REVIEWS

The diverse functions of the PD1 inhibitory pathway

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Abstract | T cell activation is a highly regulated process involving peptide–MHC engagement of the T cell receptor and positive costimulatory signals. Upon activation, coinhibitory 'checkpoints', including programmed cell death protein 1 (PD1), become induced to regulate T cells. PD1 has an essential role in balancing protective immunity and immunopathology, homeostasis and tolerance. However, during responses to chronic pathogens and tumours, PD1 expression can limit protective immunity. Recently developed PD1 pathway inhibitors have revolutionized cancer treatment for some patients, but the majority of patients do not show complete responses, and adverse events have been noted. This Review discusses the diverse roles of the PD1 pathway in regulating immune responses and how this knowledge can improve cancer immunotherapy as well as restore and/or maintain tolerance during autoimmunity and transplantation.

Self-tolerance
Broadly refers to a series of mechanisms used by the body to limit the activation of self-reactive T cells and B cells to prevent these cells from targeting and destroying self tissues.

Central tolerance
Mechanisms of tolerance that occur in the central lymphoid organs (thymus for T cells, bone marrow for B cells). Mechanisms include negative selection (for both T cells and B cells), receptor editing (for B cells) and lineage deviation (for T cells).

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The adaptive immune system has evolved to eliminate virtually any threat from the organism. Through the combined effector potentials of $CD4^+$ T cells, $CD8^+$ T cells and B cells, the adaptive immune system can inflict extraordinary damage on harmful invaders. However, the immune system must do so while sparing healthy cells and maintaining self-tolerance. This task is accomplished through multiple checks and balances on immune responses that function during lymphocyte development in central lymphoid organs (central tolerance) and the periphery (peripheral tolerance).

For T cells, several regulatory mechanisms are induced during initial antigen-mediated activation, which involves peptide–MHC engagement of the T cell receptor (TCR) and positive costimulatory signals such as interactions between CD28 on T cells and CD80 (also known as B7.1) and/or CD86 (also known as B7.2) on antigen-presenting cells (APCs). Early during the activation process, negative regulators are induced to counteract the activation programme. Cytotoxic T lymphocyte antigen 4 (CTLA4; also known as CD152) is one of the first negative regulators to be induced, and it directly competes with CD28 for the ligands CD80 and CD86 (REF. 1). Programmed cell death protein 1 (PD1, also known as PDCD1 and CD279) is also expressed during T cell activation and counters positive signals through the TCR and CD28 by engaging its ligands programmed cell death 1 ligand 1 (PDL1; also known as CD274 and B7-H1) and/or PDL2 (also known as CD273 and B7-DC) (referred to collectively here as PD1 ligands)[1–5] (FIG. 1). These 'coinhibitory' receptors function as breaks for the adaptive immune response, serving as immune checkpoints that effector T cells must pass in order to exert their full functions.

Inhibitory signals are used in many ways to maintain balance in the immune system. PD1 has become the paradigm for understanding the diverse physiological roles of inhibitory receptors. Signals through the PD1 pathway contribute to regulation of initial T cell activation, fine-tuning of T cell fate and functions, T cell tolerance and return to immune homeostasis[1,6–8]. Perturbing the PD1 pathway can profoundly impact host physiology. Mice genetically deficient in *Pdcd1* (which encodes PD1) develop accelerated autoimmunity[9–12]. Conversely, high and sustained expression of PD1 and its ligands are common during chronic infections and cancer, in which blocking the PD1 pathway can improve T cell functions and reduce viral load and tumour burden[13–19].

These observations have been translated to the clinic. In early trials, treatment with PD1 pathway inhibitors (known as 'checkpoint blockade') showed success in promoting durable antitumour immune responses, and this success led to the approval by the US Food and Drug Administration of the monoclonal antibodies nivolumab (anti-PD1; Bristol-Myers Squibb, USA), pembrolizumab (anti-PD1; Merck, USA), atezolizumab (anti-PDL1; Genentech, USA), avelumab (anti-PDL1; EMD Serono, USA) and durvalumab (anti-PDL1; AstraZeneca, UK) for therapeutic use in various cancers, including melanoma, non-small-cell lung cancer (NSCLC), head and neck squamous cell carcinoma, renal cell carcinoma (RCC), Hodgkin lymphoma, bladder cancer, Merkel cell carcinoma and microsatellite instability

Peripheral tolerance
Mechanisms of tolerance that occur in the periphery after full development of lymphocytes in the bone marrow or thymus and their egress from these sites. These mechanisms can occur during priming in the secondary lymphoid organs or in peripheral tissues.

Immune checkpoints
An alternative term for coinhibitory molecules, generally referring to inhibitory signals that immune cells must overcome to perform full effector functions.

high or mismatch repair-deficient adult and paediatric solid tumours[20,21]. Although the results are promising, most patients do not show long-lasting remission, and some cancers have been completely refractory to response with checkpoint blockade. Furthermore, toxicity and immune-related adverse events (IRAEs) have been observed, with particularly high rates occurring when PD1-targeted therapy is used in combination with CTLA4-targeted therapy (ipilimumab; Bristol-Myers Squibb)[20,22,23]. These clinical findings underscore the need for a better mechanistic understanding of why PD1 pathway modulation leads to significant clinical benefit in some patients but temporary, partial or no clinical benefit in other patients. In addition, a better understanding of the causes of IRAEs is sorely needed to guide safer use of PD1 pathway inhibitors.

Although the PD1 pathway has received considerable attention for its roles in T cell exhaustion and tumour immunosuppression, PD1 is not an exhaustion-specific molecule (BOX 1). All T cells express PD1 during activation, such that it is a marker of effector T cells. Furthermore, PD1 is expressed by subsets of tolerant T cells, regulatory T (T_{reg}) cells, T follicular helper (T_{FH}) cells, T follicular regulatory (T_{FR}) cells and memory T cells (BOX 1) and several other cell types, including B cells, natural killer (NK) cells, some myeloid cells and cancer cells (TABLE 1). $PD1^+CD8^+$ T cells can be found in the circulation of healthy humans, and these cells do not resemble exhausted T cell populations[24]. Consequently, for the PD1 pathway, context is everything. Issues of timing, location, T cell differentiation state, antigen burden, inflammation levels,

