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Uncovering the mechanisms that regulate tumor-induced T-cell anergy

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Helper T cells become hyporesponsive in the tumor microenvironment (at least in part) owing to the NFAT1-dependent expression of anergy-associated genes. Anergy constitutes a crucial mechanism to prevent tumor destruction by T cells, and hence may represent a powerful target to boost antitumor immune responses and improve the efficacy of immunotherapy.

The tumor microenvironment often promotes immunosuppression, due to (1) the recruitment of cell populations with suppressive capabilities (e.g., regulatory T cells or myeloid-derived suppressor cells); (2) the production by different cell types of immunosuppressive molecules (e.g., TGFβ, IL-10, indoleamine 2,3-deoxygenase) and (3) the expression of ligands that activate inhibitory receptors (e.g., CTLA-4 or PD-1). Altogether, these processes prevent the development of effective antitumor immune responses and constitute an obstacle against the generation of efficient cancer vaccines.[1]

T cells are one of the main targets of the tumor-associated immunosuppressive microenvironment. One of the mechanisms that can render tumor antigen-specific responses inefficient is anergy.[2] Anergy in T cells has been described as a process of self-inactivation that results from T-cell receptor (TCR) activation in the absence of appropriate co-stimulation, e.g., via CD28.[3] In the past 10 years, we have started to unravel the complex mechanisms that are responsible for the induction and maintenance of anergy in T cells. Several forms of T-cell anergy have been described, each occurring under specific conditions and in response to different kinds of stimuli. For instance, clonal anergy has been extensively characterized in vitro and is caused by the activation of a NFAT-dependent program of gene expression that is regulated by specific NFAT-containing transcriptional complexes and leads to the synthesis of negative regulators of TCR signaling and silencers of cytokine expression.[4] Other forms of T-cell anergy respond to different stimuli and are characterized by a different set of signaling alterations. For instance, adaptive tolerance develops when self-reactive T cells are transferred into lymphopenic hosts and is characterized by inhibition of TCR signaling due to defective ZAP70 activity.[5] Despite this in-depth characterization of the mechanisms that control T-cell responsiveness, the specific roles that various forms of T-cell anergy play in distinct physiological or pathological situations to regulate T-cell reactivity remain largely uncharacterized.

Anergy has been reported to occur in T cells that infiltrate tumors,[2] and the immunosuppressive nature of the tumor microenvironment is thought to be responsible for this effect. In particular, the lack of dendritic cell maturation appears to account for an inefficient presentation of tumor antigens (**Fig. 1**).[6] In turn, suboptimally presented antigens, presumably due to the presence of inhibitory signaling and/or poor co-stimulation, would lead to the induction of T-cell anergy. This process has been shown to affect both $CD4^+$ and $CD8^+$ T-cell populations, in both solid and hematological tumors.[7,8]

Using a model of melanoma (B16), we have shown that tumor antigen-specific $CD4^+$ T cells become anergic as a result of the expression of genes that have been previously characterized as part of the program that regulates clonal anergy in helper T cells.[9] Using mice whose T cells expressed a transgenic TCR specific for ovalbumin (expressed by B16 cells as a model antigen), we could detect that anergy is established in helper T cells in a tumor antigen-specific way that results in diminished proliferative responses and decreased IL-2, IFNγ and IL-17 production by tumor-infiltrating T lymphocytes and T cells isolated from tumor-draining lymph nodes. Interestingly, these cells upregulated the expression of several clonal anergy-associated genes, including *Grail*, *Itch*, *Egr2* or C*asp3*, suggesting that a process related to clonal anergy is responsible for the inactivation of helper T cells in the tumor microenvironment. In an attempt to characterize this process, we used animals deficient in NFAT1, the transcription factor that regulates the expression of anergy-associated genes in T cells. Confirming our results, the lack of NFAT1 prevented the expression of anergy genes, and *Nfat1*$^{-/-}$ T cells failed to become anergic in B16 melanoma-bearing animals.

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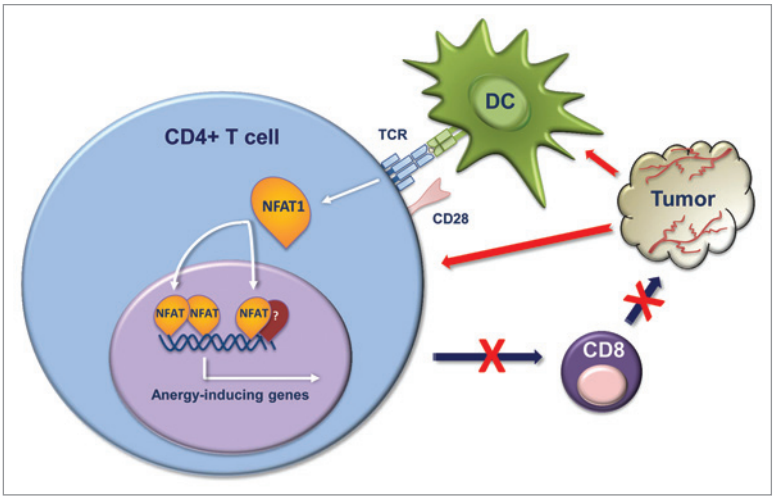

Figure 1. Tumor-induced helper T-cell anergy. The immunosuppressive tumor microenvironment induces anergy in helper T cells through the NFAT-dependent expression of anergy-inducing genes, coding for proteins that can inhibit TCR signaling and silence cytokine expression. The activation of this program can be caused by interferences with dendritic cell (DC) maturation, which might result in defective co-stimulation, although a direct role for other cells in the tumor microenvironment cannot be excluded. Anergic $CD4^+$ T cells cannot efficiently "help" $CD8^+$ T-cell antitumor responses, allowing cancer cells to escape $CD8^+$ T cell-mediated lytic responses.

One of the questions that remained unanswered by previous models was to what extent anergy contributes to the overall process of tumor escape from T cell responses. Interestingly, NFAT1 deficiency does not seem to have a marked effect on T-cell activation, possibly due to a compensatory effect by NFAT2. Nevertheless, *Nfat1*$^{-/-}$ T cells are less susceptible to anergizing stimuli.[4,10] As NFAT1-deficient cells can mount T-cell responses but fail to normally undergo anergy, we used this model as a tool to evaluate the relative contribution of helper T-cell anergy to tumor immune escape. Mice bearing anergy-resistant T cells were able to control tumor growth more efficiently than their wild type counterparts, which was reflected by a reduced tumor growth rate and hence in a delayed appearance of detectable melanoma lesions. This process was dependent of $CD8^+$ cells and likely reflected the ability of NFAT1-deficient cells to provide efficient help to tumor-specific $CD8^+$ T-cell populations. However, this benefit was eventually overcome, allowing tumors to grow. These results indicate that, as previously proposed, anergy occurs early during the establishment of antitumor T-cell responses, and that other immunosuppressive mechanisms are activated later during tumor progression to inhibit antitumor T-cell responses.[9]

Our results demonstrate that the NFAT-regulated expression of anergy-associated genes is responsible for the induction of tumor-antigen specific helper T cell anergy (**Fig. 1**) and that this process significantly limits antitumor T cell responses and promotes immune evasion early in the oncogenic process. Several therapeutic approaches have been analyzed to boost antitumor responses, ranging from the blockade of inhibitory receptors to the adoptive transfer of antigen-specific T cells. We propose that inhibiting anergy by targeting specific NFAT complexes or the downstream genes may represent a powerful means to boost antitumor T cell responses and increase the efficacy of immunotherapeutic approaches.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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