inside blood

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Mancuso et al, page 452

An endothelial metronome breast cancer?

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In this issue of *Blood*, Mancuso and colleagues present evidence that the number of apoptotic circulating endothelial cells is a promising predictor of outcome in patients undergoing metronomic chemotherapy for advanced breast cancer.

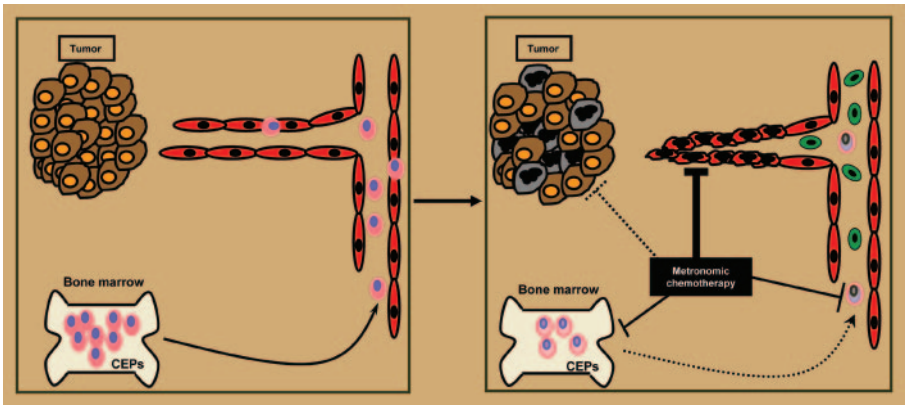
Breast cancer remains the leading cause of death in women aged 55 years or younger, and new approaches to treatment of advanced disease are urgently required. Neovascularization, or angiogenesis, is a rate-limiting step during tumorigenesis that promotes tumor cell survival and proliferation by ensuring a supply of oxygen and metabolites. In breast cancer, access to the vasculature also provides means for tissue invasion and metastasis to distant sites.

Antiangiogenic drugs and novel strategies that target the neovasculature are subjects of intense investigation and a popular focus of the drug development industry. One novel approach, termed "metronomic therapy," uses long-term administration of low doses of chemotherapy, which effectively inhibits the tumor's drug-sensitive endothelial cell compartment.[1] Preclinical models support the strong antiangiogenic effect of metronomic therapy[1]; however, classic methods for measuring regression of experimental tumors may not be appropriate for monitoring the efficacy of metronomic therapy in patients, and the development of reliable surrogate markers is required.

In this issue of *Blood*, Mancuso and colleagues quantified circulating endothelial cells (CECs) and circulating endothelial progenitors (CEPs) in the peripheral blood of patients with advanced breast cancer enrolled in a low-dose metronomic chemotherapy clinical trial.[2] Whereas baseline CECs showed no relationship with clinical benefit, univariate and multivariate analyses revealed that higher numbers of CECs after 2 months of therapy were significantly associated with longer progression-free survival and overall survival.

A novel and key aspect of the current study, in contrast to previous preclinical and clinical studies in this area, is in the analysis of apoptotic (rather than viable or total) CECs and their correlation with clinical outcome. In those patients who gained a clinical benefit, the number of CECs increased significantly, with the increase derived from apoptotic CECs. In contrast, patients who experienced no clinical benefit exhibited a decline in CECs. These results suggest that apoptotic CECs may be a useful indicator of the efficacy of metronomic therapy. However, it remains unclear whether apoptosis induced in the CECs was via direct killing by metronomic therapy or was a consequence of deprivation of survival factors such as vascular endothelial growth factor (VEGF).

Using xenograft models, Mancuso and colleagues demonstrated that apoptotic CECs were not observed in cancer-free mice treated with chemotherapy, and concluded that apoptotic CECs in xenografted mice were derived from tumor-associated blood vessels (see figure). The possibility that apoptotic CECs arose from tumor vasculature is consistent with the "vessel normalization" model proposed by Jain.[4] However, caution is necessary when extrapolating from preclinical models. Conclusive evidence of the origin of CECs in patients on metronomic therapy will depend



Revised model for the mechanism of metronomic chemotherapy. Metronomic chemotherapy may directly affect tumor cells (brown and gray ovals), may cause direct endothelial cell death or growth inhibition (red ovals), or may decrease the mobilization or viability of bone marrow–derived CEPs, which contribute to the tumor vasculature (pink ovals). The study by Mancuso and colleagues in this issue suggests that the primary mechanism of action is via induction of endothelial cell apoptosis (green ovals). Adapted from Shaked et al[3] with the authors' permission.

on the development of methods that distinguish human CECs derived from tumor and bone marrow. Elucidation of the origin of the patients' apoptotic CECs will clarify the role of host-versus-tumor differences in CEC responses, which will have implications for the broader significance of this study to other types of cancers and treatments. More extensive translational clinical trials that follow a larger number of patients for a longer-term follow-up will determine whether a simple blood test can be used to identify and monitor cancer patients who will benefit from novel antiangiogenic therapies. ■

