

## INSIGHTS

### Less cholesterol means better tumor killing for cytotoxic T9 cells

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In this issue, Ma et al. (<https://doi.org/10.1084/jem.20171576>) show that removal of cholesterol from CD8 T cells during type 9 differentiation increases their IL-9 production, persistence in vivo, and cytolytic function against tumors by preventing SUMOylation of liver X receptors.

Ma et al. (2018) show that cholesterol negatively regulates differentiation of CD8 T cells toward a type 9 phenotype through SUMOylation of liver X receptors (LXRs) and that removal of cholesterol from these cells increases their IL-9 production and antitumor activity. IL-9-producing cytotoxic CD8 T cells (Tc9) have been shown to have better tumor killing potential than the classical IFN- $\gamma$ -producing cytotoxic CD8 T cells (Tc1) in several studies and with different tumor models (melanoma, lung metastasis, lymphoma, and acute myeloid leukemia; Lu et al., 2012, 2014; Purwar et al., 2012; Ramadan et al., 2017; Ma et al., 2018).

Cancer immunotherapy was designated as the “breakthrough therapy” of the year in 2013 by *Science*, and for good reason. These therapies allow a person’s own immune cells, often T cells, to specifically target tumor cells while limiting normal tissue damage. Use of antibodies against the checkpoint molecules programmed cell death protein 1 and cytotoxic T-lymphocyte antigen 4, which tumors use to normally inhibit the immune response, increase the antitumor activity of T cells across multiple malignancies mediated primarily through CD8 T cells (Topalian et al., 2012; Eggermont et al., 2016). Cancer vaccines have been made from neoantigens from cancer cells to help target T cells against the cancer (Ott et al., 2017). Adoptive transfer of manipulated T cells through generation of chimeric antigen receptors has shown incredible promise against blood cancers and have now received Food and Drug Administration approval (Maude et al., 2014; Neelapu et al., 2017). Now, the group of Qing Yi and colleagues (see Ma et al. in this issue) report that Tc9 cells differentiated with minimal

cholesterol increases their persistence and increases their antitumor activity in vivo.

Cholesterol is important for membrane protein function, regulating transmembrane signaling, and membrane trafficking, while also acting as a metabolite for signal transduction. In T cells, cholesterol can influence TCR signaling, immunological synapse formation, and cytokine secretion, all of which can influence antitumor activity (Wang et al., 2016; Yang et al., 2016). To identify cholesterol as a negative regulator of Tc9 differentiation, Ma et al. (2018) first used Ingenuity Pathway Analysis comparing microarrays from murine cytotoxic CD8 T cells differentiated under type 1 (IL-2 and IL-12) and type 9 (TGF- $\beta$  and IL-4) cytokine conditions. They found that Tc9 cells have lower basal expression of cholesterol synthesis genes, such as *Hmgcr* and *Sqle*, and high expression of cholesterol efflux genes, such as *Abcg1* and *Abca1*, than Tc1 cells. To further characterize how cholesterol affects Tc9 cells, they added either cholesterol or cholesterol inhibitors like  $\beta$ -cyclodextrin ( $\beta$ -CD) and statins drugs to increase or decrease, respectively, the amount of cholesterol in the Tc9 cells. IL-9 expression and production, a hallmark of successful Tc9, was down-regulated with the addition of cholesterol and up-regulated with the addition of  $\beta$ -CD or statins. Importantly, this phenotype was recapitulated in human cells. Tc9 cells from *Apoe*<sup>-/-</sup> mice, a transgenic, atherosclerosis-prone mouse with high levels of cellular and plasma cholesterol, had impaired IL-9 expression and production compared with Tc9 cells from wild-type mice. The authors found that the cholesterol-mediated regulation of IL-9 is specifically dependent on LXRs because all



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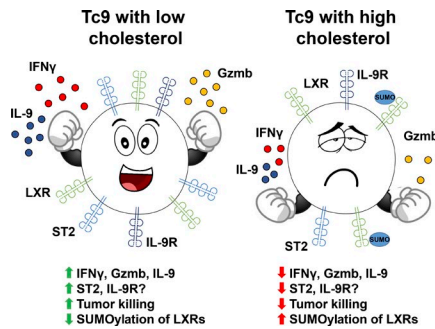
oxysterols but no other cholesterol derivatives inhibited IL-9 expression and secretion, further suggesting the inhibitory role of LXRs on IL-9 production. Synthetic LXR agonists also inhibited IL-9 production in Tc9 cells. Indeed, cholesterol and oxysterols cause SUMOylation of LXRs, leading to inhibition of IL-9 through reducing the binding of the transcription factor p65 to the *Il9* promoter in the Tc9 cells.

