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Immune checkpoint blockade in infectious diseases

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Abstract

The up-regulation of immune checkpoint molecules, such as PD-1 and CTLA4 on immune cells occur during acute infections, such as malaria, as well as during chronic persistent viral infections, including HIV and hepatitis B virus (HBV). These pathways are important for preventing immune-driven pathology, but can also limit immune-mediated clearance of the infection. The recent success of immune checkpoint blockade in cancer therapy suggests that targeting these pathways could also be effective for preventing and treating a range of infectious diseases. Here, we review our current understanding of immune checkpoint pathways in the pathogenesis of infectious diseases and discuss the potential for therapeutically targeting these pathways in this setting.

Introduction

Immune checkpoint molecules are inhibitory receptors expressed on immune cells that trigger immunosuppressive signalling pathways. These molecules are crucial for maintaining self-tolerance and for modulating the length and magnitude of effector immune responses in peripheral tissues, in order to minimize collateral tissue damage[1,2]. Signalling via these molecules can drive effector immune cells (especially T cells), into a state known as ‘exhaustion’. T cell exhaustion is defined by reduced effector function, sustained expression of immune checkpoint molecules (such as PD-1), poor recall responses and a transcriptional state distinct from that of functional effector or memory T cells[3]. There are numerous types of activating and inhibitory interactions that occur between antigen-presenting cells (APCs) and T cells, and these regulate the nature of immune responses (Figure 1). It is now clear that many pathogens and cancers promote inhibitory interactions between immune cells via immune checkpoint proteins to escape immune control.

Investigation of these immunosuppressive interactions has led to the clinical development and licensing of novel efficacious cancer treatments, which use specific antibodies to improve immune responses by blockade of checkpoint protein functions (Box 1). Antibodies targeting PD-1 (Pembrolizumab; Nivolumab), CTLA4 (ipilimumab) and PD-L1 (atezolizumab; avelumab) are currently licensed as monotherapies for various types of cancer (Box 2). In addition, combined therapeutic targeting of PD-1 and CTLA4 was shown to be more effective than either therapy alone for treatment of melanoma[4], although such

combination therapy also leads to increased toxicity in patients. Therapies targeting several other immune checkpoint pathways have also shown promise for controlling various types of cancer (Table 1 and reviewed in Ref.[2]). It is also possible to enhance immunity by directly targeting molecules on T cells which improve T cell functions (Box 1), and their clinical utility is currently being assessed in clinical trials. These antibody-mediated treatments use the individual's own immune system to eliminate or slow the growth of cancer cells and have shown remarkable success in malignancies such as melanoma.

A major challenge in immunotherapy is to understand why treatment responses are variable and thus there is a search for predictive 'biomarkers" of a favourable clinical response. PD-L1 expression on tumor cells can identify patients who would most benefit from PD-1 or PD-L1 blockade therapy[5]. There are also more complex "gene signatures" in tumours to identify patients who will show the best responses[6]. Earlier T cell expansion following anti-PD-1 therapy in small lung cancer has been associated with improved responses[7] and a composite biomarker of the T cell proliferative response together with pre-treatment tumor burden can predict response to anti-PD-1 in individuals with metastatic melanoma[8]. Given the cost and toxicity of immune checkpoint blockade, identifying biomarkers that predict a clinical response are currently a top priority. It is feasible that a composite marker of functional responses by T cells and burden of residual infection may also be relevant for infectious diseases.

Whether immunotherapies can also be effective for treating infectious diseases is less well explored. However, the fact that these inhibitory pathways are also exploited for immune evasion by pathogens suggests that blockade could be used for the prevention and treatment of infectious diseases, in either the acute or chronic phases of infection. Currently, checkpoint blockade is being evaluated for reversing T cell exhaustion that follows on from chronic disease, but there is potential for also treating acute infections to generate long term immunity[9]. The development of vaccines for a range of infectious diseases, including malaria, HBV and HIV could also potentially be enhanced through checkpoint blockade. Most importantly, given that drug resistance against malaria[10] and many other infections are rising, and that control of both HIV and HBV require life-long treatment, new strategies for treatment or potentially cure are now being considered. Furthermore, parallel searches for biomarkers which inform on the best therapy choice as well as indicate if there is a timeframe when the therapy would be most efficacious are also required. In this review, we describe in detail the impact of immune checkpoint signalling during malaria, HIV and HBV infections, as well as in tuberculosis, and we discuss the potential for therapeutically targeting these pathways in these settings.

Immune checkpoint proteins in malaria

Malaria is a mosquito-borne infectious disease of humans caused by parasitic protozoans of the genus Plasmodium. The majority of malaria infections are caused by *P. falciparum* and *P. vivax*, and in 2015, there were 212 million new cases of malaria worldwide with 429,000 deaths due to *P. falciparum* alone[11]. These parasites have a complex life cycle within the mammalian host, in which a liver stage of infection is followed by asexual and sexual blood

stages of infection; the blood stages cause the severe symptoms and high mortality associated with malaria.

Over the past 20 years, more than 100 vaccines have been developed to control malaria and clinically evaluated. Most vaccines were specifically designed to target liver or blood-stage parasites by inducing protective antibodies and $CD4^+$ T cells, although a few vaccines were designed to generate $CD8^+$ T cell responses against the liver-stage parasites. The best candidate vaccine identified to date is the RTS,S/AS01E vaccine, which will soon be administered to children in Africa; however, this vaccines had an efficacy of only 43.6% in the first year of administration and efficacy decreased to 16.8% by the fourth year[12]. This highlights the significant challenges in developing an effective malaria vaccine and suggests that new strategies that target potential mechanisms of immune evasion by parasites need consideration.

The symptoms of malaria range from asymptomatic to chronic, severe and finally lethal disease. Partial immunity is developed by those living in endemic areas only after repeated attacks of malaria over several years[13–15]. Protection against malaria is dependent on both cell-mediated and humoral immune responses. Parasites in the liver stage are known to be cleared by cytotoxic $CD8^+$ T cells and possibly $CD4^+$ T cells[16]. For blood stage malaria, antibodies have been shown to play a key role in protection as demonstrated by transfer of serum from protected adults into children[17]. Studies in experimental rodent models of malaria have shown that multiple effector responses are required to protect against blood stage malaria. T helper 1 (Th1) cell responses are critical for controlling the bulk of blood-stage parasites and thus preventing severe disease[18,19]. Antibodies are required to eliminate the remaining patent parasites[20]. Recent studies have shown that $CD8^+$ T cells are required for sterile immunity that prevents the acute infection from progressing to chronic malaria[21]. Antibodies and $CD8^+$ T cells are also required for long-term protection against re-infection[22]. Studies have also shown that malarial infections caused apoptosis of vaccine-specific memory B cells[23] because of compromised dendritic cell (DC) functions[24]. This could explain why vaccines have not been successful in the field. Several other factors also contribute to short-lived immunity against malaria (reviewed in Ref.[25]), but the role of PD-1 as a major factor in loss of immunity against malaria has risen to the forefront.

Malaria and T cell exhaustion—As vaccines have been the focus of malaria control, the study of immune checkpoint proteins in malaria infections is relatively new. Field studies in malaria-endemic Mali and Kenya, found that individuals recently infected with *P. falciparum* expressed PD-1 on $CD4^+$ [26,27] and $CD8^+$ T cells[27], implicating this molecule in immune evasion. Similarly, an increased proportion of $CD4^+$ T cells from individuals with acute-phase infections with *P. vivax*, *P. falciparum*, or both had increased expression of CTLA4, OX40, GITR, and CD69[28] suggesting a role for regulatory T cells in suppressing immunity to malaria and indicating potential targets of checkpoint control. Finally, T cell immunoglobulin and mucin-domain containing 3 (TIM3) expression was significantly increased on key populations of lymphocytes in *P. falciparum*-infected patients[29].

