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Colon cancer eradication after chemoimmunotherapy is associated with intratumoral emergence of proinflammatory myeloid cells

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Abbreviations: CPA, cyclophosphamide; MDSC, myeloid-derived suppressor cell; Treg, regulatory T cell; IMC, inflammatory myeloid cell; IL-12, interleukin-12

Interleukin-12 immune stimulation lacks efficacy in established solid tumor models. Disruption of tumor microenvironment homeostasis by low-dose cyclophosphamide prior to interleukin-12 gene therapy led to CD8$^+$ T cell-driven established tumor rejection. This only takes place when inflammatory myeloid cells infiltrate the tumor bed, and is crucial for the latter antitumor response.

Cytokine-based therapies against cancer are aimed to stimulate the host immune system and thus achieve therapeutic responses. To this end, interleukin-12 (IL-12) has been proposed as a potential anticancer drug due to its immunostimulatory capacities. However, the IL-12 effect in preclinical and clinical settings is limited by the tumor-induced immune subversion. Tumors are heterogeneous tissues composed of a variety of cell types that participate in tangled interactions with one another. Some of these cells belong to the immune system and they abrogate effector antitumor responses. For instance, in a recent report in reference 1, we showed that mice bearing MC38 established tumors accumulate regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC) in secondary lymphoid organs and tumors. Tumor MDSC displayed a monocytic and more differentiated phenotype due to the presence of tumor-derived factors, which induce their maturation toward tumor-infiltrating macrophages or dendritic cells.[2]

The modulation of the tumor microenvironment might therefore be required for the therapeutic success of cytokine-based therapies. Based on this hypothesis, we combined IL-12 gene delivery either with Treg depletion by anti-CD25 mAb or single low-dose cyclophosphamide (CPA) injection in established MC38 tumor bearing mice. As expected due to tumor size, IL-12 monotherapy did not work. Anti-CD25 combination showed poor therapeutic success contrasting with CPA+IL-12, which induced tumor eradication in 70% of treated mice.

Further insight into the mechanism of action confirmed that IL-12 effect was counteracted by the tumor microenvironment, since CD3$^+$ cells are retained at the tumor rim and tumor Treg and MDSC populations increase. This finding strengthens our previous results in *c-myc* mice bearing spontaneous hepatocarcinomas.[3] Remarkably, CPA treatment efficiently infiltrated lymphocytes—especially activated MC38 tetramer-specific CD8$^+$ T cells—within tumors when compared with anti-CD25. In fact, the antitumor effect of CPA in combination with IL-12 relies on CD8$^+$ T cell infiltration, as depletion experiments confirmed. Surprisingly, both combined therapies significantly decreased Treg and monocytic MDSC subsets within tumors, meaning that both treatments were able to modulate tumor immune suppressive populations. Strikingly, only CPA+IL-12 combined treatment resulted in the occurrence of CD11b$^+$ myeloid cells expressing high levels of Ly6C monocyte/macrophage lineage marker within the tumor bed. Giemsa staining and differential surface expression of Ly6G and Ly6C among myeloid cells discerned among neutrophils and inflammatory monocytes/macrophages; this heterogenous population was named inflammatory myeloid cells (IMC).

We noticed that the tumor microenvironment plays an active role in the infiltration of IMC, since the kinetics in the tumor is different to that observed in spleen. In vitro experiments with isolated CD11b$^+$ cells from mock- or CPA+IL-12 treated established MC38 tumors