Type 9 cells have been shown to persist long term in vivo after transfer in multiple studies (Lu et al., 2012, 2014; Ramadan et al., 2017). Ma et al. (2018) show here that Tc9 cells from Pmel-1 mice, a transgenic strain that carries a rearranged T cell receptor specific for pmel-17, an orthologue of the melanocyte differentiation antigen gp100, which is overexpressed in many human melanomas, persist longer than Tc1 cells when adoptively transferred into MC38-gp100 tumor bearing mice. Treatment of Tc9 cells with  $\beta$ -CD before adoptive transfer increased their persistence in vivo, whereas treatment with cholesterol decreased their persistence compared with untreated Tc9 cells by decreasing and increasing apoptosis, respectively. Importantly, treatment with  $\beta$ -CD increased the number of IFN- $\gamma$  and granzyme-B-producing Tc9 cells in

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Effect of cholesterol on antitumor activity of Tc9 cells. Left: Differentiation of Tc9 cells with low cholesterol decreases the SUMOylation of LXRs, leading to increased production of the cytolytic proteins IFN- $\gamma$  and Gzmb, increased IL-9 production, possibly increased IL-9R expression, and increased expression of ST2. These cells have strong antitumor activity. Right: Differentiation of Tc9 cells with high cholesterol increases the SUMOylation of LXRs, leading to decreased production of the cytolytic proteins IFN- $\gamma$  and Gzmb, decreased IL-9 production, possibly decreased IL-9R expression, and decreased expression of ST2. These cells have weak antitumor activity.

the tumor, which are required for optimal tumor killing. This led to decreased tumor growth both in this skin tumor model and in a metastatic lung tumor model. Surprisingly, the cholesterol level in the hosts did not affect the killing potential of the adoptively transferred Tc9 cells, as tumors in *Apoe*<sup>-/-</sup> hosts grew similarly to tumors in wild-type hosts when both were treated with  $\beta$ -CD-Tc9 cells. The antitumor activity of these cells is strongly dependent on IL-9 expression, as loss of IL-9 led to poor persistence and poor antitumor activity. These effects are summarized in the figure.

One of the more interesting data [Ma et al. \(2018\)](#) received from their microarray study was the increase in *Il1rl1* expression. *Il1rl1* encodes for the protein ST2, also known as the IL-33 receptor. It has recently been shown that adding IL-33 to normal type 9 differentiation promotes IL-9 production and ST2 expression on their cell surface. In mice with lymphoma or acute myeloid leukemia, these type 9 T cells with IL-33 (T9<sub>IL-33</sub>), when adoptively transferred into mice undergoing allogeneic bone marrow transplantation, exhibited a stronger antitumor effect than T9 cells without IL-33 ([Ramadan et al., 2017](#)). It

would be exciting to see if removing cholesterol during in vitro differentiation or with systemic administration of statins along with addition of IL-33 could further increase the antitumor potential of these T cells. In addition, IL-9 seems to be a driver of antitumor activity of these cells, as loss of IL-9 abrogated the effects of  $\beta$ -CD and IL-33 ([Ramadan et al., 2017](#); [Ma et al., 2018](#)). What role IL-9 has in promoting antitumor activity has yet to be elucidated. It is important that we now try to understand why IL-9 is necessary for optimal type 9 T cell antitumor function so that we may bring this type of adoptive transfer therapy closer to the clinic.

The results presented by the authors contrast the data showing that increased free cholesterol content in cells through inhibition of cholesterol esterification enhances the antitumor activity of CD8 T cells ([Yang et al., 2016](#)). Whereas bulk CD8 cells may require more cholesterol for their antitumor effect, cholesterol has been shown in various cell lines to negatively modulate TGF- $\beta$  signaling, which is necessary for type 9 T cells ([Chen et al., 2007](#)). Together, these data suggest that cholesterol and TGF- $\beta$  may have contrasting functions for Tc9 cell differentiation and function.

There are important clinical implications on modulating cholesterol during T cell culture for adoptive transfer. Although over 70% of American adults are at least overweight and almost 38% are obese ([Fryar et al., 2016](#)), treatment of overweight or obese patients with adoptively transferred Tc9 cells differentiated with minimal cholesterol may not be affected, as suggested by the experiments by [Ma et al. \(2018\)](#) that show that wild-type Tc9 cells adoptively transferred into *Apoe*<sup>-/-</sup> hosts have similar antitumor activity as when transferred into wild-type hosts. However, an important caveat of these experiments is that the adoptively transferred T cells were specific for the tumor antigen. It would be of interest to repeat these experiments using bulk Tc9 cells treated with  $\beta$ -CD or statins that are already used in clinic. Many patients who are overweight or obese also have high

cholesterol and they are treated with statins to help reduce their cholesterol. Statins have already been shown to be effective in enhancing antitumor activity through exerting anti-proliferative, proapoptotic, and anti-invasive effects of the tumor itself ([Altwaigri, 2015](#)). Perhaps, statins also increase IL-9 production in T cells, which could further explain the antitumor effect seen in patients who take statins. Adoptive transfer of both CD4 and CD8 T cells differentiated under type 9 conditioning has also recently been shown to better control lymphoma and acute myeloid leukemia after experimental bone marrow transplantation ([Ramadan et al., 2017](#)). It will be of interest to determine if adoptive transfer of T cells differentiated under type 9 conditioning can increase antitumor activity against other tumor types besides melanoma, lung metastasis, lymphoma, and acute myeloid leukemia. Collectively, the results of this study along with what is known about the antitumor capabilities of Tc9 cells will lead to more exciting research toward development of better adoptive transfer therapies against tumors.

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