T and B lymphocytes proceed through fundamentally different pathways in development. However, clonal analyses will be necessary to determine whether YS and P-Sp contain 1 precursor/HSC that generates both T lymphocyte lineages or 2 precursor/HSC, 1 for the early γδ T lymphocytes and 1 for the adult lineage.

Yoshimoto and colleagues show that the nonhematopoietic (VE-cad + CD41 −), hemogenic endothelium is the source for the T-cell activity in the YS. The authors argue that the appearance of T lymphocyte precursors precedes the development of HSCs. This argument is based on the high frequency of the T-cell activity in the YS, the low frequency of HSCs in the early embryo, the appearance of transplantable HSCs 0.5 days after the T-precursor activity, and the observation that the VE-cad + CD41 − cells seem to repopulate only T lymphocytes in neonatal hosts. The conclusion that T-lymphocyte potential develops independent of HSCs is provocative. There is precedence for the appearance of mature hematopoietic cells before HSC activity both in vivo and in vitro. Yet, alternative interpretations of the findings presented here are possible. For example, it cannot be excluded that the absence of a heartbeat in Ncx1−/− mice skews the development of the hematopoietic lineages. Moreover, the potential for differentiation is not the same as actual differentiation. Whether T lymphocytes are functionally important in the embryo remains to be determined. Yet the conclusion is inescapable that the capacity to generate T lymphocytes develops surprisingly early in development. The data will undoubtedly spark vigorous discussion and new insights into the puzzling early development of HSCs and lymphocytes.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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CD4+ T lymphocytes are as low as in AIDS. In fact, almost all circulating leukocyte subsets are low. Yet classic opportunistic infections do not occur, bacterial infections usually do not become life-threatening, and the main problematic virus is human papillomavirus (HPV), which causes large numbers of skin and anogenital warts. The mother, brother, and 2 of 3 children of this patient have a similar condition.

This patient with deficiency of both myeloid and lymphoid immunity is a classic case of a rare and fascinating disease known as WHIM syndrome. Despite neutropenia, the bone marrow contains abundant mature neutrophils, an almost pathognomonic finding known as myelokathexis (Greek for marrow retention, and the “M” in the acronym WHIM). W, H, and I refer to warts, hypogammaglobulinemia, and infections, respectively. Infections transiently elevate the ANC, promoting clinical recovery and survival into adulthood. Thus, neutropenia in WHIM syndrome is considered in part a problem of neutrophil egress from bone marrow, not neutrophil production.

This idea gained traction in 2003 when Hernandez et al discovered inherited autosomal dominant mutations in the chemokine receptor CXCR4 as the cause of WHIM syndrome. The mutations truncate the C-terminal domain, but because this region normally mediates GRK-dependent desensitization, they result in increased signaling. Because CXCR4 normally mediates bone marrow homing and retention of neutrophils, gain-of-function WHIM mutations may cause myelokathexis by simply increasing these activities. Because CXCR4 is widely expressed on all leukocyte subtypes, other cytopenias could arise from a similar mechanism in immune organs. If so, immune organs should contain increased numbers of all leukocytes deficient in blood. This hypothesis is difficult to test in patients and direct data have been lacking. In fact, apart from bone marrow biopsies, knowledge of immune organ histopathology in WHIM syndrome is limited to lymph nodes from 2 patients. In this regard, Balabanan et al blaze an important new trail with their mouse, in which the WHIM allele CXCR4<sup>1013</sup> replaces 1 wild-type mouse Cxcr4 allele, allowing study of the mutation in any tissue at any time under both homeostatic conditions and specific stresses. The mouse developed normally and phenocopied severe panleukopenia, validating the model in the
Skewed leukocyte compartmentalization in immune organs in a CXCR4<sup>1013</sup> knock-in mouse model of WHIM syndrome. NL indicate normal; and eff/mem, effector/memory.

blood. However, the tissue findings are complex, lineage-specific, and unexpected, and suggest effects of the mutation on both leukocyte trafficking and development in immune organs (see figure).

In primary immune organs (bone marrow and thymus), the tissue architecture was normal. Myelopoiesis was also normal; however, unexpectedly, the numbers of both total and apoptotic neutrophils were not increased in bone marrow. The authors concluded that CXCR4 desensitization is therefore unlikely to be the primary cause of myelokathexis. However, total bone marrow neutrophils cannot be readily measured in humans and could be normal or even decreased in WHIM patients, too. More likely, multiple mechanisms, including increased neutrophil retention in and accelerated homing to bone marrow plus a relative decrease in myelopoiesis, account for neutropenia.

