different envelopes to pseudotype the vectors for optimal target HSC binding and entry. Initially, envelopes from retroviruses were used that conferred tropism to human cells, such as the murine retrovirus amphotropic envelope, the gibbon ape leukemia virus envelope, or the feline leukemia virus RD114 envelope. The envelope glycoproteins are essentially nontoxic, which allows them to be incorporated into stable "packaging cell lines," and these pseudotypes have been most commonly used for γ-retroviral vectors in clinical trials. However, the use of vectors with these envelope pseudotypes requires surface expression of the specific cellular protein(s) that they exploit as receptors on target cells, typically amino acid or mineral transporters that may require cell activation to be induced.

The vesicular stomatitis virus (VSV-G) protein was identified as having some advantages over retroviral envelopes for pseudotyping retroviral and lentiviral vectors, including the wide species tropism it confers (from human down to zebrafish). The physical hardiness of VSV-G–pseudotyped virion allows vector concentration to high titers without significant loss of biological activity (in contrast to retroviral envelope proteins that are thought to be more fragile, leading to their loss during ultracentrifugation).[5] However, there has been a love-hate relationship with the VSV-G envelope, as it is moderately fusogenic; this causes cytotoxicity and makes it difficult to derive stable packaging lines that produce VSV-G pseudotyped virion, necessitating vector production by cumbersome transient transfection methods (although a first vector produced from a stable VSV-G packaging cell line has entered clinical trials).[6] Also, VSV-G-pseudotyped vectors can be cytotoxic to their target cells, such as HSCs, especially when used at high concentrations or with impure preparations.

Investigators in Lyon, France, reported earlier this year that the VSV-G cellular receptor, only recently identified as the low-density lipoprotein receptor (LDL-R),[7] is minimally expressed on human HSCs; induction of LDL-R expression turns out to be one of the major mechanisms by which prestimulation with growth factors augments HSC transduction.[8] They now report studies using a novel envelope pseudotype derived from the baboon endogenous retrovirus (BaEV) that appears to present an important advance for the production of vectors for gene transfer to human HSCs.[1] Vectors packaged with the BaEV envelope were shown to use 2 cellular proteins, the neutral amino acid transporters ASCT-1 and ASCT-2, as receptors, and basal expression of these receptors on freshly isolated human HSCs allowed moderately effective gene transfer without prestimulation, in contrast to the strict requirement for prestimulation for transduction by VSV-G-pseudotyped vectors (see figure). Although the titers measured for the BaEV-pseudotyped vectors were ~20-fold lower than the VSV-G pseudotypes, they were more effective when used at equivalent amounts (adjusted for virion p24 content). Using both in vitro and in vivo serial xenografts in NOD/SCID/gammaC(null) (NSG) mice as assays of human HSC transduction, the BaEV-pseudotyped vectors led to significantly higher levels of gene transfer to human HSC than VSV-G-pseudotyped vectors: up to 30% of NSG-engrafting cells without prestimulation and up to 90% with prestimulation, with vector present in all lineages of human blood cells produced. Effective transduction could be achieved using fewer HGFs than are typically used, which may contribute to better preservation of stem cell function.

Of course, the xenograft models are still surrogates for the long-term reconstituting HSCs in clinical transplants, and additional studies will need to be performed to verify and extend these findings, both in nonhuman primate models (the BaEV packaged vectors were shown to effectively transduce macaque $CD34^+$ cells) and eventually in clinical trials. However, the identification of the unique properties of the BaEV envelope holds the prospect of improving gene production methods, by allowing the development of stable packaging cell lines, as well as increasing the efficiency of gene transfer to stem cells while better preserving their function by minimizing ex vivo manipulation. This newly characterized envelope may be the award winner for Best Envelope in a Pseudotyping Role needed to take HSC gene therapy to the next level of effectiveness.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● HEMATOPOIESIS & STEM CELLS

