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associated CD20, and nearby B-cell plasma membrane are transferred from the B-cells and taken up and internalized by acceptor cells via their FcγRs.[8] The B cells then emerge alive but with considerably reduced CD20 target. Obviously this reaction can substantially decrease the efficacy of CD20 mAb–mediated therapy, and several clinical investigations and in vitro models have indeed demonstrated that after ADCC and complement are exhausted, this reaction can lead to almost complete loss of CD20 on CLL cells when patients are treated with the usual high doses of RTX or ofatumumab.[9] The role of neutrophils in these processes is uncertain, but the findings of Valgardsdottir et al clearly place neutrophils firmly on the negative side of the ledger with respect to CD20 mAb–mediated immunotherapy. Although the investigators' previous investigations as well as those of other groups suggested that neutrophils could kill CD20 mAb–opsonized B cells, the current analyses, based on combinations of time-lapse microscopy, confocal microscopy, and flow cytometry, reveal otherwise. Neither RTX nor OBZ mediate killing by neutrophils; only trogocytosis is observed.

The careful experimental design and interpretation of results in this report are important and informative and provide a cautionary note for future investigations of phagocytosis in similar systems. The investigators recognize that flow cytometry experiments can confuse phagocytosis with trogocytosis. That is, if membranes of mAb-opsonized donor cells are labeled with a fluorescent dye, and these opsonized cells are reacted with acceptor cells, then transfer of relatively small amounts of dye to acceptor cells (compared with no transfer with controls), observed over several time points, is actually diagnostic for trogocytosis. If full phagocytosis of donor cells by the acceptor cells were to occur, then acceptor cells would be expected to have fluorescence signal intensities approximately equal to those of donor cells. The investigators compared trogocytosis with another reaction, internalization, which can result in reduction of CD20 on mAb-opsonized cells. They found that over 3 hours, neutrophils promoted substantial loss of CD20 from RTX-opsonized B cells, but internalization (conducted in the absence of neutrophils) was quite modest, in excellent agreement with findings of Beum et al,[10] who reported that monocyte-mediated trogocytosis of CD20 on opsonized B cells was much faster than internalization.

FcγR-mediated trogocytosis has been demonstrated for several other mAbs and their cellular targets,[8] thus extending the importance of this report. Therefore, several research directions are indicated, with the goal of developing strategies that preserve cytotoxic mechanisms but prevent trogocytosis. First, during mAb therapy, after effector functions are exhausted, it will be important to determine which immune effector cells play the most important role in trogocytosis. Candidates now include circulating monocytes, neutrophils, NK cells, fixed tissue macrophages, and liver sinusoidal endothelial cells (LSECs).[8] In the case of CLL, circulating cells in blood samples obtained during and after CD20 mAb therapy should be examined. In particular, after trogocytosis, can CD20 and/or RTX be found inside neutrophils or other effector cells? If appropriate mouse models can be developed, analyses can be extended to include macrophages and LSECs. Although type II mAb OBZ did not promote killing of B cells by neutrophils, its ability to mediate trogocytosis was considerably less than that of RTX. To our knowledge, there have been no investigations as to whether OBZ mediates trogocytosis of CLL cells when it is used in the clinic, and this question should be addressed. Moreover, laboratory studies that compare RTX or OBZ with respect to trogocytosis mediated by other effector cells, including monocytes, macrophages, and NK cells, may be most informative.

