blood
commentary

PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Kurotaki et al, page 1803

Priority lane to cDC1 open for IRF8+ progenitors

Martin Guilliams[1,2] and Charlotte L. Scott[1-3] | [1]VIB Center for Inflammation Research; [2]Ghent University; [3]University of Glasgow

In this issue of *Blood*, Kurotaki et al demonstrate that IRF8 expression delineates a subset of lymphoid-primed multipotent progenitors (LMPPs) biased toward the generation of conventional type 1 dendritic cells (cDC1s) by epigenetically priming the precursors for the expression of dendritic cell (DC) lineage genes.[1]

cDC1s are a conventional DC subset specialized in the cross-presentation of intracellular antigens. cDC1s develop in a stepwise manner from hematopoietic stem cells to LMPPs, common myeloid progenitors, granulocyte macrophage progenitors (GMPs), common dendritic cell progenitors (CDPs), pre-cDC1s, and, finally, to cDC1s. At each stage of development, multipotent progenitors (MPPs) are thought to make a decision to progress to a more restricted progenitor and, eventually, a specific cell lineage. However, the idea of a true MPP has recently been questioned, with cellular barcoding and single-cell analyses suggesting that some LMPPs may be preprogrammed toward a specific lineage.[2-5]

Interferon regulatory factor 8 (IRF8) has long been associated with cDC1 development and maintenance. We recently proposed IRF8 to be a "terminal selector" for cDC1s, a transcription factor (TF) whose expression is continuously required to prevent loss of identity and death.[6] IRF8 is gradually upregulated during the different stages of cDC1 development, and its expression has been shown to be induced and maintained via autoactivation by the TFs PU.1 and Batf3.[7]

In this study, Kurotaki et al build on their previous work understanding the role of IRF8 in mononuclear phagocyte biology,

identifying a subset of IRF8-expressing LMPPs that are biased toward the cDC1 lineage. Mechanistically, they show that this bias toward cDC1s is not the result of an altered transcriptome in IRF8+ LMPPs compared with their IRF8− counterparts. Rather, the acquisition of IRF8 expression results in epigenetic changes in the LMPPs, leading to chromatin around genes associated with the DC lineage being made accessible and, thus, later leading to the transcriptional profile observed in DC committed progenitors and DCs themselves but not the earlier upstream progenitors, GMPs, or common monocyte progenitors (cMoPs).[1] Importantly, IRF8 itself seems crucial for introducing this bias because PU.1-IRF8 composite binding motifs were found to be enriched in the assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-Seq) peaks present in the IRF8-expressing LMPPs but not in the IRF8− LMPPs. Moreover, these epigenetic changes were absent in IRF8-null LMPPs; although formal proof for direct IRF8 involvement will come from IRF8 chromatin immunoprecipitation on LMPPs, this was confirmed in previously published data on monocyte/DC progenitors (MDPs).[1] Underlining the translational value of these findings, this report is in line with a recent study in humans in which expression of IRF8 in early MPPs marked cells biased toward the DC lineage.[4]

Several key questions arise based on these findings. First, do all cDC1s arise through an IRF8+ LMPP? Kurotaki et al demonstrate that IRF8− LMPPs also retain the capacity to generate cDC1s, although this appears slower than observed with IRF8+ LMPPs. They also show that at least some IRF8− LMPPs can acquire IRF8 expression. Perhaps this is the cause of the delayed development, in that they may need to acquire IRF8 expression first. However, alternatively, one could also envisage distinct pathways for cDC1 development: some IRF8+ precursors may preferentially become cDC1s, and some precursors may acquire IRF8 later and represent true MPPs, or perhaps precursors preferentially programmed for another lineage can still switch toward cDC1s relatively late. A second question pertaining to this is then, why does IRF8 expression in the LMPP specify cells toward the cDC1 lineage when MDPs, CDPs, and cMoPs also express IRF8 and these are not all biased toward cDC1s? In fact, IRF8 also controls a set of enhancers highly expressed in monocytes,[8] and loss of IRF8 impedes the progression from cMoPs to monocytes.[6] Thus, how does IRF8 safeguard differentiation toward other immune lineages at these later time points? Perhaps through cooperation with other TFs? Indeed, Kurotaki et al demonstrated that expression of the TF hepatic leukemia factor can skew LMPPs away from the cDC1 lineage and more toward cDC2s. The interaction with PU.1 is also important to consider, because PU.1-IRF8 composite binding motifs are (as for IRF8+ LMPPs) enriched during the progression from the GMP to MDP[8] and, hence, could be essential for safeguarding cDC1 and monocyte fate. Further work will be required to understand how IRF8 interacts with other TFs at the different stages of development to induce specification toward distinct myeloid cell lineages.

