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PD-L1 co-stimulation, ligand-induced TCR down-modulation and anti-tumor immunotherapy

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PD-1 engagement on the surface of effector T cells strongly suppresses their cytotoxic function, which constitutes a major obstacle for T cell-mediated anti-tumor activities. Surprisingly, PD-1 is strongly upregulated in T cells, engaging its ligand PD-L1 during antigen presentation. However, our recent published data may provide an explanation for this apparent contradiction.

The ultimate goal of anti-tumor immunotherapy is stimulating the immune system to specifically destroy cancer cells. The expansion of effector cytotoxic T lymphocytes is particularly important. However, immunotherapy relies on the assumption that the immune system can recognize cancer as "foreign" or "non-self". In reality, tumors are mostly immunologically silent or strongly immunosuppressive; they are usually recognized as "self" to which there is immunological tolerance. To develop effective anti-tumor therapies, we need first to understand these tolerogenic/immunosuppressive mechanisms, in order to interfere with them while minimising collateral damage.

T cell responses are key in the induction of protective and long-lasting immunity. However, if uncontrolled, effector T cells can cause significant autoreactive damage. Thus, T cell activation is regulated at multiple levels, especially during antigen presentation. T cells recognize peptide-MHC complexes expressed on the surface of antigen presenting cells such as dendritic cells (DCs) (**Fig. 1A**). Simultaneously, a range of co-stimulatory ligand-receptor interactions takes place, providing further signalling to T cells. The final outcome will depend on the integration of "positive" and "negative" stimuli.[1] One such interaction is mediated between PD-L1 on antigen presenting cells to PD-1 on T cells, which is critical to maintain peripheral tolerance.[2] PD-L1 engagement with PD-1 on effector T cells inhibits their cytotoxic activities by terminating T cell receptor (TCR) signal transduction.[3] Intriguingly, PD-1 is strongly upregulated in T cells during antigen-presentation, where it engages with PD-L1 on the surface of antigen-presenting DCs (**Fig. 1A and B**). What is the role of such a suppressive interaction in antigen presentation?

In our recent publication,[4] we argue that the answer resides on the necessity for an early control of T cell activation. For the first time we show that PD-L1 co-stimulation contributes to ligand-induced TCR down-modulation, a fundamental immunological process that regulates TCR signalling. TCRs are removed from the surface shortly after activation, limiting signal transduction and avoiding excessive responses.[5] TCR down-modulation is a transient early event in antigen presentation, and interestingly coincides with the early stages of the exponential T cell expansion[4] (**Fig. 1**). Our results indicate that PD-L1/PD-1 co-stimulation contributes to ligand-induced TCR down-modulation, by upregulating the expression of Cbl E3 ubiquitin ligases in activated T cells. Interference with PD-L1 co-stimulation led to hyperactivated pro-inflammatory TCR^{high} CD8 T cells. These effector T cells can exert autoimmune damage if directed towards an auto-antigen, but on the other hand they significantly accelerated anti-tumor immune responses. Vaccination with PD-L1-silenced DCs strongly inhibited tumor growth in a mouse model of lymphoma, and increased the lifespan of tumor-bearing mice.[4] Overall, our observations expanded the role of PD-L1 in immune regulation to a critical step in T cell activation and highlighted its interference as a key therapeutic target.

However, things were not that simple, and these accelerated anti-tumor responses did not increase cure rates in our experimental model. In fact, many tumor cells upregulate PD-L1 expression to counteract cytotoxic $PD\text{-}1^{+}$ T cells.[6] So, PD-1 expression that occurs as a physiological regulatory mechanism in antigen presentation ends up backfiring and becomes an obstacle. As a matter of fact, there is limited therapeutic efficacy in PD-L1/PD-1 interference unless combined with other strategies, as others and we have observed.[4,7] Reduced PD-L1 co-stimulation may lead to sustained TCR signalling in the immunological synapse, but does not necessarily improve T cell effector capacities. Only after a combination with selected modulators of DC signalling pathways