Valgardsdottir et al have rigorously asked and answered an important question with respect to the role of neutrophils in mediating trogocytosis of CD20 mAb–opsonized cells. Their findings may have important clinical implications and set the stage for future intriguing studies both in the laboratory and in the clinic.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● MYELOID NEOPLASIA

Comment on Liyanage et al, page 2657

A mitochondrial drug to treat AML

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In this issue of *Blood*, Liyanage et al demonstrate that acute myeloid leukemia (AML) cells exhibit increased mitochondrial DNA (mtDNA) content and that the 2′,3′-dideoxycytidine (ddC; an inhibitor of mtDNA biosynthesis already used in clinic) selectively kills leukemia cells.[1]

AML is a blood cancer wherein the uncontrolled proliferation of immature myeloid cells leads to bone marrow failure. AML is now cured in only 30% of young patients, with a worse prognosis in older patients.[2] One of the problems in improving therapy in AML is that there are multiple types of AML that differ in genetic abnormalities, immunophenotype, and clinical features.[3]

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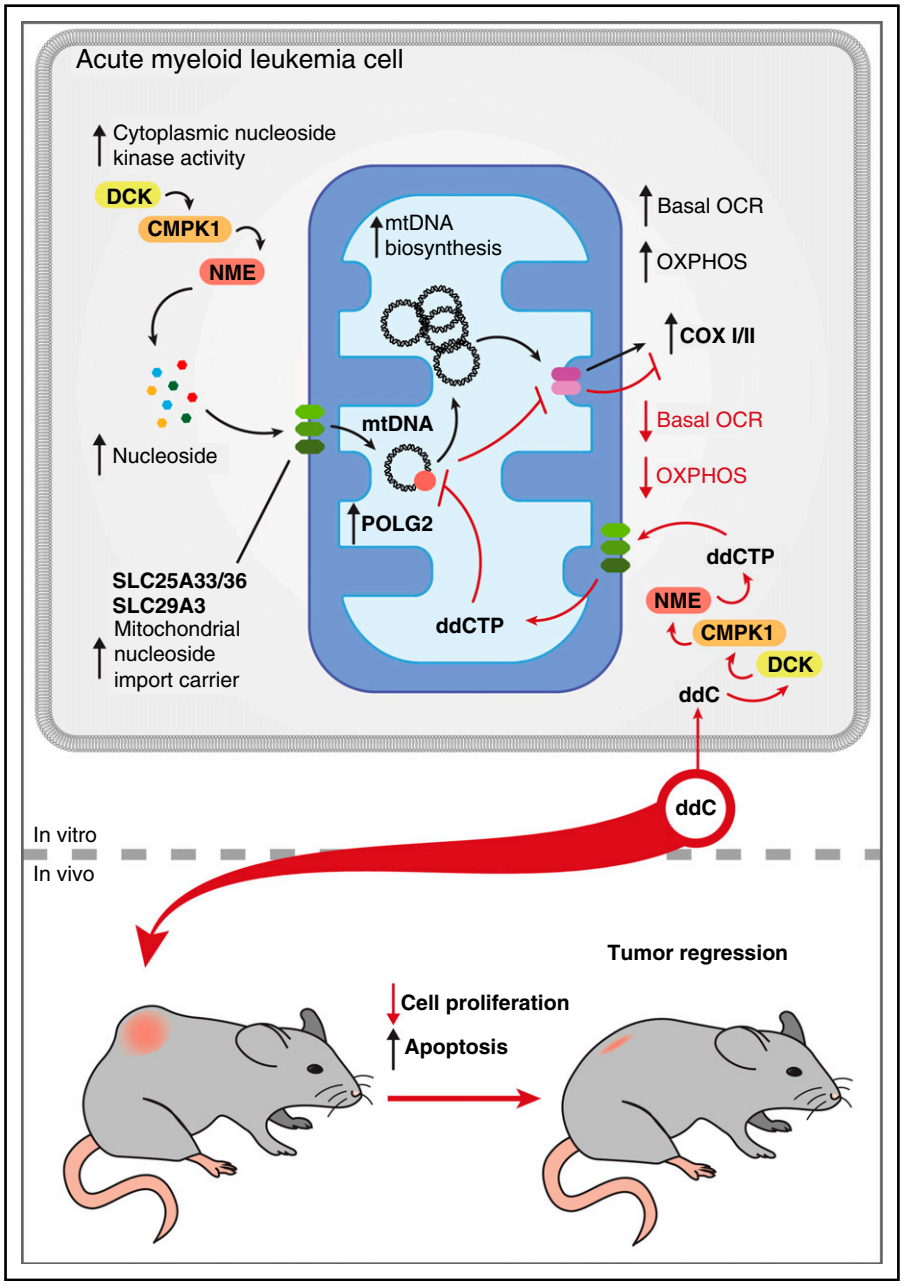

