found a correlation between the prevalence and/or titer of antibody and the severity of thrombocytopenia.

The fact that the severity of the thrombocytopenia and the duration of disease were comparable in the 2 populations reported by Fabris et al raises several interesting questions concerning the antibody-negative ITP population. Is the threshold required to clear antibody-coated platelets below the detection limits of the assay employed? If so, what caused the increase in antibody production over time in some patients but not in others? Alternatively, does somatic mutation of the autoantibodies lead to the recognition of additional epitopes, including one or more of the more prevalent platelet glycoprotein complexes that are detected in these assays, thereby enhancing platelet opsonization or further impeding platelet production? Or might T-cell–mediated cytotoxicity,[6] alterations in Fcγ receptor–mediated platelet clearance, or additional, as yet unrecognized, processes make a greater contribution to the severity of the thrombocytopenia in this subpopulation?

Although platelet antibody testing cannot as yet be recommended in routine clinical practice to either make or exclude the diagnosis of ITP, the study by Fabris et al raises the possibility that measuring platelet-specific antibodies may be of use to prognosticate the clinical course in patients with an established diagnosis. Serial prospective studies in much larger patient populations showing that "seroconversion" precedes clinical deterioration in the antibody-negative group would be required to prove this point and to identify how much warning, if any, such data provide.

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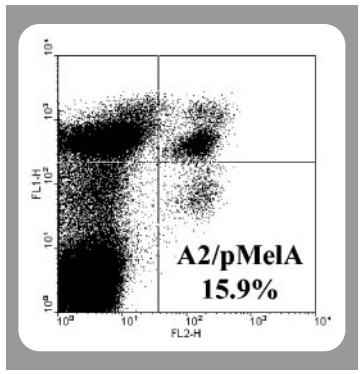
IMMUNOBIOLOGY

Generating CTL by HLA transplant

T-cell immunotherapy of human cancers is in most cases dependent on circumventing the tolerance of T cells to tumor-associated antigens. One approach to circumvent T-cell tolerance would be formed by HLA-mismatched bone marrow transplantation (BMT) coupled to donor lymphocyte infusion. Unfortunately, this approach is complicated by the fact that many of the infused cytotoxic T lymphocytes (CTLs) will recognize healthy tissues, causing severe graft-versus-host disease.[1] In this issue, Savage and colleagues (page 4613) describe an elegant and straightforward strategy to selectively generate tumor-specific CTLs from HLA-mismatched donors. Infusion of such cells holds promise for the induction of a tumor-selective T-cell attack upon HLA-mismatched BMT.

Many of the tumor antigens that can form targets for CTL attack are self-antigens, and the leukemia-associated Wilms tumor antigen (WT1) and melanoma-associated Melan-A antigens that are used by the authors are no exception.[2] If CTLs specific for these antigens can be found in patients, their affinity, and hence their activity, is often low because the high-affinity self-specific T cells have been deleted in the thymus or rendered inactive. The T cells of an HLA-mismatched individual could be a solution to this problem, as they have not been exposed to the donor's HLA and are thus not affected. However, if this heterogeneous population of T cells is transferred into the donor, the allogeneic T cells will



recognize a broad range of allo-HLA/peptide combinations, leading to a general anti-allo response.

The crucial element of the technique developed by Savage and colleagues is to selectively expose T cells of the donor to the allogeneic HLA complex that contains the tumor antigen, by conjugating these HLA-peptide complexes to donor-derived B cells. In vitro stimulation of the donor T cells with the "HLA-transplanted" B cells results in an allo-HLA–restricted response that is focused on a single tumor antigen. The resulting CTLs appear to be of high affinity, consistent with the notion that they were derived from an individual in whom tolerance was not an issue. More importantly, the CTLs also seem to be selective for this HLA-peptide complex and appear to ignore the large repertoire of other HLA-peptide combinations. This is all the more remarkable in view of the fact that the approach used to generate these cells does not actively select against cross-reactivity with other allo-HLA/peptide combinations. Hence, a more extensive analysis of this issue before clinical application seems prudent.

