# Tumor Cholesterol Up, T Cells Down

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**Highly suppressive tumor microenvironments often result in T cell dysfunction and inability to control tumors, but factors regulating this process remain elusive. A new study by Ma et al. (2019) reports that tumor cholesterol devitalizes T cells by modulating endoplasmic reticulum stress pathways, revealing a new mechanism underlying T cell exhaustion.**

T cell exhaustion is a common phenomenon in chronic infections and in the tumor microenvironment (TME). Prolonged antigen exposure and inflammation result in a dysfunctional state of T cells characterized by upregulation of inhibitory immune checkpoint receptors such as PD-1, LAG-3, TIM-3, CTLA-4, and 2B4; decreased production of effector cytokines; decreased proliferation; and altered transcriptional and metabolic profiles (Wherry and Kurachi, 2015). Targeting inhibitory checkpoints, particularly PD-1, with monoclonal antibodies is an effective strategy to reinvigorate exhausted T cells and has shown remarkable clinical success (Chinai et al., 2015). However, resistance to checkpoint blockade occurs in a large majority of cancer patients and is partially due to concomitant upregulation of multiple co-inhibitory molecules. This highlights the need to better understand the mechanisms regulating immune checkpoint expression and subsequent T cell exhaustion in the TME. Several molecular and cellular factors control PD-1 expression, including TCR activation, various transcription factors, and epigenetic components. Cancer cell-derived kynurenine metabolite in the TME is also known to promote PD-1 expression on T cells. Indeed, it is established that tumors have unique metabolic restrictions affecting T cell function. However, it is unknown whether other metabolic factors in the TME can induce immune checkpoint upregulation, the main hallmark of T cell exhaustion.

Cholesterol is an essential lipid component of the immune cell membrane. It can be acquired or synthesized by T cells and participates in cell activation, proliferation, metabolism, and anti-tumor immune response (Bietz et al., 2017). A previous study showed that cholesterol negatively regulates IL-9-producing CD8 T cell differentiation and anti-tumor activity (Ma et al., 2018). In this issue of *Cell Metabolism*, Ma and colleagues now reveal new insights into the link between cholesterol metabolism and CD8 T cell exhaustion in tumors (Ma et al., 2019). Across different murine models, they observed cholesterol was enriched in tumor tissues and tumor-infiltrating CD8 T cells (CD8 TILs), and immune checkpoint (PD-1, 2B4, TIM-3, and LAG-3) expression level on these CD8 T cells was positively associated with cholesterol accumulation in the cells (Figure 1). Consistently, they found adoptively transferred CD8 T cells accumulated cholesterol and acquired higher immune checkpoint expression upon entry into the TME, consequently undergoing dysfunction. These observations in mouse models are relevant to human as the authors confirmed that cholesterol accumulation was higher in tumor tissues and CD8 TILs and positively correlated with upregulated checkpoint expression in colon cancer patients. Additional studies will be needed to test if cholesterol levels in tumor cells or TILs can be used to predict disease progression in patients.

Through a series of *in vivo* and *in vitro* experiments, the authors found that tumor-derived or exogenous cholesterol was able to induce T cell exhaustion by upregulating immune checkpoints on CD8 T cells and promoting apoptosis. Microarray and ingenuity pathway analysis revealed disrupted lipid metabolism and increased endoplasmic reticulum (ER) stress response in cholesterol-treated CD8 T cells. In particular, the transcription factor X-box binding protein 1 (XBP1), an ER stress sensor, was strongly upregulated upon cholesterol treatment, suggesting XBP1 might account for cholesterol-induced CD8 T cell exhaustion. An important remaining question is how ER stress is induced by cholesterol in CD8 T cells. In macrophages, excess cellular cholesterol was shown to traffic to and accumulate in the ER, deplete calcium stores, activate the unfolded protein responses, and ultimately lead to apoptosis (Feng et al., 2003). Does a similar mechanism exist in T cells? The authors further identified XBP1 binding sites on both *Pdcd1* (PD-1 gene) and *CD244* (2B4 gene) promoters, and found that overexpressed XBP1 increased PD-1 and 2B4 mRNA and protein expression. Furthermore, pharmacological inhibition or genetic ablation of XBP1 reversed cholesterol-induced exhaustion and recovered anti-tumor activity of adoptively transferred CD8 T cells in a murine melanoma metastasis model. Further investigation is needed to determine if the ER-stress-XBP1 pathway is required for cholesterol-induced T cell exhaustion in humans as well. Lastly, by reducing cholesterol content in the TME with statins or blocking cholesterol synthesis in either tumor cells or CD8 T cells, the authors observed less checkpoint expression and enhanced anti-tumor activity of CD8 T cells. These novel and important findings raise several questions: Is cholesterol synthesized by tumor cells or other cells in the TME? How does it accumulate in CD8 T cells? What is the contribution of cholesterol uptake from the TME versus cellular synthesis to the overall increase? It would be interesting to further assess expression of cholesterol transporters and biosynthetic molecules in CD8 TILs.