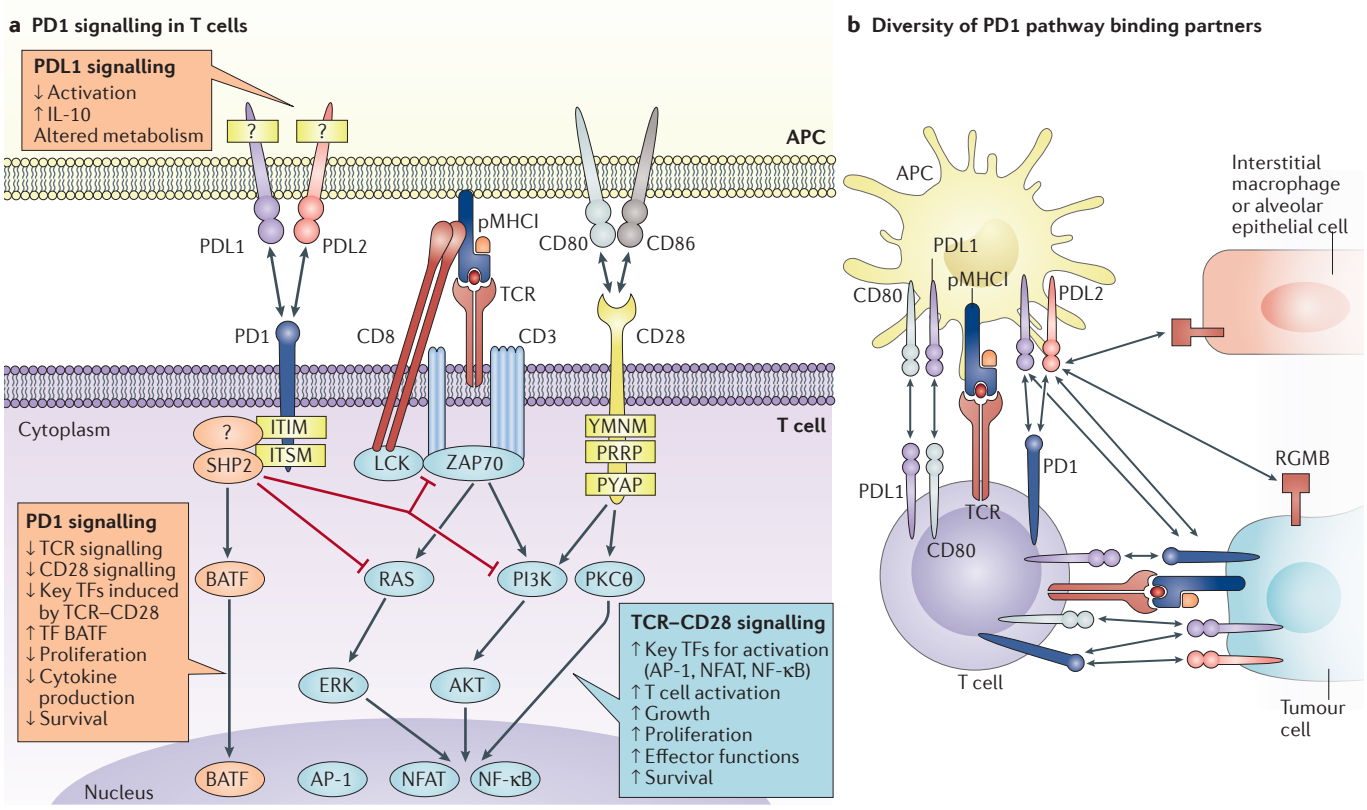

Figure 1 | **PD1 signalling and diversity of binding partners. a** | Mechanisms of programmed cell death protein 1 (PD1) signalling in T cells. For T cells, in order for PD1 to deliver an inhibitory signal, the peptide–MHC class I complex (pMHCI) must be presented by the same cell expressing PD1 ligands (programmed cell death 1 ligand 1 (PDL1) and PDL2). PD1 can inhibit T cell functions by recruiting phosphatases, including SHP2, to the immunoreceptor tyrosine-based switch motif (ITSM) in the PD1 tail. These phosphatases can counter the positive signalling events being triggered by the T cell receptor (TCR) (interacting with pMHCI) and CD28 (interacting with CD80 and/or CD86); for example, they inhibit ZAP70 and the phosphoinositide 3-kinase (PI3K)–AKT and RAS signalling pathways. Collectively, this results in decreased activation of transcription factors (TFs), such as activator protein 1 (AP-1), nuclear factor of activated T cells (NFAT) and nuclear factor-κB (NF-κB), which are important for driving T cell activation, proliferation, effector functions and survival. In addition, PD1 can inhibit T cell functions by increasing the expression of transcription factors such as basic leucine zipper transcriptional factor ATF-like (BATF), which can further counter effector transcriptional programmes. Some evidence suggests that PD1 ligands can drive signalling following their engagement with PD1, but the signalling motifs and mechanisms involved are unknown. Signalling motifs are indicated in yellow boxes; circles indicate key proteins involved in signalling pathways and key transcription factors. **b** | Diversity of PD1 pathway binding partners. PD1 can interact with either PDL1 or PDL2. Alternatively, PDL1 can also bind to CD80, and PDL2 can also bind to RGM domain family member B (RGMB). Owing to the diversity of cell types that can express these receptors and ligands (TABLE 1), there are a number of potential interactions that could occur. APC, antigen-presenting cell; ITIM, immunoreceptor tyrosine-based inhibitory motif; PKCθ, protein kinase Cθ.

Box 1 | **Diverse roles of PD1 beyond T cell exhaustion**

The programmed cell death protein 1 (PD1) pathway has received considerable attention owing to its role in regulating T cell responses during cancer and chronic infection, where persistent antigen stimulation can lead to T cell exhaustion. However, PD1 is also expressed on all conventional $CD4^+$ T cells and $CD8^+$ T cells during acute T cell activation and by some subsets of memory T cells and tolerant T cells. Consequently, PD1 is not an exhaustion-specific marker. PD1 is also expressed by regulatory T (T_{reg}) cells, B cells, natural killer cells and some myeloid cells. Taking into account the diverse roles of PD1 in these cell types is crucial for predicting the immunological changes that will occur following checkpoint blockade.

For example, the PD1 pathway has a crucial role in regulating humoral immunity. During humoral immune responses, B cells can express PD1, programmed cell death 1 ligand 1 (PDL1) and PDL2. In the germinal centre, help from T follicular helper (T_{FH}) cells, which express high levels of PD1, is essential for optimal class switching and affinity maturation[76]. T follicular regulatory (T_{FR}) cells, which regulate T_{FH} cells, also express PD1 and PDL1 (REF. 76). Consequently, diverse PD1–PD1 ligand interactions in the germinal centre are possible. Early studies blocking the PD1 pathway reported contradictory findings, with some showing attenuated humoral immune responses, whereas others showed enhanced responses[76]. These studies used either complete knockouts of the genes encoding PD1, PDL1 or PDL2, or blocking antibodies, which complicates the interpretation of the role of the PD1 pathway in individual cell types. Work using cell-type-specific deletion of the gene encoding PD1 showed that loss of PD1 in T_{FR} cells enhanced their number and suppressive capacity, suggesting that PD1 normally functions to inhibit the suppressive capacity of these cells[159]. Consequently, the ability of PD1 to not only restrain effector cells but also inhibit regulatory populations has important implications for productive immune responses.

In addition to T_{FR} cells, PD1 and PDL1 are also expressed by $CD4^+FOXP3^+CXCR5^-$ T_{reg} cells. Furthermore, ligation of PDL1 on conventional $CD4^+$ T cells ($FOXP3^-$) can promote the development of peripherally induced T_{reg} cells[160]. Considering the diversity of T_{reg} cell subsets and methods of suppressing immune responses, further work investigating the mechanisms by which the PD1–PDL1 pathway controls T_{reg} cells in different settings is crucial for the safe modulation of this pathway in the clinic.

Effector T cells
T cells that have recently encountered antigen and differentiated from a quiescent state to a fully activated state, a conversion that is accompanied by proliferation and acquisition of effector functions.

T cell exhaustion
Caused by chronic antigenic stimulation and exposure to chronic inflammation, T cell exhaustion results in a progressive loss of effector functions and potential over time. There are subsets of exhausted T cells that differ in their functionality.

Tolerant T cells
Self-reactive T cells that have been activated by cognate antigen but have been rendered hypofunctional to protect self tissues from destruction. Mechanisms include anergy, active suppression by regulatory T cells and suppression through programmed cell death protein 1 (PD1).

Regulatory T (T_{reg}) cells
Generally refers to a subset of $CD4^+$ T cells that expresses the transcription factor forkhead box protein P3 (FOXP3) and actively inhibits immune responses (through immunosuppressive cytokine production, modulating dendritic cell function, metabolic disruption and/or production of adenosine). Additional populations of T_{reg} cells include $CD8^+$ T_{reg} cells, $ROR\gamma t^+FOXP3^+$ T_{reg} cells and T regulatory type 1 (T_R1) cells.

T follicular helper (T_{FH}) cells
A subset of $CD4^+$ T cells that expresses CXC-chemokine receptor 5 (CXCR5), BCL-6, programmed cell death protein 1 (PD1) and ICOS, localizes to the B cell follicle and provides help to B cells to generate productive humoral immune responses (through CD40 and IL-21).

metabolic state and other factors all influence the functional outcome of PD1 engagement (FIG. 2).

The role of the PD1 pathway in cancer has been elegantly discussed in a number of recent reviews[13,20,21,25–27]. In this Review, we focus on the diverse roles of the PD1 pathway in regulating immunity in a number of contexts beyond cancer and chronic infection, including in normal immune physiology during acute infection, in the resolution of immune responses and return to homeostasis and in self-tolerance (FIG. 2). We discuss how this knowledge can be applied to understanding IRAEs and other consequences of PD1 pathway modulation in the treatment of cancer and chronic infection. In addition, we consider challenges and opportunities for PD1 pathway immunotherapy, including PD1 blockade to boost immune responses during chronic infections and cancer as well as PD1 engagement to restore immune tolerance during autoimmunity and transplantation.

Expression of PD1 and its ligands

PD1. The mechanisms regulating PD1 expression are best described for T cells. PD1 is not expressed by naive T cells but becomes expressed on all T cells during initial antigen-mediated activation through the TCR[28] (FIG. 2). If the activating antigen is acutely cleared, PD1 expression levels decrease on responding T cells[14,29] (FIG. 2). If the antigen is not cleared (for example, during chronic infections and cancer), PD1 expression remains high and sustained[13,14,29] (FIG. 2). Several transcription factors regulate PD1 expression in antigen-activated T cells, including nuclear factor of activated T cells, cytoplasmic 1 (NFATC1), forkhead box protein O1 (FOXO1), T-bet (also known as TBX21) and B lymphocyte-induced maturation protein 1 (BLIMP1)[6,7] (TABLE 1), as well as the serine–threonine kinase glycogen synthase kinase 3 (GSK3)[30].

Although the most important regulator of PD1 expression in T cells is TCR engagement, TCR-independent mechanisms also regulate PD1. During chronic infection, PD1 expression can be sustained after antigen withdrawal or clearance[31–33] and following re-expansion of previously exhausted $CD8^+$ T cell populations[32]. The *Pdcd1* locus shows dynamic patterns of DNA methylation during T cell differentiation, which inversely correlates with PD1 expression[34]. Studies using ATAC-seq showed a unique pattern of accessibility of the *Pdcd1* locus in exhausted T cells[33,35], and deletion of a regulatory region ~23 kb upstream of the transcriptional start site reduced PD1 expression[35]. Epigenetic modifications of the *Pdcd1* promoter in autoreactive effector $CD4^+$ T cells have also been observed during peptide-induced tolerance in a mouse model of experimental autoimmune encephalomyelitis (EAE)[36]. Thus, PD1 expression may be under epigenetic regulation, but additional work is needed to investigate how epigenetic modifications control PD1 expression.