There are four mouse models of malaria that display the major symptoms and pathology of human disease and are routinely used to study malarial pathogenesis (Box 3). A definitive

role for PD-1 in malarial pathogenesis was established when PD-1-deficient mice were shown to rapidly and completely clear *P. chabaudi* infections, which usually cause chronic malaria in mice[21]. Notably, during the acute phase of *P. chabaudi* infection, PD-1 was shown to mediate a 95% loss in the numbers and functional capacity of parasite-specific $CD8^+$ T cells, which are required to control chronic disease[21].

Recent studies of malaria using four mouse models revealed a novel regulatory function for PD-L2[9]. It was shown that while PD-L1 expressed by DCs did indeed attenuate immune responses against malaria, PD-L2 protein expressed on the same DCs improved immune responses by inhibiting PD-L1–PD-1 interactions[9]. These studies also showed that PD-L2 was essential for establishing effective Th1 cell immunity for protection against lethal malaria (Figure 2). This study also examined healthy human volunteers before and after infection with experimental *P. falciparum* malaria. They found that the expression of PD-L2 but not PD-L1 on blood DCs, decreased significantly within 7 days of infection to levels that inversely correlated with the level of parasitaemia in each individual[9]. In other words, higher PD-L2 levels correlated with lower parasitaemia indicating that this was not just a feature of mouse malaria. Overall, this study highlighted the importance of PD-L2 expression for malarial immunity.

Immune checkpoint blockade in malaria—A recent study showed that a multimeric form of PD-L2 fused with the Fc part of immunoglobulin (PD-L2-Fc), given to mice infected with lethal malaria, was sufficient to attenuate the lethal infection and mediate survival following re-infections after several months, without additional PD-L2-Fc[9] (Figure 2). Furthermore, combined blockade of the PD-L1 and LAG3 inhibitory molecules with antibodies accelerated the clearance of acute non-lethal blood-stage malaria (*P. yoelii*) by improving $CD4^+$ T cell function and increasing antibody titres[26]. Finally, antibody-mediated triggering of OX40 signalling also enhanced helper $CD4^+$ T cell and humoral immunity and thus parasite clearance during non-lethal malarial infections[30].

During *P. berghei* infections in mice resistant to cerebral malaria, antibody-mediated blockade of either CTLA4 or PD-L1, but not PD-L2, resulted in higher levels of T cell activation with enhanced interferon-γ (IFN-γ) production, but increased the incidence of cerebral malaria in these mice[31]. This was most likely because CTLA4 or PD-L1 blockade did not improve $CD4^+$ T cell functions sufficiently to control systemic parasite growth and sequestration in the brain before improved $CD8^+$ T cell functions could cause bystander pathology in the brain. In contrast, administering soluble multimeric PD-L2-Fc fusion protein reduced the incidence of cerebral malaria by 78%[9]. Similarly, blocking TIM3 signalling with antibody restored lymphocyte activity in *Plasmodium* infections resulting in accelerated parasite clearance and reduced symptoms of cerebral disease in *P. berghei*-infected mice[29]. The suppressive function of T_{REG} cells in lethal *P. yoelii*-infected mice was inhibited by GITR blockade indicating another potential target[32]. BTLA has also been associated with cerebral malaria and blockade significantly reduced the incidence of cerebral malaria compared with control mice[33]. Overall, several checkpoint proteins contribute to the pathogenesis of malaria, and further investigation of their potential as therapeutic targets is warranted. These therapies may also have the potential to be used to “re-invigorate” immune cells which are suggested to be non-responsive in endemic areas of malaria[26,27] to allow

vaccines to generate long term immunity. Alternatively, checkpoint blockade could complement malarial drugs to generate long term immunity as seen for PD-L2-Fc[9].

Immune checkpoint proteins in HIV

There are 37 million people living with HIV and each year there are 2 million new infections and 1 million deaths[34]. Antiretroviral therapy (ART) has dramatically reduced HIV-related morbidity and mortality but only 40% of people living with HIV globally are receiving ART[34] and there is no vaccine or cure. ART is required lifelong as once treatment is stopped, the virus rapidly rebounds. Given the social and economic impact of lifelong medical care required for people living with HIV, finding a cure has become a major global priority[35]. Immune checkpoint proteins have been extensively studied during HIV infection, initially in relation to natural history and T cell function, but more recently in relation to complications of HIV infection. In addition, using immune checkpoint blockade could potentially be exploited as a strategy to achieve a cure.

T cell exhaustion and immune checkpoint proteins in HIV infection—T cell exhaustion is a hallmark of many chronic viral infections, including HIV. In untreated HIV infection, there is an up-regulation of multiple immune checkpoint proteins including PD-1, CTLA4, TIM3 and LAG3 on both $CD4^+$ and $CD8^+$ T cells[36–38]. Following ART, expression of immune checkpoint proteins decline but remain elevated compared to HIV-uninfected controls[38]. Whether ART is started early (within 6 months of infection) or late (within 2 years of infection), similar levels of expression of immune checkpoint proteins persist[39]. In HIV infection, expression of immune checkpoint proteins varies on different T cell subsets. Increased expression of PD-1 is predominantly seen on central memory T cells[40], while both PD-1 and CTLA4 are expressed by regulatory T cells, and LAG3 is expressed by effector memory T cells[41] (Figure 3). In addition, PD-1 is often co-expressed with proteins that help promote T cell activation, such as CD38 and MHC class II molecules[42].

Elevated levels of PD-1 expression on total and HIV-specific $CD8^+$ T cells in untreated HIV infection was first reported over 10 years ago[36,43,44]. PD-1 is also highly expressed by cytotoxic $CD8^+$ T cells that migrate into lymphoid follicles; these follicular cytotoxic CD8+ T cells express high levels of CXCR5 and PD-1 but low levels of other immune checkpoint proteins, such as TIM3[45]. There is an inverse association between the frequency of cytotoxic $CD8^+$ T cells and HIV-infected cells in lymphoid follicles and a similar inverse relationship has been recently observed in untreated SIV infection[45–47]. Finally, *ex vivo* blockade with anti-PD-1 or anti-PD-L1 resulted in enhanced HIV-specific $CD8^+$ T cell function and killing of infected target cells[36,43,44] (Table 2), as described for cancer antigens.

Multiple observational studies have demonstrated a clear association between expression of PD-1 on either $CD4^+$ or $CD8^+$ T cells and clinical outcome. In the absence of ART, increased expression of PD-1 was associated with accelerated decline in $CD4^+$ T cells following acute infection[48] and untreated chronic infection[36]. Following ART, PD-1 expression on $CD8^+$ T cells has been associated with impaired $CD4^+$ T cell immune reconstitution[49] microvascular disease[50], elevated oxidised high and low density lipoproteins[51] and a shorter time to viral rebound once ART was stopped[52].

In vitro, HIV can establish either productive or latent infection. In latent infection, virus replication is incomplete with the virus integrating in the host genome but not proceeding to transcription, translation or production of virus particles [reviewed in Ref.[35]]. Following productive infection with HIV or other retroviruses such as feline immunodeficiency virus, PD-L1 expression is up-regulated on infected $CD4^+$ T cells which enhance $CD8^+$ T cell exhaustion and immune escape[53]. It remains unclear if PD-L1 or PD-L2 expression is increased on $CD4^+$ T cells after ART or on latently infected cells.