Comment on Lopez et al, page 1232

Hematopoietic ontogeny in the axolotl

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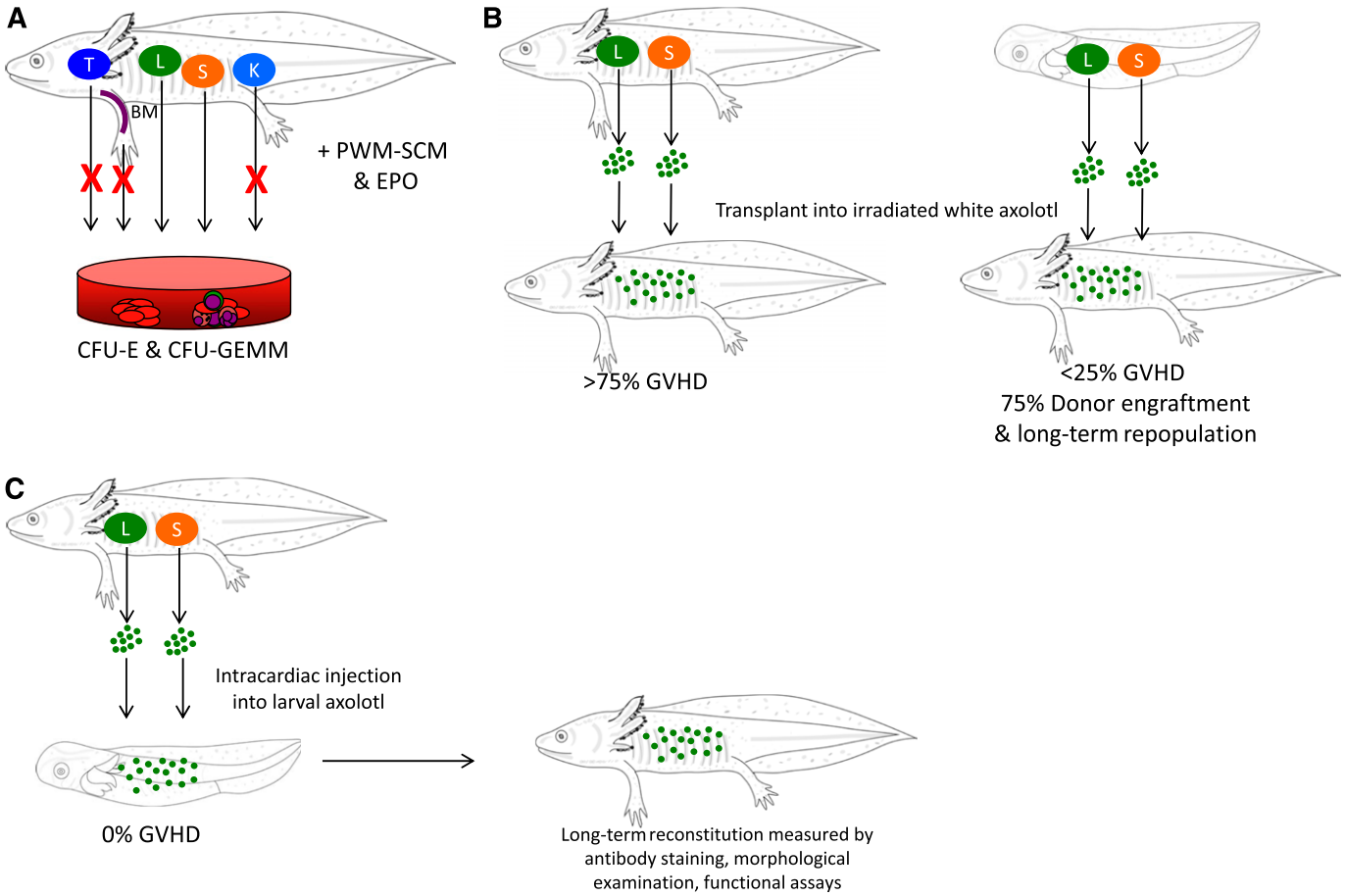
In this issue of *Blood*, Lopez et al undertake the heroic task of characterizing the blood-forming system of the axolotl (*Ambystoma mexicanum*), an aquatic salamander that provides an excellent model for tissue regeneration and scar-free wound healing.[1] Commonly referred to as the "Mexican walking fish," axolotls are not fish at all, but rather neotenic salamanders that retain many larval traits throughout their lifespan because they do not undergo a typical juvenile to adult metamorphosis. This retention of larval traits is associated with the profound ability of the axolotl to regenerate many of its tissues, including limbs, spinal cord, heart, and even parts of its brain.[2-6]

