inside blood commentary

11 AUGUST 2016 | VOLUME 128, NUMBER 6

HEMATOPOIESIS AND STEM CELLS

Comment on Brauer et al, page 783

Modeling altered human T-cell development

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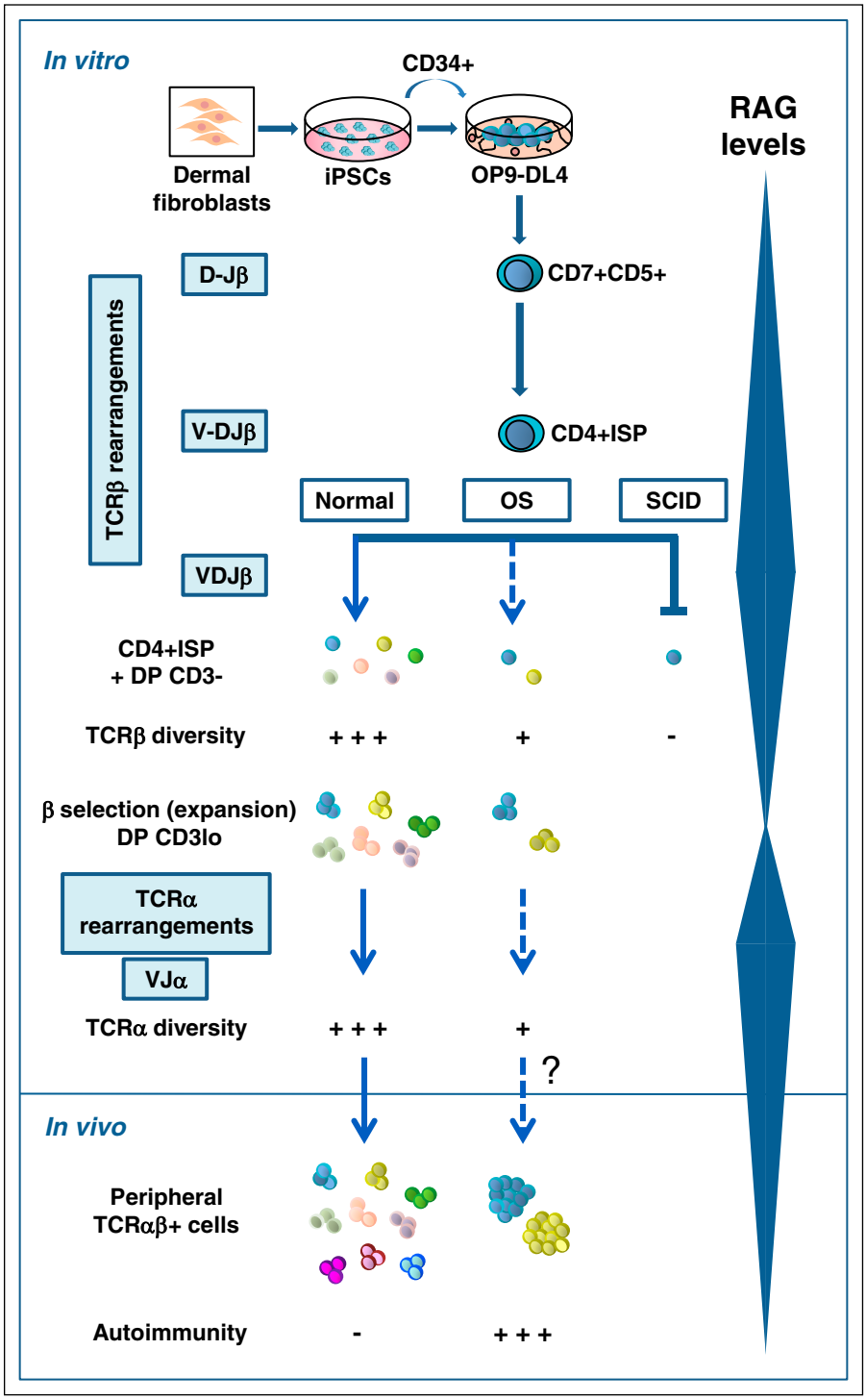
In this issue of *Blood*, Brauer et al model altered T-cell development of severe combined immunodeficiency (SCID) and Omenn syndrome (OS) patients with human induced pluripotent stem cells (iPSCs), and reveal important differences in T-cell receptor (TCR) repertoire diversity that help to understand the disparity of clinical and immunologic phenotypes that result from distinct *RAG1* mutations.[1]