Additionally, although there was a significant bias within the IRF8-expressing LMPPs toward generating cDC1s, this was not the only differentiation outcome:
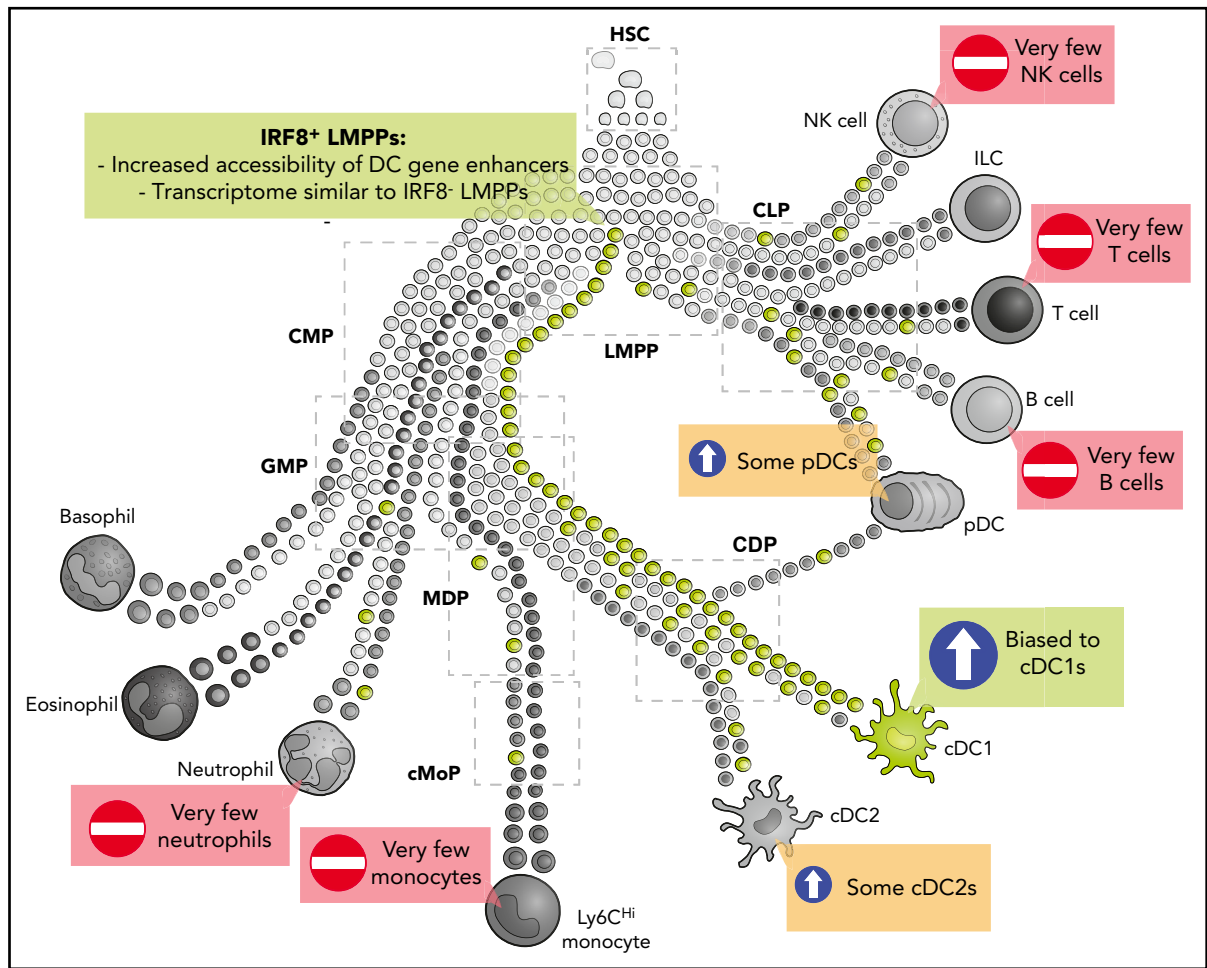
IRF8+ LMPPs are biased toward cDC1 differentiation. Schematic representation of hematopoietic development where some early progenitors are already precommitted to certain lineages (adapted from Guilliams et al[5]). Expression of IRF8 in LMPPs (green) biases differentiation toward cDC1s. Some IRF8+ LMPPs also give rise to cDC2s and pDCs, whereas a few IRF8+ LMPPs will give rise to other lineages, including monocytes, neutrophils, T cells, or B cells. CLP, common lymphoid progenitor; CMP, common myeloid progenitor; HSC, hematopoietic stem cell; ILC, innate lymphoid cell; NK, natural killer.