A cell belonging to a subtype of AML clusters which is characterized by high cytoplasmic nucleoside kinase activity (deoxycytidine kinase [DCK], CMPK1, and NME) is depicted. As a consequence, a large nucleoside pool is imported in mitochondria via the SLC25A33/36 and SLC29A3 import carriers as indicated by the green boxes on the left of the mitochondrial membrane. This pool favors mtDNA biosynthesis, oxidative phosphorylation (OXPHOS), and increased oxygen consumption rate (OCR). ddC treatment minimizes mtDNA content upon its activation in 2′,3′-dideoxycytidine triphosphate (ddCTP) (on the right) and import into mitochondria. This treatment allows the tumor regression in a mouse model. POLG2, DNA polymerase γ2.

The current study by Liyanage and coworkers focused on a subset of primary human AML cases characterized by increased mitochondrial biogenesis and reliance on oxidative phosphorylation in AML cells compared with normal progenitors. These features were linked to increased mtDNA biosynthesis. It is widely known that mtDNA content and its replication takes advantage of mitochondrial nucleotide pools; however, the large mtDNA content found in this AML subset requires the support of the nucleotides imported from the cytosolic compartment. By Affymetrix gene expression analysis, the authors observed an upregulation of the genes involved in mtDNA biosynthesis in a subset of AML samples that were not associated with known cytogenetic abnormalities. Moreover, the pattern was upregulated in leukemia cell lines but not in other cancer types. As increased mtDNA biosynthesis needs a massive infusion of nucleotides, the authors suggest that this could be accomplished by import of cytoplasmic nucleotides. Mitochondrial nucleotide transporters and cytoplasmic nucleoside kinase activity were investigated by immunoblotting and mass-spectrometry approaches. The analysis revealed increased nucleotide import from the cytoplasm due to SLC25A33, SLC25A36, and SLC29A3 activity and parallel augmented cytoplasmic nucleoside activity in AML cells compared with normal progenitors. The major question arising from these novel findings is how to use the discrepancies between AML and normal cells to selectively induce cell death (see figure).

In the last 10 years, there have only been refinements in AML chemotherapy.[4] The work of Liyanage et al has potential impact for new therapeutic approaches.

ddC is a nucleoside analog made by replacing the hydroxyl group in position 3′ of a pyrimidine. ddC is activated in ddCTP by cytoplasmic nucleoside kinases and imported into mitochondria where it is a selective inhibitor of mtDNA polymerase. The activation of ddC in ddCTP was greater in AML cells compared with control. ddC treatment was more effective in the inhibition of mtDNA content in AML cells leading to a decrease of the mtDNA-encoded COX I and COX II proteins resulting in alteration of mitochondrial morphology and activity in AML cells.

Although the clinical use of ddC is already known, these findings provide a molecular basis for future clinical studies. Since its approval in 1992, ddC has been used alone or in combination for the treatment of AIDS as a reverse transcriptase inhibitor[5]; however, severe adverse events were seen in a significant number of patients. Previous studies have shown an amplification of the mtDNA in AML[6] and suggest that this could be a therapeutic target.[7] Here, the use of ddC in an AML mouse model was encouraging, not only for the greater tumor regression obtained using ddC treatment (from 75% to a 90% of the total mass), but also for its potential effects on the leukemic stem cells and for the minimal cytotoxic effects in normal progenitor cells. Importantly, treatment with ddC did not affect normal mouse hematopoiesis, body

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weight, or behavior. Although this study has remarkable clinical implications, some issues have to be addressed. It would be interesting to investigate a putative ddC-induced resistance in the treatment of AML due to the onset of mutations in mtDNA.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● THROMBOSIS AND HEMOSTASIS

