Although mature clinical data from multiple ongoing studies have not yet been reported, it appears that, although vemurafenib can achieve rapid and clinically valuable remissions, elimination of detectable minimal residual disease (MRD) has not yet been reported even after complete response. Currently, the most sensitive standard test for HCL MRD is flow cytometry of the bone marrow aspirate. As shown in this study, the ability of bone marrow stromal cells to block the effect of BRAF inhibition suggests a possible mechanism for persistence of HCL in the bone marrow by flow cytometry after clearance of MRD by other studies. However, persistence of MRD only in the bone marrow is common in HCL after other types of treatments as well. Clinical relapse within several months has been documented after partial response to vemurafenib. How long patients can be maintained after relapse from complete or partial remission before requiring additional therapy has yet to be reported. Thus far, other treatments for HCL that are associated with elimination of HCL MRD include cladribine alone, at least in a minority of cases, purine analogs combined with rituximab in a majority of cases, and the anti-CD22 recombinant immunotoxin moxetumomab pasudotox. It will be exciting to determine whether MRD elimination and prevention of relapse in HCL can be achieved by combining targeted approaches and lead to chemotherapy-free initial and salvage treatment of this disease.

Conflict-of-interest disclosure: The author is a coinventor on the National Institutes of Health (NIH) patent for moxetumomab pasudotox, and coinventor on the National Institutes of Health immunotoxin moxetumomab pasudotox.

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Comment on Nair et al, page 1256

NKT-dependent B-cell activation in Gaucher disease

Mariolina Salio and Vincenzo Cerundolo

In this issue of Blood, Nair et al describe a new population of type II natural killer T (NKT) cells with follicular helper phenotype (TFH), which is more abundant in patients and mice with Gaucher disease (GD) and is capable of regulating B-cell activity.

It is now well established that αβ T lymphocytes recognize not only peptide epitopes but also lipid and glycolipid antigens presented by nonpolymorphic CD1 molecules. Of the 5 CD1 family members expressed in humans, CD1d molecules are highly conserved in mammalian species and present endogenous and microbial glycolipids

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to a subset of T cells known as NKT cells, so called because they express natural killer cell markers in addition to a T-cell receptor (TCR). Two subsets of CD1d-restricted NKT cells have been described: (1) invariant NKT (iNKT) or type I NKT cells, expressing a semi-invariant TCR; and (2) type II NKT cells, with a much broader TCR repertoire. The prototype lipid antigen for type I iNKT cells is α-galactosylceramide (α-GaCer), a marine-sponge–derived glycosphingolipid, and the availability of CD1d-α-GaCer tetramers has allowed a detailed understanding of the role of iNKT cells in several disease settings. Conversely, there is no such prototypic lipid antigen recognized by type II NKT cells, and because of the lack of specific tools to identify them, this population has been less characterized. The best-studied antigen for type II NKT cells to date is sulfatide, a myelin–derived glycolipid. In addition, reactivity to lysosphospholipids, which are generated during inflammatory responses following hepatitis B infection or in multiple myeloma, has been reported.

Both subsets of NKT cells are innate-like lymphocytes that rapidly produce large amounts of cytokines upon TCR engagement and play an important immune-regulatory role in inflammatory conditions, autoimmunity, and cancer. Hence, understanding the identity of antigens that trigger NKT cell activation in health and disease is of importance, as harnessing these cells in vivo may provide therapeutic opportunities to either enhance or suppress immune responses.

Dysregulation of lipid metabolism occurring in obesity and congenital metabolic disorders has the potential to affect the development and/or function of NKT cells and the concomitant chronic inflammation. Gaucher disease (GD) is a disorder of glycosphingolipid (GSL) metabolism due to an inherited deficiency of the acidic β-glucosidase enzyme, resulting in progressive lysosomal accumulation of β-glucosylceramide (βGL1) and its decacylated product, glucosylsphingosine (Lyso-GL1; LGL1). Accumulation of these lipids in GD patients is associated with chronic inflammation and B-cell activation, often manifested by polyclonal and monoclonal gammopathy. To gain insights into mechanisms underlying lipid–associated inflammation in GD patients, Nair and al set out to analyze βGL1- and LGL1-specific T-cell responses.

Using βGL1- and LGL1-loaded CD1d tetramers, the authors convincingly demonstrated a lipid–specific CD1d–restricted NKT cell population in the blood of healthy donors and the spleen and liver of wild-type mice. Unlike type I NKT cells, human βGL1- and LGL1-specific NKT cells have a naive phenotype and a higher proportion of CD8 expression. Furthermore, although a detailed analysis of the functional specificity and affinity of individual β families for βGL1- and LGL1-CD1d complexes remains to be done, analysis of TCRβ usage following cell sorting and lipid–specific expansion revealed a much broader T-cell repertoire than anticipated from previously published data on sulfatide–specific T cells. Results obtained with in vitro–expanded βGL1- and LGL1-specific NKT cells revealed that, despite a similar TCRβ usage, the 2 populations are not cross-reactive, as they can specifically recognize βGL1 and LGL1 pulsed target cells, respectively. From the broad pattern of tetramer staining observed, a wide range of binding affinities is expected, suggesting that combined biophysical and structural data will eventually elucidate the fine details of this molecular recognition.