There is increasing evidence for a connection between PD1 signalling and metabolic activity in T cells[37–44]. During T cell activation, switching from oxidative phosphorylation to aerobic glycolysis enables effector T cells to meet their energy requirements for proliferation and differentiation[45]. PD1 signalling can modulate metabolic reprogramming during initial T cell activation, inhibiting the upregulation of glucose and glutamine metabolism that is driven by TCR and CD28 signalling[41,43]. In addition, PD1 signalling can promote lipolysis and fatty acid oxidation in $CD4^+$ T cells[43]. Considering that metabolic competition in the

Table 1 | **Basic features of PD1 and its ligands**

Molecule (gene)	Binding partners	Expression pattern	Structure of gene	Structure of protein	Splice variants	Positive regulators	Negative regulators	Refs
PD1 *(PDCD1)*	PDL1 and PDL2	• Activated T cells, maturing thymocytes, B cells, NK cells, NKT cells, some myeloid and APC populations and ILC progenitors • Some cancer cells	• Five exons in both mice (chr 1) and humans (chr 2) • Only 59% conservation between cytoplasmic tails in the mouse and human genes	• Ectodomain contains a single IgV domain • Cytoplasmic tail contains ITIM and ITSM	• Four in humans: lacking exon 2; lacking exon 3; lacking exons 2 and 3; lacking exons 2–4 • The Δexon 3 variant lacks the transmembrane domain and is a soluble molecule that may interfere with PD1 binding	• T cells: TCR engagement, common γ-chain cytokines, and transcription factors FOXO1 and NFAT • B cells: BCR engagement	• T cells: Transcription factors TBET and BLIMP1 • B cells: BCR-mediated upregulation of PD1 can be inhibited by IFNγ, IL-4, LPS and CpG	6,7,13, 167, 168
PDL1 *(CD274)*	PD1 and CD80	• APCs, T cells and B cells • Thymic cortex • Non-haematopoietic lineages, including vascular endothelial cells, pancreatic islets, liver non-parenchymal cells, placental syncytiotrophoblasts, keratinocytes and the cornea • A number of cancer cell lineages	• Seven exons in both mice (chr 19) and humans (chr 9) • 23 kb away from the *Pdcd1l2* gene in mice, 42 kb in humans	Extracellular IgV and IgC domains, transmembrane domain and cytoplasmic tail	• One variant in humans that lacks the IgV domain and therefore cannot bind PD1. This variant is not found in mice	• Cytokines: type I IFNs type II IFN, TNF, IL-10, IL-27 and some γ-chain cytokines • Transcription factors: FOXA1, which can be induced by IFNβ	MicroRNAs miR-513 and miR-200	6,7, 169, 170
PDL2 *(PDCD1L2)*	PD1 and RGMB	• DCs, macrophages, some B cells, some mast cells and T_H2 cells • Thymic medulla • Some cancer cells (oesophageal, lung and kidney)	• Six exons in mice (chr 19) and seven exons in humans (chr 9) • The intracellular domain differs substantially between humans and mice	Extracellular IgV and IgC domains, transmembrane domain and cytoplasmic tail	• Three in humans that lack: the IgV domain; the IgC domain; the IgC domain and the transmembrane domain	• Cytokines: IFNγ, GM-CSF and IL-4	Unknown	6,7
CD80 *(CD80)*	CD28, CTLA4 and PDL1	• DCs, macrophages, activated T cells and activated B cells • Limited non-haematopoietic cells (for example, liver non-parenchymal cells and keratinocytes)	• Seven exons in mice (chr 16) • Eight exons humans (chr 3)	Extracellular IgV and IgC domains, transmembrane domain and cytoplasmic tail	One variant (B7-1a) lacking the second Ig-like domain	• LPS, mitogen, MHC class II ligation, CD40 ligation and some cytokines	IFNγ (decreases expression on some cell types, including Langerhans cells and peritoneal macrophages)	6,7
RGMB *(RGMB)*	PDL2, BMP2, BPM4 and neogenin	• Neural tissues, ovaries, testes and kidneys • Lung, spleen, thymus, peripheral and mesenteric lymph nodes and some cancer cells • Lung interstitial macrophages and alveolar epithelial cells	• Three exons mice (chr 17) and five exons humans (chr 5) • Mouse cDNA sequence has 89% homology with human sequence	Single chain protein attached to the membrane by a GPI anchor	Unknown	Unknown	Unknown	61, 171, 172

APC, antigen-presenting cell; BCR, B cell receptor; BLIMP1, B lymphocyte-induced maturation protein 1; DCs, dendritic cells; GPI, glycosyl phosphatidylinositol; FOXO1, forkhead box protein O1; GM-CSF, granulocyte–macrophage colony-stimulating factor; IgC, immunoglobulin constant domain; IgV, immunoglobulin variable domain; ILC, innate lymphoid cell; ITIM, immunoreceptor tyrosine-based inhibitory motif; ITSM, immunoreceptor tyrosine-based switch motif; TCR, T cell receptor; TNF, tumour necrosis factor.

T follicular regulatory (T_{FR}) cells
A subset of regulatory T cells that expresses forkhead box protein P3 (FOXP3), CXCR5, BCL-6, B lymphocyte-induced maturation protein 1 (BLIMP1), programmed cell death protein 1 (PD1) and ICOS and that attenuates humoral immunity by controlling T_{FH} cell and B cell functions.

Memory T cells
Long-lived populations of antigen-experienced T cells that persist after acute antigen is cleared. Compared with their naive counterparts, memory T cells are present in higher numbers, have a broader anatomical distribution and more rapidly differentiate into effector cells upon antigen re-encounter. Naive T cells are restricted to secondary lymphoid organs (SLOs), while central memory T cells can be found in SLOs, effector memory T cells circulate in blood and non-lymphoid tissues, and resident memory T cells permanently reside in either SLOs or non-lymphoid tissues.

DNA methylation
An epigenetic modification that results in transcriptional repression.

ATAC-seq
(Assay for transposase-accessible chromatin with high-throughput sequencing). A rapid and sensitive method of assaying chromatin accessibility that uses *in vitro* transposition of sequencing adaptors into open chromatin followed by high-throughput sequencing to determine the location of open chromatin.

Epigenetic regulation
A broad set of heritable changes in gene expression that occur independently of changes to the DNA sequence (for example, DNA methylation, histone modifications) that broadly defines the transcriptional capacity of a cell, dictating cell lineage, fate and effector potential.

tumour microenvironment can drive tumour progression by inducing a T cell hyporesponsive state through glucose deprivation[46], understanding how metabolism regulates PD1 and PD1 ligand expression and, conversely, how PD1 signalling feeds back on metabolism is becoming increasingly important. In mice transplanted with tumour cells from a sarcoma cell line, PD1 blockade altered the transcription of genes associated with metabolism in tumour-infiltrating T cells[42]. PD1 blockade was also shown to promote T cell glycolytic activity in the tumour microenvironment[46]. Of note, compounds that directly activate key metabolic pathways, including the mechanistic target of rapamycin (mTOR), AMP-activated protein kinase (AMPK) and PPARγ co-activator 1α (PGC1α) signalling pathways, can synergize with PD1 pathway blockade to improve the antitumour efficacy of this therapy[44]. These findings strengthen the rationale for combination therapies targeting PD1 and metabolic signalling pathways and point to the importance of more precisely defining the relationships between these pathways in order to develop effective combination therapies.

PD1 ligands. PDL1 is widely expressed by many different cell types and is found on both haematopoietic cells (including T cells, B cells, dendritic cells (DCs) and macrophages) and on non-haematopoietic cells (including vascular and stromal endothelial cells, pancreatic islet cells, placental syncytiotrophoblasts and keratinocytes). Pro-inflammatory signals have been shown to induce higher levels of PDL1 expression (TABLE 1). By contrast, PDL2 expression is much more restricted, and this ligand is expressed predominantly by DCs, macrophages and B cell populations (TABLE 1). Expression of PDL2 is generally low at steady state, but similarly to PDL1, it is induced by inflammatory stimuli (TABLE 1). Both PDL1 and PDL2 can be expressed by cancer cells, but PDL1 is more commonly found on these cells. PDL1 expression is often associated with ongoing inflammatory responses, although some mutations in cancer cells can cause increased PDL1 levels in the absence of high levels of inflammation[47] (TABLE 1). Cytokines are crucial regulators of PDL1 and PDL2 expression (TABLE 1), with type I interferons and type II interferon being some of the most potent drivers of PDL1 expression[6,48]. The ability of interferons to regulate PDL1 expression has contributed to the concept of 'adaptive resistance' in tumours, which proposes that the pro-inflammatory cytokines produced by infiltrating T cells further increase PDL1 expression and promote immunosuppression in the tumour microenvironment. This process represents a key example of how immune checkpoints act as a negative feedback mechanism to dampen ongoing adaptive immune responses.

PD1 signalling and molecular mechanisms

Much of our understanding of PD1 signalling comes from studies of acutely activated T cells[8]. PD1 has two tyrosine motifs in its cytoplasmic domain (TABLE 1). When engaged with a ligand, PD1 becomes phosphorylated at these tyrosine residues, leading to binding of protein tyrosine phosphatases (PTPs), such as SHP2. These PTPs can dephosphorylate kinases and antagonize positive signals that occur through the TCR and CD28, affecting downstream signalling pathways including those involving phosphoinositide 3-kinase (PI3K)–AKT, RAS, extracellular-signal-regulated kinase (ERK), VAV and phospholipase Cγ (PLCγ)[8,41,49–51]. The functional outcome of these effects is decreased T cell activation, proliferation, survival and cytokine production and altered metabolism.

