Over the past 2 decades there has been significant progress in the treatment of this disease with the introduction, first of interferon alfa, and then the nucleoside analogs, cladribine and pentostatin. The majority of patients treated with the latter drugs achieve long-lasting complete responses. This success has probably been the prime reason why further research into the biology and treatment of this rare disease has been limited in scope and conducted in a few, highly specialized centers with specific interest in HCL.

A number of studies have shed further light onto the cell of origin in HCL, as well as predictors of response to treatment with the nucleoside analogs. Using gene-expression profiling, Basso and colleagues showed that HCL expressed a homogeneous pattern of genes clearly distinct from other lymphoid neoplasms and more related to the memory (postgerminal center [post-GC]) B cells than naive (pre-GC) B cells. Others have reported heterogeneity in the cell of origin in terms of mutational status with a few patients having unmutated immunoglobulin heavy chain variable (IGHV) region gene, indicative of a pre-GC cell of origin. Furthermore, the same group has reported that patients with unmutated IGHV were more likely to fail to respond to cladribine. In a previous study, Arons and colleagues also reported that patients with VH4-34 IGHV gene rearrangement were more frequently unmutated and had a greater white blood cell count at diagnosis, significantly lower response rate, and shorter progression-free and overall survival after initial therapy with cladribine.

The ability of the immune system to recognize antigens depends on the immunoglobulins generated by the B cells and the antigen receptors on the surface of T cells. This significant diversity needed in antigen recognition is achieved by a number of well-characterized mechanisms such as the recombination of multiple V genes with D and J segments in the immunoglobulin heavy chain variable region (together with V and J recombination in the immunoglobulin light chains) that is largely responsible for the variability in the complimentary determining regions, an essential part of the antigen binding site. Further required diversity is provided by somatic mutations produced by single base pair changes within the variable regions of the immunoglobulin genes, providing further variation in antigen specificity. Membrane-bound immunoglobulin, or the B-cell receptor, then serves as the receptor for the antigens. Naive B cells that have not encountered antigen have unmutated variable region genes, while B cells that have entered the site of somatic mutation, considered to be the GC of lymph nodes, typically have acquired these somatic mutations (see figure). The origin of the neoplastic B cell reflects this mutational status and its clinical relevance has been clearly demonstrated in chronic lymphocytic leukemia (CLL) where the unmutated IGHV gene is clearly associated with a worse prognosis.

The present study by Arons and colleagues provides further evidence that in the majority of patients with classic HCL (83% of 102 cases), the cell of origin is post-GC with mutated IGHV. This contrasted with their cohort of variant HCL where > half of the patients had unmutated IGHV and with historical data for patients with CLL where, again, ~ half are unmutated. They also report higher usage of certain IGHV (and IGHD) gene families and a difference in mutational frequency among these genes. Furthermore, by demonstrating that the mutations fulfilled predefined characteristics of a canonical and nonrandom event, they provide further evidence suggestive of an antigen-driven process. As shown in studies reported earlier, the origin of the malignant cell in HCL is of clinical relevance in predicting response to treatment with cladribine (and perhaps, as the authors suggest, with CD22 directed therapy). Similarly, insights into the process of somatic hypermutation may provide more understanding of the potential triggers for the genesis of the disease.

Clearly, further research is needed into this and other aspects of the biology of the disease; for example, the interaction between the malignant cells and their microenvironment and the potential role of the B cell receptor in this process. The rarity of the disease and the success of treatment decelerated the pace of such research in the past. The recent development of the Hairy Cell Leukemia Consortium that may further stimulate such research is welcome news.

**REFERENCES**


**Comment on Salojin et al, page 4895**

**Knocking out the PCFT**

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Although hereditary folate malabsorption was first described 50 years ago, only recently has the molecular basis of this disease been determined. In this issue of Blood, Salojin et al describe the proton coupled folate transporter (PCFT) null mouse, whose phenotype resembles that of the human disease.

Five decades ago Victor Herbert performed a self-experiment to demonstrate that a diet deficient in folates could cause megaloblastic anemia. For several months he ate overcooked food (eg, thrice-boiled rice) and an occasional deep-water lobster with low folate
Histologic examination of bone marrow and spleen from the PCFT<sup>−/−</sup> mice revealed marked hyperproliferation of erythroid cells with excessive proerythroblasts and large basophilic erythroblasts. FACS analysis suggested that the erythroid cell differentiation process was blocked at the intermediate erythroblast stage. There was a significantly higher proportion of annexin V-positive apoptotic cells in the intermediate erythroblast population of the PCFT<sup>−/−</sup> bone marrow than in wild-type bone marrow. This suggests that survival of erythroblasts at the latter stages of erythroid maturation is decreased.