Immune checkpoint blockade in vivo for SIV and HIV infection—The administration of anti-PD-1 to simian immunodeficiency virus (SIV)-infected rhesus macaques resulted in rapid expansion of virus-specific $CD8^+$ T cells with improved functional quality as demonstrated by delayed time to death of the macaques and lower SIV RNA in plasma[54] (Table 2). Other beneficial effects of anti-PD-1 in the setting of SIV infection have also included reduced interferon signalling and improved gut permeability[55]. In preliminary work assessing the administration of anti-PD-1[56] or anti-PD-L1 (avelumab; [57]) to SIV-infected macaques on ART, there were no adverse effects, but in contrast to the studies in rhesus macaques off ART, there was limited expansion of SIV-specific $CD8^+$ T cells[56]. It is possible that an effective T cell response to anti-PD-1 requires the presence of antigen, and that given that ART leads to a dramatic reduction in viral antigens, the functional response to immune checkpoint blockade may be limited in this setting. Further work is needed to better understand the effects of immune checkpoint blockade on T cell function following ART.

In HIV-infected individuals who had stopped receiving ART, the up-regulation of CTLA4 expression on HIV-specific $CD4^+$ T cells was also demonstrated over a decade ago and, similarly to up-regulation of PD-1 expression, this was associated with increased HIV disease progression[37]. When SIV-infected macaques, both on and off ART, were treated with ipilimumab (anti-CTLA4), those that were off ART showed a significant increase in rates of HIV replication, presumably as a result of an increased number of activated $CD4^+$ T cells which would be targets for SIV infection[58]. In another study of SIV-infected macaques on partially suppressive ART, ipilimumab led to a modest increase in both HIV-specific $CD4^+$ and $CD8^+$ T cells and a significant reduction in cell-associated HIV RNA in lymph node[59]. These data therefore suggest that anti-CTLA4, may have a significant effect on HIV that persists on ART, through a different mechanism of action to anti-PD-1, leading to a reduction in HIV RNA in lymph node tissue. However, the mechanism of how this is achieved or whether there is antibody activity in HIV-infected subjects on fully suppressive ART remains unknown.

In HIV-infected individuals, LAG3 is also highly expressed on $CD4^+$ and $CD8^+$ T cells in lymph node and blood, and expression is directly related to levels of HIV RNA in plasma but inversely related to $CD4^+$ T cell counts[41]. Expression of T cell immunoreceptor with Ig and ITIM domains (TIGIT) is also increased on $CD8^+$ T cells in untreated and treated HIV infection compared to HIV-negative controls[38] even following early initiation of ART[60]. HIV-specific $CD8^+$ T cells were almost exclusively $TIGIT^+$, and co-expressed PD-1, CD160 and 2B4[60]. HIV-specific $TIGIT^{hi}$ cells were also negatively correlated with polyfunctionality and had reduced expression of the co-stimulatory receptor CD226. Blockade of TIGIT and

PD-1 with anti-TIGIT and anti-PD-L1 led to a significant enhancement of HIV-specific function of $CD4^+$ T cells from HIV-infected individuals on ART[38]. Antibodies to LAG3, TIM3 and TIGIT are all in early clinical development and given their more favourable safety profiles may be more suitable agents to assess in HIV-infected individuals[61].

Immune checkpoint proteins and HIV persistence—In contrast to malignant cells, which traditionally express the ligands for immune checkpoint proteins such as PD-L1[62], in HIV-infected individuals on ART, immune checkpoint proteins (e.g. PD-1) themselves identify cells preferentially infected with HIV that persist on ART (Figure 4)[63,64]. This observation is of high importance in efforts to eliminate residual virus that persists on ART as these infected cells are a major barrier to a cure. Although HIV can persist in multiple forms in HIV-infected individuals on ART, latently infected cells are the most important. Latency can be established in long-lived and proliferating central and transitional memory T cells as well as other T cell subsets including T follicular helper cells (TFH) and stem cell memory (TSCM) (reviewed in Ref.[35]).

Many studies have shown a significant correlation between the frequency of $PD\text{-}1^+$ $CD4^+$ and $CD8^+$ T cells with HIV persistence on ART in blood[63,65,66], lymph node[67] and the gastrointestinal tract, which has almost three times the frequency of $PD\text{-}1^+CD4^+$ T cells compared to lymph node or blood[68]. However, the most direct evidence of a clear relationship between HIV persistence and PD-1 expression comes from sorting $CD4^+$ T cells from blood where a 10-fold enrichment of HIV in $PD\text{-}1^{high}$ compared to $PD\text{-}1^{low}$ $CD4^+$ T cells was observed[63]. Similar findings were also reported from lymph node tissue collected from HIV-infected individuals on ART where HIV was highly enriched in cells expressing PD-1 and CXCR5, which together identify TFH[67] (Figure 3). HIV enrichment in $PD\text{-}1^{high}$ cells may be due to the inhibitory effects of PD-1 on T cell activation which would limit HIV transcription, RNA export and RNA translation, and therefore favour latent over productive infection (Figure 4).

Immune checkpoint proteins, other than PD-1 may also identify infected cells in individuals on ART. We recently demonstrated that HIV was significantly enriched in sorted cells from HIV-infected individuals on ART that expressed PD-1, TIGIT and LAG3 compared to cells that expressed none of these immune checkpoint proteins[64] (Figure 3). The relationship between CTLA4 and virus persistence on ART has been less well studied. In untreated individuals, HIV replicates preferentially in activated $CD4^+$ T cells which express high levels of CTLA4, and therefore virus is enriched in $CTLA4^+CD4^+$ T cells[69]. Rapid internalisation of CTLA4, mediated by the viral protein nef, may potentially play a role in favouring HIV persistence in these cells[70]. Whether latently infected $CTLA4^+CD4^+$ T cells in blood or tissue persist on ART is currently unclear.

These exciting observations are now being exploited by using immune checkpoint blockers to potentially reverse latency, allowing for expression of HIV proteins on the surface of the cell which would lead to immune clearance of virus or virus induced cytolysis (Figure 4). Latency reversal would be attempted in individuals on ART, so that any new virus produced could not go on to infect other cells. Using $CD4^+$ T cells from HIV-infected individuals on ART, the *ex vivo* administration of anti-PD-1 together with the latency reversing agent

bryostatin led to a significant increase in HIV RNA released into supernatant [Dr. Nicolas Chomont, personal communication]. In addition, in an HIV-infected individual on ART with metastatic melanoma, we observed a significant increase in cell associated HIV RNA following anti-CTLA4 (ipilimumab)[71] and anti-PD-1 (nivolumab)[72] The effects of other immune checkpoint blockers on latency establishment or reversal is unknown and warrants further exploration using antibodies, either alone or in combination.

Clinical trials of immune checkpoint blockade as a strategy for cure for HIV— A phase II dose escalation study of anti-PD-L1 therapy (by Bristol-Myers Squibb) was recently ceased after administration of the lowest dose to 6 individuals with HIV infection on ART[73]. The study was stopped due to retinal toxicity observed in a simultaneous macaque study. Interestingly, although there were no changes in HIV RNA or DNA, there was a clear increase in Gag-specific $CD4^+$ and $CD8^+$ T cells in 2 of the 6 participants. One of the 6 participants developed hypophysitis many months after receiving anti-PD-L1 therapy. This study remains the only trial of an immune checkpoint blocker in HIV-infected individuals without malignancy and further trials are unlikely to proceed until more safety data of these compounds are available.

To date, few individuals with HIV infection have received even the now licensed immune checkpoint blockers as individuals with HIV infection were excluded from the initial clinical trials of these agents. However, now that these drugs are licensed many individuals with HIV infection are receiving these antibodies as part of clinical care. Several clinical trials in the US and France are currently evaluating the effects of anti-PD-1 and anti-CTLA4 either alone or in combination on HIV-associated malignancies, as well as on proteins of HIV persistence and HIV-specific immunity (reviewed in Ref.[74]).