Our understanding of the mechanisms by which PD1 modulates the functions of other types of T cells (including regulatory, exhausted, memory, tolerant and anergic T cells) remains less clear. Some evidence suggests that PD1 signalling preferentially inhibits CD28-mediated signalling rather than TCR-driven pathways[50]. However, the dependence of different types of T cells on continuous TCR signalling versus CD28 signalling differs, and this probably affects susceptibility to signalling through PD1. Exhausted T cells become 'addicted' to their antigen and require continuous TCR engagement for survival[52,53]. Recent work suggests that rescue of exhausted $CD8^+$ T cells by anti-PDL1 therapy depends on CD28 signalling[54], but how PD1 specifically modulates CD28 versus TCR signalling in this setting remains to be defined. By contrast, memory $CD8^+$ T cells can be maintained without ongoing TCR signalling[55] and can be less dependent on CD28 (REFS 56–58), although CD28 has a role in some settings[59,60]. Consequently, additional work is needed to clarify the specific effect of PD1 signalling on other intracellular signalling pathways in cells of varying differentiation states.

The signalling capacity of PDL1 and PDL2 is an area of active investigation. The intracellular tails of PDL1 and PDL2 do not contain canonical conserved signalling motifs, and the intracellular domain of PDL2 differs markedly between mice and humans[4]. Growing evidence suggests that ligation of PD1 ligands can send a 'reverse' signal into PD1 ligand-expressing cells. Blocking PDL1 on tumour cell lines *in vitro* can directly affect tumour cell metabolism in the complete absence of PD1-expressing T cells[46]. In this study, tumour cell expression of glycolytic enzymes, AKT phosphorylation and glucose uptake were reduced after treatment with anti-PDL1 antibody *in vitro*[46].

In addition to interacting with PD1, PDL1 and PDL2 have other binding partners (FIG. 1; TABLE 1). PDL1–CD80 and PDL2–RGM domain family member B (RGMB) interactions seem to be generally inhibitory[61–64]. However, further characterization of when these receptor–ligand interactions are biologically active, their signalling in different types of cells and their functional effects is needed to understand the consequences of these interactions for PD1 pathway blockade. These alternative binding partners may also partially account for the differences seen in the efficacy of the different antibodies that are directed against PD1, PDL1 or PDL2 in various biological settings.

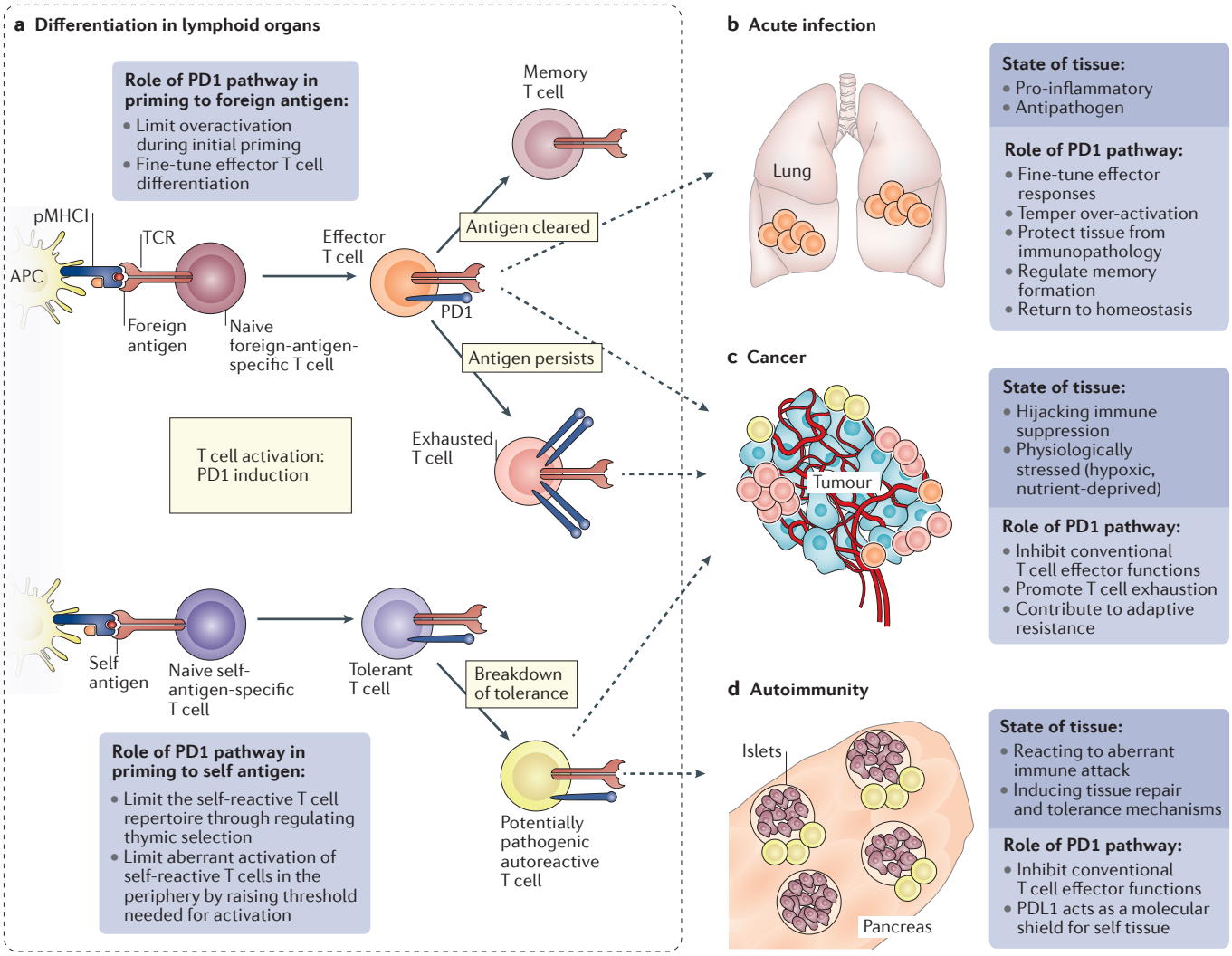

Figure 2 | **Roles of PD1 in acute infection, tolerance and cancer. a** | Programmed cell death protein 1 (PD1)-mediated immune regulation in secondary lymphoid organs. PD1 becomes expressed by all T cells during activation. Following exposure to foreign antigens (top), if antigen is cleared, memory T cells form and PD1 expression levels decrease. If antigen persists, T cell exhaustion can develop and PD1 expression levels remain high. PD1 also can be expressed following self antigen encounter (bottom) and can prevent the activation of autoreactive cells. **b–d** | The roles of PD1 in non-lymphoid tissues. **b** | During acute infection (for example, in the lung), PD1 plays roles in fine-tuning effector T cell responses, tempering overactivation, limiting immunopathology and regulating memory T cell formation and the return to tissue homeostasis. Here, the tissue is in a pro-inflammatory state; expression of PD1 ligands is a normal response to inflammation. **c** | During cancer progression, PD1 can inhibit T cell effector functions and promote T cell dysfunction. Here, elevated physiological stress (for example, hypoxia and nutrient deprivation), a number of immune suppression mechanisms, including expression of PD1 ligands, and the prevalence of dysfunctional T cell subsets (either exhausted or tolerant) serve as obstacles for protective immunity. Infiltrating T cells can also further induce programmed cell death 1 ligand 1 (PDL1) expression through pro-inflammatory cytokine production, contributing to adaptive resistance. **d** | During the development of autoimmunity, the breakdown of peripheral tolerance allows infiltration of pathogenic autoreactive T cells from the draining lymph node to the target organ (for example, the pancreatic islets). Here, PDL1 in the target tissue can act as a molecular shield to limit T cell attacks. The tissue reacts to aberrant immune attack, inducing tissue repair and tolerance mechanisms for protection. Solid lines indicate T cell differentiation; dashed lines indicate trafficking of T cells to different non-lymphoid tissues. APC, antigen-presenting cell; pMHCI, peptide–MHC class I complex; TCR, T cell receptor.

Type I interferons
Type I interferons, such as IFNα and IFNβ, are generally produced in response to danger-associated signals such as Toll-like receptors and cytosolic nucleic acid sensors. Type I interferons are produced by most cells in the body and have a variety of immunostimulatory effects and innate antiviral effects during acute infection, including inhibition of translation. By contrast, persistent type I interferon signalling during chronic viral infection can promote immune dysfunction.

Type II interferon
IFNγ, a key T cell and natural killer cell effector molecule that drives myeloid activation, MHC class I and II processing and presentation, leukocyte trafficking and pathogen replication inhibition.

PD1 and normal host physiology

The PD1 pathway is a crucial regulator of normal host physiology. PD1 is expressed by all T cells during activation and acts as a natural brake to temper overactivation of T cell responses. Without PD1, excessive immune-mediated tissue damage can lead to devastating consequences for the host. In addition, PD1 plays crucial roles in central and peripheral T cell tolerance, aiding in the protection of self tissues from autoimmune responses. Here, we discuss the roles of PD1 in normal host physiology, including in acute infection, tissue homeostasis and immune tolerance.

