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A new strategy to target regulatory T cells in solid tumors

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The depletion of regulatory T cells (Tregs) is a promising therapeutic strategy to enhance antitumor immune responses. Our recent findings indicate that low doses of arsenic trioxide can delay tumor growth in murine models of colon and breast cancer by depleting Tregs through oxidative and nitrosative bursts.

An increased number of regulatory T cells (Tregs) has been observed in the blood and tumor tissues of a high proportion of cancer patients, a phenomenon that promotes tumor progression and adversely affects prognosis.[1] Tregs express a series of markers including CD4, CD25 and the transcription factor FOXP3, which enables cells to suppress immune responses. The adverse effects of Tregs on the immune response are underscored by the observation that Treg depletion can enhance the efficacy of immunotherapeutic strategies.[2] Cyclophosphamide (CPM), a DNA alkylating agent, is used in numerous chemotherapy regimens. Clinical data reveal that although high-dose CPM exert potent immunosuppressive effects, low-dose CPM has an immunostimulatory activity. In this context, it has been shown that low-dose CPM decreases the number of Tregs and hence can enhance the antitumor activity of adoptively transferred T cells as well as of antitumor vaccines.[3]

Several strategies have been or are being developed to target Tregs.[2] Two different human anti-CD25 antibodies (basiliximab and daclizumab) and the recombinant interleukin (IL)-2-diphtheria toxin conjugate known as denileukin diftitox are currently under development. Ipilimumab, a monoclonal antibody targeting CTLA-4 (which is required for Treg function), has provided impressive results in advanced melanoma patients.[2] Finally, as outlined in a review by Galluzzi et al., several others targeted agents stimulate tumor-specific immune responses, and some of them have been associated with decreased levels of circulating or tumor-infiltrating Tregs.[4] More specifically, a recent study has revealed that targeted agents that block the vascular endothelial growth factor A (VEGFA)/ VEGF receptor 2 (VEGFR2) axis (e.g., bevacizumab and sunitinib) inhibit Treg proliferation triggered by VEGFA.[5]

Arsenic trioxide (As_2O_3) has been associated with substantial clinical efficacy in the treatment of promyelocytic leukemia patients. In addition, preclinical studies have shown that other hematological cancer and solid tumors are susceptible to As_2O_3. Although the exact mechanisms underlying the antitumor effects of this agent remain unclear, As_2O_3 has been recognized as a powerful inducer of oxidative stress in tumor cells.[6]

We recently demonstrated that low-dose As_2O_3 increases antitumor immune response in colon tumor-bearing mice by modulating Treg abundance.[7] We first observed that tumor-bearing mice display an increased proportion of Tregs among the splenic $CD4^+$ cell population, contributing to immune escape. We showed that As_2O_3 induces the selective depletion of Tregs both in vitro and in vivo. Indeed, As_2O_3 depleted Tregs in both the spleen and the tumor tissues of mice bearing murine colon carcinoma CT26 cells. Low-dose As_2O_3 was found to exert antitumor effects that are closely related to Treg depletion in both colon (Ct26 cells) and breast (4T1 cells) carcinoma murine models. As_2O_3 turned out to be able to restore the activity of immune cells adoptively transferred from donor mice, thus enhance their antitumor potential. Our results confirmed a previous study that had revealed that As_2O_3 can exacerbate immune responses against breast cancer cells.[8] In this study, As_2O_3 was shown to increase the cytotoxicity of lymphokine-activated killer (LAK) cells. Tregs were not specifically explored, but they are well known to affect the antitumor activity of multiple effector cells, including LAK cells.

