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Immune rage against MAGE unleashed

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In this issue of *Blood*, Goodyear and colleagues show that CD8 T-cell immunity against melanoma-associated antigen (MAGE)—a cancer testis antigen (CTA) not normally expressed in hematopoietic cells—is induced in leukemia patients treated with DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors azacitidine (AZA) and sodium valproate (VPA), respectively.[1] The authors report that up-regulation of MAGE in acute myeloid leukemia (AML) by AZA plus VPA is associated with major clinical responses and an increase of MAGE-specific effector T cells. Will this observation lead to testing of these drugs in immune-therapy strategies?

During the multistep pathogenesis of AML, chromosomal translocations that mostly lead to loss-of-function mutations in genes such as transcription factors and tumor suppressors, together with activating mutations in genes such as hematopoietic tyrosine kinases, cooperate to induce cell proliferation and impair differentiation resulting in the leukemic phenotype.[2,3] Epigenetic alteration of chromatin structure can also impair gene expression. Altered methylation of cytosine-phosphate-guanosine (CpG) islands, such as in the promoter region of genes, is common in many malignancies including AML and myelodysplastic syndrome (MDS), and hypermethylation can lead to inactivation, or "epigenetic silencing," of genes[4] including tumor suppressor genes. In addition, deacetylation of histone proteins facilitates binding to DNA, resulting in repression of transcription by limiting access of transcription factors to the DNA. AZA and VPA can reactivate silenced genes and induce cell differentiation, and changes in CpG methylation in AML and MDS correlate with clinical responses.[5,6] The induction of epigenetically silenced proapoptotic genes that drive differentiation and programmed cell death is the prevailing theory in explaining how DNMT and HDAC inhibitors may function in leukemia. Nevertheless, a direct cause-and-effect mechanism linking specific aberrantly expressed genes with the elimination of leukemia has not yet been shown. Therefore, the indirect mechanism proposed by Goodyear et al,[1] which links epigenetically modified MAGE expression in leukemia cells to adaptive T-cell immune responses against MAGE, is a novel and important breakthrough.

There is substantial evidence of clinically significant T-cell immunity to leukemia in the setting of allogeneic stem cell transplantation,[7,8] donor lymphocyte infusion,[9] and treatment with interferon.[10] Treatment with vaccines or with antigen-specific T cells has shown biologic and clinical activity, supporting continued investigation of immune-based treatment approaches. Target antigens recognized by T cells include minor histocompatibility antigens (mHAs), leukemia-associated antigens (LAAs), and CTAs such as NY-ESO-1 and MAGE, which are expressed only in hematopoietic tissue or are aberrantly expressed in leukemia. T-cell receptors (TCRs) on CD8 T cells recognize peptides 8- to 11-aa long that are derived from intracellular proteins, and presented on major histocompatibility complex I (MHC-I) molecules on the cell surface. Antigen overexpression can increase target cell susceptibility to T-cell recognition because surface peptide antigen density is sufficient to trigger the T-cell activation threshold. However, if TCR affinity for peptide/MHC is sufficiently high, T cells can be overstimulated and will undergo apoptosis through a process of clonal deletion.[11] This can facilitate leukemia outgrowth because residual low-affinity T cells are functionally less effective against leukemia due to antigen expression insufficient to trigger T-cell activation. Therefore, strategies to increase tumor antigen expression in leukemia may increase susceptibility to T cell–mediated killing.

It is known that CpG motifs in the promoter regions of CTAs, and of MAGE in particular, are hypermethylated in solid tumor cells, lymphoid leukemia cells, and multiple myeloma, and that expression of CTA is increased by AZA or decitabine.[5] In addition, Goodyear et al previously showed that the frequency of CTA-specific T cells in patients with multiple myeloma correlates with disease burden, and that AZA plus VPA increased recognition of myeloma cells by MAGE-specific CD8 T cells. In the current study, they found MAGE-specific T cells increased from undetectable levels in 10 of 21 patients with AML or MDS after treatment with AZA plus VPA, including in 8 of 11 patients who achieved a major clinical response after therapy.[1] T cells were enumerated with peptide/MHC tetramers and the majority of MAGE-specific T cells displayed an effector phenotype. Goodyear and colleagues also showed that MAGE-specific T cells from some patients secreted interferon-γ in response to stimulation with specific HLA-restricted MAGE-derived peptides.

The results of the current study together with previous studies support the idea that tumor antigen overexpression leads to T-cell recognition. If tumor antigens are down-regulated by epigenetic processes during pathogenesis of AML, then AML may escape immune detection. However, if antigen down-regulation can be reversed with epigenetic modification, tumor cell susceptibility could be restored, leading to proliferation of tumor antigen–specific T cells. The study by Goodyear and colleagues shows that AZA and VPA can up-regulate CTA in AML, which would be expected to increase susceptibility of leukemia cells to attack by low-affinity T cells. However, as the authors point out, other mechanisms may be operative, such as modulation of adhesion molecules or costimulatory molecules involved in T-cell activation.