some plasmacytoid DCs (pDCs) and cDC2s were also generated, as well as low numbers of neutrophils and monocytes[1] (see figure). Because the ATAC-Seq experiments identifying the altered chromatin states were performed as bulk analyses, it remains an open question whether the epigenetic landscape is the same within those IRF8-expressing LMPPs that give rise to cDC1s compared with the IRF8-expressing LMPPs that will go on to generate cDC2s, pDCs, or monocytes.

Finally, how is IRF8 induced in the LMPPs? Interferon-γ (IFN-γ) signaling represents a potential candidate.[9] Interestingly, because the investigators speculate that IRF8-expressing LMPPs could represent a "priority lane" to generating cDC1s, IFN-induced cDC1 development could represent a positive-feedback loop developed in response to evolutionary pressure from intracellular pathogens. It

has been proposed that, following infection with toxoplasma, interleukin-12 production by peripheral cDC1s induces the production of IFN-γ by natural killer cells in the bone marrow.[10] Thus, this IFN-γ might drive the expression of IRF8 in LMPPs, promoting the accelerated biased development toward cDC1s.

Thus, this report highlights a population of LMPPs expressing IRF8 that is biased toward cDC1 differentiation as the result of an altered epigenetic landscape. This highlights the importance of epigenetics and not just transcriptomics when studying cell fates and further expands recent work suggesting that we need to reconsider the nature of true MPPs.

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

REFERENCES

1. Kurotaki D, Kawase W, Sasaki H, et al. Epigenetic control of early dendritic cell lineage specification by the transcription factor IRF8 in mice. *Blood.* 2019;133(17):1803-1813.

2. Naik SH, Perié L, Swart E, et al. Diverse and heritable lineage imprinting of early haematopoietic progenitors. *Nature.* 2013; 496(7444):229-232.

3. Lin DS, Kan A, Gao J, Crampin EJ, Hodgkin PD, Naik SH. DiSNE movie visualization and assessment of clonal kinetics reveal multiple trajectories of dendritic cell development. *Cell Reports.* 2018;22(10):2557-2566.

4. Lee J, Zhou YJ, Ma W, et al. Lineage specification of human dendritic cells is marked by IRF8 expression in hematopoietic stem cells and multipotent progenitors [published correction appears in *Nat. Immunol.* 2017; 18(10):1173]. *Nat Immunol.* 2017;18(8): 877-888.

5. Guilliams M, Mildner A, Yona S. Developmental and functional heterogeneity of monocytes. *Immunity.* 2018;49(4): 595-613.

6. Sichien D, Scott CL, Martens L, et al. IRF8 transcription factor controls survival and function of terminally differentiated conventional and plasmacytoid dendritic cells, respectively. *Immunity*. 2016;45(3):626-640.

7. Grajales-Reyes GE, Iwata A, Albring J, et al. Batf3 maintains autoactivation of Irf8 for commitment of a CD8α(+) conventional DC clonogenic progenitor. *Nat Immunol*. 2015; 16(7):708-717.

8. Kurotaki D, Nakabayashi J, Nishiyama A, et al. Transcription factor IRF8 governs enhancer landscape dynamics in mononuclear phagocyte progenitors. *Cell Reports*. 2018;22(10): 2628-2641.

9. Tsujimura H, Nagamura-Inoue T, Tamura T, Ozato K. IFN consensus sequence binding protein/IFN regulatory factor-8 guides bone marrow progenitor cells toward the macrophage lineage. *J Immunol*. 2002;169(3): 1261-1269.

10. Askenase MH, Han S-J, Byrd AL, et al. Bone-marrow-resident NK cells prime monocytes for regulatory function during infection. *Immunity*. 2015;42(6): 1130-1142.

