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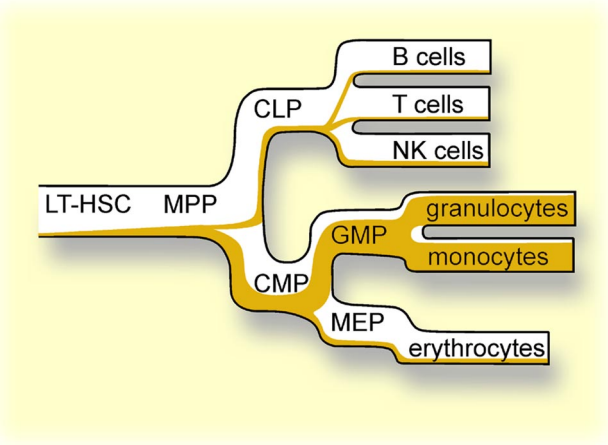

In each hematopoietic compartment, the relative amount of cells that express C/EBPα or the progeny thereof is indicated in orange.

in erythroid/megakaryocyte progenitors (MEPs) or lymphoid cells.[5] However, little was known about the precise cell type in which C/EBPα is first expressed within the hematopoietic hierarchy, and what the ultimate fate of the progeny of these C/EBPα–expressing cells would be.

Wölfler and colleagues make use of a model in which Cre recombinase is knocked-in in the *Cebpa* locus and Cre is therefore expressed under the control of the endogenous *Cebpa* promoter.[6] These mice lack one *Cebpa* allele but this does not impair myeloid development or steady-state hematopoiesis. Next, these $Cebpa^{+/Cre}$ mice were crossed with ROSA26 EYFP reporter mice, in which EYFP is only expressed after Cre-mediated recombination of *loxP* sites within the locus. Thus, as soon as the *Cebpa* promoter becomes activated, expression of Cre will allow the expression of EYFP. Not only will cells in which the *Cebpa* promoter is activated become $EYFP^+$, but also all progeny of these cells will be positive for EYFP since the *loxP* sites are irreversibly deleted, regardless of whether C/EBPα remains expressed (see figure).

Based on this mouse model several conclusions can be drawn: (1) only 4% of the most immature stem cells express C/EBPα, and the number of cells that express (or have expressed) C/EBPα increases to approximately 15% in multipotent progenitors; (2) upon differentiation along the myeloid lineage from CMP to GMPs an increasing number of cells express (or have expressed) C/EBPα, and practically all mature monocytes and granulocytes have expressed C/EBPα at least at some point during their development; and (3) despite the notion that erythroid and lymphoid cells do not express C/EBPα, it is clear from these tracing studies that at least some of these cells did express C/EBPα early in their development. In particular, this last point raises some important issues because it argues against a lineage-restrictive role for C/EBPα. Apparently, the expression of C/EBPα in immature progenitors does not prevent the differentiation toward a lymphoid or erythroid cell fate, even though the majority of C/EBPα-$EYFP^+$ progenitor cells become myeloid cells. These findings are in line with the notion that the promiscuous expression of myeloid, erythroid, and lymphoid genes precedes the actual lineage commitment.[7,8]

The $Cebpa^{+/Cre}$R26EYFP mouse model allows the identification of C/EBPα–expressing cells and particularly their progeny in an in vivo setting, but does not allow the quantification of C/EBPα expression at the protein level, which would for instance be possible in a transgenic mouse in which EYFP would be directly fused to C/EBPα. In addition, this C/EBPα-EYFP model would provide more insight into the kinetics of C/EBPα expression, and would allow the analysis of exact down-modulation of C/EBPα upon commitment along the lymphoid or erythroid lineage. Since the dosage and timing most likely matter, it will be interesting to develop those models as well.

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

REFERENCES

1. Enver T, Jacobsen SE. Developmental biology: Instructions writ in blood. *Nature*. 2009;461(7261):183-184.

2. Rieger MA, Hoppe PS, Smejkal BM, Eitelhuber AC, Schroeder T. Hematopoietic cytokines can instruct lineage choice. *Science*. 2009;325(5937):217-218.

3. Schroeder T. Hematopoietic stem cell heterogeneity: subtypes, not unpredictable behavior. *Cell Stem Cell*. 2010;6(3):203-207.