Unlike myelopoiesis, B and T lymphopoiesis both appeared to be suppressed. Single- and double-positive T cells were deficient in thymus, and bone marrow contained reduced B-cell precursors without increased apoptosis. This is surprising because the CXCR4<sup>1013</sup> knock-out mouse identified Cxcr4 as a positive regulator of hematopoiesis. Together, these results suggest that lymphopoiesis may depend on fine-tuning of CXCR4 signal strength. Despite reduced precursors, mature B-cell number was normal in bone marrow. Thus, B lymphopenia, like neutropenia, probably also depends on defective trafficking and egress.

Unlike primary immune organs, secondary immune organ architecture was grossly abnormal in the model, including absent or reduced numbers of B-cell follicles. Naive but not effector/memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells were deficient in spleen. In contrast, T-cell numbers were normal or increased in lymph nodes. In addition, there were fewer and abnormally localized lymphatic vessels in lymph node, suggesting impaired egress as a potential mechanism.

In spleen, where immature B cells from bone marrow mature further into type 1 and 2 transitional B cells and then become mature marginal zone and follicular B cells, only marginal zone B-cell number was not decreased. Interestingly, in spite of severe B lymphopenia and absence of follicles, immunoglobulin levels were normal or even high, raising questions about where B cells are being activated. In WHIM patients, hypogammaglobulinemia is the least penetrant feature, and when present may be mild. However, the durability of protective memory responses could still be affected. In this regard, there are several reports of short-lived antibody responses to vaccines and vaccination failures in WHIM patients. Importantly, the CXCR4<sup>1013</sup> knock-in mouse will now allow systematic modeling of primary and memory vaccine responses, germinal center formation, and the relative importance of lymphoid versus myeloid defects to infection susceptibility in WHIM syndrome.

Leukocytes can be mobilized to blood in WHIM syndrome not only by infection but also by plerixafor (Mozobil, AMD3100),<sup>7,8</sup> a CXCR4 antagonist approved by the US Food and Drug Administration for HSC mobilization in the setting of transplantation for specific lymphoid malignancies. Clinical trials are under way to test the safety and efficacy of plerixafor as mechanism-based therapy in WHIM syndrome; however, the source of mobilized cells has remained undefined. In this regard, plerixafor readily mobilized both neutrophils and B cells to the blood in the WHIM mouse, as it does in patients, but, surprisingly, without significantly affecting the number of these cells in bone marrow, suggesting the existence of other mobilizable storage sites. Alternatively, a small transient change in bone marrow could still potentially cause a large change in blood. Future in vivo trafficking studies may help define the source of plerixafor-mobilized cells in the model.

The CXCR4<sup>1013</sup> knock-in mouse, like transgenic zebrafish and xenotransplant mouse models of WHIM that preceded it,<sup>9,10</sup> is actually a model of “M,” not WHIM. Immunoglobulins were normal, spontaneous infections were not reported, and mice are not permissive for HPV. Nevertheless, this mouse is clearly an important tool for future studies of hematopoiesis and infection susceptibility, particularly for viruses other than HPV. It also provides a unique opportunity to test the phenotypic effects of homozygous CXCR4<sup>1013</sup> and CXCR4<sup>1013</sup> expression specifically in hematopoietic versus nonhematopoietic cells. In this regard, wart keratinocytes up-regulate CXCR4 in both immunologically healthy individuals and patients with WHIM syndrome, and a recent study revealed that WHIM receptors may directly drive keratinocyte transformation in vivo in mice, independently of immunodeficiency.<sup>11</sup> This suggests CXCR4 may play a direct role in HPV disease, and could be a target for local treatment.

For patients, the CXCR4<sup>1013</sup> knock-in mouse has confirmed that CXCR4 is the correct therapeutic target in WHIM syndrome.
and that dialing down CXCR4 signaling is an ideal therapeutic strategy. In the future, it may help accelerate preclinical development of drug candidates while continuing to reveal surprising new concepts about pathogenesis and the normal role of CXCR4 in hematopoiesis.

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Comment on Azab et al. page 5782

Metastatic myeloma?