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IMMUNOBIOLOGY

Comment on Ghassabeh et al, page 575

Macrophage diversity and polarization: in vivo veritas

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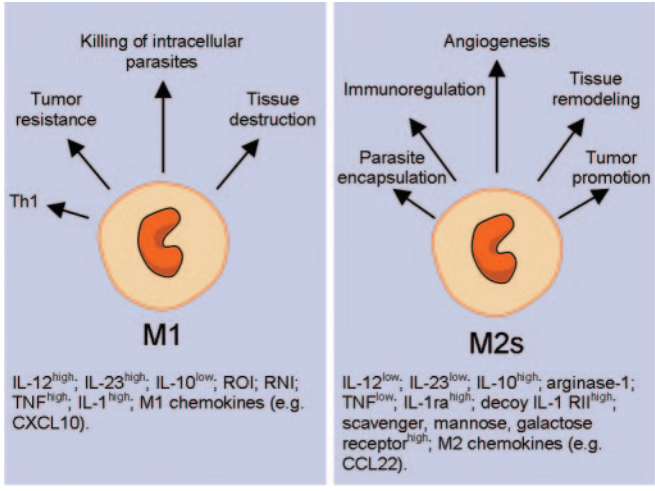
Polarized activation of cells of the monocyte-macrophage lineage into M1 and M2 cells is an operationally useful, simplified descriptor of the functional plasticity of these cells. Ghassabeh and colleagues now put to the test the actual in vivo validity and significance of the M1/M2 paradigm.

The microbial and cytokine milieu drives macrophages to express specialized and polarized functional properties.[1,2] Interferon-γ (IFN-γ), selected cytokines (granulocyte-macrophage colony-stimulating factor [GM-CSF], tumor necrosis factor [TNF]) and microbial products (lipopolysaccharide [LPS]) elicit a classic M1 form of macrophage activation. M1 macrophages are generally characterized by interleukin (IL)–12^{high}, IL-23^{high}, IL-10^{low} phenotype; produce copious amounts of reactive oxygen and nitrogen intermediates and inflammatory cytokines; are part of the afferent and efferent limb of polarized Th1 responses; and mediate resistance against intracellular parasites and tumors (see figure).

M2 is a generic name for various forms of macrophage activation other than classic M1 and includes cells exposed to IL-4 or IL-13, immune complexes, IL-10, and glucocorticoid hormones.[2] The various versions of M2 cells generally share an IL-12^{low}, IL-23^{low}, IL-10^{high} phenotype; have high levels of scavenger, mannose, and galactose-type receptors; orient arginine metabolism to ornithine and polyamine, and hence to growth promotion; and are IL-1 receptor antagonist (IL-1ra)^{high}, decoy IL-1 type II receptor^{high}, IL-1b^{low}, caspase1^{low}.[3] M1 and M2 cells express profoundly different repertoires of chemokines and chemokine receptors (the "chemokinome").[2] M2 cells are diverse, but in general are involved in T helper 2 (Th2) response; have immunoregulatory function; orchestrate encapsulation and containment of parasites; and promote tissue repair, remodeling, and tumor progression. Immature myeloid suppressor cells share functional properties and components of the transcriptome with M2 cells.[4]

The paradigm of macrophage polarization is essentially based on in vitro results. In this issue of *Blood*, Ghassabeh and colleagues report on their systematic effort to characterize macrophages from 3 models of parasite infection *(Trypanosoma brucei brucei*, *Trypanosoma congolense*, and *Taenia crassicens)* and 1 transplanted tumor. Macrophages were obtained at stages of the diseases corresponding to a predominance of M2 orientation. Myeloid cells were profiled using a differential gene expression approach. Using these diverse disease models, Ghassabeh and colleagues identify a gene signature shared by the various ex vivo–obtained M2 populations. The common signature included documented M2-associated genes (eg, arginase 1, mannose and galactose receptors, etc) as well as a new set of molecules not previously associated with M2 macrophage polarization. The functional significance and specificity of the new M2-associated genes remain to be defined. Interestingly, some of these genes could not be induced using M2 stimuli in vitro, emphasizing the importance of the in vivo approach.

This and a study on tumor-associated macrophages[4] complement results obtained in gene-modified mice reviewed in Mantovani et al[5] and provide much needed in vivo evidence of the validity of the macrophage polarization paradigm. However, these results also emphasize the diversity of the activation/differentiation states within the broad "M2" category and caution against a simplistic rigid view of the M1-M2 dichotomy. It will now be important to explore the significance of macrophage polarization in clinical conditions, where information is scanty, and the validity of new



Key properties and functions of polarized macrophages. ROI and RNI indicate reactive oxygen and nitrogen intermediates. M2s refer to diverse forms of M2 activation.