**PD1 functions by inhibiting CD28-mediated co-stimulation**

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**IMMUNE CHECKPOINTS IN CANCER IMMUNOTHERAPY**

T cells chronically exposed to antigen during cancer and persisting infections tend to undergo a phenotypic switch, in which their effector functions are significantly diminished, allowing disease to progress. These exhausted or dysfunctional T cells are unable to provide optimal control of persisting tumours and pathogens, due in part to sustained expression of co-inhibitory receptors including Programmed Death 1 (PD1). By blocking the interaction of PD1 with its ligands, programmed death ligands 1 and 2 (PDL1 and PDL2) expressed on either tumour cells or on antigen-presenting cells (APCs), the effector functions of exhausted T cells can be, at least partially reinvigorated to provide protective immunity. Within the clinic, therapeutic monoclonal antibodies targeting the PD1/PDL1 pathway have proven highly effective and consistent reports of durable responses have been reported in a number of cancer types. Although more broadly effective and manageable than the traditional and targeted cancer therapies, a significant proportion of patients display innate resistance and do not respond at all to PD1/PDL1 blockade or they acquire therapeutic resistance over time. There is a need to determine the requirements for optimal T-cell rescue not only to improve current therapies but to also identify predictive biomarkers.

**PD1 FUNCTIONS PRIMARILY BY LIMITING CD28 CO-STIMULATION**

Underlying the role of PD1 in limiting T-cell activity, was its proposed ability to inhibit T-cell receptor (TCR)-mediated signalling. This putative mechanism suggested that activated PD1 bound and sequestered Shp2 phosphatases via an immunoreceptor tyrosine-based switch motif within its cytoplasmic domain. Its association at the plasma membrane with TCR molecules was suggested to enable inhibitory phosphorylation of kinases essential for TCR-mediated activation. Despite these and other similar findings, the downstream targets and interactions of PD1 signalling intermediates remain incompletely defined, meaning that conclusions drawn regarding the biology of PD1-mediated signalling events have been at times speculative. In a recent issue of *Science*, new light has been shed on this pathway, demonstrating that the immediate downstream target of PD1-mediated signalling is CD28 and that this has important implications for the effectiveness of therapies targeting PD1/PDL1. In one study, Hui et al. suggested that while Shp2 was in fact the primary phosphatase to associate with PD1, that PD1-recruited Shp2 strongly favours dephosphorylation of the co-stimulatory receptor CD28 over TCR. In agreement with previous reports, it was also observed that some dephosphorylation of TCR components can occur, at high PDL1 levels. However, by performing direct and quantitative comparisons, it was found that the degree of TCR dephosphorylation was consistently weaker than that for CD28. Published alongside this was a second study by Kamphorst et al. in which it was demonstrated that in the absence of CD28/B7 (B7.1, CD80 and B7.2, CD86) interactions, anti-PDL1 therapy failed to rescue T cells from their exhausted state in the context of cancer and chronic viral infection. This indicated that exhausted CD8+ T cells have a cell-intrinsic requirement for CD28-mediated co-stimulation for effective responses to PD1/PDL1-targeted therapies. Further, in human non-small cell lung cancer patients, it was shown PD1+ CD8+ T cells that proliferated in peripheral blood following anti-PD1 therapy also expressed CD28, supporting the former observation. However, it was not shown whether PD1+ CD8+ T cells from non-responding patients less frequently expressed CD28; an observation which would have been highly compelling. Together, these observations were somewhat surprising. CD28 is known for its essential role during T-cell priming in binding B7 molecules expressed by APCs to provide the critical 'second signal' for T-cell activation. Under normal circumstances, T cells are not necessarily required to interact with B7-expressing professional APCs in order to carry out effector functions, perhaps suggesting a unique requirement for exhausted CD8+ T cells.

The mechanistic contribution of CD28 co-stimulation to the efficacy of anti-PD1 therapy is not immediately obvious; however, these discoveries might help to explain several recent observations in the field of cancer immunology. In at least a subset of human cancer patients, inhibition of T-cell immunity is associated with upregulation of PDL1 within tumour tissue in response to IFNγ (adaptive immune resistance). However, expression of PDL1 by tumour-infiltrating immune cells can be independently, and in some cancer types, even more predictive of clinical response than PDL1 expression by tumour cells. This is important because infiltrating immune cell subsets such as lymphocytes, monocytes and dendritic cells all express B7 molecules, while tumours themselves generally do not. If the primary target of PD1 signalling regulation is through CD28 or another co-stimulatory molecule, then effective therapeutic blockade likely reflects re-activation of co-stimulatory molecule signalling, an innovative membrane reconstitution and intact cell-based assays, the authors confirmed that while Shp2 was in fact the primary phosphatase to associate with PD1, that PD1-recruited Shp2 strongly favours dephosphorylation of the co-stimulatory receptor CD28 over TCR. In agreement with previous reports, it was also observed that some dephosphorylation of TCR components can occur, at high PDL1 levels. However, by performing direct and quantitative comparisons, it was found that the degree of TCR dephosphorylation was consistently weaker than that for CD28. Published alongside this was a second study by Kamphorst et al., in which it was demonstrated that in the absence of CD28/B7 (B7.1, CD80 and B7.2, CD86) interactions, anti-PDL1 therapy failed to rescue T cells from their exhausted state in the context of cancer and chronic viral infection. This indicated that exhausted CD8+ T cells have a cell-intrinsic requirement for CD28-mediated co-stimulation for effective responses to PD1/PDL1-targeted therapies. Further, in human non-small cell lung cancer patients, it was shown PD1+ CD8+ T cells that proliferated in peripheral blood following anti-PD1 therapy also expressed CD28, supporting the former observation. However, it was not shown whether PD1+ CD8+ T cells from non-responding patients less frequently expressed CD28; an observation which would have been highly compelling. Together, these observations were somewhat surprising. CD28 is known for its essential role during T-cell priming in binding B7 molecules expressed by APCs to provide the critical 'second signal' for T-cell activation. Under normal circumstances, T cells are not necessarily required to interact with B7-expressing professional APCs in order to carry out effector functions, perhaps suggesting a unique requirement for exhausted CD8+ T cells.