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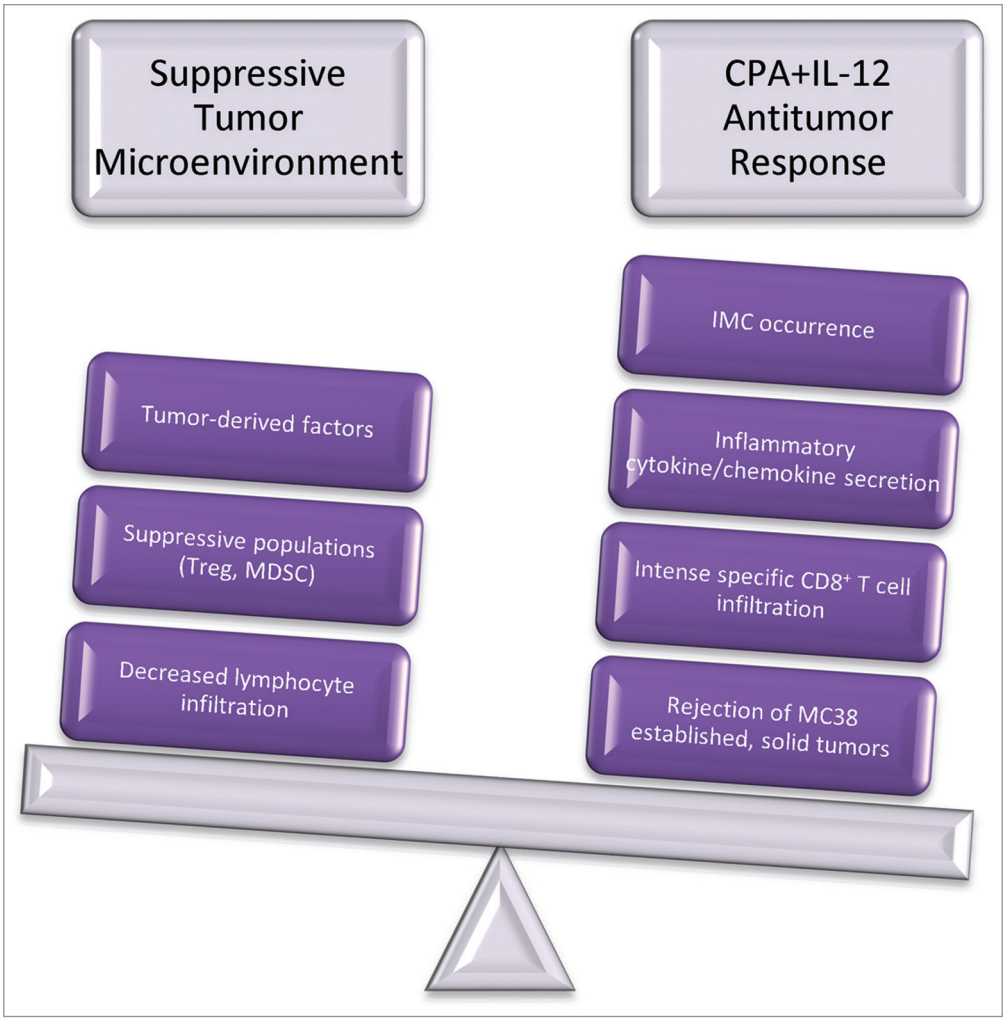

Figure 1. Schematic representation of the effect of cyclophosphamide and interleukin-12 treatment on the tumor microenvironment. CPA, cyclophosphamide; MDSC, myeloid-derived suppressor cell; Treg, regulatory T cell; IMC, inflammatory myeloid cell; IL-12, interleukin-12.

evidenced changes in the cytokine/chemokine secretion patterns. For instance, secretion of the immunosuppressive molecules interleukin 10 and nitric oxide in response to LPS was significantly higher in mock-treated monocytic MDSC than from CPA+IL-12 tumor IMC. Moreover, clear differences in the production of chemokines and cytokines by IMC and monocytic MDSC could be compared with those between M1 and M2 macrophages.[4,5]

Functional interactions with lymphocytes in vitro also reflected the inflammatory signature shown by tumor IMC. While monocytic MDSC induced conversion of naive $CD4^+$ T cells to $FoxP3^+$ Treg, IMC did not promote this conversion. We also found that the proliferative response of $CD4^+$ T cells and the production of IFNγ by $CD8^+$ T cells were higher in the presence of IMC than when compared with monocytic MDSC. Moreover, TGFβ did not affect the proliferation of $CD4^+$ and $CD8^+$ T cells when co-cultured with IMC, which was inhibited in the presence of monocytic MDSC. Altogether, IMC creates a suitable milieu to activate antitumor T cell immunity at the bench.