In summary, immune checkpoint blockers could have multiple beneficial effects to achieving a cure or allowing individuals to safely stop ART (Figure 4). First, the administration of these drugs could potentially enhance HIV-specific T cell function to eliminate HIV-infected cells. Second, they may lead to direct elimination of infected cells that express the relevant immune checkpoint marker, particularly when using a depleting antibody that activates Fc receptors as described for ipilimumab[75] and modified antibodies to PD-1[76]. Third, immune checkpoint blockers could potentially reverse HIV latency. Finally, immune checkpoint blockers can enhance vaccine responsiveness[77] and therefore could potentially be combined with other therapeutic vaccines in development.

Immune checkpoint proteins in HBV infection

HBV is a DNA virus which predominantly replicates in the hepatocytes of the liver. Following entry of HBV into a hepatocyte, there is production of intracellular covalently closed circular (ccc) DNA which produces multiple HBV proteins, including hepatitis B surface antigen (HBsAg) as well as production of HBV DNA which is required to form new infectious virions. There are 250 million people living globally with chronic HBV[78] and the main complications include end stage cirrhosis and/or hepatocellular carcinoma (HCC)[79]. Effective treatment of HBV is with self-limited interferon therapy or more commonly with long-term antiviral treatment using nucleotide/nucleoside reverse transcriptase inhibitors (NRTI). Similarly to ART in HIV infected individuals, treatment with NRTI is life-long and

there is no cure for HBV[80]. However, in contrast to HIV, approximately 10-15% of individuals can safely stop NRTI treatment for HBV without viral rebound. Inducing remission for HBV is possible, with the development of antibodies to HBsAg, commonly referred to as seroconversion[80].

T cell exhaustion and immune checkpoint proteins in HBV infection—HBV-specific T cells are important in HBV pathogenesis where they play a role in the initial clearance of acute infection, abnormal liver function commonly observed in acute and chronic infection, development of cirrhosis and successful HBsAg seroconversion following antiviral treatment (reviewed in Ref.[81]). The role and phenotype of HBV-specific T-cells is different at each of these clinical stages as circulating and intrahepatic HBV-specific T cells are infrequent in individuals chronically infected with HBV compared with in individuals who have cleared acute HBV infection[82,83].

In untreated chronic HBV infection, total and HBV-specific $CD8^+$ T cells express high levels of PD-1, CTLA4, TIM3[84–86], and in acute HBV infection circulating and intrahepatic $CD8^+$ T cells express high levels of PD-1[87] (Figure 3). The up-regulation of PD-1 in acute HBV infection is thought to limit intrahepatic inflammation[87]. The ligand for PD-1, PD-L1, has also been shown to be elevated on circulating $CD14^+$ monocytes and $CD19^+$ B cells in individuals with chronic HBV infection, liver cirrhosis and HCC and therefore may contribute to ongoing T cell exhaustion[88]. These exhausted antigen-specific $CD8^+$ T cells are prone to apoptosis through co-expression of the pro-apoptotic protein BIM[86]. In contrast, HBV-specific $CD4^+$ T cells, defined by MHC Class II tetramers and HBV core peptides, expressed increased levels of PD-1, but no increase in CTLA4, TIM3, KLRG1 and CD244[89] (summarised in Figure 3).

A recent genome-wide expression profiling study of HBV-specific $CD8^+$ T cells from individuals with acute and chronic HBV infection revealed extensive down-regulation of multiple pathways, including pathways associated with mitochondrial function, and T cells from these individuals showed functional recovery in the presence of mitochondrial-targeted antioxidants[90]. These studies demonstrated that defects in T cell function in chronic HBV infection are not limited to increased expression of immune checkpoint proteins, although mitochondrial dysfunction was clearly enriched in PD-1^{high} $CD8^+$ T cells in this study[90].

The phenotype of intrahepatic $CD4^+$ and $CD8^+$ T cells has also been extensively characterised in HBV infection. Initial descriptions of intrahepatic T cells in chronic HBV infection showed a high infiltration of total and HBV-specific $CD8^+$ T cells[91]. Intrahepatic T cells also show up-regulation of PD-1[87] and TIM3[84]. The ligand of TIM3, galectin-9 was also upregulated on Kupffer cells, perhaps allowing for persisting intrahepatic T cell exhaustion[84]. These intrahepatic $CD8^+$ T cells also express other proteins of exhaustion including BTLA and can produce IL-10 that further inhibits effective T cell function[92].

Immune checkpoint blockade as a strategy for cure for HBV—Multiple *ex vivo* studies using blood collected from individuals with chronic HBV infection have demonstrated that inhibition of PD-1, CTLA4, 2B4 and TIM3 lead to enhanced HBV-specific $CD8^+$ T cell function[83,84,86,93–96]. In contrast, only PD-1 blockade partially

improved HBV-specific $CD4^+$ T cell functions with production of IFN-γ, IL-2 and tumour necrosis factor-α (TNFα)[89]. Checkpoint blockade, used either alone or in combination may potentially enhance production of HBV-specific $CD8^+$ T cells and even production of antibody to HBsAg, but there are significant theoretical risks including increased infiltration of reinvigorated T cells into the liver which could trigger inflammation, but this has not been demonstrated in pre-clinical studies or recent clinical trials.

The effects of PD-1 blockade *in vivo*, during HBV infection, have been evaluated in mouse and woodchuck models. Blockade of the PD-1/PD-L1 or PD-L2 pathways with anti-PD-L1 and anti-PD-L2 antibodies in woodchuck hepatitis virus (WHV)-infected animals partially restored T cell function without hepatotoxicity[97]. In a separate study of WHV, the combination of antivirals (Entecavir), therapeutic vaccination and anti-PD-L1 blockade, followed by cessation of entecavir, did not result in rebound of WHV in plasma and the development of anti-WHV surface antigen, with complete viral clearance in some animals[98]. Interestingly, the addition of anti-PD-L1 to the vaccine and Entecavir arm compared to the vaccine and entecavir alone arm, led to a significantly enhanced immunological and clinical response and was not associated with hepatotoxicity[98]. These studies look very promising for similar interventions to achieve sustained remission off NRTI in human clinical trials.

A recent open label study of Nivolumab (anti PD-1; 3mg/kg), with and without a hepatitis B vaccine, involving 20 participants with virally suppressed chronic HBV infection showed that Nivolumab was safe and well tolerated, and one participant underwent HBsAg serovonversion[99]. Many other clinical trials of immune checkpoint blockade in individuals with chronic HBV, usually in the setting of HCC, are currently underway. One major study is an open-label, non-comparative study of nivolumab (anti-PD-1) or nivolumab in combination with ipilimumab (anti-CTLA4) in advanced HCC, with or without chronic HBV or hepatitis C virus (HCV) (NCT01658878). Interim results from this trial showed no significant difference in outcomes in individuals infected with HBV. Another 60-month observational study, led by the Taiwan Food and Drug Administration (TFDA), is also in progress[100]. This is a study of patients with known HBV or HCV infection, regardless of control on antiviral therapy in Taiwan and who are treated with Ipilimumab for advanced (unresectable, recurrent or metastatic) melanoma (NCT NCT02402699). Many other studies of immune checkpoint blockade for HCC, that do not specifically exclude individuals with chronic HBV, are currently enrolling participants.

In summary, immune checkpoint proteins play an important role in the natural history of HBV infection – limiting liver damage in acute infection and potentially facilitating persistent infection in chronic HBV infection. Initial studies indicate that Nivolumab is safe in chronic HBV infection, but further studies are needed to determine whether anti-PD-1 and/or other immune checkpoint blockers can be used to induce HBV remission.

