inhibitors on immune cells can influence anti-tumor immunity, a knowledge that is needed to optimally combine these inhibitors with cancer immunotherapy.

In summary, the work by Coelho et al. (2017) unveiled a novel molecular mechanism of PD-L1 regulation that is controlled by oncogenic RAS signaling. It highlights the importance of non-cancer cell-autonomous mechanisms for tumor development driven by major oncogenes like RAS. The study also opens up new avenues for the improvement of current cancer immunotherapies by targeting PD-L1 mRNA stability with small molecule inhibitors in tumors lacking genomic rearrangements of the PD-L1 3′ UTR region. As RAS mutations and PD-L1 overexpression are common in human cancer, the findings by Coelho et al. (2017) delineate therapeutic strategies with broad clinical applicability.

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Feeling Exhausted? Tuning Irf4 Energizes Dysfunctional T Cells

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The regulatory mechanisms governing T cell exhaustion remain incompletely understood. Man et al. (2017) and Wu et al. (2017) report that the T cell receptor responsive transcription factor Irf4 promotes T cell exhaustion in chronic viral infection but dampens exhaustion in response to tissue allografts.

T cell exhaustion refers to an alternative differentiation program associated with persistent antigen exposure, which is characterized by reduced effector functions, altered metabolic status, and the transcriptional induction of negative regulators of T cell function (Wherry and Kurachi, 2015). While T cell exhaustion reduces immune attack of healthy tissues, it severely limits effector responses against chronic viral infections and tumors. Accordingly, relieving exhaustion has become a prime therapeutic focus to improve immune responses to chronic infection and cancer. Conversely, promoting T cell exhaustion may be a valuable approach to prevent allograft rejection or control autoimmune diseases.

Two papers in this issue of *Immunity* address the role of interferon regulatory factor 4 (Irf4) in the T cell response to chronic viral infection (Man et al., 2017) and tissue allografts (Wu et al., 2017). Despite substantial homology with other members of the Irf family, Irf4 expression is not induced by interferons but occurs rapidly in response to T cell receptor (TCR) stimulation. The level of Irf4 expression is proportional to the affinity of the TCR for antigen and determines the extent of clonal $CD8^+$ T cell expansion and effector differentiation in response

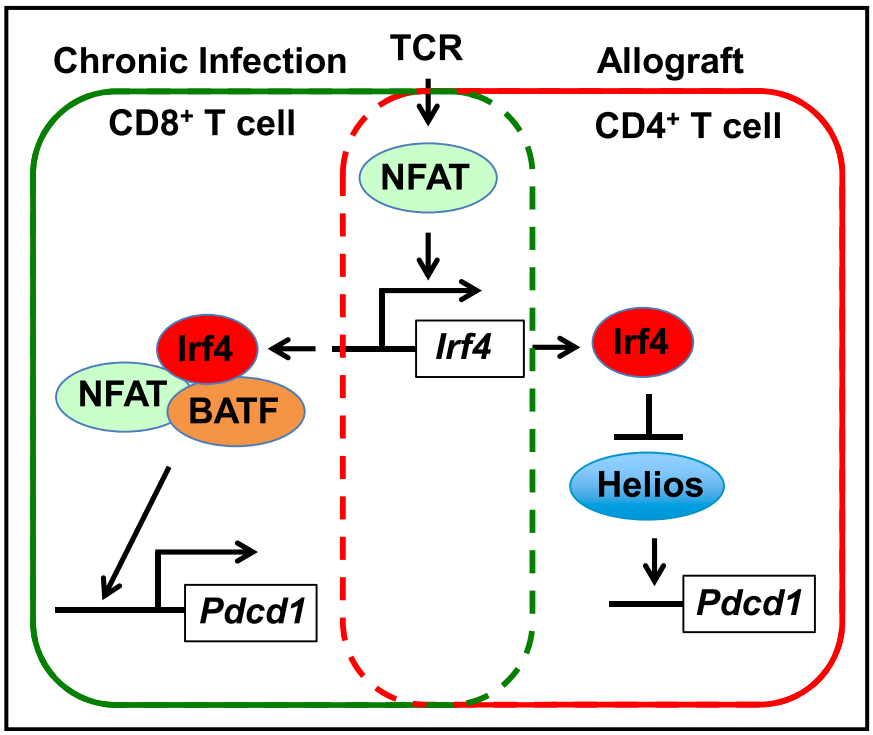

Figure 1. The Transcription Factor Irf4 Plays Opposing Roles in the Expression of Exhaustion-Associated Genes in Response to Chronic Viral Infection and Tissue Allografts
Left: In $CD8^+$ T cells responding to chronic viral infection, engagement of the T cell receptor (TCR) induces the expression of Irf4, BATF, and NFATc1, whereby NFATc1 promotes Irf4 expression. Co-operation between Irf4, BATF, and NFAT leads to the expression of exhaustion genes including *Pdcd-1* (encoding PD-1). The complete or partial absence of Irf4 reduces T cell exhaustion and PD-1 expression. Right: In $CD4^+$ T cells responding to allografts, TCR engagement induces Irf4 expression, which suppresses Helios. The absence of Irf4 de-represses Helios, which then promotes PD-1 expression.

