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Rethinking the role of myeloid-derived suppressor cells in adoptive T-cell therapy for cancer

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The expansion of cancer-induced myeloid cells is thought to be one of the main obstacles to successful immunotherapy. Nevertheless, in murine tumors undergoing immune-mediated destruction by adoptively transferred T cells, we have recently shown that such cells maintain their immunosuppressive properties. Therefore, adoptive T-cell therapy can, under certain conditions, overcome myeloid cell immunosuppression.

A myriad of studies have demonstrated the immunosuppressive capabilities of cancer-induced myeloid cells, including myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs). The general notion in the field is that when cancer immunotherapy strategies fail to cure cancer, it is very likely that MDSCs and/or TAMs are involved. However, a careful review of the literature uncovers a litany of unresolved questions regarding such a notion (see references in Ref. 1). For instance, most studies use in vitro assays to characterize the immunosuppressive properties of MDSCs/TAMs, raising questions about the extent to which such assays reflect the actual contribution of MDSCs/TAMs to a poor antitumor immune response occurring in vivo. Also, the effects of cancer-induced myeloid cells on naïve vs. effector/memory T-cells differ markedly in vitro. In vivo, MDSCs have been reported to suppress naïve T-cell responses in some models, but it is unclear how effector/memory T cells are affected, which is more relevant for tumor-infiltrating T cells.

We recently showed that adoptively transferred immune (memory) but not naïve T cells eliminated well-established tumors that expressed natural (i.e., non-artificially overexpressed) antigens.[2] However, mice bearing such tumors had increased levels of MDSCs and abundant TAMs that were strongly immunosuppressive by the standard in vitro suppression assay.[1] Conceptually, there were three possible explanations accounting for the success of T cells in eliminating tumors, despite the presence of cancer-induced myeloid cells: (i) myeloid cells were eventually abolished, (ii) myeloid cells shifted to a more "benign" phenotype, or (iii) the myeloid cells were neither eliminated nor shifted, but simply failed to prevent tumor eradication by T cells.

In prior studies from our lab, we demonstrated that stromal myeloid cells can cross-present antigen during T-cell destruction of tumor cells overexpressing artificial model antigens, a process leading to stromal cell death. In such in vivo models, myeloid cells acted as "double agents" cross-presenting antigen[3-5] to activate T cells more efficiently than cancer cells,[6] despite retaining immunosuppressive potential as characterized in vitro.[4] In other studies, tumor immunotherapy has been shown to stimulate changes in the composition of myeloid infiltrate required for optimal antitumor function of T cells. Such beneficial deviations could affect the distribution of myeloid cells in favor of more proinflammatory subsets, or alter the function of TAMs to elicit T cells to produce antitumoral cytokines (original references in Ref. 1). Nevertheless, in our native antigenic tumor model, we found that concurrently with T-cell tumor infiltration and tumor destruction, most myeloid cells retained viability, appeared relatively stable in their subset distribution and production of cytokines, and maintained their ability to suppress T-cell function in vitro.[1] Thus, our adoptively transferred T cells were able to overcome the immunosuppression imposed by the biological activity of MDSCs and TAMs present in the tumor. This finding does not necessarily dictate that the myeloid cells present during tumor destruction were the exact same that were there prior to T cell infiltration. In fact, our in vivo longitudinal imaging data have revealed morphological changes in stromal cells during tumor destruction in response to adoptively transferred T cells[1] (**Fig. 1**), possibly suggesting infiltration of new myeloid cells from the circulation. T-cell mediated tumor elimination was also characterized by the destruction of cancer cells and tumor vasculature following parallel kinetics[1] (**Fig. 1**).

