Renal cell carcinoma (RCC) exhibit a prominent immune cell infiltrate consisting of T cells, natural killer (NK) cells, dendritic cells (DCs) and macrophages. Intratumoral CD8+ T and NK cells are differentiated effector cells with lytic granules. Moreover, some CD8+ T cells express a tumor-reactive T-cell receptor (TCR) and mediate antitumor reactivity when analyzed ex vivo following exposure to interleukin (IL)-2. A high frequency of NK cells among the lymphocytic infiltrate seems to predict a better prognosis. Still, tumors grow despite the infiltration of potentially tumor-reactive cytotoxic effector cells indicating that their antitumor activity is compromised within the tumor microenvironment.

Using immune histology and ex vivo analysis of tumor-infiltrating leukocytes (TILs), we identified alterations in RCC-infiltrating T cells, NK cells and DCs that may be relevant for the loss of local immune competence and ensuing in tumor immunoescape. DCs are central regulators of immune responses with the capacity to induce immunity or tolerance depending on their differentiation state. Thus, targeting this cell population would constitute an effective means for tumors to alter the immune response toward immunosuppression. Indeed, we identified a DC subtype that is enriched within RCC (ercDC), co-expressing markers of DCs (CD209) and macrophages (CD14). Tumor-secreted factors (CXCL8 plus IL-6 and the vascular endothelial growth factor, VEGF) were sufficient to induce the ercDC differentiation state. ErcDCs were often found in close proximity to T cells; yet the absence of evidence of direct T-cell inhibition suggests that they are different from classical myeloid-derived suppressor cells. While not inhibiting T cells directly, ercDCs nevertheless showed characteristics related to tumor immunoescape: they intrinsically produced high levels of matrix metalloproteinase 9 (MMP-9) and in T-cell cross-talk experiments they induced milieu changes that are known to promote tumor cell proliferation (elevated secretion of tumor necrosis factor α, TNFα) and to limit the recruitment of T1-polarized lymphocytes (reduced levels of CXCL10, CCL5) (Fig. 1).

A T1/1T1-polarized infiltrate is associated with good prognosis in many tumor types. The immune infiltrate of RCC is indeed T1/1T1-polarized, as indicated by CXCR3 expression and the presence of lytic granules. Thus, why are RCCs not rejected? We addressed this question by analyzing the functional status of tumor-infiltrating CD8+ T cells (CD8+ TILs) and NK cells ex vivo, directly after isolation from the tumor tissue. They were non-responsive to stimulation, lacking granule mobilization, cytolytic activity and cytokine production. Deficits in the activation of TCR distal signaling molecules were found causative for the functional unresponsiveness of CD8+ TILs. Among other alterations, we observed high diacylglycerol kinase α (DGKα) expression, low basal phosphorylation of extracellular signal-regulated kinase (ERK) as well as reduced stimulation-induced activation of ERK, c-Jun N-terminal kinase (JNK) and AKT (Fig. 1). These features were caused by the tumor microenvironment as they were not observed in CD8+ T cells from normal kidney tissues (CD8+ NILs), which were functionally active. A signature similar to that observed for CD8+ TILs has been described for anergic CD4+ T cells, but it was not found in profiles of exhausted CD8+ T cells during chronic viral infection, in spite of the fact that the latter functionally resemble CD8+ TILs. DGKs are physiological inhibitors of TCR signaling. Indeed, we were able to link high DGKα levels to suppressed
IL-2 therapy has a long history in the treatment of RCC. However, response rates are low and complete responses are limited to a small subgroup of RCC patients, indicating that some mechanisms of immunoescape within the RCC environment cannot be overcome by IL-2.

Specific features of clear cell RCC, including loss-of-function mutations of the von Hippel-Lindau gene and anergic CD4+ T-cells.

ERK phosphorylation and to inhibition of CD8+ TIL function, as TILs showed stronger ERK activation and better degranulation when stimulated in the presence of a DGKα inhibitor (Fig. 1). Moreover, we observed that the in vivo-repressed CD8+ TIL functions were reversible by ex vivo culture in the presence of IL-2. IL-2 is known to regulate DGKα and to restore responsiveness of anergic CD4+ T-cells. Indeed, functional recovery of CD8+ TILs occurred concomitantly with a decrease in DGKα levels and an increase in basal and stimulation-induced phosphorylation of ERK and AKT. In addition, culture in IL-2 reduced the levels of p27KIP1 and increased those of cyclin E1 suggesting that it promotes cell cycle progression, which is critical for T cells to overcome anergy.
overexpression of carboxic anhydrases, contribute to a tumor microenvironment that is rich in lactate and acidic. We observed that tumor lactic acidosis inhibits cytokine production and impairs lytic granule exocytosis of effector lymphocytes by inhibiting the phosphorylation of JNK, c-Jun and MAPK p38, which are required for T-cell effector function. Of note, the inhibition of effector lymphocytes could be prevented by neutralizing acidosis even in the continuous presence of lactate.

Collectively, our studies provide insights into the paradoxical situation whereby RCCs are generally not rejected despite being strongly infiltrated by various immune effector cells. Among other alterations, we observed tumor-induced changes in DC differentiation and the induction of anergy-associated genes in T cells, preventing the infiltrate to mediate antitumor functions. Considering the tumor-specific immune context (i.e., type, density and functional orientation of TLs) and adjusting therapy according to the composition of the infiltrate could result in improved response rates. Patients carrying tumors with a high ercDC frequency may benefit best from the administration of tyrosine kinase inhibitors, as these agents have the potential to re-polarize myeloid cells to support antitumor responses. However, patients with high numbers of tumor-infiltrating NK and CD8+ T effector cells should be considered for IL-2 therapy. The response rates to IL-2-based approaches may be improved by DGKα inhibition and anti-acidosis treatment, as these interventions may re-establish and prolong the functional responsiveness of the cytotoxic cell infiltrate.

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