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INSIGHTS

Goldilocks and the three TILs

Hannah Dada[1] and Michael L. Dustin[2]

In this issue of *JEM*, Shakiba et al. (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20201966) tell a tale of three tumor infiltrating lymphocytes (TILs). The first TIL was too strong and became exhausted. The second TIL was too weak and became inert. The third TIL lost CD8, and this made it just right.

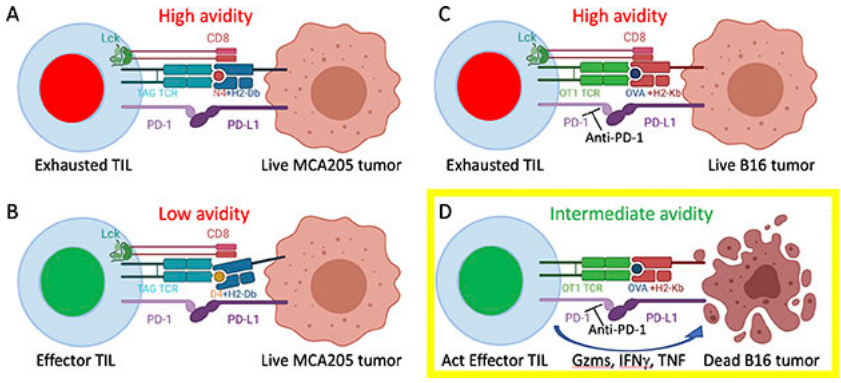
Shakiba et al. (2021) explore the relationship between TCR signal strength, gene expression programs, and tumor control in a mouse model to get at relevant mechanisms. The outcome of immunotherapy in solid tumors with T cells that can kill tumor cells in vitro is still difficult to predict, such that a better understanding of how TCR signal strength relates to tumor control is important. Their work reveals distinct genetic programs for strong and weak TCR engagement, including the coreceptor contribution. T cells with too strong a TCR entered a nonfunctional exhausted state, and T cells with too weak a TCR were stuck in a functionally capable but inert state in the tumor (see figure, A and B). They hypothesized that a balance might be struck between the triggering capacity of the strong TCR and the functional genetic program of the weak TCR to enable tumor control by TIL. Another way to test this hypothesis was to attenuate the TCR signal through elimination of the coreceptor, CD8. In combination with a classical strong TCR (OT1 TCR recognizing SIINFEKL bound to MHC class I allele H-2K^b), the loss of CD8 enables clearance of B16 mouse melanoma tumors expressing the SIINFEKL epitope in the presence of anti-PD1, which was not possible with OT1 T cells expressing CD8 (see figure, C and D). In the absence of CD8, the strong TCR activated a functional genetic program like the weak TCR with CD8, but the strong TCR was able to execute the functional program and control an aggressive tumor without CD8. This work suggests a general strategy for T cell engineering to target mutated tumor antigens for which strong TCR can develop or be engineered. The work raises a number of interesting questions.

First off, how did they look at TCR signal strength? Shakiba et al. (2021) perform the initial analysis on a mouse tumor antigen model. In this model, an H-2D^b restricted peptide SAINNYAQKL from SV40 large T antigen epitope 1 (TAG), which induces transformation of cells and is a type of viral tumor antigen, is recognized by the TAG-H-2D^b–specific TCR carried by T cells isolated from B6 background transgenic mice. Altered peptide ligands (APLs) used in addition to the strongly recognized natural peptide sequence, referred to as N_4, were F_6 (SAINNFAQKL), with an 18-fold lower potency for T cell stimulation, and low-affinity D_4 (SAIDNYAQKL), with a 560-fold lower potency. They also demonstrated that all the APLs bind similarly to H-2D^b, such that the lower potency of APLs likely relates to affinity for the TCR or other properties that impact potency (Sibener et al., 2018). The TAG APLs were expressed in the MCA205 fibrosarcoma lines for T cell activation and tumor rejection studies. Through in vivo studies using the APL expressing tumors, Shakiba et al. revealed that there is no obvious discrimination in TCR signal strength when it comes to many parameters of TCR activation, proliferation, and effector differentiation on naive tumor-specific T cells in the draining lymph node. In fact, earlier studies in acute infection models revealed highly functional responses across a range of TCR signal strengths (Zehn et al., 2009). But the situation in tumors was different. Analysis of signaling pathways and epigenetics showed discrimination based on signal strength specifically at the tumor site. These differences were translated to gene expression. N_4 and F_6 drove the expression of genes associated with T cell dysfunction and exhaustion and the down-regulation of memory/effector function. Although D_4 drove distinct gene expression consistent with functional effector capacity, the TILs were unable to kill tumor cells presenting D_4. Together, these findings astutely uncover two nonredundant mechanisms of tumor escape at high and low TCR strength (see figure, A and B). Negative regulatory receptors PD1 and LAG1 were expressed in both settings, but 2B4 and CD39 served as surface markers for the exhausted phenotype. The effector program elicited by D_4 included GZMA, a key component of the cytotoxic machinery (Bálint et al., 2020).

