

# Crucial role of inflammatory monocytes in antitumor immunity

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Although the role of myeloid cells in oncogenesis and tumor progression remains poorly understood, these cells are mainly ascribed with pro-tumor properties. We have recently unveiled a tumoricidal activity of inflammatory monocytes that can be counteracted by CD4<sup>+</sup> regulatory T cells.

In recent years, broad classes of cells derived from the mononuclear phagocytic lineage, including myeloid-derived suppressor cells<sup>1</sup> and tumor-associated macrophages,<sup>2</sup> have been intensively investigated and found to promote the growth and metastatic dissemination of malignant cells. Conversely, the potential antineoplastic activity of monocytic cells has been largely disregarded.

We have recently deciphered the role of inflammatory monocytes and inflammatory dendritic cells (DCs) in the early steps of tumor progression in a model of spontaneous uveal melanoma driven by the *RET* oncogene.<sup>3</sup> In this model (MT/ret mice, harboring *RET* under the control of the metallothionein 1 promoter/enhancer), tumor cells disseminate early, but remain dormant for several weeks.<sup>4</sup> Thereafter, MT/ret mice develop cutaneous metastases and finally distant metastases. Interestingly, one third of mice spontaneously develops vitiligo, which is associated to a decreased occurrence of cutaneous metastases.<sup>5</sup> This model is thus relevant for the study of antitumor immune responses throughout carcinogenesis and tumor progression, in the presence or in the absence of a concomitant autoimmune disease.

Inflammatory (Ly6C<sup>high</sup>) monocytes are innate immune cells that are well known for their anti-infectious properties.

As these cells express high levels of chemokine (C-C motif) receptor 2 (CCR2), their egression from the bone marrow as well as their recruitment into tissues is largely based on the CCL2/CCR2 signaling axis. Ly6C<sup>high</sup> monocytes are normally recruited to inflamed tissues where they produce high levels of tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin (IL)-1 and reactive oxygen species (ROS), earning them the appellation of “inflammatory monocytes.” Of note, inflammatory monocytes can differentiate into inflammatory dendritic cells (DCs), which preserve the capacity to produce TNF $\alpha$  while becoming able to capture and present antigens.<sup>6</sup>

Our study provides new and unexpected insights into the mechanisms involved in the control of metastatic spread by highlighting the antitumor properties of inflammatory monocytes and DCs. Based on antibody depletion experiments and on the generation of MT/ret mice lacking T cells, we indeed demonstrated that CD8<sup>+</sup> T cells and natural killer (NK) cells are not implicated in the control of tumor spread in the skin. Instead, inflammatory monocytes and DCs appear to be major actors in this setting. In fact, the absence of these cells from birth leads to the rapid death of a high fraction of MT/ret mice. Of note, MT/ret mice that survive the depletion of inflammatory monocytes exhibit an increased occurrence of

both cutaneous and distant metastases. Conversely, interventions that provoke a surge in circulating inflammatory monocytes and DC levels lessen the frequency of cutaneous and distant metastases spontaneously developing in MT/ret mice. Our data are consistent with a key role of inflammatory monocytes in the control of tumor progression. In particular, inflammatory monocytes appear to play a more crucial function than T and NK cells in limiting tumor spread in our model (Fig. 1A).<sup>3</sup>

