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Polyamine blockade promotes antitumor immunity

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Abbreviations: ARG1, arginase 1; DFMO, α-difluoromethylornithine; IDO1, indoleamine-2,3-dioxygenase 1; MDSC, myeloid-derived suppressor cell; ODC, ornithine decarboxylase; PBT, polyamine blocker therapy; PTS, polyamine transport system; Treg, T regulatory cell

The levels of polyamines are elevated in neoplastic lesions as compared with normal tissues, and cancer cells tend to manifest a robust dependence on these compounds for proliferation and survival. We have recently demonstrated that a novel approach to polyamine depletion suppresses tumor growth in a T cell-dependent manner, highlighting a poorly appreciated role of polyamines as strong modulators of antitumor immune responses.

Malignant cells have evolved sophisticated survival mechanisms to suppress tumor-specific immune responses. Part of such an "immune-sculpting" process involves the establishment of an immunosuppressive tumor microenvironment, standing out as the main cause of the failure of most anticancer immunotherapeutic regimens tested so far in clinical trials.[1] Tumor-associated immunomodulation occurs via a variety of mechanisms including the altered expression of tumor-associated antigens and MHC class I molecules on the surface of malignant cells, the production of pro- (T_H1) and anti-inflammatory (T_H2) cytokines, the reduction in antigen-presenting cell activity, and the recruitment of immunosuppressive cells, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), all of which disable antitumor effector T cells. Recent studies have unveiled an additional group of immunomodulatory mechanisms, those mediated by small molecule metabolites.[2] Our work adds polyamines to these immunomodulators, which also include adenosine, indoleamine-2,3-dioxygenase 1 (IDO1), and arginase 1 (ARG1). The development of novel therapeutic approaches that can act broadly to defeat tumor-elicited immunosuppression is urgently needed. In particular, the in-depth knowledge of immunomodulatory pathways that can be targeted to improve T-cell responses in conjunction with existing anticancer therapies is key for the achievement of long-lasting clinical responses.

Recently, we have explored the role of tumor-associated polyamines as immunosuppressive metabolites in oncogenesis and tumor progression. Polyamine metabolism is a mature area of cancer research, and polyamine-targeting drugs are already available for preclinical and clinical application. However, despite some insightful suggestions,[3] this area has seen little investigation with regard to antitumor immunity. Polyamines are amino acid-derived polycations required for cellular proliferation and are involved in a wide variety of physiological functions including signal transduction and gene expression. There is a dramatic increase in polyamine levels within human tumors, in which the activity of the rate-limiting enzyme for polyamine synthesis (i.e., ornithine decarboxylase, ODC) is upregulated by several oncogenic factors including MYC. This said, the administration of α-difluoromethylornithine (DFMO), an ODC inhibitor, to cancer patients had limited therapeutic effects.[4] It is now clear that tumors can satisfy their increased need for polyamines by upregulating the uptake of these molecules from the microenvironment. Thus, specific polyamine transport systems can be upregulated by malignant cells to supplement their requirements and take up polyamines that derive from the diet and the gut flora. To starve neoplastic lesions of polyamines, we have employed a novel polyamine blocker therapy (PBT) based on DFMO (to block polyamine biosynthesis) coupled with AMXT1501, an inhibitor of polyamine transport.[5]

Our results indicate that PBT not only blocks tumor growth but also promotes anticancer immune responses.[5] This work builds on earlier studies based on the skin-specific overexpression of ODC, demonstrating the inflammation-dependent, wound-induced formation of cutaneous tumors[6] and the suppression of hapten-induced contact hypersensitivity responses,[7] and hence highlighting the role of polyamines in carcinogenesis-associated immune dysfunction. Since PBT does not inhibit tumor growth in athymic nude mice (lacking T lymphocytes), we hypothesize that

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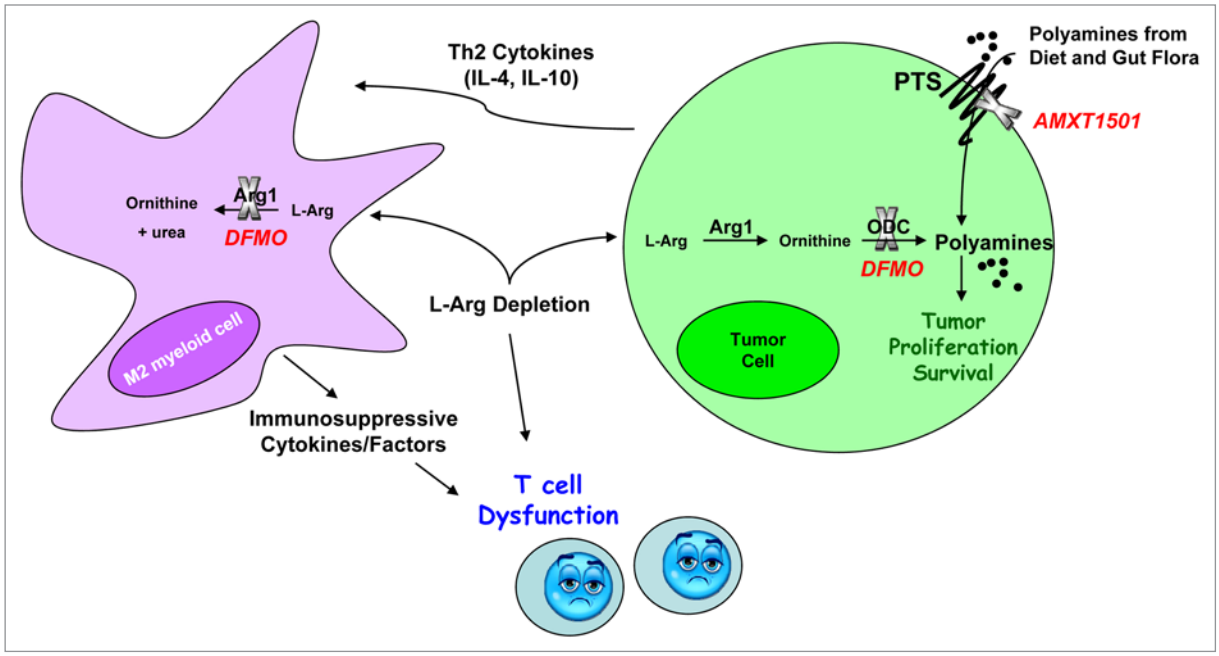