4. Zhang P, Iwasaki-Arai J, Iwasaki H, et al. Enhancement of hematopoietic stem cell repopulating capacity and self-renewal in the absence of the transcription factor C/EBP alpha. *Immunity*. 2004;21(6):853-863.

5. Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature*. 2000;404(6774):193-197.

6. Wolfler A, Danen-van Oorschot AA, Haanstra JR, et al. Lineage-instructive function of C/EBPalpha in multipotent hematopoietic cells and early thymic progenitors. *Blood*. 2010;116(20):4116-4125.

7. Miyamoto T, Iwasaki H, Reizis B, et al. Myeloid or lymphoid promiscuity as a critical step in hematopoietic lineage commitment. *Dev Cell*. 2002;3(1):137-147.

8. Huang S, Guo YP, May G, Enver T. Bifurcation dynamics in lineage-commitment in bipotent progenitor cells. *Dev Biol*. 2007;305(2):695-713.

● ● ● IMMUNOBIOLOGY

Comment on Peffault de Latour et al, page 4175

Abnormalities in Th17 T cells in aplastic anemia

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In this issue of *Blood*, Peffault de Latour et al demonstrate that interleukin-17 (IL-17)–producing Th17 T cells are increased in the peripheral blood and bone marrow of patients with aplastic anemia, compared with healthy controls. They also provide evidence that IL-17 contributes to the severity of marrow failure at an early stage. This work advances our overall understanding of the mechanisms of immune-mediated hematopoietic suppression and may ultimately have important clinical implications for the treatment of aplastic anemia.

Idiopathic aplastic anemia is characterized by pancytopenia and bone marrow hypoplasia, resulting from immune-mediated suppression of hematopoiesis.[1] Although the management of aplastic anemia is challenging and the outcome frequently fatal,

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advances in our understanding of the immune pathophysiology of the disease over the years have led to improvements in the immunosuppressive regimens used for its treatment and, in some cases, improved outcomes and survival.[1,2]

Over the past 3 decades, extensive work has established that cytokines play key roles in the suppression of hematopoiesis seen in aplastic anemia.[2] Observations made first in the 1980s suggested a disease model in which overproduction of myelosuppressive cytokines by activated cytotoxic T cells results in immune-mediated hematopoietic destruction.[3,4] The principles established by such original observations remain essentially unchanged, but subsequent work has expanded on them and defined the mechanisms of immune deregulation seen in aplastic patients. An important observation in recent years was the demonstration that the T-bet transcription factor, which binds to the promoter of the IFNγ gene, is up-regulated in T cells from aplastic anemia patients, resulting in enhanced gene expression and overproduction of myelosuppressive cytokines.[5] Other recent studies have shown that regulatory T cells (Treg) are decreased in the peripheral blood of aplastic anemia patients at diagnosis,[6] suggesting a mechanism of escape of autoreactive T cells during the development of the disease.

Th17 immune responses play important roles in the pathogenesis of several autoimmune disorders and syndromes,[7] but their roles in the pathogenesis and pathophysiology of aplastic anemia have been unclear and undefined. There had been some clues that Th17 cells may have been involved in the pathogenesis of aplastic anemia. From previous work we know that Tregs are suppressed in aplastic anemia,[6] and a dichotomy in the development of pathogenic Th17 cells and regulatory ($Foxp3^+$) Treg cells has been shown.[8] In addition, there has been some recent evidence for increased expression of IL17A mRNA in bone marrow and peripheral blood mononuclear cells of aplastic anemia patients.[9]

In this issue, Peffault de Latour et al examined the patterns of expression of Th17 cells in patients with aplastic anemia and compared them to those seen in normal controls.[10] Increased numbers of $IL\text{-}17^+$ cells were found in aplastic bone marrows, while the percentage and overall absolute numbers of $CD3^+CD8^-IL\text{-}17^+$ T cells were elevated in the peripheral blood of 21 newly diagnosed patients with aplastic anemia, compared with 10 healthy donors. Although the numbers of Th17 cells were not predictive of response to immunosuppression, patients in complete remission after treatment had lower numbers and percentages of Th17 cells than newly diagnosed patients and an inverse relationship with Treg cells in such patients was documented. To further define the role of Th17 cells in the pathophysiology of aplastic, the authors used an experimental mouse model of bone marrow failure involving infusion of allogeneic lymph node cells into sublethally irradiated recipients. Their data demonstrate that, in addition to the classical Th1 response, $CD4^+$ and $CD8^+$ IL-17–producing T cells were present—albeit to a lesser extent—in such mice. Remarkably, early treatment of such mice with an anti–IL-17 antibody resulted in reduced areas of hemorrhage in the marrow and improved overall bone marrow cellularity.

