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Chemotherapeutic targeting of myeloid-derived suppressor cells

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Abbreviations: IDO1, Indoleamine 2,3-dioxygenase 1; MDSC, myeloid-derived suppressor cell; ROS, reactive oxygen species

Myeloid-derived suppressor cells (MDSCs), which expand in cancer-bearing hosts, contribute to the escape of malignant cells from immune destruction and impair the efficacy of immunotherapeutic interventions. We have recently demonstrated that the conventional chemotherapeutic agent doxorubicin selectively eliminates MDSCs, hence promoting the activity of immune effector cells and improving the therapeutic profile of adoptively transferred helper T lymphocytes.

The expansion of myeloid-derived suppressor cells (MDSCs) has been documented in patients with different types of cancer as well as in animal tumor models.[1] Endowed with the capacity to inhibit innate and adaptive immunity, MDSCs significantly contribute to the immunosuppressive environment associated with developing cancers and compromise the efficacy of various forms of immunotherapy.[1] MDSCs can also promote tumor angiogenesis and enhance the metastatic potential of malignant cells.[2] So far, 2 main populations of MDSCs have been characterized in mice: monocytic $CD11b^+Ly6G^-Ly6C^{high}$ and granulocytic $CD11b^+Ly6G^+Ly6C^{low}$ MDSCs, each of which is equipped with specific immunosuppressive machineries and exerts different functions. Human MDSCs are usually defined by a $CD33^+CD11b^+HLA\text{-}DR^{neg/low}$ phenotype. In most cases, solid tumors preferentially induce the expansion of granulocytic MDSCs.[1] The accumulation of these cells in the blood, lymphoid organs and neoplastic lesions results from the stimulation of myelopoiesis and from a blockade in the differentiation of myeloid cells by several tumor-derived soluble factors. These signals also stimulate the expression of immunosuppressive molecules by MDSCs.[1] Expectedly, multiple studies conducted in rodents and humans have demonstrated that the elimination or inhibition of MDSCs promotes antitumor immunity and enhances the efficacy of immunotherapy.[3]

Although primarily designed as cytotoxic or cytostatic molecules that directly target cancer cells, some conventional chemotherapeutic agents can enhance antitumor immunity by triggering an immunogenic type of cell death, promoting the function of immune effector cells, impairing immunosuppressive cells and/or negatively interfering with immunosuppressive pathways.[4] Gemcitabine, 5-fluorouracil and docetaxel have been reported for their ability to (more or less selectively) deplete MDSCs.[1,5-8] In a recent study,[9] we highlight a novel immunomodulatory property of the anthracycline doxorubicin: its ability to partially override cancer-induced immunosuppression by eliminating and inactivating tumor-associated MDSCs. Using 2 murine models of breast cancer (4T1 and EMT-6 cells) and a murine thymoma (EL-4 cells), we demonstrated that doxorubicin depletes granulocytic MDSCs that have accumulated in the spleen, blood and neoplastic lesions of cancer-bearing animals. Of note, the effects of doxorubicin on MDSCs were transient and these cells eventually reconstituted. Nonetheless, the proliferative potential, activation status and cytokine secretion activity of natural killer (NK) cells as well as $CD4^+$ and $CD8^+$ T lymphocytes were augmented in mice treated with doxorubicin. In addition, increased frequencies of perforin- and granzyme B-expressing NK cells and $CD8^+$ T lymphocytes were detected in tumor-bearing animals upon treatment with doxorubicin. The preferential targeting of MDSCs by this agent translated into a substantial increase in effector T lymphocyte- or NK cell-to-MDSC ratios, an essential prerequisite for successful immunotherapy. Such a highly selective effect of doxorubicin on MDSCs represents an important feature of this drug that may be exploited clinically.

The mechanisms underlying the peculiar property of doxorubicin to selectively target tumor-accosited MDSCs were further explored. We established that doxorubicin preferentially triggers the apoptotic demise of MDSCs but not of T or NK cells. These results were further substantiated by the observation that doxorubicin exerts selective cytotoxic effects on MDSCs isolated from cancer patients. Further