Kenneth Shain Moffitt Cancer Center

Evidence suggests that multiple myeloma cells are constantly invading new regions within the bone marrow through induced systemic re-circulation. In this issue of Blood, Azab and colleagues demonstrate that regional bone marrow hypoxia promotes the dissemination of myeloma cells in a manner similar to solid tumor metastasis. This spread is mediated by a hypoxia-induced epithelial-to-mesenchymal cell transition (EMT)–like phenotype.1

Multiple myeloma is an exclusively bone marrow–localized malignancy generating from normal plasma cell differentiation. Plasma cell maturation involves migration of pre-germinal center (GC) B cells from the bone marrow to secondary lymphoid organs and return to the bone marrow as mature plasma cells. Post-GC homing is mediated by CXCR4-induced homing to CXCL12/SDF-1–rich regions of bone marrow niche. Within the bone marrow plasma cell adherence to ECM, bone marrow stromal cells (BMSCs) and other juxtaposed cells lead to the production of crucial factors for bone marrow homeostasis. Unlike their normal counterparts, myeloma cells benefit from the normal effectors within the bone marrow niche, and also hijack and contribute to the environment promoting tumorigenesis, altered bone metabolism, neo-

vascularization, drug resistance, and as now shown, myeloma metastasis.1 The expansion of plasma cells and aberrant neovascularization promotes localized hypoxic subniches.1 Studies in both solid and hematopoietic models suggest that hypoxia contributes directly to cancer survival, migration, and metastasis.2,3 The effect of reduced tissue oxygen is primarily mediated by hypoxia inducible factors (HIFs), a family of heterodimeric basic helix-loop-helix transcription factors consisting of an oxygen sensitive α subunit (HIF-1α, HIF-2α, and HIF-3α) and a constitutively expressed β subunit (HIF-β). HIF’s subunits are rapidly degraded under normoxic conditions via a von Hippel-Lindau–dependent ubiquitin-proteosome cascade. Under hypoxic conditions the α subunits stabilize facilitating translocation to the nucleus, heterodimerization with β subunits, and transcriptional activity.

Within solid tumors regional hypoxia exists due to chaotic vasculature, poor oxygen diffusion, irregular blood flow, and tumor necrosis.4,5 Intratumoral hypoxia and stabilization of HIFα subunits initiates an adaptive transcriptional program inducing epithelial cells to acquire a mesenchymal cell phenotype termed EMT.2,4 EMT is a highly conserved process integral in embryogenesis, normal tissue development, response to injury, organ fibrosis, as well as critical events in solid tumor invasion, metastasis, and the acquisition of stem cell–like properties.1,2,4,5 EMT–triggering signal pathways involve TGF-β1 and Notch1 as well as the transcription factors SNAI1, TWIST1,Slug/SNAI2, SIP1, ZEB1, and FOXC2, among others.1,2,4 A major biologic consequence of these transcriptional events is the repression of the cell adhesion molecule E-cadherin and cellular release from confines of the local microenvironment.2,4

In multiple myeloma the bone marrow microenvironment promotes proliferation and resistance to chemotherapy. As such, it has been proposed that the bone marrow microenvironment may be a target for new therapeutic agents in the treatment of myeloma.6,7 Supporting this idea, Ghobrial and colleagues have previously demonstrated that targeting of CXCR4 with AMD3100–altered myeloma homing to the bone marrow, increased circulating myeloma cells, and increased sensitivity to cytotoxic agents.8,9 These and other studies examining the CXCL12/CXCR4 axis have resulted in ongoing clinical trials. In addition, these studies indicated that myeloma cells are not statically confined to the bone marrow. Instead, populations of myeloma cells are migrating from one region of the bone marrow to another. As such, myeloma cells “invade” and “metastasize” to new sites of bone marrow.

Azab et al hypothesized that this migration was induced by hypoxia associated with progression of disease burden in a manner similar to that of solid organ tumors. Azab et al describe a novel mechanism by which increasing bone marrow tumor burden results in regional hypoxia, inducing dissemination of multiple myeloma cells into peripheral circulation via a hypoxia–induced EMT–like phenotype. Consistent with the EMT phenotype, loss of the E-cadherin correlated with increased expression of SNAI1, FOXC2, and TGF-β1 and
Unexpected developments in immune organs in WHIM syndrome

Philip M. Murphy and David H. McDermott