Balancing immunity and immunopathology. Although the PD1 pathway has received considerable attention for its role in regulating T cell exhaustion, PD1 is also expressed on all conventional CD4$^+$ and CD8$^+$ T cells during initial priming and activation (BOX 1) and has a crucial role in shaping the initial magnitude of the T cell response, in fine-tuning T cell differentiation and effector T cell fate and in the development of immunological memory[4,6,65–67] (FIG. 2). Loss of PD1 signalling often increases immune control of diverse types of infection, including viral, fungal and bacterial infections[68–72]. During the effector phase of an immune response, loss of PD1 expression can lead to marked increases in T cell proliferation and/or some T cell effector functions[65,69,71–73]. However, PD1 loss sometimes impairs host immunity[74,75]. These distinct outcomes could be due to differences in the type of infection and the timing and/or method of PD1 deletion, and the differing roles of PD1 in regulating crucial cell types[76] (BOX 1). The ability of PD1 to regulate diverse cell types highlights the importance of understanding the basic mechanisms of PD1 action in different biological contexts.

A crucial function of the PD1 pathway is to limit immunopathological responses in host tissues by promoting the resolution of inflammation and restoration of immune homeostasis (FIG. 2). If CD8$^+$ T cell responses are not adequately controlled, severe immunopathology can result from the production of pro-inflammatory cytokines (including IFNγ and tumour necrosis factor (TNF)) and/or aberrant cell killing. The crucial role of the PD1 pathway in controlling immune-mediated tissue damage is illustrated by the lethal immunopathology that develops in PD1-deficient or PDL1-deficient mice following infection with strains of lymphocytic choriomeningitis virus (LCMV) that cause chronic infection[14,72,77]. This fatal immunopathology depends on CD8$^+$ T cells and can involve killing of vascular endothelial cells in a perforin-dependent manner[72]. The PD1 pathway also has a role in regulating pro-atherogenic inflammatory responses, as mice deficient in the low-density lipoprotein receptor develop increased atherosclerotic lesions if they also lack PDL1 and PDL2 (REF. 78). The compromised vascular integrity that occurs in the absence of PD1 signalling represents a potential challenge for PD1 immunotherapy, as blocking PD1 may alter the permeability of the vascular barrier and increase the risk of heart attacks, strokes and oedema.

The PD1 pathway also regulates memory T cell differentiation and responses. Multiple factors that occur during the initial effector T cell response contribute to the size and quality of the memory T cell pool that forms following antigen clearance. Such factors include the affinity of the TCR for the peptide–MHC complex, the duration of TCR signalling and the composition of the inflammatory milieu[79]. Overactivation of T cells during the primary response may lead to suboptimal memory responses during subsequent challenges[80,81]. Because PD1 fine-tunes TCR signalling, the PD1 pathway is positioned to shape memory T cell responses. Experiments in mixed bone marrow chimeric mice infected with vaccinia virus showed that, compared with wild-type T cells, PD1-deficient T cells expressed higher levels of CD62L and CC-chemokine receptor 7 (CCR7) and were skewed towards a central memory T cell phenotype[65]. When wild-type and PD1-deficient memory T cells from these experiments were transferred into wild-type recipients that received a secondary challenge with vaccinia virus, the PD1-deficient memory T cells showed greater proliferation, which is consistent with a central memory phenotype[65]. Additional studies using vaccinia virus infection showed that PD1 blockade during secondary challenge could rescue defects in CD8$^+$ T cell responses generated without CD4$^+$ T cell help[66]. During viral infection in the lung, PD1 blockade during the secondary response also boosted CD8$^+$ T cell functions[71]. The effects of PD1 blockade during primary versus secondary challenges may affect the quantity and quality of memory T cell responses and depend on disease context. Understanding the effect of PD1 pathway modulation on T cell memory development, maintenance and recall potential will be crucial for cancer immunotherapy, for which protective anamnestic responses are highly sought.

Regulating autoimmunity. The PD1 pathway contributes to self-tolerance[9–12]. During T cell development in the thymus, PD1 signalling regulates the TCR signalling threshold during positive selection; consequently, loss of PD1 signalling at this stage results in more double-positive T cells[82,83]. How the PD1 pathway regulates thymic selection and how PD1 pathway modulation affects the repertoire of T cells entering the periphery remains poorly defined.

The PD1 pathway also has multiple roles in regulating autoreactive T cells in the periphery. Loss of PD1 through genetic knockout or antibody administration accelerates autoimmunity in disease models, including EAE, non-obese diabetic (NOD) mice and autoimmune enteritis[11,84,85]. PD1 restrains the activation, proliferation and differentiation of autoreactive CD4$^+$ T cells and CD8$^+$ T cells in antigen-draining secondary lymphoid organs (SLOs)[6,85–91] and regulates chemokine receptor expression by these cells[90]. Endothelial cell expression of PDL1 (REFS 48,72,92) may limit T cell trafficking into nonlymphoid tissues. Additional studies are needed to determine the effects of PD1–PDL1 signalling on T cell migration.

In mouse models, autoreactive T cells in the target organ express high levels of PD1 (REFS 6,90,91,93). Inflammation induced during destruction of the target tissue can increase PDL1 expression[11,94], providing an opportunity for regulation of autoreactive T cells. In NOD mice, PDL1 expression in pancreatic islets is more important than PDL1 expression on haematopoietic cells for protecting against diabetes onset[95]. Two-photon microscopy of CD4$^+$ T cells in pancreatic islets showed a direct role for PD1 in ongoing immunosuppression within the tissue microenvironment[88]. However, the pancreatic lymph nodes clearly have an important role in priming of autoreactive T cells during type 1 diabetes in NOD mice, as removal of these lymph nodes

Adaptive resistance
Mechanisms by which a tumour adapts to tumour-specific immune responses, leading to the upregulation of immunosuppressive molecules, such as programmed cell death 1 ligand 1 (PDL1), in an attempt to evade host immunity.

Anergic T cells
A form of peripheral tolerance induced at priming, generally resulting from high levels of antigen being recognized with inadequate amounts of costimulation and/or inflammatory cytokines. Anergic T cells persist in a functionally hyporesponsive state, but functionality can be restored if proper signals are provided.

at 3 weeks of age largely prevented the development of disease following the administration of anti-PDL1 antibody[89]. However, removal of the pancreatic lymph nodes at 11 weeks of age had no effect on the ability of anti-PDL1 antibody to promote disease, suggesting that T cells in the pancreas are sufficient to induce diabetes at this later time point[89]. Collectively, these data support a model in which the PD1 pathway controls autoreactive T cells at multiple stages of development: first, during the development of the thymic T cell repertoire; second, during the priming and differentiation of effector T cells in SLOs; and last, during the acquisition of effector functions in target organs (FIG. 2).

Timing has a crucial role in determining whether PD1 blockade leads to the breakdown of self-tolerance. In NOD mice, PD1 blockade induces diabetes more rapidly in older mice than in younger mice, probably due to the increased T cell infiltration and PDL1 expression that is seen in the pancreas as NOD mice age[84]. By contrast, in a model of intestinal tolerance where $CD8^+$ T cells are rendered nonresponsive to a target antigen expressed in intestinal epithelial cells, blocking PD1 soon after the transfer of target antigen-specific T cells increased T cell activation and severe autoimmune enteritis[85], whereas blocking PD1 later after T cell transfer, during the maintenance phase of tolerance, had little effect[93]. Additional studies are needed to define the stability of dysfunctional T cell states to determine when PD1 immunotherapy may (or may not) durably reprogramme these cells.

In humans, single nucleotide polymorphisms (SNPs) in *PDCD1* have been associated with autoimmune diseases[6,7], but it is not yet clear if these SNPs are causative or simply correlative. Also, it is unclear whether these SNPs will impede strategies to engage PD1 in patients with autoimmune diseases and/or can predict IRAEs in patients with cancer that are receiving PD1 pathway inhibitors.

Therapeutic implications. Understanding why PD1 blockade can precipitate autoimmunity in some settings but not others will be crucial for minimizing IRAEs during cancer immunotherapy. Context will probably be key. In autoimmunity, the relative frequencies of various $CD4^+$ T cell and $CD8^+$ T cell populations are not clear. Exhausted $CD8^+$ T cells have a unique epigenetic landscape compared with effector T cells and memory T cells[33,35,96], and anti-PDL1 antibody treatment does little to change this global epigenetic landscape[33,97]. Determining whether tolerance in $CD4^+$ T cells and $CD8^+$ T cells also has a notable epigenetic component will be important for determining the plasticity of these cells following immunotherapy. In one study in which tolerant $CD8^+$ T cells were transferred into a lymphopenic environment, tolerance could be broken and functionality restored[98]. However, this functional boost was transient, and tolerance was re-established after lymphorepletion, suggesting that tolerance may be stable, at least in this setting[98]. It is currently unclear whether IRAEs arising after immunotherapy in patients with cancer are caused by a pre-existing autoimmune response that is worsened by checkpoint blockade or whether IRAEs are caused by priming of naive autoreactive lymphocytes. Determining the immunological aetiology of IRAEs will aid in increasing the safety of checkpoint blockade therapies.