Immune checkpoint proteins in tuberculosis

Mycobacterium tuberculosis, the causal agent of tuberculosis (TB), is among the 10 most fatal diseases worldwide, with 10.4 million symptomatic infections and 1.8 million deaths (including 0.4 million among people with HIV) recorded in 2015. *M. tuberculosis* characteristically infects the lungs, but can also affect any other organ of the body. TB is

treatable and curable if the active, drug-susceptible infection is treated with a standard 6-month course of four antimicrobial drugs. However, the prevalence of multidrug-resistant TB is now increasing and requires longer treatment and more complicated antibiotic regimens.

T cell exhaustion and immune checkpoint proteins in TB infection—$CD4^+$ T cells are required for host resistance to *M. tuberculosis*. TB-specific $CD4^+$ T cell responses in individuals with active TB infection were found to produce IFN-γ, IL-2 and TNF and express PD-1[101]. However, PD-1-expression levels on total $CD4^+$ T cells from individuals with active infection were only slightly higher than cells from healthy donors[102]. Notably, HIV-TB co-infection was consistently and independently associated with a reduced frequency of $CD4^+$ IFN-γ and IL-2-dual secreting T cells and the proportion correlated inversely with HIV RNA in plasma[101].

Mouse models used to determine the contribution of PD-1 to TB pathogenesis have had conflicting results. Surprisingly, mice with a PD-1 deletion showed increased pathology and PD-1 deficient $CD4^+$ T cells are sufficient to trigger early mortality[103,104]. The lungs of the PD-1 deficient mice showed uncontrolled bacterial proliferation and focal necrotic areas with predominantly neutrophilic infiltrates, but a lower number of infiltrating T and B cells[105]. Pro-inflammatory cytokines, such as TNFα and IL-6 were significantly increased in the lung and sera of these mice, consistent with an aberrant inflammation[105]. Significantly, TB-specific T cell proliferation was dramatically reduced in PD-1 deficient mice compared with controls due to an increased numbers of T_{REGS} and recruitment of mesenchymal stem cells[104]. Similarly, functionally exhausted $TIM3^+$ T cells were shown to accumulate during chronic TB, and TIM3-blockade restored T cell functions and improved control, of the bacterial load in chronically infected susceptible mice[106].

The treatment of multi-drug-resistant tuberculosis (i.e., the resistance *in vitro* to at least isoniazid and rifampicin) is complicated. To obtain a clinical and a microbiological cure, patients are treated for long periods because of the lesser effectiveness of the second- and third-line drugs[107]. The prolonged exposure to these drugs, characterized by a poor safety and tolerability profile, reduces the adherence by patients. The combination of these drugs with checkpoint inhibition may allow immunity to develop when the bacterial burden is under even partial control. For these reasons, checkpoint blockade during chronic TB requires further consideration.

Conclusion

Studies of the interplay between immune activation and suppression have shown an important role for immune checkpoint proteins in the pathogenesis of infectious diseases and malignancies. Notably, immune checkpoint blockade has revolutionized the treatment of cancer with remarkable success. A number of studies have suggested that immune checkpoint blockade may also be highly relevant for treating several infectious diseases, including malaria, HIV infection, HBV infection and TB (Table 2); in these diseases where drug resistance remains a challenge, effective vaccine development has not been possible or lifelong drug treatment is necessary. It should however be recognized, that immune

checkpoint blockade may also cause immune-related adverse events as CTLA4, PD-1, LAG3 and TIM3, are also involved in the regulation of peripheral tolerance to prevent autoimmunity (Box 4). Furthermore, whether there will be variable responses to immune checkpoint blockade and clinical outcomes for infectious diseases also remains to be determined. Despite this, immune checkpoint blockade may be an important new strategy for tackling chronic infections for which we are still lacking effective therapies or vaccines.

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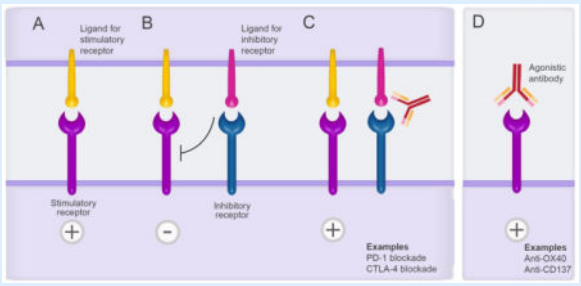
Box 1

Immunotherapy for treating cancer

Immunotherapy is a type of cancer treatment designed to boost the body's natural immune response to fight the cancer. There are currently two main types of immunotherapy. (A-C) Antibodies can block immunosuppressive interactions between antigen presenting cells or cancer cells with effector cells (e.g. T cells), to improve immune responses. For example, (A) stimulatory signals to T cells by corresponding ligands are (B) attenuated by when TCR signalling is coincident with inhibitory receptors interacting with their ligands, which decreases the magnitude of responses by T cells. (C) Antibody blockade of the inhibitory receptor/ligand interaction(s) reinstates T cell functions. The two best examples are blockade of PD-1 and CTLA4 which are both expressed on effector T cells and mediate exhaustion when in contact with their ligands, PD-L1 and CD80/CD86 respectively. (B) Antibodies that directly "activate" T cells via activating receptors. Two examples of this type of therapy are targeting OX40 and CD137 on T cells to improve their respective functions. While the development of immunotherapy has been restricted to the treatment of cancer, there is high interest in the role of these antibodies for infectious diseases.



Box 2

Overview of immune checkpoint molecules currently being targeted in cancer therapy

Below, we describe three checkpoint proteins that are currently targeted for cancer therapy and one being clinically trialled. Therapies targeting several other immune checkpoint pathways have also shown promise for controlling various cancers and some of these drugs have progressed to clinical trials (Table 1, and reviewed in detail in Ref. [108]).

CTLA-4 is a member of the immunoglobulin superfamily which is expressed by activated T cells along with the T cell co-stimulatory protein, CD28. Both molecules bind to CD80 (B7-1) and CD86 (B7-2), on DCs but CTLA-4 binds with greater affinity and avidity than CD28. While CD28 transmits a stimulatory signal[109], CTLA-4 is able to outcompete CD28 for CD80/CD86 binding to inhibit T cell functions[110]. Of note, CTLA-4 expressed on effector T cells, is increased only after T cell receptor (TCR) and CD28-mediated T cell activation to permit downstream control of immunity.

PD-1 has potent inhibitory effects on immunity. PD-1 is expressed on T cells, B cells, natural killer T cells; DCs, and activated monocytes[111,112]. PD-1 has two ligands, PD-L1 (also known as B7-H1) and PD-L2 (also known as B7-DC). PD-1 is up-regulated on the surface of T cells, within 24 hours of stimulation and the effects of PD-1 ligation can be seen within a few hours[113]. Significantly, signalling T cells through the PD-1 inhibitory receptor by PD-L1 expressed on DCs and tumor cells, attenuates TCR signals and inhibits T cell expansion, cytokine production and cytolytic function[114]. New studies demonstrate that the CD28/B7 co-stimulatory pathway is essential for effective PD-1 therapy in tumor-bearing mice and during chronic viral infection[115].

PD-L1, which drives PD-1-mediated immune-inhibition, is constitutively expressed on T cells, B cells, macrophages, DCs[116], non-lymphoid tissues such as heart and lung[111], parenchymal cells[117] as well as the surface of tumor cells[118]. PD-L1, but not PD-L2, is also detected at low levels on cardiac endothelium, pancreatic islets, and syncyciotrophoblasts in the placenta, highlighting a role for PD-L1 in immunological tolerance[119]. PD-L1 blockade has also demonstrated efficacy in lung, bladder and other cancers[120–122].