T and B lymphocytes are critical components of adaptive immunity, capable of recognizing millions of antigens with their surface antigen receptors (TCR and B-cell receptor, respectively). Their variable regions are encoded by exons assembled during lymphocyte development from the combinatorial joining of one of multiple germ line variable (V), diversity (D), and joining (J) gene segments.[2] VDJ recombination is thus of fundamental importance for the generation of diverse antigen receptor repertoires and is absolutely dependent on the synergistic activity of the recombination-activating genes 1/2 (*RAG1/2*),[3] which are regulated in a lymphoid and developmental stage-specific context. In humans, mutations of the *RAG* genes are associated with distinct clinical and immunologic phenotypes, which reflect the severity of the VDJ recombination defect and are characterized by variable association to infections and/or autoimmunity.[4] Null *RAG* mutations totally abolish the VDJ recombination and result in a complete block of B- and T-cell development at the progenitor stage and thus in SCID, which represents approximately half of the human T^-B^- SCIDs. In contrast, hypomorphic *RAG* mutations support modest, but residual, recombination activity and cause OS, a rare autosomal recessive SCID associated with an oligoclonal expansion of autoreactive peripheral T cells that infiltrate and damage peripheral organs, whereas B cells are typically absent.[4]

Progress in our knowledge of the molecular mechanisms accounting for the phenotypic heterogeneity of human RAG deficiency derives from recent advances in structural modeling of the RAG complex and the development of novel cellular platforms to measure the expression and function of mutant RAG proteins in vitro.[4] However, the functional impact of the diverse *RAG* mutations on T-cell development cannot be retrospectively studied in patients, and thus the developmental pathophysiology of the resulting disease remains ill-defined. To fill this gap, Brauer et al have now taken advantage of recent studies showing that T cells can be generated in vitro from iPSCs cocultured onto OP9 stromal cells expressing the delta-like 1 (DL-1) Notch ligand,[5] and demonstrating that the model faithfully recapitulates the early T-cell developmental arrest that suffer X-linked SCID patients carrying an *IL2RG* mutation.[6] Brauer et al extend this work to *RAG1* mutant patients and validate the suitability of the model to elucidate the causality of SCID vs OS phenotypes. Their assay recapitulates T-cell developmental transitions in $CD34^+$ hematopoietic progenitors obtained from SCID or OS dermal fibroblast-derived iPSCs harboring null or hypomorphic RAG1 mutations, which support undetectable or very modest (3.5%) recombination activity in vitro, respectively. The system also allows tracing TCR repertoire diversity generated at sequential stages of normal and defective T-cell development (see figure).

Development of αβ T cells is a complex multistep process that involves the sequential rearrangement of genes encoding the β and α chains of the TCR at 2 consecutive developmental checkpoints characterized by maximal expression levels of *RAG* genes.[7] In humans, DJ rearrangements at the *TCRB* locus first occur in $CD7^+CD5^+$ pro-T cells at the $CD4^-CD8^-$ double-negative thymocyte stage, whereas VDJ *TCRB* gene rearrangements and expression of full-length *TCRB* transcripts start in $CD4^+CD8^-$ immature single-positive (ISP) (ie, $CD4^+$ISP) thymocytes, and become common in a downstream $CD4^+CD8^+$ double-positive (DP) thymocyte subset that is highly enriched in large cycling cells expressing low CD3 levels ($CD3^{lo}$), characteristic of pre-TCR^+ cells. Therefore, the ISP to DP $CD3^{lo}$ transition represents the critical checkpoint at which developing thymocytes with a successful *TCRB* gene rearrangement downregulate RAG expression and undergo β-selection by signaling through the pre-TCR heterodimer (TCRβ/pTα), which promotes the clonal expansion of DP $CD3^{lo}$ pre-T cells.[8] Thereafter, DP thymocytes downregulate the pre-TCR, stop cycling, and re-express RAG genes to induce *TCRA* gene rearrangement and finally TCR-αβ expression.[9]

