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.

REFERENCES
1. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25): breakdown of the negative selection mechanism and reproductive autoimmunity. J Immunol. 1995;155:1151-1164.
2. Szanya V, Ermann J, Taylor C, Holness C, Fathman CG. The subpopulation of CD4+ CD25+ splenocytes that delays adoptive transfer of diabetes expresses L-selectin and high levels of CCR7. J Immunol. 2002;169:2461-2465.
3. Taylor PA, Panoskaltsis-Mortari A, Sweden JM, et al. L-Selectin(hi) but not the L-selectin(lo) CD4+25+ T regulatory cells are potent inhibitors of GVHD and BM graft rejection. Blood. 2004;104:3804-3812.
4. Ermann J, Hoffmann P, Edinger M, et al. Only the CD62L+ subpopulation of CD4+ CD25+ regulatory T cells protects from lethal acute GVHD. Blood. 2005;105:2220-2226.

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 (As2O3). This finding has a high translational impact.

Arsenic trioxide (As2O3) is a poison that has been used successfully to treat acute promyelocytic leukemia (APL). At low doses (< 0.5 μM), the drug induces differentiation of APL cells, whereas at higher doses it triggers apoptosis by activating the mitochondrial pathway. In addition, As2O3 promotes the degradation of the PML–RARα fusion protein over a wide range of concentrations.

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 mortality. 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. In addition, MRL/lpr mice display abnormally expanded TCRαβ+, CD3+, CD4−, CD8− T cells, also known as double-negative (DN) T cells. 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).

Bobé and colleagues injected As2O3 intraperitoneally (5 μg/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. As2O3 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 As2O3 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-γ, IL-18, TNF, and IL-10, as well as those of nitrite (a marker of nitric oxide synthase activity), were reduced by As2O3 treatment. The authors conclude that As2O3 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 As2O3.

Altogether, As2O3 dampens autoantibody production by inhibiting IL-10 production, decreases nitric oxide production by down-regulating the synthesis of both IFN-γ 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 As2O3 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 As2O3. 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 As2O3 may differ from that of MRL/lpr mice; the intraperitoneal route is not applicable to humans, and the appropriate schedule must be identified; and As2O3 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.
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 al1 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.2 The various NPM1 mutations identified in AML are heterogeneous 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.3

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.1,3

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.4 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.5 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.1,3,5 Together, these observations lend support to the recognition of a new category of AML.

The WHO classification has represented disease entities that demand differing management strategies.6 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.7 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 approaches8; 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.

REFERENCES
1. Falini B, Mecucci C, Tacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med. 2005;352:254-260.
2. Grisendi S, Bernardi R, Rossit M, et al. Role of nucleophosmin in embryonic development and tumorigenesis. Nature. 2005;437:147-153.
3. Falini B, Boldi N, Shan J, et al. Both carboxy-terminus NES motif and mutated tryptophan(s) are crucial for aberrant nuclear export of nucleophosmin leukemic mutants in NPMc + AML. Blood. 2006;107:4514-4523.
4. Pearce IDJ, Tausig D, Zibara K, et al. AML engraftment in the NOD/SCID assay reflects the outcome of AML: implications for our understanding of the heterogeneity of AML. Blood. 2006;107:1166-1173.
5. Thiede C, Koch S, Crestizig E, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood. 2006;107:4011-4020.
6. Mrozek K, Marucci G, Paschka P, Whitman SP, Bloomfield CD. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood. Prepublished on September 7, 2006, as DOI 10.1182/blood-2006-06-001149.
7. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of myeloid neoplasms. Blood. 2002;100:2292-2302.
Poisoning autoimmunity

Vito Pistoia