appears to play the most significant role in the capacity of the AFT024 cell line to support HSC function. Even exogenously added DPT enhanced the survival and proliferation ex vivo–cultured HSCs.

This study raises many questions. How does DPT, as an extracellular protein, mediate its effect on HSC survival? DPT is expressed by osteoblasts, but not significantly by "bone marrow" cells (http://biogps.org/#goto=genereport&id=56429). Do cells in the bone marrow express DPT and what is its spatial distribution in the marrow? Is DPT yet another component of the ever more complex HSC niche in the bone marrow?[4] Because AFT024 cells can also support human HSCs, do the findings presented here translate to human stem cells?

The AFT024 cell line was established from the fetal liver of the mouse.[3] Unlike the adult bone marrow where most, if not all, long-term HSCs are quiescent, the HSC population in the fetal liver rapidly expands in number before transitioning to the bone marrow. This rapid increase is likely related not only to increased cell cycling of fetal HSCs,[5] but also to the progressive expansion of the remodeling vascular niche,[6] and to the influx and maturation of a wave of pre-HSCs.[7] It would be of interest to determine whether DPT is expressed in the fetal liver and if it facilitates fetal HSC expansion. Given the rare number of HSCs normally present during fetal and adult life, their enhanced ex vivo survival and maintenance mediated by DPT represents a first step toward the holy grail of in vitro HSC expansion to facilitate the provision of adequate numbers of HSCs from nonadult sources and the engineering of "designer" HSCs for research and therapeutic purposes.

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

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IMMUNOBIOLOGY

Comment on Kordasti et al, page 1193

How deep can you go into Tregs?

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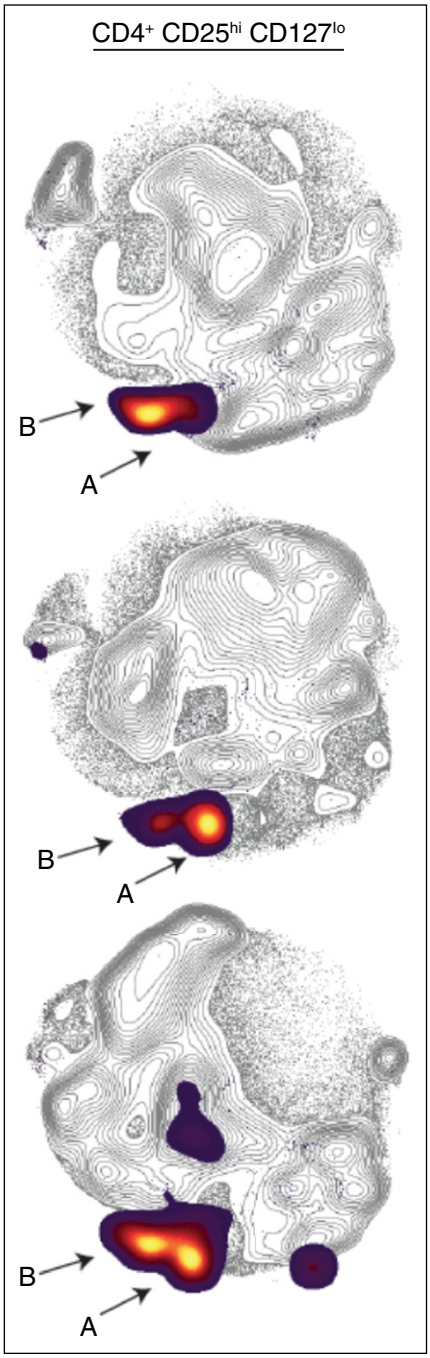
In this issue of *Blood*, Kordasti et al report findings on deep immunophenotyping which revealed a distinct population of regulatory T cells (Tregs) that associated with disease onset and response to immunosuppressive therapy in aplastic anemia (AA). This population, named Treg subset B, showed greater immunoregulatory properties compared with Treg subset A which were identified using robust deep-phenotyping techniques. This observation adds to the wealth of data which shows an altered immune system in AA.[1]