Comment on Yang et al, page 2667

Indoxyl sulfate, a uremic trigger for platelets

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In this issue of *Blood*, Yang et al explore the effects of the uremic toxin indoxyl sulfate (IS) triggering platelet hyperactivity in chronic kidney disease (CKD). In their mouse model of disease, the aging suppressor protein Klotho acts as an antidote for the toxin effects.[1]

Hemostasis is in a delicate equilibrium in CKD patients. Deficient hemostasis coexists paradoxically with accelerated atherosclerosis and an enhanced thrombotic risk. Uremic bleeding is multifactorial and has been attributed to platelet dysfunction, impaired platelet–vessel wall interactions, and altered rheological properties of the blood flow. Treatment with erythropoiesis-stimulating agents was introduced in the mid-1980s with a very favorable impact, not only in reducing the frequency of bleeding, but also in improving patients' overall quality of life.[2] The prothrombotic state in CKD may be related to an imbalance between coagulation factors and coagulation inhibitors, decreased fibrinolytic activity, platelet hyperactivity, and endothelial dysfunction. At present, although the incidence of bleeding is apparently decreasing, the thrombotic complications have become the main causes of mortality in this population.

Accelerated atherothrombosis in CKD has a complex etiology (see figure). Endothelial dysfunction coexists with a chronic inflammatory state and oxidative stress in uremic patients.[3,4] In the development of these pathological processes, there are at least 2 components: humoral and cellular. The humoral component consists of the presence of uremic toxins and those factors released by the activation of blood cells. The uremic toxins are present in patients with CKD, independent of treatment with renal replacement therapies (RRTs), and alter the function of the different cell populations involved in hemostasis (endothelial cells, platelets, and leukocytes). These activated cellular elements release cytokines that enrich the humoral component. In addition, RRTs themselves promote cell activation and further production and release of cytokines, further propelling the inflammatory reaction. Uremic toxins can be classified into 3 main groups: small water-soluble compounds, middle molecules, and protein-bound solutes, with the common characteristic of being difficult to eliminate by conventional RRTs.[5] IS, a protein-bound uremic toxin derived from the amino acid tryptophan, is produced by the intestinal flora and is 1 of the clinical factors thought to contribute to CKD progression.

Yang et al conducted a series of experiments to examine IS triggering of platelet activation and thrombosis both in vitro and in vivo in a mouse model. The authors present convincing data of platelet hyperactivity caused by IS, as demonstrated by an enhanced response to the platelet agonists thrombin and collagen; increased P-selectin expression; release of platelet microparticles; formation of heterotypic platelet–monocyte aggregates; and higher platelet adhesion in a thrombosis model of carotid artery occlusion. The platelet effect of IS, which is an activator of oxidative stress, seems likely to be mediated through the production of reactive oxygen species (ROS) and the activation of the inflammation-related protein p38 MAPK.

Furthermore, the authors show that Klotho protein modulates the effect of IS on platelet hyperactivity and thrombus formation, protecting against IS-induced atherosclerosis in apoE null mice. The role of Klotho in this setting is intriguing. Klotho is known to be an aging suppressor protein that acts as a scavenger for ROS overproduction. It is highly expressed in the kidney, and CKD is a state of Klotho deficiency, with negative systemic effects on numerous organs, including the cardiovascular system. Results provided by Yang et al, together with recently generated evidence,[6] indicate that this protein could be a future prophylactic and therapeutic target to modify or prevent the progression from acute to CKD with its associated cardiovascular risk.

There are a number of publications exploring the effect of IS on different cell types, such as endothelial cells, myoblast cells, and smooth muscle cells. IS is a protein-bound toxin, and high free concentrations of these compounds and/or excessively low albumin concentrations may distort the interpretation of the results obtained and therefore overestimate the toxic impact of IS.[7]

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A mitochondrial drug to treat AML

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