PD-L2 is also an immune checkpoint inhibitor, but its function is not as well understood as PD-L1, and thus its clinical utility is still being explored. The engagement of PD-1 by PD-L2 dramatically inhibits TCR-mediated proliferation and cytokine production by $CD4^+$ T cells[123]. Antigen presenting cells (APC) from PD-$L2^{-/-}$ mice demonstrated an enhanced potential to activate T cells, both *in vitro* and *in vivo*[124], suggesting that PD-L2 has an inhibitory role similar to PD-L1. However, recent studies showed PD-L2 expressed as an aggregated form on DCs could inhibit PD-L1/PD-1-binding, and increase CD3 and Inducible T-cell COStimulator (ICOS) expression on T cells possibly via a putative second receptor[9]. Previous studies also showed that PD-L2 could improve T cell

function via a PD-1 independent mechanism[125–127]. Thus PD-L2 has a complex function and PD-L2 proteins are being investigated in clinical trials.

Box 3

Mouse models of malaria

Mouse models of malaria have been more informative on the extent to which checkpoint proteins inhibit natural immunity. Four of the most commonly used mouse species and strains of *Plasmodium* show distinct biology and pathogenicity. *P. yoelii* 17XNL and *P. chabaudi* blood-stage infections are non-lethal with the latter causing chronic disease with intermittent parasitemia for up to 200 days. In contrast, *P. yoelii* YM and *P. berghei* ANKA are severe, lethal infections with the latter sequestering from the blood into deep tissues including the brain, leading to lethal cerebral disease.

Box 4

Adverse reactions and limited durability associated with immune checkpoint blockade

Blocking of checkpoint protein-interactions with antibodies has shown remarkable anti-tumour immunity; however this immunity can be accompanied by immune-related adverse events, resembling autoimmune diseases[128]. Immune-related adverse events can occur in up to 90% of patients treated with an anti-CTLA4 antibody[129] and 70% of patients treated with anti-PD-1/PD-L1 antibodies[130,131]. These immune-related adverse events typically originate in the skin, gastrointestinal tract, liver, and endocrine system, although other organ systems may also be affected[132]. While treatment with immunosuppressive drugs such as prednisolone is effective and usually resolves the symptoms, these adverse effects can be fatal. Therefore, significant concerns remain around using these antibodies in otherwise healthy individuals living with HIV or HBV. Autoimmune toxicities such as colitis, myocarditis and pneumonitis occur, more commonly with anti-CTLA4 than anti-PD-1, and whether these can be reduced through modifications of the antibodies and/or reduction in dosage needs to be considered when exploring their use for infectious diseases.

Other factors which could reduce efficacy of immunotherapies include non-responsiveness to treatment and limited durability of restored T cell functions. Blockade of the PD-1/PD-L1 pathway has shown durable benefit in melanoma and other cancers[130,131,133] but >50% of patients do not respond or develop resistance to anti-PD-1 therapy. PD-1 also plays a role in the setting of both acute and chronic infections. While PD-1 transcription is rapidly down-regulated in functional antigen-specific $CD8^+$ T cells that develop during acute infection, persistent TCR ligation during chronic viral infections maintains increased levels of PD-1 transcription and generation of a distinct lineage of non-functional “exhausted” antigen-specific $CD8^+$ T cells[134,135]. These changes in T cell functions are persistent as a result of epigenetic modification, specifically demethylation of the promotor of PD-1. These epigenetic changes persist, even with reduction in antigen, as seen following effective antiviral therapy for HIV infection or following anti-PD-1 therapy[136–139]. Thus, for infectious diseases, immunotherapy may have optimal effects when used with a vaccine, to minimise immune suppression and thus permit vaccine responses to develop. Alternatively, immunotherapy used in conjunction with antimicrobial agents could allow long term immunity to develop when the immediate threat of a lethal infection had passed.

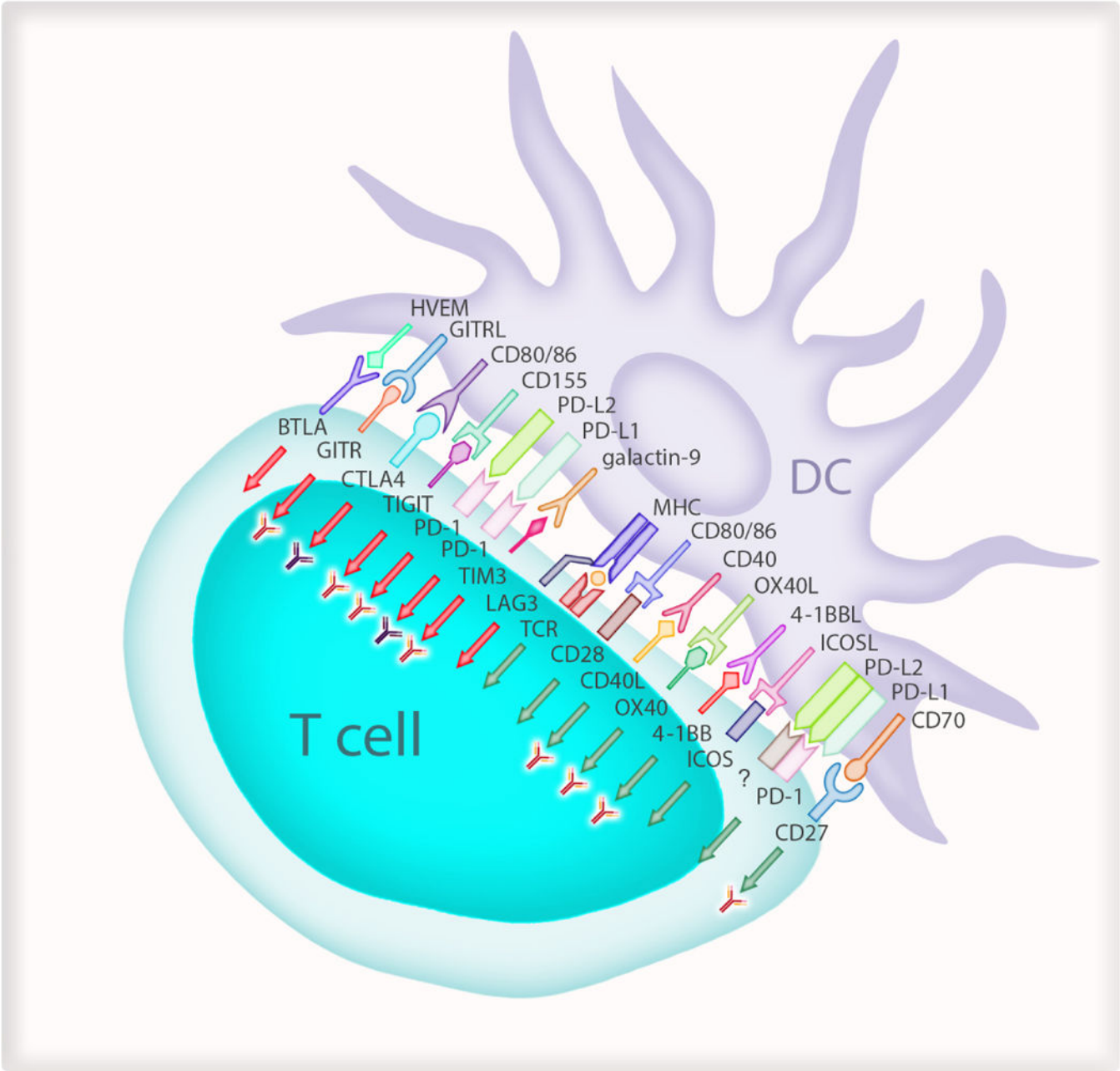


Figure 1. Interactions that regulate T cell responses

Antigen presenting cells such as dendritic cells (DCs) regulate T cell response to specific pathogens or antigens from malignant cells. The T cell receptors (TCR) on antigen-specific T cells first recognise their cognate antigen via the major histocompatibility complex (MHC) molecules on antigen presenting cells. This step has to be followed by signals to CD28 on T cells from CD80 on the APC and is described as “signal 2”. Several different ligands on DCs then provide signals to T cells which decide the quality and duration of the effector response (green arrows). These include CD40/CD40 ligand (CD40L); OX40/OX40 ligand (OX40L); 4-1BB (CD137)/4-1BB ligand (41BBL; CD137 Ligand); ICOS (Inducible T-cell COStimulator; CD278)/ICOS Ligand (ICOS-L); CD27/CD70. There are also signals to

