

## ● ● ● HEMATOPOIESIS & STEM CELLS

Comment on Amabile et al, page 1255

# Tu-mor(e) blood cells from human pluripotent stem cells

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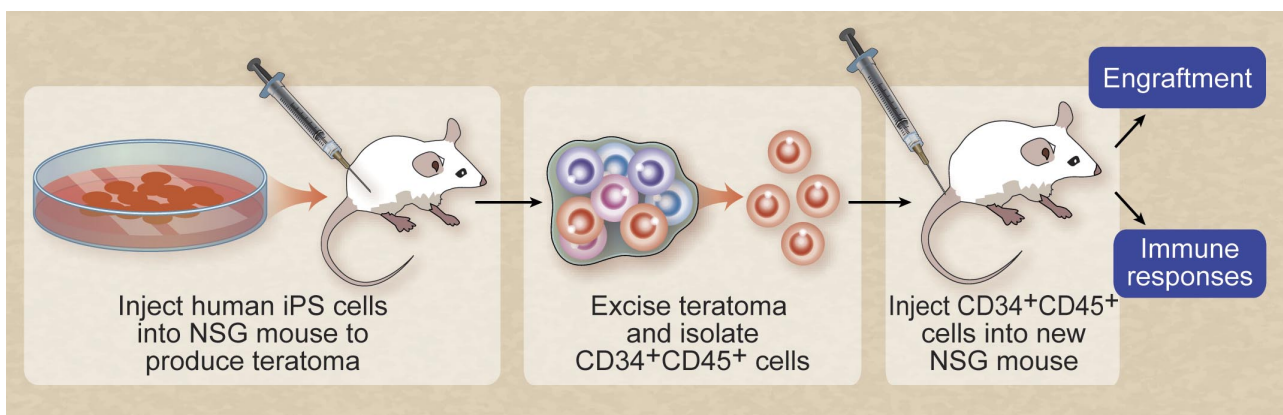
Studies by Amabile et al reported in this issue of *Blood* use a novel strategy of teratoma formation from human induced pluripotent stem cells (iPSCs) to isolate hematopoietic stem/progenitor cells (HSPCs) capable of *in vivo* engraftment and producing functional lymphocytes.<sup>1</sup>

The original report describing derivation of human embryonic stem cells (ESCs) used teratoma formation in immunodeficient mice as a key means to demonstrate pluripotency of these cells.<sup>2</sup> The ability of human ESCs or iPSCs to produce teratomas with elements of all 3 embryonic germ layers (endoderm, ectoderm, and mesoderm) remains a fundamental assay of human pluripotent stem cells.<sup>3</sup> Intriguingly, one figure in that original publication of human ESCs shows a piece of bone with hematopoietic cells inside. While these cells tantalizingly resembled human bone marrow, it was not determined if these blood cells were of human or mouse origin.

Use of human ESCs or iPSCs to derive hematopoietic cells capable of long-term, multilineage engraftment *in vivo* remains a key challenge. While several studies examine transplantation of human ESC or iPSC-derived hematopoietic cells into immunodeficient mice or fetal sheep models, the overall level of engraftment is typically low and primarily consists of myeloid cells.<sup>4-6</sup> In addition, while strategies such as overexpression of *HoxB4* in mouse ESCs leads to development of transplantable hematopoietic stem cells, *HOXB4* does not have the same effect when expressed in human ESCs.<sup>4</sup>

Here, Amabile and colleagues more closely examine the hematopoietic potential of teratomas produced from human iPSCs that are grown subcutaneously and intramuscularly in immunodeficient NOD/SCID/IL2R $\gamma$ <sup>-/-</sup> (NSG) mice (see figure). They found many CD34<sup>+</sup> and CD45<sup>+</sup> cells within the teratomas (1.5% CD45<sup>+</sup> cells after 8 weeks). These hematopoietic cells included myeloid, lymphoid, and erythroid cells, as well as putative HSPCs. In addition, the spleens and lymph nodes of these mice were enlarged and contained human CD45<sup>+</sup> cells.

These studies go on to demonstrate at least 3 key insights. First, co-injection of OP9 murine bone marrow stromal cells with the iPSCs during teratoma formation leads to substantially increased numbers of hematopoietic cells. In addition, use of OP9 cells that produce Wnt3a or expressed the Notch ligand Delta-like 1 produce even more hematopoietic cells within the teratomas. Indeed, use of these cells model what is known about normal human hematopoiesis, with OP9-Wnt3a cells leading to more CD19<sup>+</sup> B cells and OP9-DL1 stimulating development of more CD3<sup>+</sup> T cells. This strategy demonstrates this model can be used to further dissect stromal elements of the human hematopoietic niche



Schematic of studies by Amabile et al to demonstrate development of transplantable hematopoietic cells from human pluripotent stem cells. These studies first produce teratomas from human iPSCs injected into immunodeficient NSG mice. Human CD34<sup>+</sup> and CD45<sup>+</sup> cells can be isolated from these tumors. These hematopoietic cells are then transplanted into new NSG mice where they demonstrate effective engraftment and functional human immune responses. Figure adapted from the article by Amabile et al that begins on page 1255. Professional illustration by Debra T. Dartz.

that regulate normal (and potentially malignant) hematopoiesis.