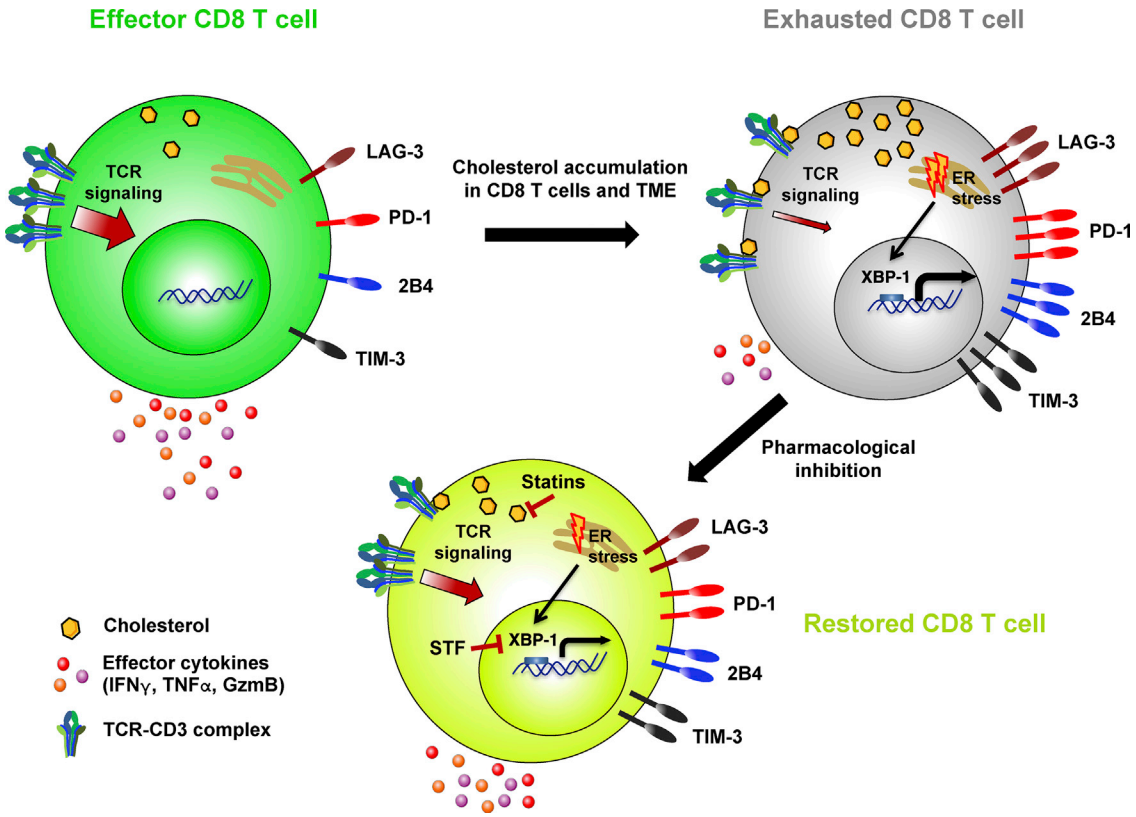


**Figure 1. Cholesterol Is a New Regulator of CD8 T Cell Exhaustion in Tumors and Is a Potential New Target for Cancer Immunotherapy**
Cholesterol enriched in the tumor microenvironment (TME) induces CD8 T cell exhaustion through an ER-stress-XBP1 pathway-dependent manner. In parallel, cholesterol inhibits TCR signaling by binding to transmembrane region of TCRβ chain and disrupting TCR clustering. Pharmacological inhibition of cholesterol (statins) and ER stress (STF) in the TME restores anti-tumor activity of CD8 T cells.