An immunologic disarray forms the basis of bone marrow destruction in severe AA resulting in profound cytopenias and substantial morbidity/mortality if marrow function is not improved. Over the years, several methodologies have been applied to characterize this perturbation that culminates in marrow failure. In the early days of flow cytometry, Zoumbos et al showed an increased proportion of interferon-γ (IFNγ)-producing T cells in the blood and bone marrow of patients with AA.[2] Subsequently, IFNγ and other cytokines such as tumor necrosis factor β (TNFβ) were implicated in hematopoietic suppression by upregulation of Fas in marrow progenitor cells leading to apoptosis.[3] Later, advances in flow cytometric protocols allowed for the characterization of skewing of the T-cell repertoire in AA and patient-specific clonotypes were identified by T-cell receptor sequencing which tracked with disease activity.[4] More broadly, the Treg subset was shown to be decreased in AA whereas the more proinflammatory TH17 subset and TH1-associated proteins increased at diagnosis.[5]

The refinement of these techniques has allowed greater depth in molecular and phenotypic characterization of the genetic, molecular, and T-cell subset alterations in severe AA (SAA). Next-generation sequencing has been applied to hundreds of patients with SAA with important insights into the extent of clonal hematopoiesis and its implication in outcomes.[6,7] A deeper immune subset analysis is now possible with single-cell mass cytometry (cytometry by time-of-flight [CyTOF]) which, instead of fluorescence-based flow cytometry, applies transition element isotopes to label antibodies which are analyzed by a time-of-flight mass spectrometer.[8] The tagging of antibodies to rare heavy metals in CyTOF allows for the analysis of dozens of parameters simultaneously providing multidimensional data with little to no background (given the minimal autofluorescence) and no need for compensation.

Kordasti et al applied CyTOF in a 31-patient cohort providing an in-depth characterization of the Treg subset in AA. Two distinct Treg subsets named A and B were defined based on an extended phenotypic profile (12 parameters), gene expression, expandability, and suppression function. Treg B was significantly lower at diagnosis compared to healthy donors and tended to improve among responders to immunosuppression. Furthermore, subset B had more IFNγ- and TNFα-suppressing properties than subset A. There was minimal clonotypic overlap between the 2 Treg subsets, suggesting distinct origins. A higher Treg B population at baseline correlated with hematologic recovery following immunosuppression with this subpopulation increase likely contributing to regulation of autoimmunity in AA in recovering patients



The CyTOF density plots (visual stochastic neighbor embedding [viSNE]) revealed 2 subpopulations within Tregs, namely A and B. Their frequencies were distinct between healthy donors and patients with AA. Furthermore, patients with a higher Treg A frequency were less likely to respond to immunosuppression, and conversely, those with a higher Treg B frequency more likely to have a hematologic recovery following therapy. See Figure 2D in the article by Kordasti et al that begins on page 1193.

(see figure) although the number of patients in this correlative clinical analysis was small.

The more in-depth characterization of molecular and immunologic disarrangements in SAA has created a challenge in applying the wealth of information into the clinic. One of the more immediate goals with this data is to predict short- (hematologic response) and long-term outcomes (relapse and clonal evolution) following immunosuppressive therapy. Prognostication has long been sought in all fields of medicine as evidenced by the thousands of publications on the topic.[9] However, very few change practice. Many are not applied for not being practical, reproducible, robust, or cost-effective.[9] Although certain genetic defects (mutations in *ASXL1*, *DNMT3A*) and immunologic findings such as expansion of oligoclones can correlate with outcomes, these observations are highly variable, suggesting a more intricate relationship between these findings and outcomes. A more functional assessment of these findings, in the context of immunosuppression failure for example, might be more revealing. Thus, discrimination of Treg subsets with CyTOF is not likely to be applied routinely in the clinic as a prognosticator. However, this important observation continues to strengthen the wealth of data detailing the aberrant immune system in AA. Finally, recent reports with a 3-drug immunosuppression regimen which included eltrombopag along with horse antithymocyte globulin plus cyclosporine have shown very high overall hematologic response rates (around 90%) with excellent survival to date, making the prediction of response less critical.[10] Predicting durability of response and the occurrence of late events such as clonal evolution with these more sophisticated tools will become more pressing as this information could have an impact on treatment decisions.

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● ● ● LYMPHOID NEOPLASIA

Comment on Chong et al, page 1206

PDL structural rearrangements in B-cell NHL

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In this issue of *Blood*, Chong et al report a comprehensive landscape of structural rearrangements (SRs) of the programmed death ligand (PDL) locus (9p24.1), which harbors PDL1 (*CD274*) and PDL2 (*PDCD1LG2*) providing novel information about the breakpoint anatomy of the SRs in a large cohort of B-cell lymphomas (eg, non-Hodgkin lymphoma [NHL]). Importantly, the studies were performed by using DNA obtained from formalin-fixed paraffin-embedded tissues.[1]