severe and early onset form of disease (recurrent TINF2 mutation carriers), or families with cancer who were later found to have DC (see figure). There is considerable ascertainment bias in both cohorts toward patients with a high disease penetrance, an early onset of disease, a more severe disease presentation, and an increased incidence of cancer.

Risk estimates depend on the characteristics of the studied population. While this study includes a large DC patient population, the genetic heterogeneity of DC was not taken into account. Despite these limitations, the study has many important findings, highlighting the need for careful cancer surveillance and the spectrum of cancers characteristic of patients with DC. In addition, the study outlines the need to identify new DC-specific hematopoietic stem cell transplantation (HSCT) approaches to improve long-term survival, and the need to explore new therapeutic agents that modify the course of disease. However, DC is a disease with a wide spectrum of features that are likely linked to the presence of specific gene mutations and other as yet poorly understood genetic modifiers and environmental exposures. Thus, not all DC patients will develop manifestations as severe as those described in the current study. When counseling DC patients regarding survival or possible treatment outcomes, it is important that these issues be considered to avoid generating unnecessary fear of dying, suffering from cancer, or withholding medical treatments that might offer a cure for this disease.

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REFERENCES
Despite the promise of human embryonic stem cells (hESCs) for producing therapeutically relevant hematopoietic cells, there have been few concrete demonstrations of the capacity of hESCs to produce functional mature hematopoietic progeny. The majority of hematopoietic differentiation studies on hESCs have focused on cell morphology, surface marker or gene expression, colony formation, or repopulation potential in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mouse transplants. But they have not assessed whether the mature cells are capable of the full range of functions at the level of their normal peripheral blood hematopoietic counterparts. In this issue of Blood, Yokoyama et al.,1 in addition to a recent publication by Saeki et al,2 offer the first demonstrations of functional mature neutrophils being obtained from hESCs following differentiation in vitro. Notably, Yokoyama et al describe a method of highly directed terminal differentiation from immature hESCs into final cultures containing 70% to 80% mature neutrophils (stabs and polymorphonuclear cells) and 10% to 20% granulocytic metamyelocytes, with the remainder consisting of other myelomonocytic lineage cells.

Yokoyama et al provide the first demonstration that these hESC-derived mature neutrophils are capable of similar levels of superoxide production, phagocytosis, bactericidal activity, and chemotaxis functions as peripheral blood neutrophils obtained from healthy human subjects.

The ability to obtain functional hematopoietic progeny from hESCs is a major step toward the eventual use of hESCs or other pluripotent stem cells for the treatment of hematopoietic disorders. However, there are still major hurdles before that goal can be achieved. First, this differentiation protocol results in a relatively low (1.7-fold) increase in the number of neutrophils obtained relative to the initial number of hESCs, while the protocol described by Saeki et al2 resulted in roughly equal numbers of mature neutrophils as input hESCs. As such, the effective scaling-up of the differentiation procedure to obtain sufficient cell numbers for a therapeutic benefit remains a very formidable issue. In addition, while transplant of terminally differentiated mature hematopoietic cells could allow for short-term clinical benefit, the ability to engraft cells differentiated to a hematopoietic stem cell stage could allow for therapeutic long-term hematopoietic repopulation. While a few studies have demonstrated hematopoietic engraftment in NOD/SCID mice of apparent hematopoietic stem cells derived from in vitro differentiation of hESCs,3-5 normal functionality of the mature progeny of those engrafting cells has not yet been demonstrated. Consequently, a differentiation protocol that produces both efficient hematopoietic stem cell engraftment and functional progeny has yet to be conclusively demonstrated. Also, of critical importance for safety, undifferentiated hESCs have the potential to form teratomas. Therefore, differentiation cultures that result in even a small number of residual undifferentiated cells may pose a tumor risk in a transplantation setting.

Combined with the establishment of hematopoietic differentiation protocols for hESCs, the recent breakthroughs in creating induced pluripotent stem cells (iPSCs) also hold great potential for the treatment of neutrophil disorders and other hematopoietic diseases by providing highly expandable patient-specific stem cells. Human iPSCs can be derived from terminally differentiated cells by the introduction of combinations of exogenous transcription factor genes including OCT3/4, SOX2, c-MYC, KLF-4, NANOG, and LIN28, delivered using integrating gamma-retrovirus6 or lentivirus7 gene transfer vectors, or using non-integrating episomal vectors for transient gene transfer.8 Typically skin fibroblasts have been used for this purpose, but a recent paper in this journal demonstrated that peripheral blood cells could also be used as a starting point.9