to acute resolving infections (Man et al., 2013; Yao et al., 2013).

In one of these new studies, Man et al. (2017) show that Irf4 is overexpressed in chronically activated as compared to acutely stimulated $CD8^+$ T cells. The elevated levels of Irf4 drive $CD8^+$ T cell exhaustion, as complete or partial ablation of Irf4 in T cells in mice by conditional gene deletion reduces the surface expression of multiple inhibitory receptors, increases anabolic metabolism, and improves the production of effector cytokines such as IFN-γ and TNF-α. Lowered Irf4 expression does not improve virus control, which may be explained by the reduced initial expansion of virus-specific cells that also depends on Irf4. However, lowered Irf4 expression increases immune pathology, indicating that residual $CD8^+$ T cells exert heightened effector function (Man et al., 2017). Thus, elevated Irf4 expression promotes $CD8^+$ T cell exhaustion in chronic viral infection.

In the other study, Wu et al. (2017) examine the role of Irf4 in heterotopic heart allograft transplantation. In this model, cessation of heartbeat serves as a convenient and relevant readout for graft rejection. It is important to note that $CD4^+$ rather than $CD8^+$ T cells are necessary and sufficient for allograft rejection in this model. Like $CD8^+$ T cells in chronic infection, graft-infiltrating $CD4^+$ T cells express high amounts of Irf4. Ablation of Irf4 in T cells completely prevents heart allograft rejection. This observation is notable as there are very few single gene knockouts in which the rejection of fully allogeneic transplants is completely prevented. Regulatory T (Treg) cells do not explain the absence of graft rejection, as Irf4-deficient Treg cells are less suppressive than wild-type and their depletion does not result in rejection. Rather, the authors demonstrate that alloantigen-specific Irf4-deficient $CD4^+$ T cells become dysfunctional. Specifically, they overexpress a subset of molecules associated with $CD4^+$ T cell exhaustion, including PD-1, CD160, and Helios (*Ikzf2*). Checkpoint blockade using PD-L1- and CTLA-4-specific antibodies improves the abundance, proliferation, and effector cytokine production by alloreactive $CD4^+$ T cells lacking Irf4. In addition, a significant fraction of mice reject allografts when treated with anti-PD-L1 alone or combined with anti-CTLA-4, at least when treatment is initiated during the first week following transplantation (Wu et al., 2017). Thus, reduced Irf4 expression promotes $CD4^+$ T cell exhaustion and this results in stable allograft acceptance.

Incidentally, checkpoint blockade at a later stage does not lead to graft rejection, indicating that *Irf4*-deficient $CD4^+$ T cells undergo progressive dysfunction that becomes refractory to checkpoint intervention (Wu et al., 2017). This type of resistance is not apparent in chronic infections as PD-1 blockade can still reinvigorate exhausted $CD8^+$ T cells at even later time points. Understanding this additional tolerance mechanism is of considerable interest as it may provide further targets to promote allograft tolerance.

Why does elevated Irf4 expression promote exhaustion in chronic infection and prevent exhaustion in response to allografts? Irf4 binds the consensus "GAAA" DNA sequence but requires co-factors for high-affinity binding. In T cells, Irf4 co-operates with heterodimeric Jun-Basic leucine zipper transcriptional factor ATF-like (BATF) complexes, which bind AP-1-Irf4 composite DNA elements (AICEs). Irf4 can associate with additional transcription factors, including NFAT and others and, depending on the respective interactions and the cellular context, can mediate transcriptional activation or repression (Huber and Lohoff, 2014). Man et al. (2017) confirm that exhausted $CD8^+$ T cells overexpress several TCR-responsive transcription factors including Irf4, BATF, and NFATc1. Further, they identify a feed forward loop in which NFATc1 promotes Irf4 expression. Finally, the analysis of publically available genome-wide binding data shows that Irf4, BATF, and NFATc2 are often recruited to adjacent binding sites and preferentially co-occupy genes that constitute the exhaustion-specific gene signature, which includes *Pdcd-1* (encoding PD-1) (Figure 1). Functionally, Irf4 and BATF deficiencies lead to a corresponding

reversal of CD8$^+$ T cell exhaustion (Man et al., 2017), which is similar to earlier NFAT loss-of-function experiments (Martinez et al., 2015), indicating that CD8$^+$ T cell exhaustion is driven by the co-operation of a set of TCR-responsive transcription factors.