The regenerative mechanisms of the axolotl have been reasonably well studied, and the involvement of specific hematopoietic lineages in these processes has been documented.[7,8] However, there remains a lack of knowledge regarding the full spectrum of hematopoietic cells present, techniques to mark these cells, and assays to test their functions. Although the axolotl generates blood cell lineages similar to other vertebrates,[8,9] where, when, and how these cells arise have remained enigmatic.

In this study, Lopez et al present a first characterization of the embryonic and adult hematopoietic systems of these animals (see figure for an overview). They show that, like blood cells of mammals and zebrafish, axolotl blood cells can be characterized and isolated by fluorescence activated cell sorting based on their size and granularity. Morphological analyses of these cells suggest that the major blood cell lineages found in other vertebrates are present, including lymphocytes, monocytes, macrophages, mast cells, and neutrophils. Importantly, they also present assays to test the function of hematopoietic stem and progenitor cells (HSPCs), which they use to investigate the sites of hematopoiesis in the adult animal. Interestingly, unlike mammals and teleosts, there exist 2 sites of hematopoiesis in the adult axolotl. By developing colony-forming unit (CFU) assays to reveal erythroid and multilineage potential, the authors demonstrate that both the liver and spleen harbor robust CFU activity. In contrast, the bone marrow, thymus, and kidney generated no hematopoietic colonies. The authors subsequently developed transplantation assays to test each tissue for long-term repopulating hematopoietic stem cells (HSCs). Consistent with the CFU results, only the liver and spleen contained HSCs, as evidenced by long-term, donor-derived hematopoiesis in irradiated recipient animals. Importantly, the authors identify an array of lineage-specific antibodies that can be used to demonstrate multilineage reconstitution. Finally, they show convincing evidence that ablation of the liver or spleen by targeted irradiation led to anemia and subsequent death of the animals, supporting the key roles of each tissue in maintaining the hematopoietic program.

After characterizing the hematopoietic system in the adult axolotl, the authors examined HSPC ontogeny during development. Transplantation of green fluorescent protein–positive (GFP^+) cells from transgenic donor embryos demonstrated that the blood islands lacked HSC activity. Interestingly, the first organ to harbor HSCs



Hematopoietic assays developed by Lopez et al in the axolotl.[1] (A) CFU assays indicate that CFU-E and CFU-GEMM colonies are generated from adult axolotl liver (L; green) and spleen (S; orange), but not thymus (T; dark blue), bone marrow (BM; purple), or kidney (K; light blue), when stimulated with pokeweed mitogen-stimulated axolotl spleen cell-conditioned media (PWM-SCM) and human EPO. (B) After the sites of hematopoietic progenitors in adult axolotls were identified, GFP^+ hematopoietic stem and progenitor cells were isolated from the liver (L; green) and spleen (S; orange), and engrafted in white adult axolotls. (Left) However, rates of graft-versus-host disease (GVHD) were high. (Right) Harvesting GFP^+ hematopoietic stem and progenitor cells from 3- to 7-month-old axolotls reduced the incidence of GVHD, leading to long-term engraftment of progenitors. (C) Because irradiation can impair regeneration in axolotls, the authors developed an intracardiac injection assay. GFP^+ hematopoietic stem and progenitor cells were isolated from adult axolotl and injected into larvae before 3 months of age. This allowed long-term reconstitution of recipients (up to 2 years later) that could be measured by antibody staining, morphological examination of hematopoietic cells, and functional assays. Importantly, the incidence of GVHD using this assay was reduced to 0%.

capable of long-term engraftment was the embryonic liver, followed by the embryonic spleen.