Brauer et al have directly addressed when and how this developmental pathway is altered in OS and SCID patients by coculturing their iPSC-derived $CD34^+$ progenitors on OP9 cells expressing the DL-4 Notch ligand (OP9-DL-4). The authors show that the critical transition from $CD4^+$ISP to DP $CD3^{lo}$ pre-T cells observed in



Modeling altered T-cell development of SCID and OS patients with dermal fibroblast-derived iPSCs.

control cultures is severely impaired for both SCID and OS. Accordingly, no patient DP cells with a $CD3^{lo}$ phenotype characteristic of pre–TCR-expressing pre-T cells are expanded in vitro, confirming a specific blockade at the β-selection checkpoint, which concurs with the severe lymphoid depletion observed in the thymus of *RAG*-mutant patients. However, both OS and SCID $CD7^{+}CD5^{+}$ pro-T cells produce in vitro considerable, yet reduced, numbers of CD4ISP and DP $CD3^{-}$ pre-T cells with no detectable intracellular TCR-β expression, which show a drastically reduced proportion of *TCRB* (and *TCRA*) rearrangements and a severe restriction of *TCRB* (and *TCRA)* repertoire diversity, with preferential usage of few VDJ genes, compared with controls. In the case of SCID samples, *RAG1* activity is severely affected in vitro, and extremely rare cells are thus expected to accomplish functional *TCRB* rearrangements and pre–TCR-mediated β-selection. OS pre-T cells, in contrast, contain residual RAG activity that supports some DNA recombination and a higher propensity for DNA breaks. Unexpectedly, they do not display an enhanced ability to progress beyond the β-selection checkpoint when compared with SCID cells in vitro, likely because, as the authors suggest, most of them accumulate single-strand DNA breaks that eventually may lead to cell death. In vivo, however, a few OS pre-T cells must succeed at some *TCRB* rearrangement, traverse the β-selection checkpoint to accomplish *TCRA* rearrangement, and migrate to the periphery as an oligoclonal population of T cells with a restricted TCR-αβ repertoire that may escape central tolerance, as such cells are readily detected and have been shown to contribute to autoimmunity in OS patients.[4]

Although the OP9-DL-4 system could not support the expansion and developmental progression of OS surviving thymocytes, it served to demonstrate their unique differences in VDJ diversity that may explain the phenotypic diversity of RAG1-associated immunodeficiency (ID). This highlights the applicability of iPSCs to model altered early T-cell development in human ID.

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

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DOI 10.1182/blood-2016-06-723908