There is great interest in translating PD1 pathway engagement to induce tolerance in patients with autoimmunity and transplant rejection. One feature of the PD1 pathway that makes it an attractive therapeutic target is that PD1 is only expressed on T cells that are responding to antigen; consequently, PD1 pathway modulation could selectively affect ongoing immune responses compared with other therapies that broadly affect host immune cells (for example, corticosteroids and cyclophosphamide). Despite this promise, engaging the PD1 pathway to inhibit T cell functions has been challenging, partially due to the cellular requirements for PD1–PD1 ligand signalling (in *cis* with the TCR, requiring PD1 ligand and peptide–MHC expression on the same cell)[99] (FIG. 1). Several strategies have tested the effects of PD1 pathway engagement in preclinical models, including DCs engineered *ex vivo* to express either PDL1 and peptide–MHC complexes[100] or surface-bound PD1 agonists[101]. Surface-bound agonists could be particularly effective for inducing transplant tolerance (BOX 2) because donor cells could be transduced before transplantation. It will be essential to ensure that PD1 agonists restore immune tolerance but do not compromise protective immunity.

Hijacking the PD1 pathway for immune evasion

The PD1 pathway has been exploited by chronic pathogens and tumour cells to promote immune evasion. Blockade of PD1–PD1 ligand interactions in these settings can improve T cell functions and reduce viral load or tumour burden, as we discuss below.

Lessons from chronic infection. The importance of PD1 expression in regulating virus-specific T cells was first described during chronic LCMV infection in mice[14] and was subsequently reported in humans infected with HIV, hepatitis C virus (HCV) or hepatitis B virus[13]. PD1 pathway blockade after the onset of $CD8^+$ T cell exhaustion during LCMV infection increased virus-specific $CD8^+$ T cell proliferation, cytokine production and killing capacity, and decreased viral load[14]. Studies in nonhuman primates (infected with simian immunodeficiency virus or HCV) and humans (infected with HCV) showed similar results[102–104].

The timing of PD1 modulation is particularly important for 're-invigorating' exhausted T cells. PD1 is not required for the induction of T cell exhaustion[73] but it has a role in maintaining exhaustion, as PD1 blockade after the onset of exhaustion can boost effector functions in exhausted T cells[14]. On a population level, this re-invigoration results from the proliferation of a subset of T cells in response to PD1 blockade[105–107]. During chronic infection, a 'progenitor-like' subset of T cells that is preferentially sensitive to PD1 blockade retains higher proliferative capacity and greater capacity to produce effector cytokines,

Neoantigen-specific T cell
A T cell that recognizes antigens in the tumour that have been mutated so that the antigen no longer resembles self antigens, theoretically making these antigens more immunogenic because they are less affected by central tolerance.

and is less terminally differentiated[106–108]. The 'terminally differentiated' subset of T cells that responds poorly to PD1 blockade is less proliferative and produces lower amounts of effector cytokines, but retains higher cytotoxic potential and is the predominant T cell subset in non-lymphoid tissues[106,108]. The progenitor-like subset expresses lower levels of PD1 (REFS 105–107), suggesting that high levels of PD1 expression do not necessarily correlate with potential for re-invigoration. Importantly, proliferation of the progenitor-like T cell population (either through PD1 blockade or antigen-driven proliferation) induces conversion to the terminally differentiated phenotype[105–108]. Whether this conversion contributes to resistance to PD1 immunotherapy is unclear.

The progenitor-like T cell population is almost exclusively found in SLOs, not in non-lymphoid tissues[106,108], raising the question of whether exhausted T cells in non-lymphoid tissues can be re-invigorated by blocking PD1. However, several studies have shown that PD1 pathway signalling in non-lymphoid tissues and tumours can be functionally relevant[67,77,88,95,109–112]. When evaluating sensitivity or resistance to immunotherapy, it will be important to consider the range of T cell effector functions in different anatomical locations. For example, memory $CD8^+$ T cell subsets in SLOs and non-lymphoid tissues have notable functional differences. Splenic memory $CD8^+$ T cell populations expand more than memory T cell populations from the small intestine (intraepithelial lymphocytes) following secondary challenge[113]. However, memory $CD8^+$ T cells found in non-lymphoid tissues generally retain higher levels of expression of cytotoxic molecules compared with splenic T cells[113,114]. Thus, whereas central memory T cells in SLOs may be more proliferative, they take longer to differentiate into effector T cells and migrate, whereas tissue-resident T cell populations can immediately respond to secondary challenge[115]. Improved understanding of the effect of PD1 on T cells in different anatomical locations and their residual functional capacities will inform PD1 immunotherapy approaches.

Inhibiting antitumour immunity. Cancer provides a unique context for PD1-mediated inhibition of immune responses, as many diverse T cell types may be present in patients who respond to PD1 blockade in different ways. PD1 can potentially restrain trafficking to the tumour and effector functions in T cells that have recently been activated by tumour-antigen-bearing APCs in the SLOs (FIG. 3). The relative contribution of the PD1 pathway to regulating immune responses at each of these steps probably varies with tumour type[109–112,116], given the substantial heterogeneity among tumour microenvironments and tissue-specific tolerance mechanisms. As malignant cells arise from normal host cells, cancer has an element of self. The PD1 pathway may regulate T cells that cross-react with tumour-specific and self antigens (FIG. 2). Mutations in the tumour can be interpreted by the immune system as a form of 'altered self', which may result in more robust immune responses because the neoantigen-specific T cell repertoire should not be as limited by central tolerance. There are a number of types of neoantigens, including those generated by somatic mutations, viral antigens in virally induced cancers and cancer and/or testis antigens[117]. A number of strategies are currently being implemented to boost neoantigen responses (for example, inducing mutations with radiation or chemotherapy) in patients to circumvent overt autoimmunity. However, neoantigen-specific T cells that successfully differentiate into effector cells and traffic into the tumour are subject to PD1-mediated regulation. Furthermore, these cells may become exhausted owing to chronic antigen stimulation and the inflammatory tumour environment, in which pro-inflammatory cytokines produced by tumour-infiltrating T cells can further drive PDL1 expression and promote adaptive resistance and

Box 2 | **PD1 in transplant and fetomaternal tolerance**

An important unrealized clinical opportunity for immune checkpoint modulation is in the restoration of immunological tolerance during allogeneic and/or xenogeneic tissue transplantation. Transplantation is commonly used clinically to replace or restore organ functionality. Transplanted allogeneic grafts induce a pro-inflammatory environment that can lead to graft injury or loss (host versus graft) or graft-versus-host disease (GVHD). Currently, transplant patients receive high doses of immunosuppressive drugs to suppress graft rejection. There is increasing interest in restoring immunological tolerance in these patients to reduce or eliminate the need for widespread immunosuppression.

The programmed cell death protein 1 (PD1) pathway has an important role in mitigating tissue damage during allotransplantation. Blockade of the PD1 pathway exacerbates tissue rejection in acute GVHD[161]. Programmed cell death 1 ligand 1 (PDL1) upregulation in transplanted tissue is more important for mediating tolerance and allograft acceptance than PDL2 (REF. 161). PD1 on donor T cells can be important for tolerance in some settings, whereas in other settings, PDL1 on donor T cells may have a role[161–163]. Interactions between PDL1 and CD80 may also be important[163]. Further work is needed to define the dynamics of each of these interactions on effector T cells, regulatory T (T_{reg}) cells and B cells during transplantation, as there may be distinct and sometimes opposing effects (BOX 1).

The PD1 pathway also has a crucial role in maintaining fetomaternal tolerance during pregnancy. Pregnancy resembles carrying a semi-allogeneic graft, containing half maternal and half paternal genetic material. In mice, PDL1 expression is increased during allogeneic but not syngeneic pregnancies[164]. PDL1 expression is lower in the first trimester than the second and third trimesters, and fetal cells in contact with maternal blood and tissues (for example, the villous syncytiotrophoblast and basal plate cytotrophoblast) express increased levels of PDL1 (REF. 165). Blockade of the PD1 pathway during pregnancy in mice increases T cell infiltration and cytokine production in the placenta and decreases litter size and pup survival[164]. PDL1 expression on T_{reg} cells also may be important in this setting[166]. Further work is needed for clinical translation and the development of immune modulation strategies that engage the PD1 pathway to restore tolerance.