suppress immune responses (red arrows) to maintain self tolerance and limit the duration of immune responses to minimize bystander damage to host tissue. These include LAG3 (lymphocyte activation gene 3); MHC class II; TIM3 (T cell immunoglobulin and mucin-domain containing-3; HAVCR2 in humans)/galectin-9; PD-1 (programmed cell death-1)/PD-L1 (programmed cell death-1-ligand 1) and PD-L2 (programmed cell death-1-ligand 2); TIGIT (T cell immunoreceptor with Ig and ITIM domains)/CD155; CTLA4 (cytotoxic T-lymphocyte-associated protein 4)/CD86 or CD80; GITR (Glucocorticoid-induced TNFR-related protein)/GITR-L (GITR-ligand) and BTLA (B and T lymphocyte attenuator)/HVEM (Herpesvirus entry mediator). Antibody symbol represents pathways being tested in current clinical trials. The “?” refers to an unknown receptor which “activates” T cells. The “red” antibodies indicate pathways undergoing clinical trials for cancer and the “dark coloured” antibodies indicate clinical use.

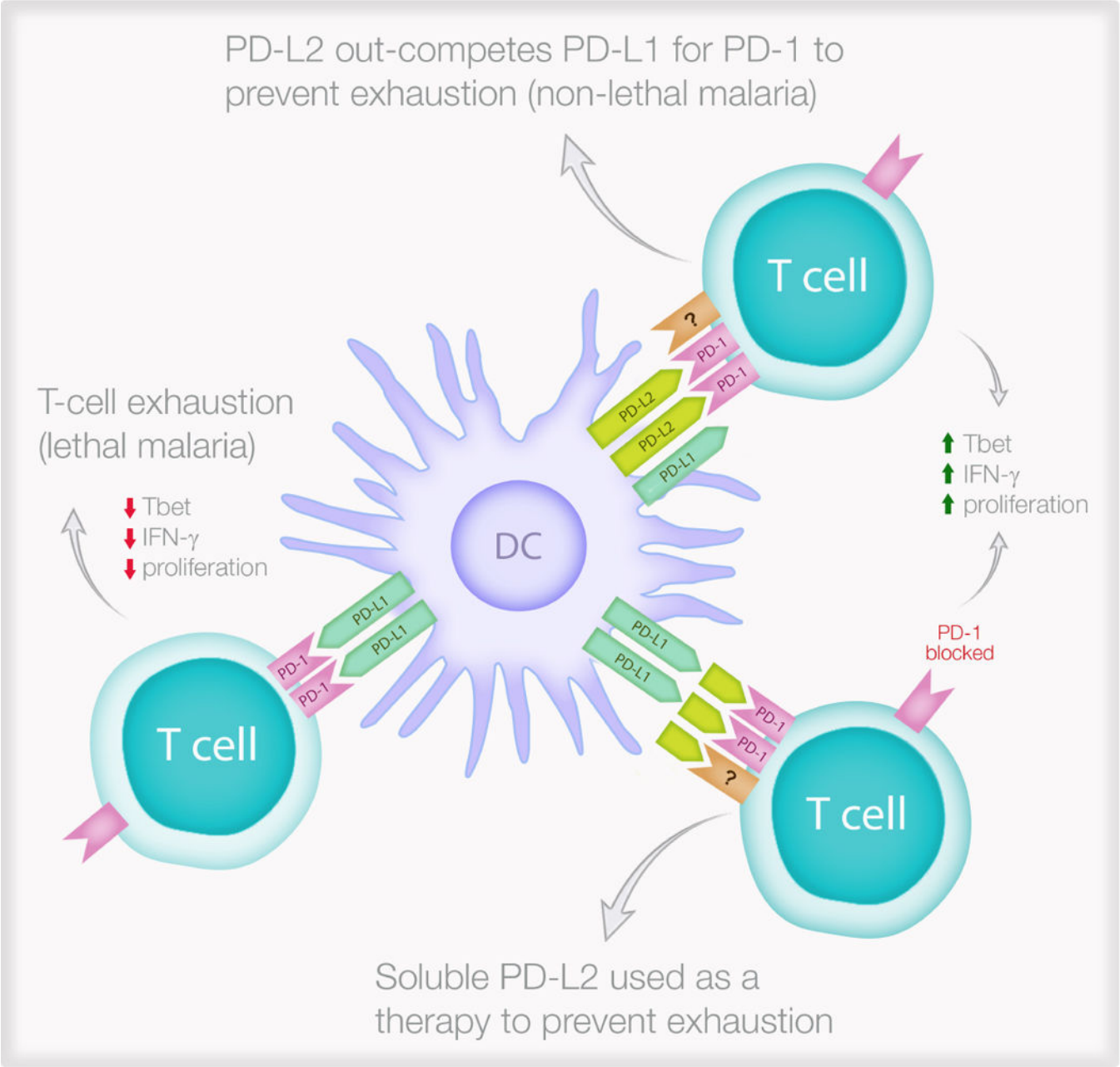


Figure 2. PD-L2 protects against lethal malaria and has translational potential

The expression of PD-L2 on dendritic cells determines effector T cell function following a PD-1/PD-L1 interaction. During non-lethal malaria (top right), DCs express PD-L2 which inhibits the immunosuppressive PD-1/PD-L1 interaction while interacting with an unknown receptor (?) to improve T cell functions. This leads to protective immunity characterised by increased T-box transcription factor TBX21 (Tbet) expression, increase interferon-γ (IFN-γ) secretion and better proliferation in response to the parasite. In contrast, during lethal malaria (left), PD-L2 expression is low or absent and this allows the immunosuppressive PD-1/PD-L1 interaction to generate exhausted T cells which do not express Tbet, do not

secrete IFN-γ and cannot proliferate in response to the parasite. Soluble PD-L2 administered to mice infected with lethal malaria (lower right) can prevent T cell exhaustion.