Altogether, the findings of this study provide evidence for an important role of Th17-mediated responses in the development and/or progression of early stages of aplastic anemia. They also suggest a synchronized Th1/Th17 response during development of marrow failure, associated with Treg deficiency. Beyond advancing our understanding of the immune pathophysiology of marrow failure, this work raises the prospect of future approaches to optimize immunosuppression regimens for the treatment of aplastic anemia patients, by targeting the Th17 response. There are already clinical trials aiming to block the Th17 response using anti–IL-17 monoclonal antibodies for autoimmune diseases. Efforts to incorporate monoclonal anti–IL-17 antibodies or other means to target Th17 T cells in current immunosuppressive regimens, such antithymocyte globulin and cyclosporine A, may provide a nonoverlapping approach to enhance responses and optimize immunosuppression. Such an approach may be particularly relevant for the treatment of patients with moderate aplastic anemia and efforts in that direction are warranted.

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

REFERENCES

1. Young NS. Acquired aplastic anemia. *Ann Intern Med.* 2002;136(7):534-546.
2. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. *Blood.* 2006;108(8):2509-2519.
3. Zoumbos NC, Gascon P, Djeu JY, Young NS. Interferon is a mediator of hematopoietic suppression in aplastic anemia in vitro and possibly in vivo. *Proc Natl Acad Sci U S A.* 1985;82(1):188-192.
4. Zoumbos NC, Gascon P, Djeu JY, Trost SR, Young NS. Circulating activated suppressor T lymphocytes in aplastic anemia. *N Engl J Med.* 1985;312(5):257-265.
5. Solomou EE, Keyvanfar K, Young NS. T-bet, a Th1 transcription factor, is up-regulated in T cells from patients with aplastic anemia. *Blood.* 2006;107(10):3983-3991.
6. Solomou EE, Rezvani K, Mielke S, et al. Deficient CD4+CD25+FOXP3+ T regulatory cells in acquired aplastic anemia. *Blood.* 2007;110(5):1603-1606.
7. Crispín JC, Tsokos GC. Interleukin-17-producing T cells in lupus. *Curr Opin Rheumatol.* 2010;22(5): 499-503.
8. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature.* 2006;441:235-238.
9. Gu Y, Hu X, Liu C, Qy X, Xu C. Interleukin (IL)-17 promotes macrophages to produce IL-8, IL-6 and tumour necrosis factor-alpha in aplastic anaemia. *Br J Haematol.* 2008;142(1):109-114.
10. Peffault de Latour R, Visconte V, Takaku T, et al. Th17 immune responses contribute to the pathophysiology of aplastic anemia. *Blood.* 2010;116(20):4175-4184.

● ● ● RED CELLS & IRON

Comment on Sangokoya et al, page 4338

Micro-mismanaging sickle cell stress

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SCD (or "HbSS") can vary markedly in its clinical manifestations[1]: in HbSS cells, regulatory factors that skew in association with disease severity may present new prognostic and/or therapeutic opportunities. In this issue of *Blood*, Sangokoya et al have applied unsupervised miRNA profiling to reveal elevated microRNA-144 levels in a severe anemia subset of SCD patients (despite an essential lack of mRNA transcripts, erythrocytes can retain miRNAs).[2,3]

Evidence further is provided that the CNC-bZip transcription factor *NRF2* is a target for decay by miR-144. NRF2 is known to activate the expression of several antioxidant encoding genes (eg, *SOD1*, *CAT*, *GCL2*) in part via antioxidant response elements. Increased miR-144 levels (and consequentially decreased NRF2 levels) therefore

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