needed for signs or symptoms of congestive heart failure should they occur.

Umit Tapan
Section of Hematology-Oncology, Boston University School of Medicine, Boston, MA

David C. Seldin
Section of Hematology-Oncology and Amyloid Treatment and Research Program, Boston University School of Medicine, Boston, MA

Kathleen T. Finn
Section of Hematology-Oncology and Amyloid Treatment and Research Program, Boston University School of Medicine, Boston, MA

Salli Fennessey
Section of Hematology-Oncology, Boston University School of Medicine, Boston, MA

Anthony Shelton
Section of Hematology-Oncology, Boston University School of Medicine, Boston, MA

Jerome B. Zeldis
Celgene Corporation, Summit, NJ

Vaishali Sanchorawala
Section of Hematology-Oncology and Amyloid Treatment and Research Program, Boston University School of Medicine, Boston, MA

Acknowledgment: This clinical trial was supported by Celgene Corporation.

Conflict-of-interest disclosure: J.B.Z. is employed by Celgene Corp, whose product (lenalidomide) was studied in the present work. The remaining authors declare no competing financial interests.

Correspondence: Vaishali Sanchorawala, MD, Section of Hematology/Oncology, FGH 1007, 820 Harrison Ave, Boston, MA 02118; e-mail: Vaishali.Sanchorawala@bmc.org.

References

To the editor:

Double-negative T cells are non–ALPS-specific markers of immune dysregulation found in patients with aplastic anemia

We read with interest the recent article by Dowdell and colleagues exploring somatic FAS gene mutations among patients with autoimmune lymphoproliferative syndrome (ALPS), a syndrome characterized by defective CD95 (Fas) cell surface-mediated apoptosis. In assigning the diagnosis of ALPS, demonstration of a population of peripherally expanded CD4+/CD8−, double-negative T lymphocytes (DNTs; >1.5% of normal lymphocytes) that express αβ T-cell receptors (α-β TCRs), is widely considered a key laboratory criteria. As indicated by the authors, the pathogenic nature of DNT cells remains a matter of debate. Indeed, patients with rare autoimmune diseases, including systemic lupus erythematosus (SLE) and immune thrombocytopenic purpura (ITP), have demonstrated mild elevations, suggesting that DNTs may perhaps be more common among immune disorders.2,5

Aplastic anemia (AA) is characterized by an acquired, progressive loss of hematopoietic function thought to result from an immune-mediated reaction that targets the hematopoietic stem cell. We undertook a retrospective study of 22 pediatric patients with idiopathic AA of varying disease severity, consecutively diagnosed at our institution between 2007 and 2010. Study approval was obtained by the Oregon Health & Science University Institutional Review Board. In the 11 patients evaluated most recently we performed a detailed immunophenotypic analysis of lymphocyte subsets as part of the diagnostic workup. Remarkably, our study showed an elevated proportion of double-negative T cells (range, 4.3%-9.1% of CD3+ lymphocytes) in 9 patients (Table 1).

Further subfractionation revealed that 10 of our 11 patients (including 2 with DNT just below the upper limit of normal) demonstrated a predominant elevation of γδ TCR—rather than the more common α-β TCR—expressing DNTs. Peripherally expanded DNTs are considered pathogenic in the peripheral cytopenias of patients with ALPS. However, our findings suggest that their occurrence may not be limited to this syndrome and their role might be more complex than the destruction of mature cells in

Table 1. Immunophenotypic analysis of T-cell subpopulations

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age at diagnosis, y</th>
<th>Sex</th>
<th>α-β TCR (0%-1.5%)†</th>
<th>γδ TCR (0%-2%)</th>
<th>CD4+/CD8- (0%-4%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAA</td>
<td>15 F</td>
<td></td>
<td>2.3</td>
<td>4.5</td>
<td>7</td>
</tr>
<tr>
<td>MAA</td>
<td>16 F</td>
<td></td>
<td>1.1</td>
<td>6.4</td>
<td>7</td>
</tr>
<tr>
<td>SAA</td>
<td>4 M</td>
<td></td>
<td>2.2</td>
<td>1.6</td>
<td>4.6</td>
</tr>
<tr>
<td>SAA</td>
<td>11 F</td>
<td></td>
<td>1</td>
<td>4.8</td>
<td>3.8</td>
</tr>
<tr>
<td>SAA</td>
<td>10 M</td>
<td></td>
<td>1.6</td>
<td>2.5</td>
<td>3.7</td>
</tr>
<tr>
<td>SAA</td>
<td>10 M</td>
<td></td>
<td>2.2</td>
<td>4.2</td>
<td>5.5</td>
</tr>
<tr>
<td>SAA</td>
<td>3 F</td>
<td></td>
<td>2.7</td>
<td>4.6</td>
<td>7.3</td>
</tr>
<tr>
<td>SAA</td>
<td>15 F</td>
<td></td>
<td>0.8</td>
<td>5.1</td>
<td>6.6</td>
</tr>
<tr>
<td>SAA</td>
<td>14 M</td>
<td></td>
<td>1.5</td>
<td>5.2</td>
<td>6.7</td>
</tr>
<tr>
<td>SAA</td>
<td>12 M</td>
<td></td>
<td>1.1</td>
<td>8</td>
<td>9.1</td>
</tr>
<tr>
<td>SAA*</td>
<td>16 F</td>
<td></td>
<td>1.3</td>
<td>3</td>
<td>4.3</td>
</tr>
</tbody>
</table>

MAA indicates moderately severe aplastic anemia; SAA, severe aplastic anemia.