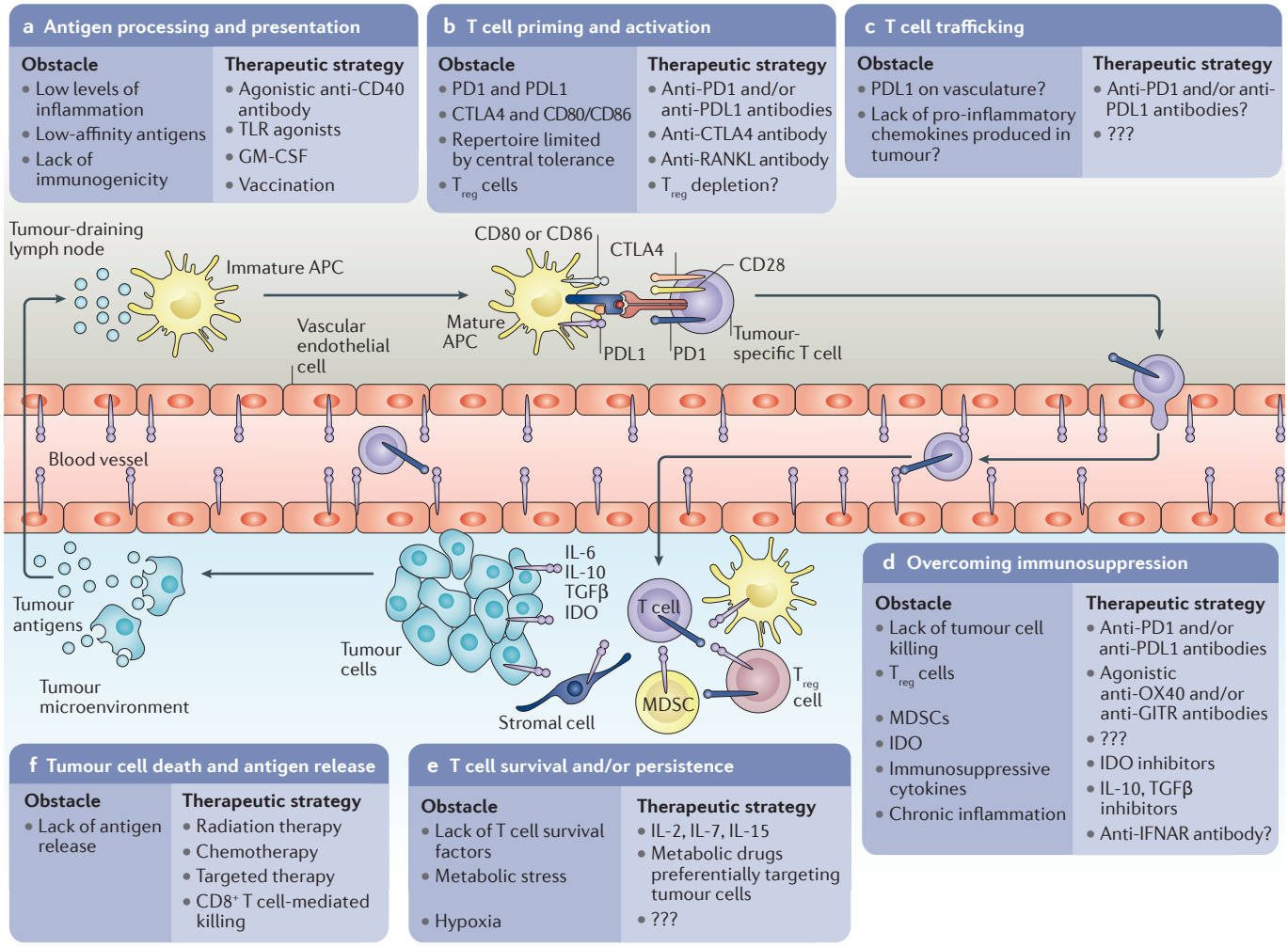

Figure 3 | **Use of PD1-based combination therapy to promote anticancer immunity. a** | Antigen processing and presentation by antigen-presenting cells (APCs) in the tumour-draining lymph node. **b** | T cell priming and activation in the tumour-draining lymph node. **c** | T cell trafficking from the tumour-draining lymph node into the tumour. **d** | Overcoming diverse forms of immunosuppression in the tumour microenvironment. **e** | T cell survival and persistence in the tumour microenvironment. **f** | Tumour cell death and antigen release. Antigen can drain into the tumour-draining lymph node through the lymphatics to get picked up and processed by APCs in the lymph node, or it can get taken up by APCs in the tumour and then be carried by the APCs into the lymph node. This step feeds back into part **a** of the cycle. Bold headings indicate the key steps that are required for productive antitumour immunity. CTLA4, cytotoxic T lymphocyte antigen 4; GM-CSF, granulocyte–macrophage colony-stimulating factor; IDO, indoleamine 2,3-dioxygenase; IFNAR, IFNα/β receptor; MDSC, myeloid-derived suppressor cell; PD1, programmed cell death protein 1; PDL1, programmed cell death 1 ligand 1; TGFβ, transforming growth factor-β; T_{reg} cells, regulatory T cells; TLR, Toll-like receptor.

immunosuppression. Inhibitors of the PD1 pathway have the potential to overcome these crucial barriers to effective antitumour immunity (FIG. 3).

Early studies in mouse models of cancer showed a role for the PD1 pathway in regulating antitumour immunity[15–19]. In many mouse models, tumour-infiltrating lymphocytes overexpress PD1 (REFS 118–121) and different types of tumours express PD1 ligands[4,15]. Mouse cancer models show varying degrees of sensitivity to PD1 blockade when used as a single agent. MCA sarcoma and MC38 adenocarcinoma show better responses to PD1 blockade than B16 melanoma and MB49 bladder cancer[42,118,119]. Most tumours in mice require some form of combination therapy for complete eradication[25,26].

Many factors contribute to sensitivity versus resistance to PD1 therapy in mouse cancer models. These factors should be considered when designing experiments to address mechanistic questions. For example, tumour size can critically influence sensitivity to PD1 blockade, with smaller tumours responding better[110,118]. Further work is needed to increase the breadth of clinical scenarios that can be modelled in mice — including mutational and/or neoantigen burden, rate and method of transformation, mutations associated with tumorigenesis, location of the tumour and metastasis — to extend observations from the bedside to the bench and back to the patient. In addition, it would be useful for the field to develop models that better reflect the different immune

microenvironments that can be found in the tumour; for example, the 'suppressed infiltrate', where T cells enter the tumour but become functionally suppressed; the 'excluded infiltrate', where T cells migrate to the tumour but cannot infiltrate the tumour; and the 'immune desert', where T cells fail to traffic to the tumour.

Implications for cancer therapy

Blocking antibodies that target PD1 or PDL1 have demonstrated remarkable success in clinical trials[20,22,122–130]. However, most patients have not shown stable remission after PD1 therapy. More basic and clinical research is needed to determine why PD1 blockade is effective in some patients but not others.

The dynamic interplay between the immune system and cancer is complex, partly because both the tumours and the corresponding immune responses are diverse and heterogeneous. The immune system has the ability to combat tumour cells, but some cells of the immune system promote transformation and tumour growth[131]. As both the tumour and the immune infiltrate influence responses to therapy, both need to be considered when developing combination therapies.

Correlates associated with clinical outcomes. Substantial efforts are underway to define biomarkers to predict which patients will benefit from PD1 pathway blockade as a monotherapy and also to develop effective combination therapies for patients who do not respond to monotherapy. For PD1 pathway blockade monotherapy, the presence of $CD8^+$ T cells at the invasive tumour margin[132] and high levels of PDL1 expression in the tumour[122,127,130,132,133] correlate with better response rates. In many cases, PDL1 expression in tumours correlates with $CD8^+$ T cell infiltration[47,132,134], suggesting the presence of an ongoing inflammatory response. However, PDL1 expression does not always correlate with therapeutic outcome: some $PDL1^+$ tumours respond poorly to PD1 pathway blockade[22,122,130], whereas some $PDL1^-$ tumours show good responses[22,135]. In some tumours, mutations can cause constitutive expression of PDL1, even in the absence of an inflammatory response[47]. Consequently, multiple biomarkers are likely to be more effective at predicting responses to PD1 monotherapy than PDL1 expression level alone[136].

In addition, efforts are increasing to develop genetic signatures predictive of the response to checkpoint blockade. For example, in RCC[137], certain metabolic signatures (associated with nutrient and solute transport and clearance of toxins and lipophilic chemicals in normal kidney epithelial cells) correlated with treatment failure, possibly owing to increased metabolic fitness of the tumour cells. Conversely, immunological signatures containing *BACH2*, which encodes a transcription factor that regulates the differentiation and function of effector T cells and memory T cells, and *CCL3*, which encodes a chemokine involved in leukocyte migration, correlated with treatment success in $PDL1^+$ RCC tumours[137]. Recent data examining metastatic melanoma lesions showed that 'mesenchymal' and 'suppressive inflammatory' transcriptional phenotypes are associated with resistance to PD1 inhibitors[138]. Moving forward, coupling high-dimensional imaging and flow cytometry with genomic analyses should clarify optimal biomarkers for response to therapy. Temporal analyses of samples from different types of tumours will also be crucial to better predict the response to immunotherapy.