Insights from Hannah Dada and Michael L. Dustin.

[1]National Institutes of Health Oxford-Cambridge Scholars Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD; [2]Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK.

Michael L. Dustin: michael.dustin@kennedy.ox.ac.uk.



Finding the Goldilocks zone for TILs. **(A)** Exhausted TILs elicited by strong antigen recognition fail to control MCA205 fibrosarcoma. **(B)** Weak antigen recognition leads to effector TILs but can't trigger MCA205 killing. **(C)** Replication of result in A with B16 melanoma model in the presence of anti-PD1. **(D)** Loss of CD8 from A resulted in just-right effector TILs that were able to kill B16 in the presence of anti-PD1—the Goldilocks zone.

Given these two extremes of tumor immune evasion, the authors reasoned that there could be a Goldilocks zone of intermediate strength of TCR-pMHC interaction where tumor-specific T cells could maintain their functionality and not be driven to rapid exhaustion, but the TCR signal would retain the ability to activate the effector programs needed for tumor control or eradication. Such peptides may exist between F_6 and D_4, but it would be a large undertaking to identify them in this new APL system and it might not lead to an actionable therapeutic strategy. So they didn't formally identify this sweet spot, but it's possible that finding this Goldilocks zone for TCR–peptide-bound MHC (pMHC) is a requisite for autoimmunity (Correa and Dustin, 2021; Dressel et al., 1997).

How did they find the Goldilocks zone? Faced with the challenge of reducing TCR signal strength that could be applied in a T cell immunotherapy setting, the authors asked if removal of the CD8αβ coreceptor would generate the necessary attenuation of the strong TCR signal to find the Goldilocks zone. This would then provide an opportunity for any high-affinity T cells generated against mutated self-proteins, where high affinity is likely, to be rescued. The current model for CD8 function is that it generates a relatively long-lived tetrameric complex of TCR, pMHC, Lck, and CD8 itself that enhances the TCR signaling platform (Stepanek et al., 2014) and enables single molecule sensitivity of the TCR (Irvine et al., 2002). Using CRISPR, Shakiba et al. knocked out the CD8α gene in the OT1 T cells, a distinct TCR transgenic system that recognizes chicken egg OVA peptide SIINFEKL presented by MHC class I allele H-2K^b, which is widely used as a model antigen. Interestingly, the attenuation of the dose response for the SIINFEKL peptide due to loss of CD8 in the OT1 T cells is similar to the difference between the N_4 and F_6 peptides in the presence of CD8 for the TAG T cells (Shakiba et al., 2021). However, the shape of the response curve is different with greater cooperativity evident for SIINFEKL in the absence of CD8, which is characteristic of TCR signaling without a coreceptor (Irvine et al., 2002). Thus, there are differences in the quality of the signal that may make the loss of CD8 more impactful than a simple tuning down of TCR–pMHC affinity by ∼10-fold. This was clearly the case, as the gene expression program of the CD8-deficient OT1 T cells responding to SIINFEKL-H-2K^b was similar to the CD8-sufficient TAG T cells exposed to the low-strength D_4 ligand. However, its known that OT1 T cells can kill targets expressing the SIINFEKL-H-2K^b complexes in the absence of CD8 (Yachi et al., 2006). Consistent with this, CD8-deficient OT1 T cells were able to control OVA expressing B16 tumors, in the presence of anti-PD1 antibodies, confirmed that they had found the Goldilocks zone for this system (see figure, C and D). While PD1 blockade was required for control of B16 tumor in this setting, the CD8 expressing OT1 T cells were unable to control the tumor growth even in the presence of anti-PD1 due to their exhausted epigenetic program. Of course, it will be interesting to determine in the future if this can be generalized to strong polyclonal T cell repertoires against tumors expressing mutated proteins. Additionally, there may be further potential to use CD8 engineering to potentially boost low-strength TCR–pMHC into the Goldilocks zone from the other direction (Clement et al., 2021). This may be helpful in situations where the TCR repertoire available in a patient is skewed toward lower affinity overexpressed self-antigens rather than higher affinity TCR to mutated proteins.

The finding that T cell responses against a tumor can be improved by removing CD8 would have seemed counter-intuitive before the finding that there are two distinct failure modes faced by TILs (Shakiba et al., 2021). This finding may also have implications for design of chimeric antigen receptor T cells (CAR-T), which are subject to exhaustion (Waldman et al., 2020). Synthetic biologists have started to engineer coreceptors to work with CAR-T, and this effort might be shaped by Shakiba et al.'s findings; it may be useful to have custom synthetic coreceptors that complement a given CAR-tumor antigen system to ensure they operate together to keep the T cells in the Goldilocks zone. Could particular anti-CD8 antibodies help to move endogenous TILs with strong TCR signaling into this Goldilocks zone? Schietinger and colleagues have provided an interesting new paradigm for T cell dysfunction in tumors that challenges current thinking and opens new opportunities.

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