To appreciate how important IMC generation is in supporting CPA+IL-12-mediated tumor rejection, intratumoral depletion of granulocytes and monocytes was performed 24 h after giving CPA combination. These mice were unable to reject MC38 solid tumors. In fact, in vivo IMC depletion led to a lack of lymphocyte infiltration within tumors, involving a drastic reduction of tumor infiltrating $CD4^+$ and $CD8^+$ T cells. Thus, IMC upsurge at the tumor site is essential for eradication of MC38 established tumors by low-dose cyclophosphamide plus IL-12 treatment.

In conclusion, our recent report showed how tumor immune modulation followed by the enhancement of host immunity clearly affects the tumor site. In our case, both combinations of IL-12 either with anti-CD25 mAb or low-dose CPA resulted in a drastic reduction of tumor Treg and MDSC. However, only CPA+IL-12 reflects an influx of innate immune cells within the tumor which led to a strong T_H1 pro-inflammatory response, resulting in tumor rejection (**Fig. 1**). Our work correlates tumor eradication with the transition from an immune suppressive tumor microenvironment toward an inflammatory milieu. Although the rapid turnover of neutrophil and monocytic populations

adds a degree of complexity when monitoring their occurrence in antitumor responses, compelling evidence is being obtained in both preclinical and clinical situations concerning how innate immune stimulation is pivotal for orchestrating tumor rejection.[6-8] Therefore, forthcoming clinical trials of immunotherapy should pay attention to the dynamics of the intratumoral innate immune system.

References

1. Medina-Echeverz J, Fioravanti J, Zabala M, Ardaiz N, Prieto J, Berraondo P. Successful colon cancer eradication after chemoimmunotherapy is associated with profound phenotypic change of intratumoral myeloid cells. J Immunol 2011; 807-15; DOI:10.4049/jimmunol.1001483.
2. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. J Immunol 2008; 181:5791-802; PMID:18832739.
3. Zabala M, Lasarte JJ, Perret C, Sola J, Berraondo P, Alfaro M, et al. Induction of immunosuppressive molecules and regulatory T cells counteracts the antitumor effect of interleukin-12-based gene therapy in a transgenic mouse model of liver cancer. J Hepatol 2007; 47:807-15; PMID:17935823; DOI:10.1016/j.jhep.2007.07.025.
4. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 2004; 25:677-86; PMID:15530839; DOI:10.1016/j.it.2004.09.015.
5. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002; 23:549-55; PMID:12401408; DOI:10.1016/S1471-4906(02)02302-5.
6. Berraondo P, Nouze C, Preville X, Ladant D, Leclerc C. Eradication of large tumors in mice by a tritherapy targeting the innate, adaptive and regulatory components of the immune system. Cancer Res 2007; 67:8847-55; PMID:17875726; DOI:10.1158/0008-5472.CAN-07-0321.
7. Jiang Q, Weiss JM, Back T, Chan T, Ortaldo JR, Guichard S, et al. mTOR kinase inhibitor AZD8055 enhances the immunotherapeutic activity of an agonist CD40 antibody in cancer treatment. Cancer Res 2011; 71:4074-84; PMID:21540234; DOI:10.1158/0008-5472.CAN-10-3968.
8. Gonzalez-Aparicio M, Alzuguren P, Mauleon I, Medina-Echeverz J, Hervas-Stubbs S, Mancheno U, et al. Oxaliplatin in combination with liver-specific expression of interleukin 12 reduces the immunosuppressive microenvironment of tumours and eradicates metastatic colorectal cancer in mice. Gut 2011; 60:341-9; PMID:20855451; DOI:10.1136/gut.2010.211722.