Commentary

Tumor-infiltrating T cells are invigorated by modulating cholesterol metabolism

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CD8$^+$ cytotoxic T cells are fundamental immune cells required for tumor suppression. Under infectious conditions, these cells respond to their corresponding antigen, differentiating to cytotoxic effector T cells. Upon resolution of infection, most effector T cells will undergo contraction while a subset remain, forming immunological memory to protect from subsequent infection. During chronic infection, such as infection with chronic lymphocytic choriomeningitis virus (LCMV), HIV or hepatitis, responding CD8$^+$ T cells will undergo what has been termed 'exhaustion', in which these cells rapidly lose effector function (1). Importantly, in the context of the tumor microenvironment, where tumor self-antigen can be considered 'chronic', CD8$^+$ T cells also become exhausted, losing effector function and expressing inhibitory receptors such as PD-1 and CTLA-4 (1,2). Reversing exhaustion of tumor infiltrating effector T cells is considered fundamental for tumor eradication and a key focus of cancer immunotherapy (3).

In recent years, cancer immunotherapy has concentrated on increasing immune activation to enable the patient's own immune system to attack tumors, leading to dramatic results for patients with some types of tumors (4,5). Much effort has been focused on immune checkpoint inhibitors, a class of drugs that can that 'take the breaks off' the immune system by inhibiting the interaction of checkpoint receptors, including PD-1 and CTLA-4, with their ligands thus preventing inhibitory signaling through these checkpoint receptors (4,5). Treatment with immune checkpoint inhibitors has led to long-term durable responses for many patients with melanoma. However, across tumor types a majority of patients fail to respond to immune checkpoint blockade therapy (6). Furthermore, immune checkpoint blockade therapy does not fully reinvigorate the immune response (1). Thus, an effort is underway to identify methods that, when used in combination with checkpoint blockade therapy, further enhances the immune response to tumors. These efforts have included investigating methods to increase stimulation or co-stimulation of T cells. A recent study by Yang *et al*. provides a novel method by which to do this (7).

While the important role of metabolism in the effector T cell response is now well established (8,9), Yang and colleagues explore one intriguing area of metabolism, namely cholesterol metabolism, in the context of the effector T cell response and demonstrate that modification of cholesterol metabolism can increase anti-tumor response in these cells (7). In healthy mammalian cells, cholesterol is a critical component of the plasma membrane and often clusters at lipid rafts (10). In T cells, lipid rafts cluster at sites of T cell receptor (TCR) signaling and cholesterol is considered a critical factor required for TCR signaling (10,11). Free cholesterol can be esterified by cholesterol enzymes, ACAT1 and ACAT2, resulting in cholesteryl esters, which can then be stored in the cell (12). Yang *et al*. focus on inhibition of these cholesterol enzymes to augment the CD8$^+$ anti-tumor response (7).

Using cholesterol esterification inhibitors including CP-113,818 the authors showed mouse and human CD8$^+$ T cells produced more IFN-γ and TNF-α *in vitro*, while use of inhibitors of cholesterol biosynthesis resulted in

reduced cytokine production (7). Using T cell specific conditional ACAT1-deficient mice, Yang and colleagues went on to show that genetic ablation of ACAT1 in T cells recapitulated the *in vitro* results observed with the ACAT inhibitors (7). Moreover, cell survival and proliferation were increased in the absence of ACAT1. The authors demonstrated this result was restricted to $CD8^+$ T cells, postulating that ACAT2 may be compensating in $CD4^+$ effector cells. Using three tumor models, a skin melanoma model, a lung metastasis melanoma model, and the Lewis lung carcinoma model, Yang *et al*. showed loss of ACAT1 in T cells increased survival times and resulted in more $CD8^+$ T cell activation and cytotoxicity as well as inflammatory cytokine production (7). Interestingly, ACAT1 T cell deficiency also appeared to limit number of metastases in the lung metastasis melanoma model. Importantly, ACAT1 is a potential target for cancer immunotherapy. Administration of the ACAT1 inhibitor, avasimibe, a drug previously used to treat atherosclerosis in humans (13), to mice bearing established tumors similarly limited tumor progression, improved survival, enhanced T cell effector function and importantly, synergized with anti-PD1 therapy (7). Indeed, combined avasimibe and anti-PD1 therapy was more efficacious than either therapy alone. As the data showed that each therapy acted independently, the authors concluded that combination therapy had an additive anti-tumor effect (7). Importantly, both IFN-γ and TNF-α production increased in human $CD8^+$ T cells in the presence of avasimibe (7).

The authors went on to show that ACAT1-deficiency or inhibition led to higher plasma membrane cholesterol levels, and this correlated with higher TCR signaling levels upon stimulation with anti-CD3 and anti-CD28 as determined by increased phosphorylation of ZAP70, LAT and ERK (7). ACAT1-deficiency also resulted in more compact immunological synapses that formed at a faster rate. The authors postulated that improved immunological synapses in ACAT1-deficient $CD8^+$ T cells led to improved killing. To determine if increased plasma membrane cholesterol levels could account for the increased TCR clustering and effector functions in ACAT1-deficient $CD8^+$ T cells, the authors investigated the effect of addition of cholesterol to WT T cells or depletion of cholesterol from ACAT1 deficient T cells. They found that increasing cholesterol levels in WT T cells increased TCR clustering and enhanced cytokine production and cytotoxicity, while depletion of cholesterol from ACAT1-deficient cells limited these functions (7).

The increased plasma membrane cholesterol concentration apparently results from both loss of cholesterol esterification and an increase in cholesterol biosynthesis, due to increased expression of genes involved in cholesterol biosynthesis (7). The mechanism by which ACAT1 deficiency led to increased cholesterol biosynthesis remains an open question, although it is easy to imagine a system by which a certain concentration of cholesteryl esters feeds back to limit cholesterol biosynthesis. In the era of cancer immunotherapy, Yang *et al*. provide precedent for modification of T cell cholesterol metabolism in order to improve patient outcomes. Importantly, inhibition of ACAT with avasimibe is already in use in clinical trials for both heart disease and Alzheimer's disease (13,14). Yang and colleagues show combined immunotherapy using both avasimibe and anti-PD1 may be key to improving the $CD8^+$ T cell immune response to tumors.

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Footnote

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