The Yokoyama et al hESC differentiation protocol provides a road map to determine whether human iPSCs may also produce functional hematopoietic progeny using a similar protocol. A number of investigators have noted that various hESC lines differ in their capacity for efficient differentiation into particular mature lineages including hematopoietic lineages. Saeki et al indicate that of the 3 hESC lines developed at Kyoto University that they examined (KhES-1, KhES-2, and KhES-3), only the KhES-3 line demonstrated efficient hematopoietic differentiation.2 These subtle differences in the in vitro differentiation capability of different hESC lines require further investigation. It is of note that Yokoyama et al also used the KhES-3 line for their studies. As with hESCs, it is likely that various iPSCs cloned lines derived even from the same patient also may demonstrate differences in
their capacity for hematopoietic differentiation in vitro. Nonetheless, if human iPSCs can likewise give rise to functional hematopoietic progeny, it raises the possibility of developing patient-customized stem cells for autologous transplants to treat hematopoietic diseases in cases in which matched donor transplants are not available. For diseases arising from genetic defects, this process could involve obtaining mature cells from the patient, then using reprogramming factors to create iPSCs. Due to their capacity for prolonged (potentially indefinite) proliferation in an undifferentiated state, these iPSCs would be an attractive target for gene correction or repair, with subsequent cellular expansion to provide a larger pool of corrected cells than is achievable with current hematopoietic stem cell gene therapy. In vitro differentiation of gene-corrected iPSCs could then provide hematopoietic stem cells for autologous transplantation, resulting in long-term repopulation with functional progeny to treat the hematopoietic disorder following a theoretical schema (see figure).

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REFERENCES

UCB transplantation: miRNA involvement

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In this issue of Blood, Weitzel and colleagues show the regulation of NFAT1 expression, an important transcription factor in T cells by microRNA-184, advancing our understanding of autoimmunity and GVHD.

Allogeneic hematopoietic stem cell transplantation is routinely used in clinical protocols for the treatment of high-risk hematologic malignancies following intensive chemotherapy and/or radiation. However, the limited number of available compatible unrelated donors is one of the major limitations to wider application, especially for ethnic minorities. Umbilical cord blood (UCB) transplantation is an alternative strategy and has been successfully implemented for both pediatric and adults recipients. Two of its advantages are rapid access and availability of cord blood units, and the less frequent and severe acute graft-versus-host disease (aGVHD) in a partially unmatched transplantation setting. Some major drawbacks, however, are limited numbers of nucleated cells per UCB unit, prolonged time to engraftment, and slow recovery of immunity, resulting in higher risk of severe infections 3 to 4 months after transplantation.1

Donor T-cell activation and secretion of proinflammatory cytokines play an important role in the development of aGVHD. The nuclear factor of activated T cells (NFAT) family of proteins are important regulators of T-cell activation, differentiation, and self-tolerance.2 They also are indirectly inhibited by cyclosporin A and FK506. These immunosuppressive agents are widely used for preventing and counteracting severe aGVHD. NFAT1 protein-deficient mice show decreased IFN-γ production in response to T-cell receptor ligation. Since UCB T cells have a lower capacity for cytokine production than T cells derived from adult blood, it was striking that Weitzel et al identified a lower expression of NFAT1 protein in UCB CD4+ T cells as compared with adult CD4+ T cells in their previous work.3,4 However, it remained mysterious as to why NFAT1-mRNA was not expressed at a markedly lower level in these cells. In this issue of Blood, Weitzel et al now report the translational repression of the NFAT1 protein by a microRNA, miR-184, in cord blood T cells, explaining this “contradiction.”5

MicroRNAs (miRNAs) constitute a large class of endogenous noncoding RNAs and are involved in a wide variety of processes including cell differentiation, apoptosis, and metabolic pathways.6 They are 19 to 24 nucleotides in length, are part of a ribonucleoprotein complex and direct this complex mostly to the 3′ UTR of the targeted mRNA. This results in translational repression of the mRNA by a wide variety of molecular mechanisms including destabilization of the mRNA and shuffling of the mRNA to structures without access to the ribosomal translation machinery.7

Weitzel et al nicely demonstrate that the translational repression of NFAT1 is most prominent in umbilical cord blood–derived CD4+ T cells with low NFAT1 levels and high miR-184 expression compared with adult blood-derived CD4+ T cells. However, this effect might also be due to the lower fraction of naive T cells in the adult CD4+ compartment, since the authors show that the miR-184 expression is higher in this naive fraction. Upon stimulation, inhibition in UCB CD4+ T cells is relieved by 2 different mechanisms. At the earlier time points, miRNA expression is reduced, resulting in higher NFAT1 protein levels, while at later time points, the NFAT1 mRNA is transferred from silenced cell structures to the polyribosomal fraction where the message is translated. The factors involved in the latter remain to be elucidated, but competing regulators binding to mRNA have been identified to counteract mRNA action in other cell types including human hematoma cells, rat neurons, and fly olfactory neurons.8 The authors go on to define the binding site of the miRNA to the 3′ UTR on a molecular level, using a dual luciferase assay system and selectively inhibiting or overexpressing the miR-184 as well as mutating the binding site.
Functional neutrophils from human ES cells

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