CLINICAL TRIALS AND OBSERVATIONS

Comment on Howard et al, page 1865

Primary polymerization prevention

Martin H Steinberg | Boston University School of Medicine

In this issue of *Blood*, Howard et al report a phase 1/2 study of voxelotor (GBT440) in sickle cell anemia. This drug binds to the α-globin chain of hemoglobin and increases hemoglobin-oxygen affinity, thereby decreasing the polymerization tendency of deoxygenated sickle hemoglobin (HbS). Treated patients had a median 1 g/dL increase of hemoglobin concentration, and markers of hemolysis were decreased.[1]

Hydroxyurea is the only approved drug that modifies the course of sickle cell disease. Based on controlled clinical trials in children and adults, it has become the standard of care for this disease, and nearly all patients should receive this drug starting in the first 2 years of life.[2] It works primarily by inducing higher levels of fetal hemoglobin (HbF). HbF prevents HbS polymerization because of its exclusion from the polymer. Although most patients, especially when they are young, have an increase in HbF, the benefits of hydroxyurea are not uniform, and 5% to 15% of patients, mainly adults, do not respond or respond inadequately. Voxelator, because it also directly effects HbS polymerization, is another potential disease-modifying treatment. If the results reported here are confirmed, this agent could be employed with hydroxyurea in a combination-therapy approach targeting HbS polymerization; it might even be used as stand-alone treatment in patients who do not respond to or cannot take hydroxyurea.

Sickle cell disease pathophysiology is triggered by HbS polymerization (see figure). Erythrocytes damaged by HbS polymer lead directly and indirectly to sickle vaso-occlusion and hemolytic anemia.[3] Many clinical subphenotypes characterize sickle cell disease, and although vaso-occlusion and hemolysis are interrelated at some level, the complications of disease tend to be associated with 1 or the other of these 2 pathophysiologic branches. Hemolytic anemia, particularly that component of hemolysis that occurs intravascularly, is associated with a suite of complications that develop over years and include pulmonary and systemic vascular disease, cerebrovascular disease, and sickle nephropathy, and patients with the most intense hemolysis have increased mortality.[4] Reducing the rate of hemolysis, as appears to be the case with voxelator, is consequently an attractive approach to treatment.

However, there is reason for caution. Higher hemoglobin levels might have adverse effects on the complications of disease closely linked to sickle vaso-occlusion. Perhaps this is a result of increased blood viscosity due to the longer survival of erythrocytes that still contain mainly HbS. Compared with simple homozygotes for the HbS gene, the one-third of sickle cell anemia patients who have α thalassemia in addition to sickle cell anemia have ~0.5 to 1 g/dL higher hemoglobin levels because they have less hemolysis due to reduced mean cell HbS concentration. Nevertheless, they have an increased risk of certain vaso-occlusive viscosity-related complications like osteonecrosis and painful episodes. Beneficially, they have a reduced propensity to stroke, sickle nephropathy, leg ulcers, and priapism.[5] These observations were reinforced by the results of a phase 3 clinical trial of senicapoc, an inhibitor of potassium traffic across the erythrocyte membrane via the Gardos channel. The "rehydrated" sickle erythrocytes of treated patients had reduced HbS concentration, and like patients with sickle cell anemia–α thalassemia, hemolysis was decreased and hemoglobin concentration increased by ~0.6 g/dL. Unfortunately, the trial had to be stopped prematurely as an interim analysis portended futility.[6] In some subgroups, drug-treated patients had an increased rate of vaso-occlusive events. The short duration of this trial made it unlikely that any improvement of the hemolysis-associated complications of disease would be observed. Similarly, it might take years before any benefit of voxelator on these complications can be appreciated. In contrast to the observations in sickle cell anemia–α thalassemia, although hemolysis is reduced and hemoglobin is increased by 0.5 to 1 g/dL when patients are treated with hydroxyurea, the rate of acute sickle vaso-occlusive events falls. This could be the result of direct inhibition of HbS polymerization by HbF compared with the indirect effects of improved cell hydration where there is no hemoglobin modification. It remains unclear how the increased hemoglobin level that results from voxelator treatment will affect sickle vaso-occlusive events. The optimistic view is that as this agent directly modifies hemoglobin as does HbF, higher hemoglobin levels that result from treatment will not favor vaso-occlusion.

This study included 40 patients who received active drug for 4 to 12 weeks. Only 4 continued treatment of up to 6 months, so long-term safety cannot be assumed. Regulation of oxygen delivery is a fundamental physiologic process, and peril could lurk if this is compromised. Although exercise testing results did not differ between treated cases and controls and oxygen delivery seemed unimpaired, longer-term adverse effect