Some studies have shown that increased TCR clonality in the tumour correlates with better responses to PD1 inhibitors[132,139], suggesting that less TCR diversity or a more clonal T cell population can correlate with better antitumour immunity. Somatic mutations within tumours represent an opportunity for the immune system to respond to epitopes that are distinct from self antigens, which potentially increases the immunogenicity of the tumour. Preclinical work supports the idea that neoantigens increase tumour immunogenicity[42,117,140]. Comprehensive analyses of human tumours have revealed a wide range in the number of somatic mutations, from low (for example, in acute myeloid leukaemia, acute lymphocytic leukaemia and glioblastoma) to high (for example, in melanoma and NSCLC)[141]. Clinical reports have shown varying degrees of correlation between mutational burden and clinical outcomes following checkpoint blockade (of PD1 or CTLA4 signalling)[138,142–145]. Melanoma and NSCLC generally have high mutational burdens due to chronic exposure to mutagens and have some of the highest response rates to PD1 inhibition[20,122–124,128–130]. In NSCLC, higher rates of nonsynonymous mutations correlated with durable clinical benefit following pembrolizumab treatment (defined as a partial or stable response lasting longer than 6 months)[142]. However, a subset of patients with NSCLC (5 of 18) with high mutational loads did not have a durable response to therapy[142]. In patients with metastatic melanoma, whole-exome sequencing of tumour samples before treatment (with nivolumab or pembrolizumab) showed that the overall somatic mutational burden did not significantly correlate with responsiveness to anti-PD1 antibody treatment (where responders were defined as patients with complete, partial or stable disease and nonresponders were defined as those with progressive disease by irRECIST). However, higher mutational load correlated with increased survival after anti-PD1 antibody therapy[138]. A subset of patients with low mutational burdens also responded to anti-PD1 antibody therapy[138]. These data support the notion that somatic mutations can correlate with better responses to PD1 immunotherapy, but low mutational loads do not necessarily preclude benefit[138]. Given that intratumour heterogeneity and clonal versus subclonal levels of neoantigens may affect sensitivity to checkpoint blockade[146], mutational analyses of limited tumour biopsy samples should be interpreted with caution. T cell responses to neoantigens provide an opportunity to promote potent antitumour immunity while avoiding issues such as autoimmunity. Thus, strategies to increase neoantigen responses through vaccination[42,147], radiation[140] or targeted therapies (for example, drugs targeting kinases or growth factor receptors) have great potential to increase response rates to checkpoint blockade. However, many tumour antigens probably have high

Immunogenicity
The ability of an antigen to stimulate an immune response; highly immunogenic antigens are generally recognized by the immune system as foreign (or distinct from self) and their recognition is accompanied by inflammation.

irRECIST
(Immune-related response evaluation criteria in solid tumours). Similar to RECIST, where a total of five malignant lesions, two per organ, are measured unidimensionally, with shared response criteria (complete response, partial response, stable disease and progressive disease defined), but the physician waits up to 12 weeks to confirm progressive disease to account for the flare effect.

similarity to self antigens. Considering the crucial role of the PD1 pathway in maintaining self-tolerance, PD1 inhibitors may work in part by boosting autoreactive T cells that are specific for self antigens in the tumour, which might explain the responsiveness to PD1 therapy even in tumours with a low mutational burden.

Combination therapies. Combination therapies represent the next wave of clinical cancer treatment. For rational combination therapies, it is important to consider how treatments converge to influence the antitumour immune response and the tumour itself, as both responses will dictate the clinical outcome. It is crucial to understand how therapies target intracellular signalling pathways in tumour and immune cell types, diverse cell types and differentiation states, or anatomical locations (for example, SLOs versus the tumour microenvironment) (FIG. 3). Strategies for combination therapies with PD1 inhibitors include blocking other inhibitory receptors (such as LAG3, TIM3 or TIGIT); blocking immunoregulatory cytokines (such as IL-10 and chronic type I interferon production); delivering agonists for costimulatory molecules (such as CD40 or ICOS); administering homeostatic cytokines (such as IL-2, IL-7 or IL-15); vaccinating to boost T cell responses[25,26]; and delivering engineered T cells (FIG. 3).

A deeper understanding of basic mechanisms underlying clinical successes versus failures is needed to better predict which combination therapies will work best for which patients. Some pathways pose intrinsic risks because of context-dependent roles in host immunity. For example, clinical trials using pegylated type I interferon in patients with cancer (including lymphoma, melanoma, leukaemia and Kaposi sarcoma) from the 1970s onwards showed mixed efficacy. It is now appreciated that type I and type II interferons have complex roles in host immune responses. Interferons can augment antitumour immunity by promoting DC functions, T cell priming and tumour cell death[148]. However, IFNγ produced by tumour-infiltrating lymphocytes can drive adaptive resistance[121,134]. Furthermore, chronic type I interferon signalling can have potent immunosuppressive effects[149,150], and prolonged IFNγ signalling in mouse cancer models can drive increased resistance to checkpoint blockade[151]. Consequently, it is unclear whether blocking or administering interferons to patients with cancer will augment checkpoint blockade. Considering recent studies indicating that checkpoint blockade can be less effective when interferon signalling is impaired or defective[152–154], additional studies are needed to clarify the intersection between these two pathways, particularly when interferon signalling is acute versus chronic.

In addition to combination therapies targeting immunological pathways directly, there is increasing interest in targeting metabolism and epigenetic regulation, owing to their importance in determining cell fate and function. Pharmacological agents targeting metabolism (such as methotrexate, dichloroacetate and isocitrate dehydrogenase inhibitors) and the epigenome (such as histone deacetylase inhibitors, including vorinostat and romidepsin, and DNA methyltransferase inhibitors, such as 5-azacitidine and decitabine) are in clinical use or in clinical trials for treatment of cancer, although their effects on the immune system are only beginning to be elucidated. There seems to be promising synergy between PD1 inhibitors and metabolic pathway modulators[39,40,44,155]. Whether combination therapy using PD1 inhibitors and global epigenetic modifying agents will show increased clinical efficacy remains to be determined. Anti-PDL1 antibody as a monotherapy cannot globally change the epigenetic landscape of exhausted T cells in LCMV and tumours[33,97], but combination therapy with epigenetic modifying agents might enable reprogramming of these cells.

One of the most important barriers to *de novo* immune responses to tumour antigens is antigen presentation in the appropriate inflammatory context. Many forms of targeted therapy (such as cetuximab, dabrafenib and trametinib), chemotherapy (such as 5-fluorouracil, doxorubicin and oxaliplatin) and radiotherapy can have immunostimulatory effects by increasing tumour immunogenicity, thereby overcoming this barrier (FIG. 3). Dosing and timing are likely to be crucial factors for optimizing responses. High doses of chemotherapy and/or radiation therapy may have adverse effects on immune cells. Additional studies are needed to determine which therapies synergize with or antagonize immunotherapies and how to optimize their doses and sequence to improve patient outcomes.

Conclusions

PD1 is a truly unique inhibitory receptor due to the wide-ranging functions of its regulatory signals in immune homeostasis, resolution of inflammation, tolerance, chronic infections and cancer (FIG. 2). Because PD1 regulates T cells not only in SLOs but also in non-lymphoid tissues and tumours, PD1 modulation can exert its effects in several ways. Although our understanding of the PD1 pathway has been translated to therapies that benefit many patients with cancer, most patients do not respond to PD1 blockade alone. Anti-PD1 antibody therapy has become a foundational building block for combination therapies aimed at increasing the number of patients who respond (FIG. 3), but further work is needed to determine which combinations will work best for which patients.

To guide rational PD1-based combination therapy, several key questions need to be addressed. First, how does PD1 function change in conventional $CD4^+$ T cells and $CD8^+$ T cells at different stages of their differentiation (naive versus effector versus memory cells)? One of the most highly sought-after targets of adaptive immunity in cancer immunotherapy is immunological memory, given its potential to protect patients from relapse. However, it is currently unclear if durable responses to anti-PD1 antibody therapy in patients with cancer are due to the development of an antitumour memory T cell response. Here, it will be essential to determine whether dysfunctional antitumour T cells can be durably reprogrammed into memory cells or if the memory response that forms is due to enhanced priming of new effector T cells.

Second, beyond conventional CD4+ T cells and CD8+ T cells, what are the functions of PD1 on T_{reg} cells, B cells, myeloid cells and NK cells? Additional work is needed to understand how PD1 controls these cells, as systemic modulation of the PD1 pathway will broadly impact all immune cell subsets.

Third, what are the unique and overlapping functions of PD1 compared with other inhibitory receptors (for example, CTLA4, LAG3, TIM3 and TIGIT)? It is not clear if there is a hierarchy by which inhibitory receptors operate, such that if one receptor is lost, other receptors will compensate. Also unclear is how to optimize synergies between inhibitory receptors. Do multiple receptors need to be expressed on the same cell, or is better synergy observed if different or overlapping signalling pathways are targeted? We have a very rudimentary understanding of these signalling pathways, let alone how blockade of one receptor impacts the signalling capacity of another.

Fourth, is it possible to uncouple the antitumour activity of immune checkpoints from the activity of these checkpoints in maintaining self-tolerance to limit IRAEs? Autoreactive T cell clones can be found in the circulation of all healthy people[156,157]. Because of the indispensable roles of PD1 in peripheral tolerance, boosting autoreactive T cells is a challenge for PD1-targeted cancer immunotherapy. One potential solution is local delivery of checkpoint inhibitors to the tumour microenvironment. This approach might limit IRAEs, but could also potentially reduce antitumour efficacy by excluding recruitment of tumour-specific T cells from the SLO into the response. Recent work has proposed gene modules of T cell exhaustion that are distinct from general T cell activation[158]. Similar approaches could potentially identify targets to boost antitumour immunity independently from autoreactive T cell responses.

Fifth, how can PD1-mediated inhibitory signals be engaged to promote tolerance? This is a major unrealized clinical opportunity, given that the PD1 pathway can shield tissues from potentially pathogenic effector cells.

Further understanding of the PD1 pathway will shape the future of PD1 modulators in the clinic, whether they are designed to block the PD1 pathway to boost immune responses during cancer and/or chronic infections or engage the pathway to suppress pathogenic immune responses during autoimmunity and/or transplant rejection. Efforts from both the basic science and clinical arenas will be essential to answer these questions and provide insight into optimal ways to modulate the PD1 pathway for sustained patient benefit.

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