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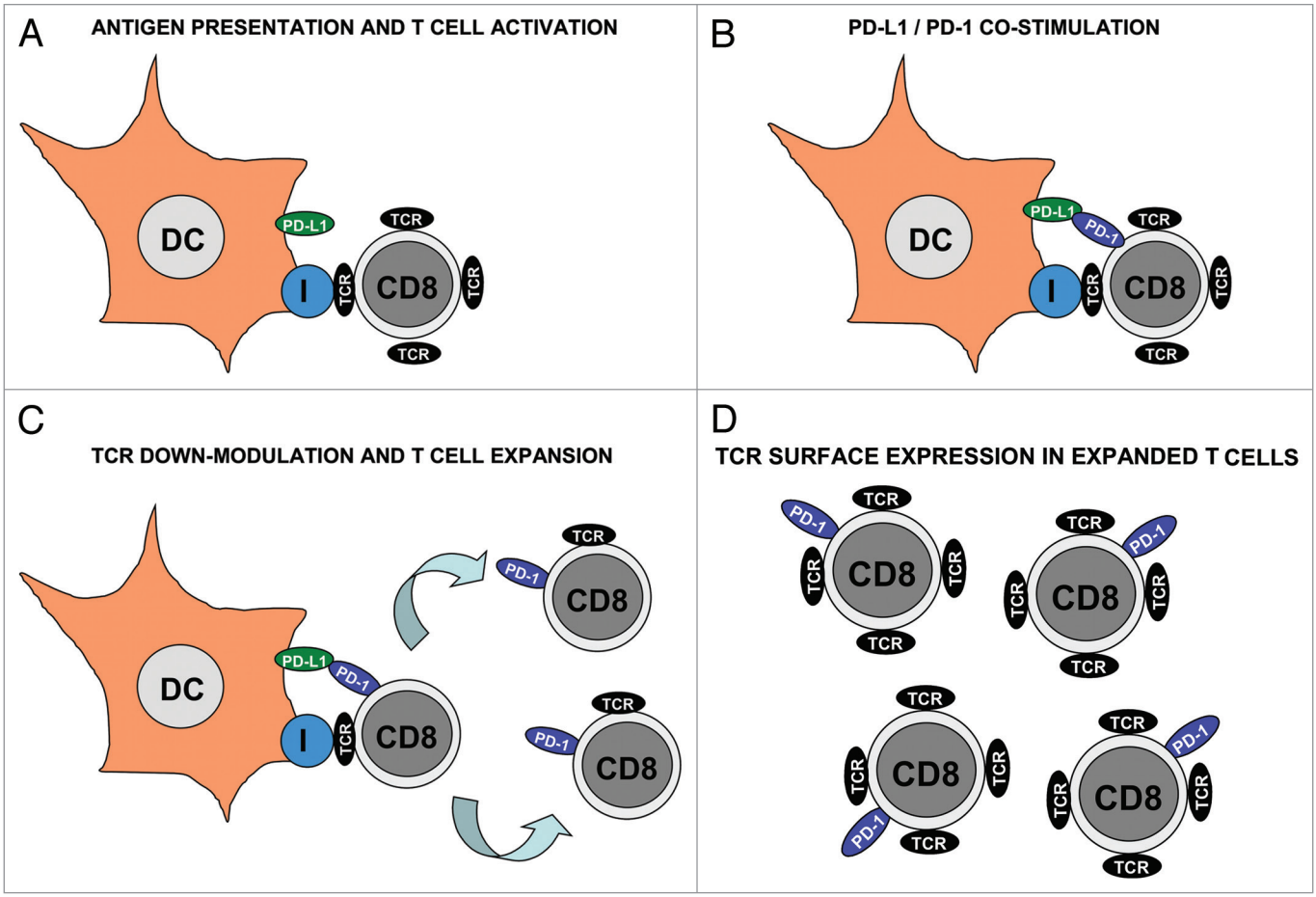

Figure 1. Regulatory role of PD-1 during T cell activation after antigen presentation. (A) Dendritic cells (DC, as indicated) present antigenic peptide associated with MHC class I (encircled "I") to specific CD8 T cells. DCs also express PD-L1 (green ovoid) on their surface. CD8 T cells associate with DCs through their TCR (black ovoid) as shown in the figure. These CD8 T cells are TCR^{high} and do not express PD-1. (B) After antigen recognition, T cells express PD-1 on their surface (blue ovoid), where it engages with PD-L1 on the DC surface. Binding of PD-1 reduces TCR signal transduction and (C) down-modulates TCR levels in CD8 T cells. Activated CD8 T cells proliferate as indicated by arrows. Please note that these proliferating CD8 T cells are TCR^{low} $PD\text{-}1^{+}$. It is tempting to speculate that low TCR and high PD-1 may inhibit autoreactive cytotoxicity while effector CD8 T cells are undergoing expansion. (D) Expanded effector CD8 T cells gradually recover their TCR surface expression while keeping PD-1 expression. It could be argued that this increase in TCR levels may "arm" cytotoxic T cells against their targets.

delivered with lentivectors, PD-L1 silencing was clearly effective. These modulators consisted of a constitutive activator of mitogen activated protein kinase (MAPK) p38,[8] and an inhibitor of MAPK ERK.[9] These were chosen according to their capacity to induce DC maturation by upregulating expression of co-stimulatory and adhesion molecules such as CD80, CD40 and ICAM I. Particularly, when the MAPK ERK inhibitor was expressed in PD-L1-silenced DCs, we increased survival and reduced DC vaccination doses up to 1,000-fold compared to standard experimental protocols.[4]

Concluding, our results highlight the contribution of PD-L1/PD-1 co-stimulation to ligand-induced TCR down-modulation in the immunological synapse. Interference with this pathway results in hyperactivated TCR^{high} CD8 T cells. These CD8 T cells significantly accelerate anti-tumor immune responses, but are nevertheless insufficient to increase long-term survival. According to our data, and from a therapeutic point of view, clinically relevant PD-L1/PD-1 blocking antibodies could be co-administered in combination with, for example, inhibitors of the Ras/Raf/MEK/ERK pathway. Certain kinase inhibitors currently used in human chemotherapy already possess strong adjuvant capacities.[10] The combination of these inhibitors with PD-L1/PD-1 interference may lead to effective anti-tumor immunotherapy. However, would therapeutic strategies such as these cause autoimmune disease?

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