There are several practical implications arising from these findings. First, the observation that MDSCs and TAMs can exhibit strongly immunosuppressive activities in vitro does not necessarily mean that they actually will be immunosuppressive in vivo. Concerns about the applicability of findings derived from in vitro murine suppression assays to the

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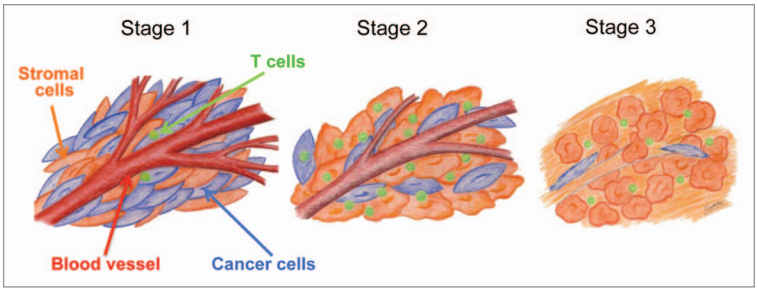

Figure 1. Sequence of events during immune-mediated destruction of 8101 tumors by adoptively transferred immune T cells. Three distinct temporal stages can be distinguished after adoptively transferred 8101-immune T cells infiltrate well-established 8101 tumors by cytofluorimetric analysis and longitudinal in vivo microscopy.[1] During "Stage 1" (days 7–8 after adoptive transfer), the first T cells appear in the tumor. The vasculature is branched and tortuous, and cancer and spindle-shaped stromal cells are tightly packed. During "Stage 2" (days 10–12 after adoptive transfer), T cell infiltration reaches its peak. The blood vessels are visibly damaged. Stromal and cancer cells are more loosely connected and stromal cells adopt a rounder shape. Most cancer cells are no longer viable. Despite their morphological change, stromal cells maintain immunosuppressive properties in vitro. During "Stage 3" (days 14–17 after adoptive transfer), the vasculature and cancer cells have been destroyed. Only round and motile stromal cells remain, interacting with T cells. (Drawings by Leticia Corrales and Ainhoa Arina.)

pathophysiology of patient responses in the clinic have been raised.[7] In support, a recent study demonstrated that myeloid cell immunosuppression in vitro does not equate with the degree of T-cell responsiveness in several SV40 T-antigen driven autochthonous mouse cancer models. In this particular study, monocytic MDSCs suppressed cytotoxic T lymphocyte (CTL) function in vitro, regardless of whether the mice exhibited tumor-induced tolerance and general CTL hyporesponsiveness, neonatal tolerance to tumor antigens, or systemic immunity against the tumor.[8] Therefore, T-cell suppression assays in vivo would be more informative and should be used to conclusively determine the relative contribution of MDSCs and TAMs to tumor-induced immunosuppression.

Among other practical repercussions, our work also suggests that the failures of some adoptive T-cell therapy strategies may be related more to the suboptimal tumoricidal properties of T cells than to a strong opposition by cancer-induced myeloid cells. Our mouse-model studies have provided important clues about the conditions that T cells must meet in order to successfully eradicate established tumors. In our hands, T cell preimmunization against tumor antigens is essential.[2] The age of the T cell donor is also critical, since T cells derived from middle-aged and elderly mice failed to reject tumors that were eliminated by T cells obtained from young animals.[2] Interestingly, the age of donor cells at the time of donation ultimately determined success or failure, such that even if mice were immunized at a young age, the T cells were ineffective if the mice became old prior to use. Furthermore, the collaboration of $CD8^+$ and $CD4^+$ T cells is crucial for the optimal efficacy of antitumor T-cell responses,[9] and our ongoing work supports that this paradigm also holds true in the case of adoptive T-cell therapy (unpublished observations). Finally, the choice of antigen to be targeted by $CD8^+$ T cells is of utmost importance. In these regards, we have recently shown that upon adoptive T cell transfer, only peptides with high-affinity for MHC class-I are efficiently cross-presented by tumor stroma, thereby inducing T cell cytokine secretion and associated stromal cell death, and culminating in relapse-free regression of tumors.[5] New methods to identify mutated epitopes based on whole-exome sequencing of tumors will permit the identification of truly tumor-specific antigens[10] with the highest potential affinity for MHC class-I, leading to a new generation of engineered T cells for cancer immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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