Despite expression of the transcription factor PLZF, a distinct transcriptional profile marks GL1- and LGL1–specific NKT cells in comparison with type I NKT cells, with a prominent Th-17 and a Th11 signature. Interestingly, the Th11 signature is present also at steady state, unlike type I NKT cells, a fraction of which acquire it only upon antigen stimulation. Experiments performed in wild-type mice revealed that in vivo activation of βGL1- and LGL1–specific T cells with their cognate antigens led to the induction of a germinal center B-cell response and lipid–specific antibodies. Likewise, in vitro activation of human βGL1- and LGL1–specific T cells induced plasmablast differentiation from cocultured autologous B cells. However, it remains to be determined whether the B memory responses elicited by type II NKT cells are short lived, as seen in the case of type I NKT Tfh.

These results are of great interest, as the authors report an increased frequency of LGL1–specific T cells in a mouse model of GD and in patients with GD (over 20-fold in mice and 3-fold in humans). Notably, the frequency of type I NKT in GD mice is significantly reduced, in agreement with data showing impairment in their selection in mouse models of lysosomal storage disorders. The mechanisms by which the frequency of LGL1–specific T cells is selectively enhanced in GD patients and mice remain unclear. However, it is tempting to speculate that enhanced availability of LGL1, and/or factors regulating its loading or intracellular trafficking (in the presence of lysosomal GSL accumulation), may affect the density of LGL1–CD1d complexes presented by antigen-presenting cells. Determination of the TCR usage of LGL1–specific T cells in GD patients and their affinity of binding to LGL1–CD1d complexes will also provide important insights into the understanding of their selective expansion and their potential role in GD. Interestingly, LGL1–specific T cells in GD patients display a memory phenotype, consistent with in vivo antigen exposure, and their increase correlates with clinical disease activity and serum levels of inflammatory cytokines. However, it remains to be determined whether antibodies to βGL1 and LGL1 are detectable in the serum of mice and humans with GD and whether their titers correlate with frequencies of lipid–specific NKT cells and disease activity. Selective deletion of CD1d expression on B cells or myeloid cells in murine models of GD will be of importance to provide conclusive evidence in support of the hypothesis that βGL1- and LGL1–specific T cells might modulate B-cell activation and chronic inflammation (see figure). The imbalance between type I and type II NKT cells, together with chronic inflammation, may also be contributing to the onset of hematologic malignancies, often associated with the progression of GD. Further elucidation of the role of type I and type II NKT cells will advance our understanding of the pathophysiology of GD and associated disorders and possibly open new therapeutic strategies, in association with the currently available enzyme-replacement therapy and substrate-reduction therapy.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Comment on Shaham et al, page 1292, and Wang et al, page 1302

A 2-way miRror of red blood cells and leukemia

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In this issue of Blood, the articles by Shaham et al1 and Wang et al2 are the first to identify microRNA 486 (miR-486) as a requisite oncomiR and credible therapeutic target in myeloid leukemia of Down syndrome (ML-DS) and chronic myeloid leukemia (CML) by showing that these 2 leukemias co-opt miR-486 functions in normal erythroid progenitor progrowth and survival activity.

The figure summarizes these 2 independent reports, which delineate the mechanisms leading to the aberrant overexpression of miR-486 in ML-DS and CML (panel A). Their highlights are described in greater detail below. In sum, the articles clearly demonstrate that miR-486 directs erythroid differentiation of normal hematopoietic cells involving activation of the AKT pathway (panel B), which is mirrored by a similar erythroid phenotype signaled through miR-486/AKT in leukemia cells that also acts to promote cell survival (panel C). The extensive and congruent results using human and mouse in vivo and in vitro models combined with primary human leukemia and normal hematopoietic cells underscore the importance of miR-486 as a conserved mediator of erythropoiesis and leukemogenesis. Moreover, these studies provide the initial proof of principle for miR-486 as a therapeutic target in CML and ML-DS, laying the groundwork for follow-up in vivo preclinical testing.

Infants and children with Down syndrome (DS) have significantly increased risk of developing transient myeloproliferative disorder (TMD), which sometimes transforms to myeloid leukemia (ML-DS), the most common subtype being acute megakaryoblastic leukemia (AMKL).3 Acquired somatic mutations in the megakaryocyte/erythroid-lineage specifying transcription factor GATA1 generate a short isoform (GATA1s) that cooperates with trisomy 21 early on in the evolution of TMD and ML-DS.4 Reported herein, microRNA (miRNA) expression analyses on bone marrow from patients with ML-DS, non-DS AMKL, or remission samples led to the discovery by Shaham et al that miR-486 is uniquely overexpressed in ML-DS patients (panel A). On the other hand, Wang et al independently discovered that miR-486 is the most highly expressed miRNA in their cohort of patients with CML, a molecularly, pathologically, and phenotypically distinct myeloid neoplasm from ML-DS.5 The Philadelphia chromosome t(9;22) rearrangement generating the BCR-ABL tyrosine kinase fusion protein is the most common and the earliest initiating event in CML pathogenesis.

What is driving miR-486 expression? Because GATA1 mutations are exclusively found in ML-DS, the expression pattern of miR-486 hinted that GATA1s might be its upstream regulator in normal and malignant hematopoiesis. Indeed, Shaham et al uncover that (1) miR-486 is encoded within the GATA1 target gene ANK12; (2) miR-486 positively correlates with GATA1s in primary ML-DS; and (3) miR-486 expression changes concordantly with manipulation of GATA1 or GATA1s in human ML-DS cell lines. Conversely, in CML, Wang et al find that expression of BCR-ABL leads to significant


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