Next, the authors tested the HSPC potential of teratoma-derived CD34<sup>+</sup>CD45<sup>+</sup> cells by transplanting them into new NSG mice. While only relatively few of these cells could be isolated from the tumors, they engrafted at approximately the same efficiency as CD34<sup>+</sup> cells isolated from human cord blood. In addition, these teratoma-derived cells are capable of engraftment in secondary recipients, although at very low frequency. Also of interest is the lack of malignant appearance of the human hematopoietic cells in the teratomas or transplanted mice. Indeed, teratomas from human ESCs and iPSCs are almost always benign tumors without evidence of malignant germ cell tumors.<sup>3</sup>

Third, mice engrafted with human iPSC teratoma-derived hematopoietic cells produced human B and T cells capable of functional immune responses. Mice immunized with viral antigens produced human IgG. Human T cells isolated from these engrafted mice were stimulated with IL2 and anti-CD3 beads to produce human cytokines. In addition, human CD15<sup>+</sup> cells isolated from these mice were able to phagocytize latex beads similar to normal myeloid human peripheral blood CD15<sup>+</sup> cells.

Of course, there are limits to this teratoma-based model of human hematopoiesis. While this strategy will be valuable to more closely examine regulation of early human hematopoiesis and potentially to produce fully human antibodies from the B cells, the limited size of the tumors and the xenogeneic environment precludes use of this system to derive transplantable HSPCs suitable for human clinical use. In addition, the *in vivo* engraftment studies find the teratoma-derived cells remain biased toward myeloid (CD15<sup>+</sup>) cells in both primary and secondary recipients. Furthermore, globin gene expression was not analyzed in these studies. To date, most analyses of human pluripotent stem cell-derived erythroid cells find they produce primarily embryonic ( $\epsilon$ ) and fetal ( $\gamma$ ) globin genes, with little  $\beta$ -globin production.<sup>7</sup>

Other recent studies also demonstrate exciting advances in use of human ESCs and iPSCs to study hematopoiesis. For example, a recent report by the Keller group using human ESCs and iPSCs identifies an *in vitro* system to better produce human hematopoietic cells with potent T cell potential.<sup>8</sup> Another study

recently reported at the 54th annual meeting of the American Society of Hematology used over-expression of *RUNX1a* in human ESCs and iPSCs to markedly improve hematopoietic development, including cells that engraft efficiently in NSG mice.<sup>9</sup> However, further characterization of these cells is pending. Human iPSCs also provide a unique means to model human genetic diseases that affect hematopoietic development.<sup>10</sup> Together, the continued rapid developments in this field emphasize human pluripotent stem cells (both ESCs and iPSCs) are expanding as a valuable resource to study mechanisms that mediate development of human hematopoietic and other cell lineages, as well as an important resource for drug screening and potential therapies.

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

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## ● ● ● IMMUNOBIOLOGY

Comment on Chi et al, page 1357

# Autoantibodies against cytokines: back to human genetics

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In this issue of *Blood*, Chi et al have discovered that the occurrence of autoantibodies against IFN- $\gamma$ , which trigger mycobacterial diseases and mimic inborn errors of IFN- $\gamma$  immunity, is genetically determined.<sup>1</sup>

It has long been known that infections can trigger autoimmunity. Paradoxically, autoimmunity has also recently been shown to precipitate infectious diseases. Indeed, autoantibodies against certain cytokines have been shown to underlie the pathogenesis of specific infectious diseases. This is best illustrated by autoantibodies against IFN- $\gamma$ , which were first described in 2004-2005 in 5 adults with no significant medical history who developed disseminated disease caused by environmental and tuberculous mycobacteria.<sup>2-4</sup> A 25-year-old woman from Thailand<sup>2</sup> and a 47-year-old man from the Philippines<sup>3</sup> died of mycobacterial disease. The other 3 patients, 2 British

women aged 46 and 59 years, respectively, and a 32-year-old South African man, slowly improved after treatment with antibiotics and, in 1 case, recombinant IFN- $\gamma$ .<sup>4</sup> Neutralizing anti-IFN- $\gamma$  autoantibodies have been reported now in approximately 130 individuals. Most patients initially presented as adults with disseminated environmental mycobacterial infections.<sup>2-5</sup> Strikingly, these patients are late-onset clinical phenocopies of patients with Mendelian susceptibility to mycobacterial disease (MSMD) carrying inborn errors of IFN- $\gamma$  immunity.<sup>6</sup> This observation alone strongly suggested that autoantibodies against IFN- $\gamma$  were the cause rather than a