Figure 1. Interaction between polyamine and arginine metabolism and its possible impact on T-cell dysfunction in the tumor microenvironment. Arginase 1 (ARG1) is upregulated in tumor-infiltrating myeloid cells by tumor-secreted T_H2 cytokines, including interleukin (IL)-4 and IL-10. ARG1 is associated with the polarization of the tumor infiltrate toward an M2 phenotype, which is thought to promote tumor progression by inhibiting the activation of cytotoxic T cells and stimulating angiogenesis, metastatic dissemination, and resistance to therapy. ARG1 hydrolyzes *L*-arginine (*L*-Arg) to *L*-ornithine and urea. Extracellular arginine can be further depleted by the increased ARG1 activity in tumor cells in response to the increased consumption of ornithine for polyamine biosynthesis. Polyamines are essential for the proliferation and survival of malignant cells. Arginine depletion suppresses T-cell responses and favors tumor-associated immunosuppression. The inhibition of ornithine decarboxylase (ODC) with α-difluoromethylornithine (DFMO) increases ornithine levels while limiting ARG1 activity through feedback inhibition. DFMO can also directly inhibit ARG1 activity. The inhibition of polyamine biosynthesis by DFMO upregulates the polyamine transport system (PTS), which allows for the uptake of polyamines from the diet and the gut flora by cancer cells. Polyamine blockade in tumors can be achieved by the co-administration of DFMO (to inhibit polyamine biosynthesis) and AMXT1501 (to inhibit the PTS).

effectors of the anticancer activity of PBT are $CD8^+$ and/or $CD4^+$ T cells. Tantalizing data indicating that PBT can promote a durable anticancer immune response that protects mice against a re-challenge with neoplastic cells of the same type suggest that PBT may enhance the efficacy of other tumor immunotherapies. Ongoing experiments are investigating the mechanism(s) that underlie PBT-elicited antitumor immune responses, which may involve an improved activation and/or cytotoxic activity of T cells.

Perhaps it is not surprising that different immunomodulatory metabolic pathways bisect and influence each other. Because polyamine biosynthesis depends on the activity of ARG1, which supplies ornithine to ODC, perturbations of polyamine metabolism will impact arginine metabolism within the tumor microenvironment. Arginine metabolism is known to play a central role in the immune system. In particular, the depletion of this amino acid suppresses T-cell immune responses and favors tumor-associated immunosuppression (**Fig. 1**). Immunosuppressive tumor-infiltrating myeloid cells, including $Gr\text{-}1^+CD11b^+$ MDSCs, granulocytes, immature dendritic cells, and Tregs, profoundly impair the activity of T cells via the ARG1-mediated depletion of arginine.[8,9] In line with this notion, the inhibition of myeloid cell-associated ARG1 limits the immune dysfunction that generally accompanies oncogenesis and tumor progression, hence mediating antineoplastic effects.[8,9] An increased biosynthesis of polyamines (coupled to a robust consumption of ornithine) promotes the activity of ARG1 in tumor cells, and may also contribute to the induction of immunosuppressive ARG1-expressing tumor myeloid cell populations. DFMO inhibits the activity of ARG1 directly[10] and/or via a feedback circuitry that involves the inhibition of ODC and the consequent increase in ornithine levels. By limiting the activity of ARG1 in neoplastic lesions, PBT may restore the responsiveness of tumor-infiltrating T cells. In addition, we have shown that DFMO blocks the interleukin-4-dependent induction of ARG1 activity in macrophages, which is associated with their polarization toward an M2 phenotype and hence with tumor-promoting functions. It is also possible that PBT may skew the polarization of tumor-infiltrating myeloid cells from an M2 to an M1 pro-inflammatory phenotype.

PBT stimulates the apoptotic demise of murine cancer cells in vivo, but has little or no effects on their proliferative index, suggesting that the therapeutic efficacy of PBT depends on its ability to re-condition the tumor microenvironment rather than on its direct anti-proliferative effects. The antineoplastic activity of PBT may rely on its ability to promote the immunogenic death of cancer cells or to selectively eliminate immunosuppressive cell populations

such as MDSCs and Tregs. Given the strict requirement for polyamines by most, if not all, solid tumors, this new strategy, resulting in the inhibition of tumor-elicited immunosuppressive mechanisms, may constitute a broad approach for the treatment of multiple types of cancer. In particular, polyamine deprivation via PBT may be particularly suitable to improve the efficacy of conventional chemotherapeutic agents or immunotherapeutic regimens. Future work will investigate in detail the mechanisms whereby PBT can reverse the immunosuppressive microenvironment of solid neoplasms and stimulate tumor-specific immune responses.

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Disclosure of Potential Conflicts of Interest

SK Gilmour is a co-inventor on a patent application describing the therapeutic use of PBT. MR Burns is employed as President and CEO of Aminex Therapeutics, Inc. and also has ownership interest (including patents) in the same. No potential conflicts of interest are disclosed by CS Hayes.