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**ESAM: adding to the hematopoietic toolbox**

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In this issue of *Blood*, Yokota and colleagues define ESAM as a novel marker that facilitates isolation of multipotential lympho-myeloid hematopoietic stem and progenitor cells in mice throughout ontogeny.

**Distinction of hematopoietic stem cells (HSCs) from differentiated cells is essential for improving our understanding of HSC development and unique biological properties and for purification of HSCs for therapeutic applications. However, most HSC markers are developmental stage, context and species dependent, and multiple surface markers need to be combined to provide significant enrichment for HSCs (see figure; reviewed in Mikkola and Orkin).** Sc1, the classical mouse HSC marker, is expressed only in certain mouse strains, and its expression is not fully penetrant in newly emerged HSCs. Conversely, CD41, the first embryonic HSC/progenitor marker, is only expressed on nascent HSCs. Many traditional endothelial markers are expressed on HSCs as they emerge from the hemogenic endothelium. But they become down-regulated when HSCs colonize the liver (VE-cadherin) or in early postnatal life when the highly proliferative fetal HSCs switch to a quiescent adult phenotype (CD34). In addition to the phenotypic evolution that occurs throughout ontogeny, the surface markers on HSCs change on activation in culture or in response to mobilization or myeloablative treatment. Recently, SLAM markers CD150 and CD48 were shown to facilitate enrichment of HSCs from the fetal liver stage onwards, including mobilized and aging HSCs, whereas the analysis of their expression in nascent HSCs is still pending. Furthermore, despite the general conservation of hematopoietic sites, HSC/progenitor hierarchy, and transcription factors between mice and humans, the surface markers that facilitate identification of HSCs in the 2 species have turned out to be strikingly different. CD41, Sc1, and SLAM markers are relevant for HSC purification in mice but not humans, whereas CD34+CD38+, the classical human HSC profile, does not select comparable hematopoietic populations in mice. The complexity of HSC surface markers makes it difficult to study progressive evolution of HSCs from one developmental stage or environment to another, and across species.

This study by Yokota et al defines endothelial cell selective adhesion molecule (ESAM) as a novel marker that enriches for multipotential hematopoietic stem and progenitor cells in mice throughout ontogeny. ESAM belongs to the family of transmembrane proteins with immunoglobulin-like extracellular domains, and its expression has been documented in various species in endothelial cell junctions, platelets, and most recently in HSCs. The authors identified ESAM as a candidate HSC marker from a microarray screen that used Rag1-GFP reporter strain to separate the ckit+ Sc1 Rag1− HSC subset from the ckit− Sc1+ Rag1− early lymphoid progenitors in midgestation fetal liver. Further studies showed that expression of ESAM was highly correlated with HSC phenotype, and high levels of ESAM expression could alone be...
used as means to isolate both multipotential progenitors as well as transplantable HSCs in the fetal liver.

In the AGM (aorta-gonad mesoderm region) where HSCs emerge, ESAM<sup>+</sup> cells coexpressed c-kit and the endothelial markers Tie2, CD34, andPECAM that are known to be present in fetal HSCs. Interestingly, ESAM<sup>+</sup> cells formed a distinct subpopulation that selected all hematopoietic progenitors with lymphoid potential, which is of importance as the establishment of lymphoid potential during the onset of fetal hematopoiesis distinguishes the true multipotential hematopoietic stem and progenitor cells from the earlier, yolk sac--derived progenitors that have limited lifespan and developmental potential. Comparison with the yolk sac further suggested that ESAM<sup>Hi</sup>c-kit<sup>Hi</sup>Tie2<sup>Hi</sup> cells represent emerging HSCs whereas the myeloerythroid progenitor cells are harbored in ESAM<sup>Lo</sup>c-kit<sup>Lo</sup>Tie2<sup>Lo</sup> fraction. In contrast to many endothelial markers that become downregulated in HSCs later during development, ESAM expression was not only maintained in the Lin<sup>−</sup>c-kit<sup>−</sup>Sca1<sup>Hi</sup> fraction in the adult bone marrow, but its expression level even increased in aging mice.

The finding that ESAM is faithfully expressed in long-term repopulating HSCs and their immediate precursors throughout ontogeny may have many important applications. Purification of hematopoietic cells based on ESAM expression may facilitate investigation of the mechanisms that dictate cell fate toward multipotential HSCs rather than short-lived myeloerythroid progenitors during embryogenesis, and assessment of the development of these distinct hematopoietic programs in vivo as well as in vitro from embryonic stem cells or induced pluripotent cells. Furthermore, the high level and fairly specific expression of ESAM in multipotential hematopoietic cells could potentially be used for tracking HSCs during development and localizing them in distinct cellular niches by various imaging techniques. In addition, unraveling the molecular networks that regulate ESAM expression may give important clues about the cell intrinsic programs that govern the identity and functional properties of multipotential hematopoietic stem and progenitor cells. The importance of the findings in this study is further enhanced by recent findings that ESAM expression in HSCs appears to be conserved between different mouse strains and across species, as Human HSCs were also shown to express this marker. Another important question is whether ESAM is functionally required for establishing and maintaining HSC properties, unlike most surface markers associated with HSC potential. Although ESAM<sup>−/−</sup> mice are viable and fertile and do not exhibit major hematopoietic failure, their hematopoietic lineage distribution is skewed and the number of their HSCs appears to be even slightly higher, implying that ESAM may be functionally involved in HSC-niche interactions in the bone marrow.

REFERENCES

CLINICAL TRIALS

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Where is the start line?

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In this issue of Blood, Mielcarek and colleagues report on the results of a retrospective analysis of the outcomes of initial acute GVHD therapy.

Despite advances in the management of complications related to hematopoietic cell transplantation, treatment of acute graft-versus-host disease (GVHD) remains suboptimal. Corticosteroids are the primary front-line therapy for acute GVHD at a standard dose of 2 mg/kg per day prednisone, with response rates of about 50%.<sup>1-3</sup> In this issue of Blood, Mielcarek et al report on the outcomes of initial acute GVHD therapy among 773 transplantation patients at the Fred Hutchinson Cancer Research Center from 2000 to 2005.<sup>4</sup> Patients were treated with either the standard dose of 2 mg/kg per day prednisone or low-dose prednisone (1 mg/kg per day) at the discretion of the attending physician. By day 100 after initiating therapy, patients treated in the low-dose group received a mean cumulative dose of 44 mg/kg compared with 87 mg/kg in the standard-dose group. Adjusted outcomes between the 2 groups were not statistically different. In multivariate analysis, treatment with low-dose steroids was associated with a reduction in prolonged hospitalization and a trend toward lower risk for invasive fungal infections. The authors conclude that initial treatment with low-dose prednisone did not compromise acute GVHD control or mortality and was associated with decreased toxicity.

This article addresses an important question regarding the preferred initial steroid dose for patients with acute GVHD. It is clearly desirable to use as low a dose of steroids as possible to reduce the risk of toxicity. However, this goal must be balanced with the need to attain early and durable control of acute GVHD. This single
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