*Repeat bone marrow evaluation revealed monosomy 7, consistent with the diagnosis of MDS.
†Normal range for test.
circulation. Double-negative T cells observed in our study population may represent a pathophysiologically important T-cell subpopulation involved in the characteristic progressive loss of hematopoietic stem cells from the bone marrow microenvironment in AA. Related, others have previously shown that T lymphocytes are a potent source of inflammatory and regulatory cytokines, including interleukin 10 (IL-10), reported to be elevated in a subgroup of patients with severe AA and more recently in patients with ALPS.3,7 Alternatively, abnormal T-cell biogenesis is merely an epiphenomenon in AA, and perhaps in ALPS, that indicates a common pathway of dysregulation leading to autoimmunity.

In conclusion, our retrospective analysis for the first time demonstrates the existence of a sizable population of double-negative T cells in AA patients, suggesting that DNTs represent a sensitive but not necessarily specific marker of ALPS. Further investigation may demonstrate previously unrecognized overlap in pathogenesis of AA and other immune cytopenias. Clearly, our observation of peripheral expansion of γ-δ TCR–expressing DNTs in AA warrants the prospective study of a larger cohort and may lead to additional diagnostic and therapeutic approaches.

Thomas B. Russell
Department of Pediatrics, Oregon Health & Science University, Portland, OR

Peter Kurre
Departments of Pediatrics and Cell & Developmental Biology and Papel Family Pediatric Research Institute, Oregon Health & Science University, Portland, OR

To the editor:

IDH mutation analysis is not suitable for the routine molecular diagnostic algorithm in myeloproliferative and myelodysplastic neoplasms

The Janus kinase 2 V617F mutation (JAK2V617F) was the first common molecular marker for the characterization of Philadelphia chromosome–negative myeloproliferative neoplasms (Ph− MPN).1 Depending on the entity, 50% to 95% of Ph− MPN are JAK2V617F-positive.1,2 Over the past few years, several other molecular defects have been described but these aberrations are detectable only in a smaller subfraction of Ph− MPN cases (<10%), for example, myeloproliferative leukemia virus oncogene (MPL), tet oncogene family member 2 (TET2) or additional sex combs like 1 (Drosophila) (ASXL1). However, approximately 50% of these cases are also JAK2V617F-positive,2,3 making these infrequent markers not useful for routine diagnostics.

Recently, mutations in the isocitrato dehydrogenase 1 (IDH1) and IDH2 have been reported in Ph− MPN, myelodysplastic neoplasms/syndromes (MDS) and acute myeloid leukemias.4-7 These mutant enzymes exhibit decreased affinity to isocitrate, which is decarboxylated to 2-oxoglutarate by the wild-type enzymes. Mutant IDH exhibits an aberrant catalytic activity toward α-ketoglutarate, which results indirectly in accumulation of 2-hydroxyglutarate and activation of hypoxia inducible factor 1α subunit (HIF-1α).5 The IDH mutation and JAK2V617F can occur in parallel in one aberrant hematopoietic stem cell clone.5 In Ph− MPN, the mutation frequency is 0.8% for essential thrombocytemia (ET), 1.9% for polycythemia vera (PV), 4.2% for primary myelofibrosis (PMF),6 and 3.6% for MDS.7

The aim of our study was to evaluate the usefulness of IDH mutation analysis as an additional marker for routine molecular diagnostics of chronic stage myeloid neoplasms, particularly Ph− MPN and MDS.

DNA samples (n = 326) from total bone marrow cells were analyzed with a Pyrosequencer assay (Biotage).2 A representative number of samples from ET (n = 93), prefibrotic stage PMF (n = 72), fibrotic stage PMF (n = 52), PV (n = 46), MDS (n = 45), and nonneoplastic controls (n = 18) were evaluated. Ph− MPN and MDS samples were derived from previously characterized cohorts (98% JAK2V617F PV, 48% JAK2V617F ET and PMF).2,9 Bone marrow samples were formalin-fixed and paraffin-embedded and were retrieved from the tissue archive of the Institute of Pathology (Hannover Medical School). The retrospective analysis had been approved by the local ethics committee.

None of the 263 Ph− MPN and 45 MDS cases showed detectable IDH mutations in codons R132 and R172 in IDH1 and IDH2, respectively (Figure 1). In Ph− MPN, JAK2V617F as well as IDH132H can be acquired in a small subfraction of cells and can comprise a range of 5% to 10% of mutant alleles and < 5% of cells,10 respectively.2 Thus, we cannot exclude that some cases with a very low mutant allele burden or cases with other IDH aberrations were not detectable by this methodology.

A molecular marker such as JAK2V617F supports the diagnosis of a histomorphologically based Ph− MPN diagnosis and thus is very helpful in distinguishing reactive from neoplastic myeloproliferation. However, due to the low occurrence of mutations, the screening for IDH1 and IDH2 aberrations is not a

References


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