of environmental factors produced throughout the embryonic program, classically defined inflammatory intermediates, such as PGE2, nitric oxide, granulocyte colony-stimulating factor (G-CSF), IFNs, tumor necrosis factor α (TNF α), Toll-like receptor 4 (TLR4), CNR2, and EETs, are crucial during HSC development [12]. Using morpholinos to screen against cytokines and pro-inflammatory signals in zebrafish embryos, Dr. North et al. determined that loss of IFN γ , TNF α , and IL-1b significantly impaired HSC formation during development. They also found that IFN-mediated signaling by IFN α , IFN γ receptor (IFN γ r), or IFN α receptor (IFN α r) is essential for HSC self-renewal capacity and specification into functional lymphoid progenitors, using limiting-dilution transplantation and co-culture assays, respectively. The reverse scenario, in which AGM explants were cultured in the presence of IFNs, confirmed that levels of inflammatory cytokines directly modulate HSPC development in the embryo, using the transgenic Ly6a-GFP mouse model to track HSCs. Importantly, Dr. North et al. also determined that IFN response genes are expressed in human and murine HSPCs, highlighting the fact that pro-inflammatory signals are active in mammalian embryos [12]. Despite functionally characterizing the relevance of pro-inflammatory signals in HSC development and homeostasis, the origin of these extrinsic cues during embryogenesis in the context of

normoglycemia remained to be investigated. To address this crucial question, Dr. North et al. looked for cell types expressing IFN γ r and downstream targets in the embryo. They identified endothelial cells, macrophages, and HSCs as the source of inflammatory cytokines in murine and zebrafish embryos [12]. Using morpholinos against myeloid-derived cytokines (TNF α , IL-1b), they also demonstrated that primitive myeloid cells are required for embryonic HSPC formation in the absence of injury or infection, which mimics the effect of IFN γ knockdown [20]. Accordingly, using morpholinos targeting IFN γ in zebrafish embryos, Dr. North's team confirmed that the expression levels of embryonic inflammatory cytokines produced by myeloid cells modulate HSPC development [12].

Together, the results discussed by Dr. North highlight the complex synergistic and compensatory mechanisms by which inflammatory responses regulate hematopoiesis during embryonic development of vertebrates [12,20–22]. These fundamental metabolic-sensing mechanisms are crucial for the maintenance of embryonic growth and viability, by enabling the embryo to adjust HSC function in response to environmental changes. In a nutshell, inflammatory cytokines and other extrinsic cues affect developing HSPCs by modulating the metabolic machinery, which plays a crucial role in hematopoietic output and homeostasis during embryogenesis. These results have great implications for the use of umbilical cord blood (UCB) as a valuable source of HSCs for allogeneic transplantations, which is the last-resort option in the absence of a suitable adult donor. Despite being publically accessible, these UCB units have low HSC content, which is associated with high graft failure rates and early mortality of adult recipients. Hence, the studies presented by Dr. North describe the beneficial impact of pro-inflammatory cues on HSC development, highlighting the possibility of expanding HSCs from UCB units in culture prior to transplantation [23]. In light of this information, we can speculate that this alternative strategy may radically change the current practice, which will ultimately improve the outcome for patients undergoing allogeneic transplantation.

Dr. Markus G. Manz: The role of inflammatory signals in adult HSC function

On the basis of clinical observations, it is well established that systemic bacterial infection results in peripheral blood neutrophilia and enhanced myelopoiesis in the bone marrow. This is regarded as a feedback mechanism allowing rapid replenishment of innate immune cells, efficient clearance of ongoing infection, and ultimately, reestablishment of homeostasis. Recent findings suggest that the hematopoietic response to infection and inflammation is more complex than previously anticipated and is likely to involve both direct and indirect mechanisms [1,24]. In this webinar, Dr. Manz discussed recent results from his laboratory, giving new insights into how HSPCs sense an ongoing infection and the signaling pathways involved in this process.

The concept of indirect activation of the hematopoietic system implies the existence of specialized cell types (hematopoietic or nonhematopoietic) that are able to sense the presence of pathogens and consequently release cytokines to stimulate emergency granulopoiesis. In line with this, Dr. Manz et al. described the involvement of endothelial cells in this process. Indeed, endothelial cells are positioned ideally to mark the threshold between local and systemic infection. Using tissue-specific knockout mouse models, Dr.