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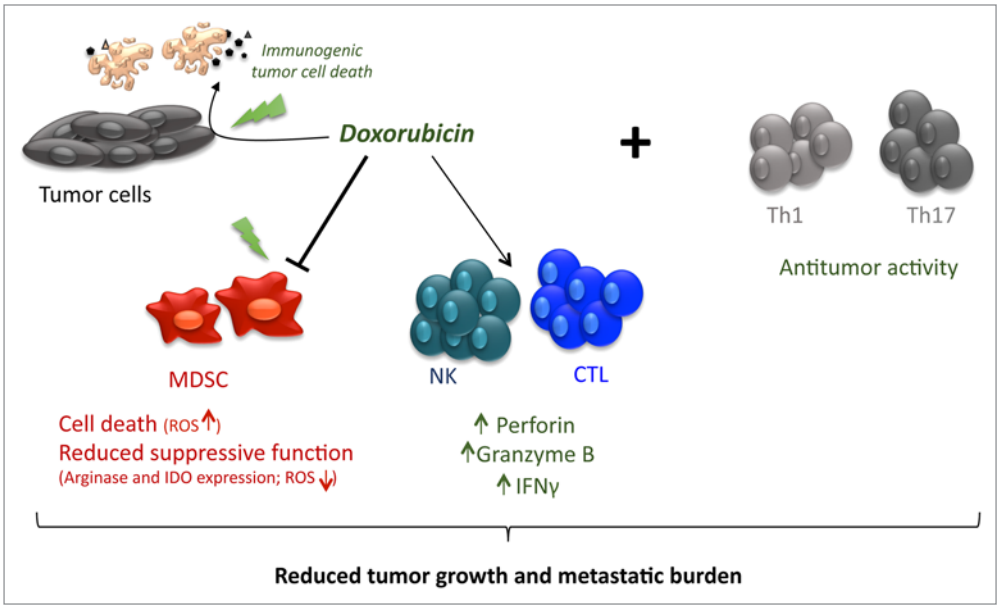

Figure 1. The multifaceted effects of doxorubicin on antitumor immunity. As it depletes myeloid-derived suppressor cells (MDSCs), promotes the immunogenic demise of cancer cells, and improves the activity of effector lymphocytes, doxorubicin may be efficiently combined with the adoptive transfer of helper T cells.

studies suggested that reactive oxygen species (ROS) may play a role in this process. The ability of doxorubicin to induce apoptosis through the overproduction of ROS has been previously described in malignant cells as well as in normal cells, such as cardiomyocytes. Our results indicate that the effects of doxorubicin on MDSCs were mitigated in tumor-bearing *Cybb*$^{-/-}$ mice (lacking the Gp91^{phox} glycosylated subunit of the NADPH oxidase flavocytochrome b558, which is responsible for the production of the superoxide ion $O_2\cdot^-$). In addition MDSCs from *Cybb*$^{-/-}$ mice were less sensitive to the cytotoxic effects of doxorubicin in vitro. These results strongly suggest that, early after its administration, doxorubicin may induce a ROS-dependent apoptotic program in MDSCs, resulting in the rapid contraction of their abundance. Of note, the MDSCs that were not eliminated and could be detected at later time points upon doxorubicin administration exhibited a defective activity, which was associated with the impaired expression of the immunosuppressive enzymes indoleamine 2, 3-dioxygenase 1 (IDO1) and arginase 1. Consistent with this result, the exposure of MDSCs isolated from (untreated) 4T1 tumor-bearing mice to non-cytotoxic concentrations of doxorubicin in vitro impaired their immunosuppressive activity.

The effects of the relatively recently identified T_H17 lymphocyte subset on oncogenesis and tumor progression have been subject of an intense debate. While several reports demonstrate that these cells can promote anticancer immune responses, other studies indicate that T_H17 cells may exhibit tumor-promoting functions. This functional dichotomy may be related to the versatile nature of these cells, which are capable of differentiating into either pro-inflammatory T_H1 cells, either immunosuppressive FOXP3$^+$ regulatory T cells (Tregs), or hybrid T-cell subsets, depending on microenvironmental conditions.[10] We reasoned that by curtailing the number and immunosuppressive activity of MDSCs (and hence restoring the function of effector lymphocytes), doxorubicin may create a microenvironment that supports immunotherapeutic interventions. The efficacy of a chemoimmunotherapeutic regimen consisting of doxorubicin coupled to the adoptive transfer of T_H1 or T_H17 lymphocytes was therefore explored. Doxorubicin was administered in conjunction with T_H1 or T_H17 lymphocytes (generated in vitro from naïve CD4$^+$ T cells) to animals bearing established 4T1 tumors. The combinatorial therapy robustly impaired tumor growth, significantly reduced the number of metastatic lung nodules, and improved the survival of tumor-bearing mice, correlating with increases in effector CD4$^+$ and CD8$^+$ T lymphocytes (**Fig. 1**). Of note, the number of MDSCs remained low over time in animals receiving the combinatorial treatment, whereas these cells re-expanded de novo in mice administered with doxorubicin alone.

These observations highlight doxorubicin as a selective MDSC-targeting agent, and further underscore the value of this drug as a potent immunomodulatory molecule for chemoimmunotherapeutic anticancer regimens.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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