We observed immunostimulatory effects at low As_2O_3 concentrations (0.5–1 μM) in vitro and with a single 1 mg/Kg dose in vivo. In previous studies, As_2O_3 doses ranging from 2 to 6.5 mg/Kg/day for one to six weeks were required to obtained therapeutic effects against solid tumors.[9] The immunogenic effects of low-dose As_2O_3 appear to be related to the high sensitivity of Tregs to this agent. It has previously been shown

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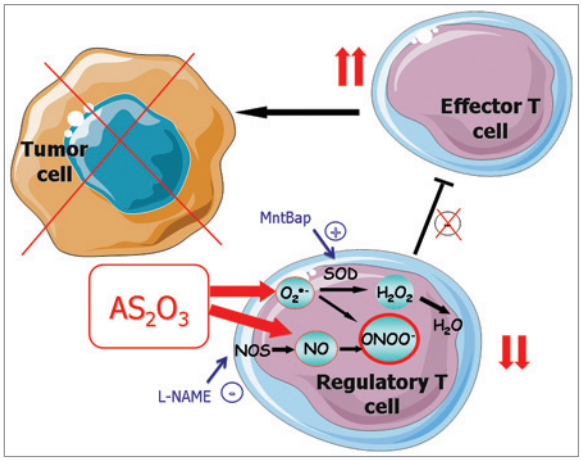

Figure 1. Arsenic trioxide can deplete regulatory T cells through the production of $ONOO^-$, thus improving the antitumor activity of effector T cells. Previous studies have shown that arsenic trioxide (As_2O_3) is able to induce the intracellular accumulation of superoxide anion ($O_2^{\bullet-}$) and nitrite oxide (NO). Superoxide dismutases (SODs) catalyze the dismutation of $O_2^{\bullet-}$ in hydrogen peroxide (H_2O_2), which is converted in H_2O by catalase and glutathione peroxidase. $O_2^{\bullet-}$ may also react with NO to form peroxynitrite ($ONOO^-$). Our data suggest that the depletion of regulatory T cells (Tregs) as induced by As_2O_3 is related to $O_2^{\bullet-}$ and NO production, resulting in $ONOO^-$ accumulation, as it is limited by the NO synthase (NOS) inhibitor NG-nitro-l-arginine methyl ester (L-NAME) and by the SOD mimic manganese [III] tetrakis-(5,10,15,20)-benzoic acid porphyrin (MnTBAP).

that low-dose CPM similarly decreases the number of Tregs. However, CPM may exert a toxic effect on other lymphocyte populations. Indeed, we observed that CPM induces a splenocyte depletion that could not be observed with As_2O_3. These data suggest that the specificity of As_2O_3 for Tregs may be higher than that of CPM.

Finally, we demonstrated that the depletion of Tregs as induced by As_2O_3 is mediated by the generation of reactive oxygen and nitrogen species. We showed that As_2O_3 promotes the accumulation of hydrogen peroxide (H_2O_2) and/or peroxynitrite ($ONOO^-$) in Tregs, as demonstrated via the fluorescent probe H_2DCFDA (2'7'dichlorodihydrofluorescein diacetate). Our data strongly suggest that Treg depletion as induced by As_2O_3 is related to $ONOO^-$ production, since it was inhibited by the nitric oxide (NO) synthase inhibitor NG-nitro-l-arginine methyl ester (L-NAME) and by the superoxide dismutase mimic manganese [III] tetrakis-(5,10,15,20)-benzoic acid porphyrin (MnTBAP) (**Fig. 1**). Thus, Treg depletion as induced by As_2O_3 is related to superoxide and NO production resulting in the accumulation of $ONOO^-$. In contrast, As_2O_3 failed to induce $ONOO^-$ accumulation, in non-Treg $CD4^+$ cells. Such differential effects of As_2O_3 on Treg and non-Treg $CD4^+$ cells could be related to differences in the redox status of these cells. The threshold for toxicity might be more easily reached in Tregs upon As_2O_3 exposure because of higher basal levels of NO and $ONOO^-$ in these cells, resulting in the overwhelming of antioxidant defenses. This redox-based differential effect of As_2O_3 has previously been observed in acute promyeolocytic leukemia cells,[10] which would be more susceptible to As_2O_3 cytotoxicty because of their relatively high basal NADPH oxidase activity.

In conclusion, we suggest for the first time that low doses of As_2O_3 may constitute a new strategy to deplete Tregs in colorectal tumors. Indeed, we have recently shown that As_2O_3 can deplete Tregs through oxidative and nitrosative bursts, thus improving antitumor immune responses. Our results offer a new opportunity to use low doses of As_2O_3 to enhance the antitumor activity of adoptive immunotherapy against human cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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