LYMPHOID NEOPLASIA

Comment on Nicolay et al, page 805

DMF: a promising therapeutic option in CTCL

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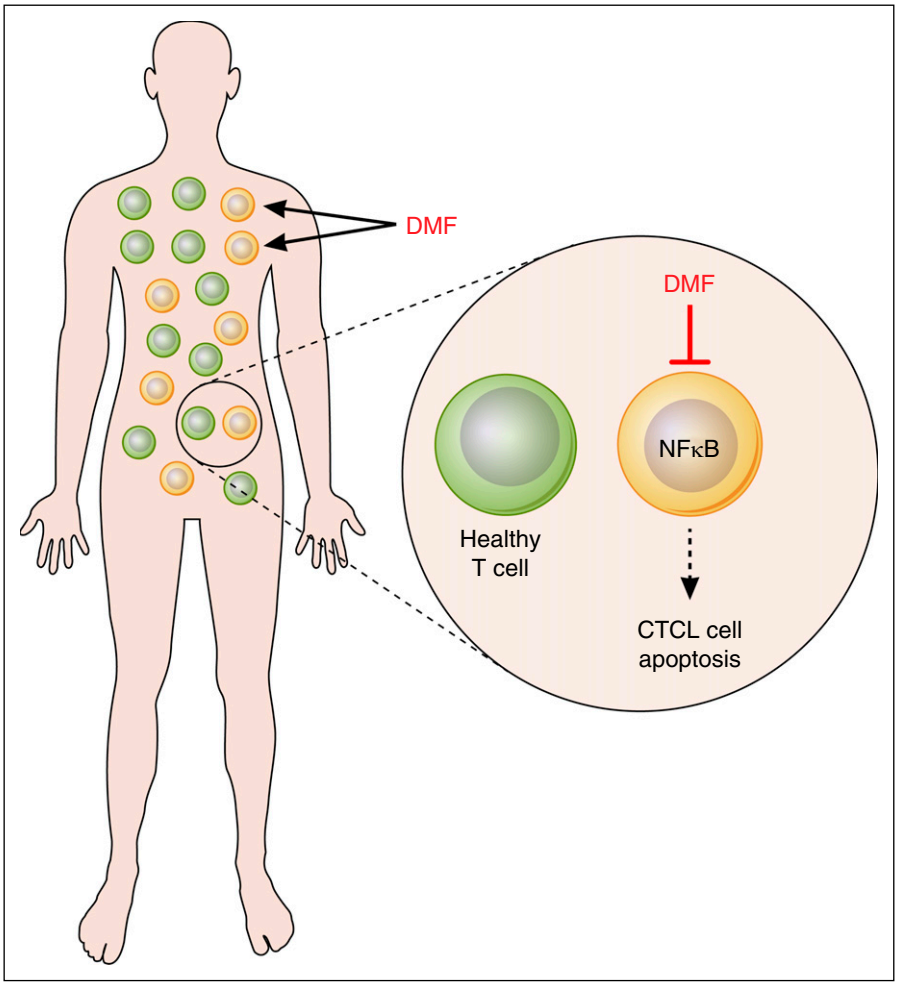
In this issue of *Blood*, Nicolay et al present their exciting findings that show that dimethyl fumarate (DMF) specifically inhibits NF-κB activity in cutaneous T-cell lymphoma (CTCL) cells, thus inducing their apoptosis, while having minimal effect on healthy T cells (see figure). Their study suggests that DMF may represent a new promising therapeutic option in CTCL.[1]



Mechanism of DMF action in CTCL. DMF specifically inhibits NF-κB activity in CTCL cells, thus inducing their apoptosis, while having minimal effect on healthy T cells. Professional illustration by Patrick Lane, ScEYEnce Studios.

CTCL represents a heterogeneous group of rare lymphoproliferative disorders that are characterized by monoclonal proliferation of malignant T lymphocytes primarily homing to the skin.[2] Several therapeutic options exist, but none of these represent a curative approach.[3,4] Therefore, there is an urgent need for the development of novel therapeutic options with higher efficacy rates, curative potential, and milder toxicity profiles.

A characteristic feature of the malignant T cells in CTCL is their resistance toward cell death due to the constitutive activation of the transcription factor NF-κB.[5-7] In addition to inducing expression of antiapoptotic genes, NF-κB induces expression of immunosuppressive genes in CTCL cells,[8,9] thus inhibiting immune responses and contributing to the immunosuppressive nature of CTCL. Therefore, NF-κB represents an important therapeutic target in CTCL, as an NF-κB–directed therapy would leave bystander T cells largely unaffected.

DMF is a potent inhibitor of NF-κB signaling in activated cells, whereas it has a marginal effect on resting cells. It has been approved and clinically used for the treatment of psoriasis and multiple sclerosis.

DMF has relatively mild side effects, which makes it a fairly well-tolerated drug. This is especially attractive for the clinical prospects of DMF as a potential medication for CTCL because many other treatment options are limited by acute or cumulative toxicity or adverse effects. Against this background, Nicolay et al investigated the effects of DMF on CTCL cells. The rationale of this study was to use the NF-κB inhibitory properties of DMF to restore the apoptotic sensitivity in malignant T cells. Indeed, using a combination of elegant in vitro and in vivo experiments, the results of Nicolay et al suggest that DMF may successfully expand the spectrum of systemic treatment options for CTCL.

First, the authors showed that DMF induces cell death in CTCL patient-derived CD4 cells as well as in CTCL cell lines, whereas it has a minimal effect on control T cells. Their in vitro results indicate that the mechanism responsible for the DMF-induced apoptosis in CTCL cells consists of the inhibition of NF-κB activity and NF-κB–dependent transcription. Interestingly, 1 of the genes that was most

2016 128: 743-745
doi:10.1182/blood-2016-06-723908

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.