Although this study focused on the effect of epigenetic modifications on peripheral T-cell immunity, there are interesting parallels to epigenetic mechanisms thought to be important for regulating antigen expression during T-cell selection in the thymus.[12] Autoimmune regulator (*AIRE*) is a transcriptional regulator expressed in thymic medullary epithelial cells (MECs) that promotes clonal deletion of self-reactive thymocytes by inducing ectopic expression of a large repertoire of transcripts encoding proteins normally restricted to differentiated organs in the periphery.[12] Studies suggest that *AIRE* impacts transcription at several levels, including epigenetic modifications of genes encoding peripheral tissue antigens (PTAs).[12] PTAs are processed

and then presented on surface MHC molecules on thymic MEC. Mature thymocytes percolate through the medulla, and, if their TCRs recognize a PTA/MHC complex in the appropriate affinity/avidity window, they will be overactivated and deleted from the repertoire. The results for MAGE by Goodyear et al[1] suggest that thymic expression of PTAs might be regulated by epigenetic modifiers. For instance, it is conceivable that pharmacologic approaches to up-regulating islet cell antigens to promote negative selection of autoreactive T cells in a still-functioning thymus could be tested to prevent diabetes mellitus or other autoimmune diseases.

The question of whether CTAs are differentially up-regulated in AML and leukemia stem cells compared with normal cells and hematopoietic stem cells remains unanswered. Furthermore, do the effects on antigen expression extend to other tumor antigens, such as LAA and mHA, and to what extent are other self-antigens up-regulated? Importantly, this study suggests a new strategy to identify tumor antigens by determining which genes are aberrantly expressed in cancer cells after exposure to agents that induce epigenetic modifications. Ultimately, the results reported by Goodyear and colleagues raise many additional questions and highlight the exciting possibility of future trials to test combinations of antigen-specific immune treatments with agents that epigenetically modify target antigen expression.

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

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MYELOID NEOPLASIA

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BRAF, a piece of the LCH puzzle

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LCH is a rare disorder affecting patients of all ages that is characterized by the pathologic accumulation of immature LC and other inflammatory cells in organs such as skin, bone, liver, lungs, bone marrow, and brain. Although first described over a century ago, the etiology of LCH has remained elusive. In this issue of *Blood*, Badalian-Very and colleagues provide exciting new insights into LCH by demonstrating that a subset of cases exhibit somatic activating mutations in the proto-oncogene *BRAF*.[1]

The clinical manifestations of Langerhans cell (LC) histiocytosis (LCH) are remarkably variable with some patients exhibiting localized involvement of specific sites and others developing a disseminated form of disease that mirrors acute leukemia. Although the majority of the patients with localized LCH may be cured of their disease, the outcome for those with systemic involvement is suboptimal with 20% to 50% of patients dying despite the use of intensive multiagent chemotherapy and/or stem cell transplantation. To improve the outcome for patients with LCH, particularly those with high-risk disease, understanding the pathogenesis of this puzzling disorder is imperative.

Researchers have long debated whether LCH represents a true malignancy or a reactive immune condition. Resolving this issue is important as the answers are likely to have consequences in terms of diagnostic testing, outcome prediction, therapy, and patient counseling. Studies in favor of the notion that LCH is a malignancy include the demonstration that LC from nonpulmonary lesions are monoclonal based on a pattern of highly skewed X-chromosome inactivation.[2,3] Other supportive findings include the immature appearance of lesional LC, the existence of rare familial clusters, evidence of cell-cycle dysregulation within lesions, and the presence of significant telomere shortening of LCH cells compared with LC from other inflammatory lesions. Epidemiologic data also suggest a close association between LCH and cancer, particularly lymphoma and acute lymphoblastic leukemia. In support of a potential clonal relationship among the disorders are reports documenting identical molecular changes in the lymphoma or leukemia and the LCH.[4-6] In contrast, supporters of the opinion that LCH is a reactive process emphasize that clonal cell populations are commonly present within the immune system and that phenotypically immature LC often accumulate in areas of chronic inflammation (eg, dermatopathic lymphadenitis). The lesional expression of inflammatory chemokines and cytokines (most recently, interleukin 17, a key cytokine in several autoimmune disorders) has been reported, although conflicting data exist.[7,8] Finally, although high levels of p53 protein have been described in LCH, no mutations in the *TP53* gene have been reported, nor have recurrent chromosomal translocations or other genomic abnormalities been consistently described.[9]

In this study, Badalian-Very and colleagues demonstrate that 35 (57%) of 61 LCH specimens exhibit mutations in *BRAF* that encode a known oncogenic V600E form of the BRAF protein. In contrast, the related disorders