As the authors state, these lymphocytes can be given following HLA-mismatched transplantation in leukemia patients to reduce chances of relapse. In addition, a combination of an HLA-mismatched BMT plus alloreactive tumor-specific CTLs might be worth pursuing for tumors like melanoma, for which conventional BMT has not

proven to be beneficial. Alternatively, this technology may be used to generate a collection of high-affinity tumor-specific T-cell receptors (TCRs). In the future, such TCRs may conceivably be used to endow autologous T cells with a new tumor specificity.[3] Regardless of how these cells are used, the bottom line of these studies appears to be that the successful generation of tumor-specific CTLs can start with transplanting HLA molecules.

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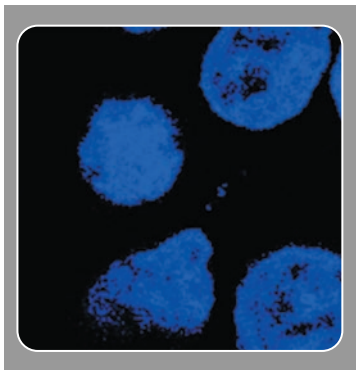
NEOPLASIA

SMRT interactions, repression, and Flt3-ITD

It is well established that mutations of the Fms-like tyrosine kinase 3 (Flt3) receptor tyrosine kinase occur in acute myelogenous leukemia (AML).[1-3] Approximately 20% to 30% of AML cases are characterized by the presence of mutant Flt3 proteins with internal tandem duplications (ITDs) in the juxtamembrane domain of Flt3 (reviewed in Levis and Small,[1] Stirewalt and Radich,[2] and Gilliland and Griffin[3]). The presence of ITD mutations in Flt3 (Flt3-ITD) results in dimerization and phosphorylation of this receptor tyrosine kinase and ultimately the generation of downstream signals that promote leukemic cell growth.[1-3] Such constitutive activation of Flt3 in leukemias expressing Flt3-ITD appears to have important pathogenetic implications and contributes to the development of the leukemic phenotype. Flt3-ITD mutations alone are not sufficient to cause AML. The presence of additional molecular abnormalities appears to be necessary, as shown by studies demonstrating that ectopic expression of Flt3-ITD in mouse bone marrow cells, followed by bone marrow transplantation, results only in the



development of a chronic myeloproliferative disease.[4] Nevertheless, expression of Flt3-ITD mutations has prognostic significance in AML and correlates with decreased response to chemotherapy and poor prognosis.[3]

Because of the importance of Flt3-ITD mutations, extensive efforts have been made over the years to understand the mechanisms of generation of cellular signals by the constitutively activated Flt3 tyrosine kinase. Several mitogenic signaling pathways have been shown to act as downstream effectors for the Flt3 receptor tyrosine kinase,[1-3] but the precise contribution of such signaling cascades in the development of the leukemic phenotype remains to be determined. In this issue of *Blood*, Takahashi and colleagues (page 4650) provide evidence for a novel mechanism by which Flt3-ITD mutations regulate induction of mitogenic signals. Their data provide the first evidence that Flt3-ITD regulates the function of the promyelocytic leukemia zinc finger (PLZF) transcriptional repressor and demonstrate that in the presence of Flt3-ITD mutations, the interaction of silencing mediator of retinoic and thyroid hormone receptors (SMRT) with the PLZF transcriptional repressor is blocked. Moreover, the authors establish that PLZF-mediated transcriptional repression is diminished and the suppression of leukemic cell growth by PLZF is abrogated. These data provide important new insights into the mechanisms of leukemogenesis by Flt3-ITD and suggest that inhibition of the function of PLZF and other transcriptional repressors that interact with SMRT may be of importance in leukemogenesis. A similar mechanism of reversal of PLZF repression may also apply in transformation by AML1-ETO,[5] further indicating the functional relevance of SMRT-regulated repressors in the control of leukemic cell growth.

Altogether, the data from Takahashi et al raise the possibility that, beyond direct activation of mitogenic cascades, a primary mechanism by which abnormal oncoproteins promote leukemic cell proliferation is inhibition of the function of transcriptional repressors. It will be important to expand on these studies and, in future studies, determine whether other well-known oncoproteins also negatively regulate transcriptional repression. Further work in that direction may provide important new insights into the pathogenetic mechanisms of hematologic malignancies and facilitate the development of future translational approaches for the treatment of leukemias.

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