

reproduced in a large-scale setting, the strategy described in this paper would allow the production of large numbers of relatively pure Treg cells for adoptive transfer.

The author declares no competing financial interests. ■

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## IMMUNOBIOLOGY

Comment on Bobé et al, page 3967

# Poisoning autoimmunity

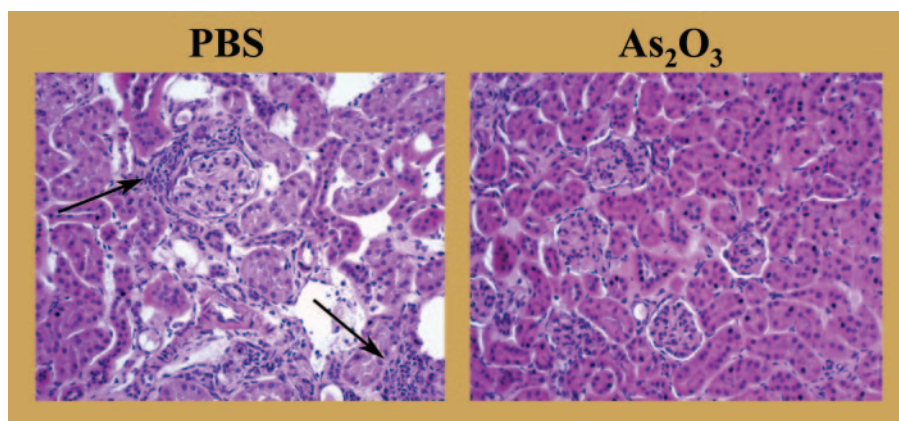
Vito Pistoia G. GASLINI INSTITUTE

In this issue of *Blood*, Bobé and colleagues demonstrate that the lymphoproliferative and autoimmune syndrome developing spontaneously in the MRL/*lpr* mouse, a model of human systemic lupus erythematosus (SLE), can be cured by arsenic trioxide ( $As_2O_3$ ). This finding has a high translational impact.

Arsenic trioxide ( $As_2O_3$ ) is a poison that has been used successfully to treat acute promyelocytic leukemia (APL).<sup>1</sup> At low doses ( $< 0.5 \mu M$ ), the drug induces differentiation of APL cells, whereas at higher doses it triggers apoptosis by activating the mitochondrial pathway. In addition,  $As_2O_3$  promotes the degradation of the PML-RAR $\alpha$  fusion protein over a wide range of concentrations.<sup>2</sup>

The Fas-deficient MRL/*lpr* mouse is widely used as a model of systemic lupus erythematosus (SLE), a disease that still exhibits considerable morbidity and mortal-

ity. Cutaneous lesions, multiorgan lymphoid infiltrates, immune complex glomerulonephritis, high-titer anti-DNA autoantibodies, and vasculitis manifesting spontaneously in MRL/*lpr* mice recapitulate many of the clinical features of SLE.<sup>3</sup> In addition, MRL/*lpr* mice display abnormally expanded TCR $\alpha\beta^+$ , CD3<sup>+</sup>, CD4<sup>-</sup>, CD8<sup>-</sup> T cells, also known as double-negative (DN) T cells.<sup>4</sup> These cells, which are virtually absent from normal peripheral blood, may be increased in SLE patients and are a hallmark of the human autoimmune lymphoproliferative syndrome (ALPS).



Kidney sections from 4-month-old MRL/*lpr* mice treated daily for 2 months with  $As_2O_3$  ( $5 \mu g/g$ ) or PBS. Lymphoid infiltrates (arrows) are detected in PBS-treated (left panel), but not  $As_2O_3$ -treated (right panel), mice.

Bobé and colleagues injected  $As_2O_3$  intraperitoneally ( $5 \mu g/g$  per day) into MRL/*lpr* mice at different ages. The drug halted disease progression when administered as preventive regimen and cured mice with overt lymphoproliferation.  $As_2O_3$  reduced cutaneous lesions, lymphoid infiltrates in the lung and the kidney (see figure), and immune complex deposition in kidney glomeruli, significantly prolonging survival.

DN T cells were selectively forced by  $As_2O_3$  to commit suicide through up-regulation of activated caspases-2, -8, and -9. Expression of FasL, which is up-regulated in MRL/*lpr* mice, was dampened by the drug. Serum levels of IFN- $\gamma$ , IL-18, TNF, and IL-10, as well as those of nitrite (a marker of nitric oxide synthase activity), were reduced by  $As_2O_3$  treatment. The authors conclude that  $As_2O_3$  also targeted activated T helper-1 (Th1) cells, natural killer (NK) cells, and macrophages through caspase-independent mechanisms. The low endogenous levels of GSH were up-regulated by  $As_2O_3$ .

Altogether,  $As_2O_3$  dampens autoantibody production by inhibiting IL-10 production, decreases nitric oxide production by down-regulating the synthesis of both IFN- $\gamma$  and NOS, and restores tissue protection from free radical-induced damage by increasing GSH levels.