Manz found that endothelial cells are able to detect systemic infection and activate granulopoiesis through the release of large amounts of G-CSF [25]. In a search for cytokines that may recruit HSCs into the cell cycle on infection, Dr. Manz et al. investigated how different clinically employed cytokine/chemokine receptor agonists/antagonists affect the proliferation and self-renewal of HSCs in vivo [26]. For this, they used an in vivo assay based on 5 (and 6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) labeling to track individual HSC cell divisions. Using this assay, the Manz laboratory had previously proposed the dynamic repetition model of HSC cycling. According to this model, under steady-state conditions, HSCs alternate between states of quiescence and fast cell division in which they more actively contribute to hematopoiesis. This model also predicts, and it was actually demonstrated for LPS, that in stress situations such as infection, quiescent HSCs are recruited into proliferation and self-renewal [7]. From the different cytokines/chemokines now investigated, only the cMpl agonist and thrombopoietin analog romiplostim resulted in the loss of quiescent (cells with none or one division after treatment) HSCs. Importantly, HSCs that underwent two to four cell divisions after romiplostim treatment retained long-term reconstitution potential with no changes in lineage distribution. This contrasts with dividing HSCs from mice treated with the G-CSF analog filgrastim, the Cxcr4 antagonist plerixafor, or Flt3L, which were no longer able to reconstitute secondary recipient mice [26].

On the other hand, HSPCs may also directly sense an ongoing infection. In support of this, HSPCs are known to express specific pathogen recognition receptors such as TLRs, which act as “smoke detectors of infection.” TLR4, for example, is able to recognize lipopolysaccharide (LPS) from gram-negative bacteria, and its activation can trigger proliferation, migration, and differentiation of HSPCs [27–29]. To investigate the role of TLR4 in the HSC response to LPS, Dr. Manz et al. generated bone marrow chimeric mice with wild-type and *Tlr4*^{-/-} hematopoietic cells in a 1:1 ratio, which were then stimulated with LPS. These experiments revealed that indeed HSCs directly respond to LPS stimulation through TLR4. In agreement with involvement of other pathways in this process, *Tlr4*-deficient HSCs were not fully impaired in their capacity to respond to LPS (Takizawa and Manz, unpublished observations). Analysis of mice deficient for *Myd88* and *Trif*, two downstream components of the TLR4 signaling pathway, revealed that the enhanced cycling of HSPCs in response to TLR4 activation is mediated dominantly by Trif (Takizawa and Manz, unpublished observations).

These observations also raised the possibility that under steady-state conditions, the microbiome may also continuously contribute to stimulate early hematopoiesis. This hypothesis was tested by comparing the HSPC and myeloid compartments of regular specific pathogen-free (SPF) and germ-free mice. Interestingly, germ-free mice have reduced numbers of granulocytes and monocytes, as well as Lin⁻Sca1⁺c-Kit^{hi} (LSK) cells and common myeloid progenitors (CMPs), but not common lymphoid progenitors (CLPs). This reduction was also observed after treatment with broad-spectrum antibiotics. The enhanced myelopoiesis in mice with an intact microbiome is driven by heat-stable microbial compounds and through TLR4 stimulation, as treatment of germ-free mice with heat-inactivated serum from SPF mice restores the numbers of immature myeloid cells [30].

Although short-term TLR4 stimulation likely has a beneficial effect allowing rapid production of innate immune-competent cells, chronic or sustained LPS challenge was found to have detrimental effects. Continuous TLR4 activation leads to a disadvantage in HSC repopulation capacity. In agreement, in a setting of chronic inflammation, *Tlr4* deficiency renders HSCs insensitive to LPS, conferring them in this way reconstitution advantage over wild-type cells. In addition to *Tlr4*, *Trif* deficiency also confers reconstitution advantage in a setting of chronic infection. Importantly, LPS–TLR4–Trif-induced HSC dysfunction can be pharmacologically prevented by respective signaling pathway inhibitors (Takizawa and Manz, unpublished observations).

The results discussed by Dr. Manz have great implications for our understanding of how hematopoiesis is regulated in stress situations and in the development of hematologic malignancies. This is especially relevant in the light of studies indicating increased inflammation and increased clonal hematopoiesis with age, which also correlates with increased incidence of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) [31]. Furthermore, population studies from the Swedish cancer registry have also found that a history of infectious or autoimmune diseases is associated with increased propensity for AML and MDS [32]. In this light, it can be speculated that enhanced cycling of HSPCs as a result of chronic or repetitive inflammation may increase the likelihood of genetic lesions, which in an inflammatory environment might be rescued and lead to the development of hematologic malignancies [1].

Summary

Collectively, these studies highlight the fundamental role that inflammatory signals play in the ontogeny and maintenance of HSPCs. Potential applications of this knowledge include directed differentiation of pluripotent stem cells to hematopoietic lineages and improved treatment of blood disorders that arise from dysfunctional HSPCs. What is critical to understand is the balancing act that HSCs undergo in response to inflammatory signals, harmonizing the need to respond to these signals to effectively fight immune stresses and regenerate the hematopoietic system with regulating proliferative cell divisions, which may ultimately select for transformative genetic mutations (Fig. 1).

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Figure 1.
Positive and negative effects of inflammatory signaling on HSC function. BM = bone marrow.