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rather than TCR signalling alone, at least in the context of chronic antigen stimulation (Figure 1).

With respect to CD8 T cells themselves, a recent publication demonstrated that the proliferative burst of CD8 T cells commonly observed following effective anti-PD1/PDL1 therapy was provided by a specific subset of stem-cell-like TIM-3<sup> hi </sup>PD1<sup> hi </sup>TCR<sup> hi </sup>CD8<sup> hi </sup>T cells.<sup>15</sup> These cells express much higher levels of co-stimulatory molecules such as CD28, ICOS, OX40 and LIGHT, and much lower levels of inhibitory receptors compared to their non-proliferative TIM-3<sup> hi </sup>PD1<sup> hi </sup>TCR<sup> hi </sup>CD8<sup> hi </sup>T-cell counterparts.<sup>15</sup> Although the roles of co-stimulatory molecules other than CD28 were not directly evaluated by Kamphorst et al.,<sup>11</sup> their findings clearly demonstrated that CD28 signalling plays a major, if not dominant, and non-redundant role for responses to PD1/PDL1 blockade. These studies likely suggest that even in the presence of PD1-targeted therapies that PD1<sup> hi </sup>CD8<sup> hi </sup>T cells require activating stimulation beyond that provided simply by encounters between TCR and peptide MHC (Figure 1).

**UNANSWERED QUESTIONS AND PERSPECTIVES**

Together, these studies raise several interesting questions relating broadly to the role of PD1 in cancer immunology. First, within tumour microenvironments sensitive to anti-PD1/PDL1 therapy, which cell type(s) is responsible for providing B7-co-stimulation? If this phenomenon is determined to be a derivative requirement of exhausted CD8<sup> hi </sup>T cells, answering this question could provide a highly reliable biomarker in addition to PDL1 expression, to stratify patients prior to treatment with anti-PD1/PDL1 therapies. Second, were tumour-specific CD4<sup> hi </sup>T cells also found to be similarly effected by loss of B7-co-stimulation following therapy? CD4 T cells can play a major role in tumour immunotherapy,<sup>16</sup> and determining the extent to which CD28 co-stimulation is required for their activity, might have serious implications for future therapeutic development. Third, would therapeutic strategies capable of promoting the influx of B7-expressing immune cells potentially synergise with PD1/PDL1 blockade? One approach explored to a limited extent has been local administration of poly-IC RNA, which can promote the activity and infiltration of a wide variety of myeloid and lymphoid cell types within tumours to enhance sensitivity to PD1-targeted therapies.<sup>17</sup> Such approaches might be of great importance within tumours resistant to anti-PD1 therapy in which selective exclusion of APCs is mediated by upregulation of β-catenin signalling.<sup>18</sup> Fourth, is expression of either B7.1 or B7.2 more important for infiltrating APCs? Answering this question might provide insight into which APCs are of greatest benefit to promote into tumour tissue. Fifth, alternatively, could anti-PD1/PDL1 therapies be combined with CD28 agonists in humans? Despite the reported systemic toxicities of CD28 agonists, it might be reasonable to predict, that if their distribution could be restricted to tumour tissue, their efficacy might be conserved, to improve responses to anti-PD1/PDL1 therapy.<sup>19</sup> Answers to these questions could be important for increasing the efficacy of anti-PD1/PDL1 therapies.

For many cancer patients, immunotherapy represents their best shot at recovery; however, resistance remains a challenge. The findings of the two studies discussed here by Hui, and Kamphorst et al.,<sup>11</sup> might have significant implications for the use and development of new combination therapies that aim to prevent therapeutic resistance.<sup>20</sup> For example, if this phenomenon is more broadly investigated in human cancers, and it is found that CD28 co-stimulation is essential for the efficacy of anti-PD1 therapy, more accurate screening techniques may be developed. In addition, a major focus in the field of cancer immunotherapy has been the development of therapies capable of promoting CD8<sup> hi </sup>T-cell infiltration into tumour tissue. These studies, however, suggest that a more diverse infiltrate might be preferable, provided that such cells are capable of promoting CD28 signalling. In any case, investigations of this type in which basic biology is compared with and complemented by therapeutic observations, are highly exciting.

**CONFLICT OF INTEREST**

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