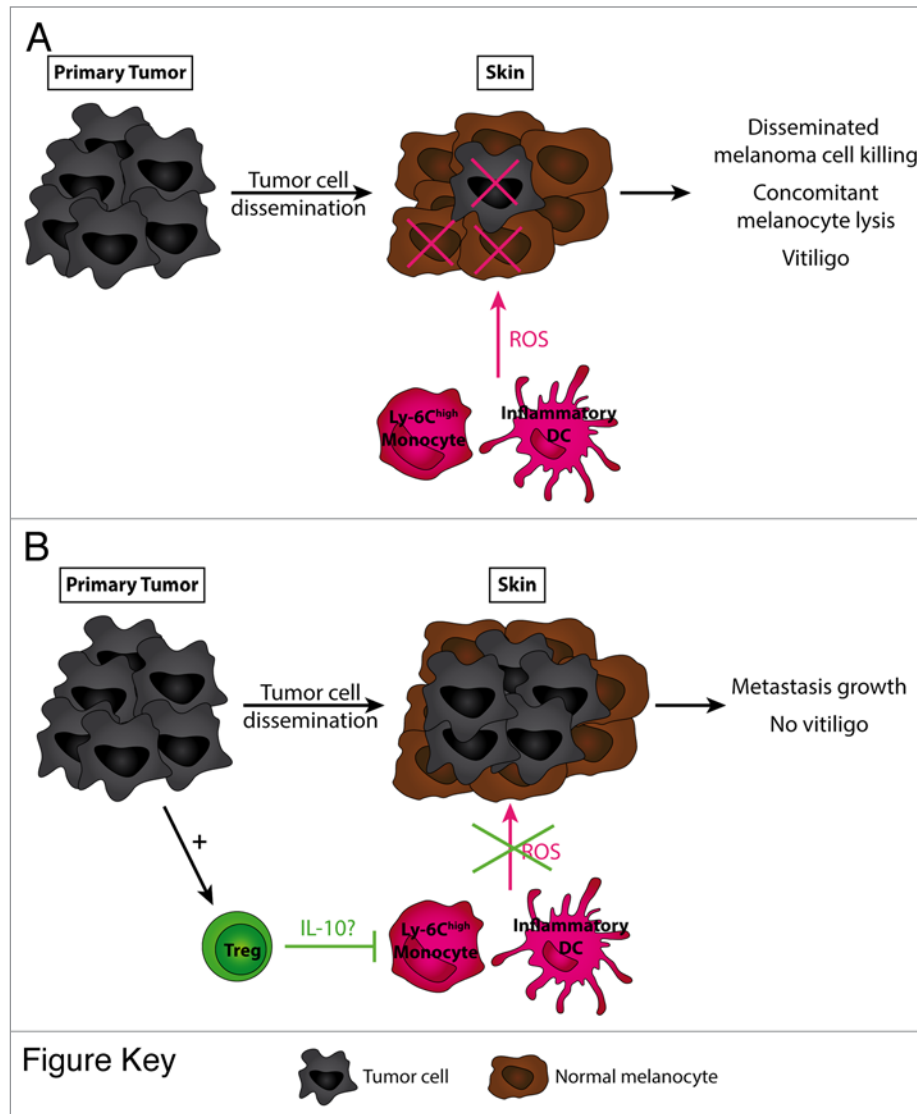
Distinct mechanisms underlying the tumoricidal activity of monocytes have been highlighted, mostly by using melanoma cell lines as targets. Such mechanisms are either related to the direct recognition and killing of target cells or to their antibody-dependent lysis.<sup>7</sup> In our recent paper, we found that inflammatory monocytes/DCs limit the growth of melanoma cells in vitro by producing ROS, with TNF $\alpha$  playing a minor role in this process. Moreover, we demonstrated that the neutralization of ROS in vivo favors the metastatic dissemination of malignant cells. Altogether, our findings suggest that inflammatory monocytes and DCs exert their antitumor effects that are mediated, at least in part, by a ROS-dependent mechanism (Fig. 1A).<sup>3</sup>

Next, we hypothesized that the dissemination of cancer cells within the skin favors

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**Figure 1.** Inflammatory monocytes and dendritic cells exert antitumor functions that can be counteracted by regulatory T cells. **(A)** Inflammatory monocytes and dendritic cells (DCs) can kill disseminated tumor cells in the skin via a reactive oxygen species (ROS)-dependent mechanism and cause the bystander lysis of normal melanocytes (vitiligo). **(B)** Regulatory T cells (Tregs) can inhibit inflammatory monocytes and DCs, in part via the secretion of interleukin-10 (IL-10).

the recruitment of inflammatory monocytes and DCs that may lyse not only transformed melanocytes but also normal ones (by a bystander effect), hence causing vitiligo. In line with this hypothesis, inflammatory monocytes and DCs accumulate in the skin of MT/ret mice with active vitiligo. Interestingly, we found an inverse correlation between the relative abundance of CD4<sup>+</sup> regulatory T cells (Tregs) and that of Ly6C<sup>high</sup> monocytes among skin-derived cells, suggesting that the former may interfere with the antitumor effects of the latter (Fig. 1B). Indeed, the depletion of Tregs

reduces the incidence of cutaneous metastases spontaneously developing in MT/ret mice and increases the frequency of animals that manifest vitiligo.<sup>3</sup>

Tregs exert immunosuppressive effects via a wide range of mechanisms, including the release of IL-10. Treg-derived IL-10 has been shown to inhibit skin inflammation<sup>8</sup> and to affect the recruitment of inflammatory monocytes to the liver as well as their differentiation into inflammatory DCs in the course of *Trypanosoma* infection.<sup>9</sup> The neutralization of IL-10 in MT/ret mice delays the development of

cutaneous metastases and increases the occurrence of vitiligo. Moreover, both the depletion of Tregs and the blockade of IL-10 increase the abundance of inflammatory monocytes and DCs in the skin. Thus, we speculate that Treg-derived IL-10 inhibits the recruitment of monocytes to the skin and/or their local differentiation into inflammatory DCs (Fig. 1A). Of course, IL-10 produced by other cell types might also be implicated in the control of antitumor responses. Further investigation is required to specifically assess this hypothesis.<sup>3</sup>

Until now, monocytic cells have been widely considered as pro-tumor cells as they facilitate the metastatic spread of malignant cells (by promoting their extravasation) and dampen antitumor immune responses. Our recent results rather suggest that inflammatory monocytes and DCs play a key role in controlling tumor dissemination. Consistent

with our data, it has recently been shown that inflammatory DCs are crucial for the induction of antitumor immune responses in the course of chemotherapy.<sup>10</sup> Our findings also suggest a new role for Tregs, which may favor the metastatic spread of cancer cells by inhibiting the recruitment and/or differentiation of inflammatory monocytes. Thus, likewise

the majority of immune cells, myeloid cells may display either pro- or anti-tumor properties, presumably depending on contextual variables including tumor type and stage.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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