Because cholesterol is a key component of membrane lipids, other groups have studied its role in TCR clustering, signaling, and T cell activation (Schamel et al., 2017). Two studies found that TCR signaling was inhibited by cholesterol or cholesterol sulfate by either binding to the TCRβ transmembrane region (Swamy et al., 2016) or disrupting TCR multimers (Wang et al., 2016), respectively (Figure 1). However, a third study reported plasma membrane cholesterol enhanced TCR nanoclustering and formation of the immunological synapse, thereby resulting in better anti-tumor activity of CD8 T cells (Yang et al., 2016). In the current study, total cellular cholesterol was measured, but the plasma membrane level versus the intracellular level remained unassessed. It remains to be seen if cholesterol accumulation in CD8 TILs can also modulate TCR conformation and signaling, which might lead to upregulation of immune checkpoint expression independent of the ER-stress-XBP1 pathway. Overall, this study sheds a new light on the interplay between cholesterol metabolism and the anti-tumor response, highlighting XBP1 and cholesterol as potential effective targets to improve efficacy of T cell-based immunotherapy against cancer.

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## REFERENCES

Bietz, A., Zhu, H., Xue, M., and Xu, C. (2017). Cholesterol metabolism in T cells. Front. Immunol. *8*, 1664.

Chinai, J.M., Janakiram, M., Chen, F., Chen, W., Kaplan, M., and Zang, X. (2015). New immunotherapies targeting the PD-1 pathway. Trends Pharmacol. Sci. *36*, 587–595.

Feng, B., Yao, P.M., Li, Y., Devlin, C.M., Zhang, D., Harding, H.P., Sweeney, M., Rong, J.X., Kuriakose, G., Fisher, E.A., et al. (2003). The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. Nat. Cell Biol. *5*, 781–792.

Ma, X., Bi, E., Huang, C., Lu, Y., Xue, G., Guo, X., Wang, A., Yang, M., Qian, J., Dong, C., and Yi, Q. (2018). Cholesterol negatively regulates IL-9-producing CD8$^+$ T cell differentiation and antitumor activity. J. Exp. Med. *215*, 1555–1569.

Ma, X., Bi, E., Lu, Y., Su, P., Huang, C., Liu, L., Wang, Q., Yang, M., Kalady, M.F., Qian, J., et al. (2019). Cholesterol induces CD8$^+$ T cell exhaustion in the tumor microenvironment. Cell Metab. *30*, this issue, 143–156.

Schamel, W.W., Alarcon, B., Höfer, T., and Minguet, S. (2017). The allostery model of TCR regulation. J. Immunol. *198*, 47–52.

Swamy, M., Beck-Garcia, K., Beck-Garcia, E., Hartl, F.A., Morath, A., Yousefi, O.S., Dopfer, E.P., Molnár, E., Schulze, A.K., Blanco, R., et al. (2016). A cholesterol-based allostery model of T cell receptor phosphorylation. Immunity *44*, 1091–1101.

Wang, F., Beck-García, K., Zorzin, C., Schamel, W.W., and Davis, M.M. (2016). Inhibition of T cell receptor signaling by cholesterol sulfate, a naturally occurring derivative of membrane cholesterol. Nat. Immunol. *17*, 844–850.

Wherry, E.J., and Kurachi, M. (2015). Molecular and cellular insights into T cell exhaustion. Nat. Rev. Immunol. *15*, 486–499.

Yang, W., Bai, Y., Xiong, Y., Zhang, J., Chen, S., Zheng, X., Meng, X., Li, L., Wang, J., Xu, C., et al. (2016). Potentiating the antitumour response of CD8(+) T cells by modulating cholesterol metabolism. Nature *531*, 651–655.