The study by Bobé et al is impressive, but some crucial issues (such as the molecular basis of  $As_2O_3$  selectivity for DN T cells, and the mechanisms of drug-induced caspase activation in DN T cells and of elimination of activated Th1 cells, NK cells, and macrophages) warrant further investigation.

In APL studies, liver toxicity was the main side effect following intravenous administration of  $As_2O_3$ .<sup>1</sup> The findings of Bobé et al support the feasibility of a clinical trial in treatment-resistant patients with SLE or possibly ALPS. However, patient response to  $As_2O_3$  may differ from that of MRL/*lpr* mice; the intraperitoneal route is not applicable to humans, and the appropriate schedule must be identified; and  $As_2O_3$  toxicity in patients with systemic autoimmunity may differ from that reported in APL patients. In spite of these caveats, a new star for the treatment of systemic B-cell autoimmunity is born and, hopefully, will grow up soon.

The author declares no competing financial interests. ■

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## ● ● ● NEOPLASIA

Comment on Pasqualucci et al, page 4146

# *NPM1* mutation in AML: WHO and why?

David Grimwade KING'S COLLEGE LONDON SCHOOL OF MEDICINE

In this issue, Pasqualucci and colleagues show that the nucleophosmin (*NPM1*) mutation can be found in multiple myeloid lineages in AML, carrying implications for its cellular origins. They also suggest the recognition of this subset of AML in the World Health Organization (WHO) classification of myeloid neoplasms.

Our understanding of acute myeloid leukemia (AML) has improved dramatically in the past 2 years with the discovery by Falini et al<sup>1</sup> that *NPM1* is mutated in around half of cases with a normal karyotype, making it the most common molecular lesion identified in this disease to date. Although the functions of *NPM1* are not completely understood, it is thought to play an important role in centrosome assembly, and has RNA binding and chaperone activity. The latter regulates the Arf-p53 pathway, suggesting that *NPM1* has tumor-suppressor activity, a hypothesis supported by murine models of *NPM1* loss of function.<sup>2</sup> The various *NPM1* mutations identified in AML are heterozygous and involve the C-terminal region encoded by exon 12. These not only disrupt key tryptophan residues that are required for localization to the nucleolus, but also generate a nuclear export signal leading to delocalization of mutant *NPM1* to the cytoplasm where it sequesters residual wild-type protein from the nucleus.<sup>3</sup> As an alternative to sequence analysis, the presence of an underlying *NPM1* mutation can be inferred by immunohistochemistry, which shows an abnormal cytoplasmic localization of the protein in leukemic blasts.<sup>1,3</sup>

In this issue, Pasqualucci and colleagues have used antibodies that specifically detect wild-type or mutant forms of *NPM1* in bone marrow trephine biopsy sections and have undertaken sequence analysis of DNA derived from laser-microdissected cells to investigate the cellular origins of the *NPM1*-mutated

clone. Granulocytic, monocytic, erythroid, and megakaryocyte series were found to be involved, with at least 2 lineages harboring the mutation in more than 60% of the 161 *NPM1*-mutated cases analyzed. These findings are consistent with the *NPM1* mutation arising in myeloid or multipotent progenitors and raise the distinct possibility that this may be a primary lesion in AML, present in the leukemic stem cell population. There is already some circumstantial evidence to support such a notion. Primary *NPM1*-mutation-positive leukemic cells can engraft in immunocompromised mice.<sup>4</sup> Moreover, in cases where *NPM1* and *FLT3* are both mutated, multiple *FLT3* internal tandem duplications (ITDs) can be detected within leukemic subpopulations on the background of a single *NPM1* mutation, implying that the latter was the first lesion to arise.<sup>5</sup> This is consistent with the observation that *NPM1* mutation status tends to be more stable over the disease course than that of *FLT3*. *NPM1* mutation is only rarely observed in AML with balanced translocations and is associated with distinct biologic features, gene expression profile, and a relatively favorable prognosis in the absence of *FLT3-ITD*.<sup>1,5,6</sup> Together, these observations lend support to the recognition of a new category of AML.

The WHO classification has represented an important step forward, taking into account major advances in understanding of the etiology and molecular basis of AML, recognizing disease entities that demand differing manage-

ment strategies.<sup>7</sup> These include cases with chimeric oncoproteins generated as a result of recurrent cytogenetic abnormalities, which are currently restricted to t(8;21)/*AML1-ETO*, inv(16)/*CBFB-MYH11*, t(15;17) and variants, and t(11q23)/*MLL* rearrangements and which together account for approximately a third of cases arising in children and younger adults.<sup>7</sup> It would seem to be in the spirit of the WHO classification to extend this category to include a wider range of molecular lesions that are fundamental to AML pathogenesis and relevant to its treatment. Hence, there appears to be a strong rationale for the development of a more inclusive category of "recurrent cytogenetic/molecular abnormalities." Molecular screening for the *NPM1* mutation is rapidly being introduced into the diagnostic workup of AML to enhance risk-stratified treatment approaches<sup>6</sup>; this will make this molecularly defined and cytogenetically cryptic entity, which is detected in approximately a third of AML cases, a prime candidate for recognition in future revisions to the WHO classification of myeloid neoplasms.

The author declares no competing financial interests. ■

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