These studies open the door for further in-depth analyses of the axolotl hematopoietic system. By using GFP^+ transgenic donors, hematopoietic cells can be easily visualized in adults, which is advantageous for future studies characterizing the role of specific cell types in wound repair and regeneration, as well as visualizing homing and engraftment. Additionally, the authors show that HSPC populations are roughly 1000-fold enriched in the lymphoblastic population of the spleen, positioning this fraction for further prospective isolation approaches. In addition, their finding that HSPCs reside in the peripheral layer of the liver indicates that the axolotl liver and spleen likely provide different hematopoietic niches for different functions. Comparison of the signaling processes occurring between these 2 sites, as well as among other niches in different vertebrate animals, may provide insight into the conserved core network of HSC support molecules. Finally, the axolotl is likely an excellent system to understand the role of hematopoietic cells not only in the regeneration of limbs, spinal cord, and other organs, but also in the regeneration of the hematopoietic system itself. For example, as mammals age, they have less ability to robustly generate lymphoid cells, with HSCs becoming skewed toward myeloid outputs. It would be interesting to investigate if this trend exists in the neotenic axolotl, which in the adult form retains juvenile traits. Overall, this pioneering work in the axolotl now provides another excellent model system in which to compare and contrast the evolution of vertebrate hematopoiesis.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● CLINICAL TRIALS & OBSERVATIONS

Comment on Fernandez et al, page 1266

Asparaginase allergies: it's all in the genes

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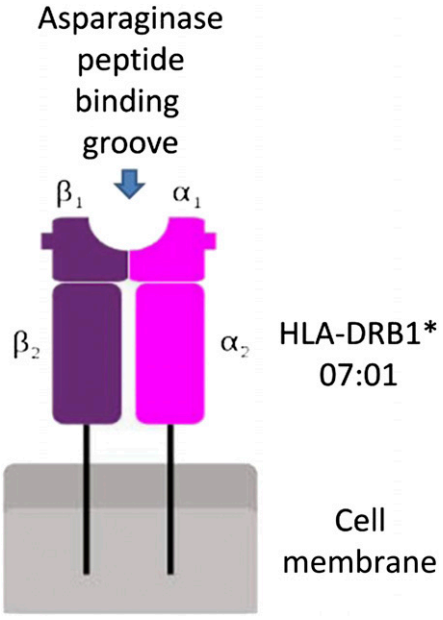
In this issue of *Blood*, Fernandez et al demonstrate that human leukocyte antigen (*HLA*) *DRB1* alleles confer high-affinity binding to asparaginase epitopes, leading to higher frequency of allergic reactions.[1] The authors initially examined *HLA* data from European ancestry patients enrolled onto St. Jude Children's Research Hospital (n = 541) and the Children's Oncology Group (n = 1329) clinical trials and identified a higher incidence of allergic reactions and anti-asparaginase antibodies in patients with *HLA-DRB1*07:01* alleles. They then analyzed the structure of the HLA protein to show high-risk amino acids located within the binding pocket (see figure), possibly affecting the interaction between asparaginase epitopes and the HLA-DRB1 protein.

A relationship between genetic aberrations and adverse drug reactions is not novel, as was demonstrated for 6-mercaptopurine and its metabolizing enzyme, thiopurine *S*-methyltransferase.[2] Specifically, defects in the thiopurine *S*-methyltransferase gene lead to decreased inactivation of 6-mercaptopurine and enhanced toxicity, which can cause severe life-threatening myelosuppression. Further, *HLA-B* alleles were associated with



Schematic representation of the interaction between HLA-DRB1 and asparaginase epitopes.

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