To obtain evidence for systemic folate deficiency in the PCFT heterozygous and null mice, serum and tissue folate levels were measured. Plasma folate was 40% lower and almost nondetectable in the PCFT<sup>−/−</sup> and the PCFT<sup>−/+</sup> animals, respectively. Liver and kidney tissue levels of folate were markedly decreased in the PCFT<sup>−/−</sup> mice. Patients who are folate deficient usually have elevated concentrations of plasma total homocysteine because of impairment of the remethylation pathway in the methionine cycle. The plasma total homocysteine concentration was approximately 3-fold higher in the PCFT<sup>−/−</sup> mice compared with wild-type animals.

The above observations suggest that the PCFT null mouse is a good model for impaired folate absorption and systemic folate deficiency. Furthermore, this work strongly supports the earlier observation that the PCFT is the primary transporter of dietary folates during absorption. Future studies will hopefully delineate the role of the PCFT in other tissues. The intriguing observation that patients with HFM have low concentrations of folate in their spinal fluid accompanied by severe neurologic complications suggests that the PCFT may be involved in delivery of folates to the brain. This animal model of HFM may be pivotal in understanding the mechanism of folate delivery to other tissues, both normal and abnormal, in the body.

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REFERENCES


Comment on Isern et al, page 4924

Erythropoiesis: early, not primitive

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In this issue of Blood, Isern and colleagues provide a comprehensive picture of the highly complex development of primitive erythroid cells from yolk sac over fetal liver to circulation at cellular and molecular resolution.¹

Erythropoiesis is one of the best-studied processes in the whole field of cell biology. From the landmark determination of hemoglobin structure by Max F. Perutz,² to work on globin locus control regions (LCRs),³ to the relatively recent discovery in 2005 of the Jak2 mutant V617F as a leading cause of erythrocytosis,⁴ the field has always remained active. Despite all these successful efforts, there are still considerable gaps in our knowledge. One of these relates to primitive erythroid cells (EryP). Their emergence as nucleated progenitors in the yolk sac, developmental transitions toward coexistence with definitive erythrocytes (EryD) in the fetal liver and enucleated erythrocytes in the bloodstream, as well as their ultimate fate is incompletely understood.

The current article by Isern et al describes the corresponding expression profiling and validation route taken and presents some unexpected results regarding necessary signaling and physiologic adaptations distinguishing EryP progenitors from EryDs.¹ Because there are no reliable surface markers for embryonic erythroid cells, isolation of the minute number of committed progenitors from an E7.5 embryo (~ 200) was made possible through availability of the e-globin–promoter-GFP transgenic mice and the fact that this promoter is already active in the yolk sac of E7.5 embryos, nicely shown in supplemental Video 1.³ After sorting for green cells, by clonogenic assays this cell population could be verified as true erythroid-committed progenitors that are already lost at E9.0 with onset of circulation and their movement to the embryo proper. To study the development of EryP progenitors to the ensuing EryPs, cell populations were isolated by FACS at daily intervals from E7.5 until E12.5, further sampling being precluded by nuclear condensation/enucleation.

The data from Illumina arrays showed that the number of significant changes in transcript abundance—up or down—peaked with entry of EryPs into circulation at E9 and the beginning of the fetal liver stage at E12. Hierarchical clustering demonstrated regulation of almost all usual suspects typical for progressive erythroid maturation, for example, induction of genes for heme synthesis or iron metabolism and down-regulation of mRNAs associated with DNA replication or ribosome biogenesis. Validation of array data by qPCR and flow cytometry was performed for an impressive battery of ~ 20 mRNAs/proteins.

Contemporary profiling papers are made complete with functional assays, opening avenues for further studies. To pick out just 2 eventual future starting points, there were, for example, indications that the extremely rapid proliferation (4-hour doubling time) of EryP precursors in the yolk sac is linked to medium/high intensity of TGF–β1 signaling, maybe involving an autocrine loop. There are only a few reports on such an autocrine stimulation in hematopoiesis. The closest counterpart may be the development of very early chick embryo erythroid progenitors, whose proliferation is accelerated by TGF–β1 while withdrawal promotes differentiation.⁶ Given the pleiotropic effects of TGF–β on apoptosis, transformation, differentiation, inflammation, proliferation, etc, already this finding is worth a follow-up. A second observation can lead to an “ah, yes, of course” in hindsight. The yolk sac is an inherently hypoxic environment. Given the lack of circulation, oxygen supply depends on diffusion. Several genes from the profiling results, many involved in glucose metabolism, are known to be up-regulated by hypoxia. And indeed, in vitro cultivation of erythroid progenitors at low oxygen (2%–5%) kept these target genes highly active and proliferation rates high, whereas atmospheric oxygen levels resulted in down-regulation and smaller colonies. This may resemble what EryPs experience during entry into circulation, migration to fetal liver and adaptation to the new environment. To give the present