In contrast, Wu et al. (2017) did not find evidence for Irf4 association with the *Pdcd-1* locus in activated wild-type CD4$^+$ T cells. Rather, increased PD-1 expression correlates with the upregulation and binding of Helios (*Ikzf2*) to the *Pdcd-1* locus. Indeed, enforced Helios expression promotes PD-1 expression in activated wild-type CD4$^+$ T cells. As Irf4 represses Helios, PD-1 expression is not induced in activated wild-type CD4$^+$ T cells (Figure 1; Wu et al., 2017). The notion that Irf4 plays distinct roles for exhaustion of CD4$^+$ versus CD8$^+$ T cells is supported by additional evidence. On one hand, Wu et al. (2017) mention that unlike the absence of Irf4, the absence of BATF does not promote allograft tolerance, indicating that Irf4 acts independently of BATF in CD4$^+$ T cell exhaustion. On the other hand, Helios expression is a feature of exhausted CD4$^+$ but not CD8$^+$ T cells (Crawford et al., 2014). Collectively, these data suggest that Irf4 plays opposing roles in T cell exhaustion in CD4$^+$ versus CD8$^+$ T cells. However, it remains to be addressed whether differences in antigen distribution (systemic versus non-hematopoietic tissue) and the inflammatory milieu also influence the role of Irf4.

Until recently, it was thought that chronic viral infection precludes the formation of CD8$^+$ T cell memory. Contrary to that assumption, it was recently shown that a subset of antigen-specific CD8$^+$ T cells, which express the transcription factor Tcf1, sustain the response in chronic infection by continuously producing exhausted cells lacking Tcf1 (Utzschneider et al., 2016; Im et al., 2016). How this memory-like compartment is generated and maintained is an obvious further question. Man et al. (2017) find that exhausted cells express significantly more Irf4 than memory-like cells. Lowering Irf4 expression (using *Irf4*$^{+/-}$ T cells) profoundly reduces the abundance of exhausted cells but has a lesser impact on memory-like cells (Man et al., 2017). Similarly, reduced Irf4 expression had a more substantial impact on the abundance of terminal effector as compared to memory precursor CD8$^+$ T cells in acute resolving infection (Nayar et al., 2014). Thus TCR-responsive Irf4 appears to be particularly important to drive conventional and exhaustive CD8$^+$ effector T cell differentiation. In addition, memory-like CD8$^+$ T cells mediate the proliferative burst in response to PD-1 blockade (Utzschneider et al., 2016; Im et al., 2016). Wu et al. (2017) show that checkpoint blockade increases the proliferation of alloreactive CD4$^+$ T cells lacking Irf4. This raises the question of whether this proliferative burst is also dependent on a subpopulation of CD4$^+$ T cells with memory-like properties.

The fact that Irf4 is now shown to promote or repress dysfunctional T cell differentiation may open opportunities to prevent, reverse, and/or induce exhaustion more specifically in T cells responding to antigen. Along this line, Man et al. (2017) show that the NFAT inhibitor Cyclosporin A reduces Irf4 expression in activated CD8$^+$ T cells, and there is evidence that this immunosuppressive drug reduces the induction of PD-1 in CD8$^+$ T cells. Wu et al. (2017) screened cytokines and small molecule inhibitors for their capacity to tune Irf4 expression. They find that the MEK1/2 inhibitor Trametinib reduces Irf4 and upregulates Helios and PD-1 expression in activated CD4$^+$ T cells. Moreover, Trametinib significantly extends allograft survival in wild-type mice (Wu et al., 2017). Further investigation will be needed to address to what extent the reduced rejection is due to the induction of T cell exhaustion. Irrespectively, these data provide a proof of concept that pharmacological inhibition of Irf4 is feasible.

Taken together, the new results reveal unexpected insights into the regulation of T cell exhaustion. While de-repressing exhausted T cells to enhance tumor immune responses is a medical success story, these new studies raise the prospect of inducing T cell exhaustion to obstruct unwanted immune responses in order to reduce transplant rejection or autoimmunity.

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