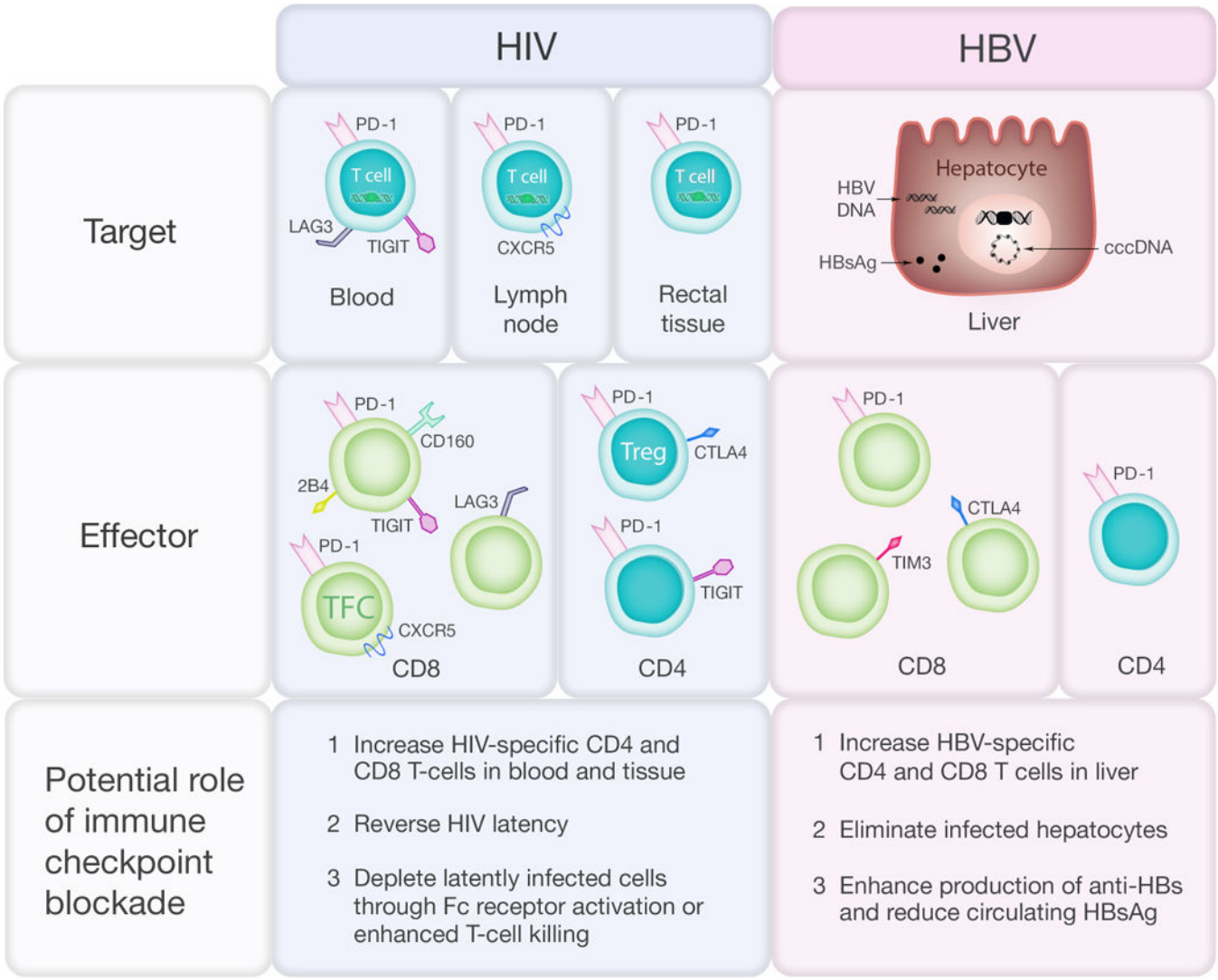


Figure 3. Immune checkpoint protein expression in HIV and HBV infection and potential effects of immune checkpoint blockade

In HIV infection, the virus persists on antiretroviral therapy (ART) in latently infected $CD4^+$ T-cells that contain integrated provirus (green box) and express PD-1 and other immune checkpoint markers in blood, lymph node and rectal tissue. Expression of immune checkpoint markers on total and HIV-specific $CD4^+$ and $CD8^+$ T cell subsets include central memory (CM), effector memory (EM), T follicular cytotoxic (TFC) and T regulatory (Treg) T cells is associated with T-cell exhaustion and reduced T-cell function. In HBV infection, HBV persists on treatment as extrachromosomal closed covalent circular (ccc) DNA and integrated HBV DNA (black box) and there is ongoing production of HBV surface antigen (HBsAg). Increased expression of immune checkpoint markers on $CD8^+$ T cells and increased expression of PD-1 on $CD4^+$ T-cells reduce T cell function.

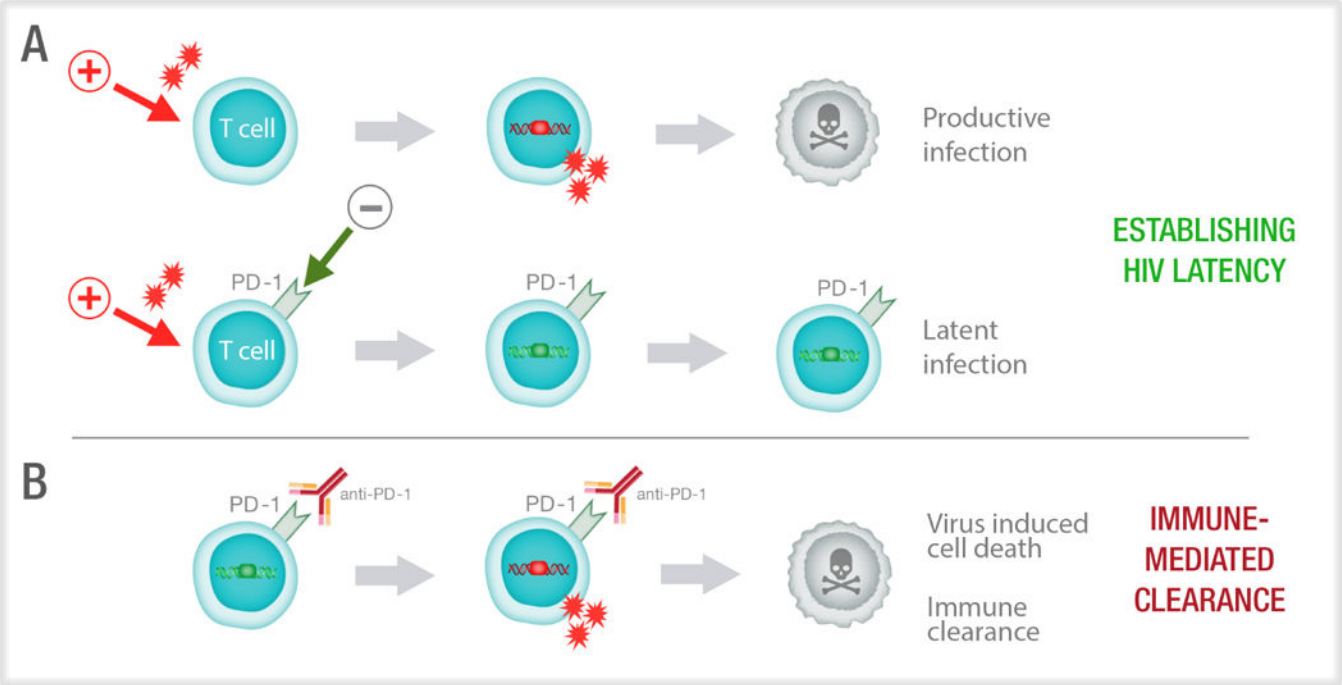


Figure 4. Proposed role of PD-1 in the establishment and reversal of HIV latency

(A) HIV preferentially infects activated CD4+ T cells which have been stimulated via T cell receptor engagement or a mitogen (red arrow). Following HIV integration, the productively infected cell (red box) usually dies by virus mediated cytolysis. Up-regulation of immune checkpoint markers such as PD-1, could potentially limit T-cell activation favouring latent over productive infection, where there is integration (green box) but no virus production. (B) Latently infected cells express immune checkpoint markers, including PD-1. The administration of anti-PD-1, or other immune checkpoint blockers, leads to activation of the T cells and increased expression of transcription factors that can enhance production of virus from latency. This leads to either immune mediated clearance or virus induced cell death.

Table 1

Summary of other major immune checkpoint pathways

Checkpoint Receptor	Cell type affected	Ligand	Notes	Ref.
T cell immunoglobulin and mucin-domain containing-3 (TIM3; HAVCR2 in humans)	Th1-T cells	Galectin-9 on APC	Galectin-9 induces intracellular calcium flux, aggregation and death of Th1 cells *in vitro*. Co-expression of TIM3 and PD-1 identifies $CD8^+$ T cell in mice with an exhaustion phenotype. Targeting TIM3 and PD-1 pathways can reverse T cell exhaustion and restore anti-tumor immunity.	140–142.
Lymphocyte activation gene 3 (LAG3; CD223)	iTreg, nTreg	MHC Class II on APC	LAG3 enhances the function of regulatory T cells. LAG3 and PD-1 are commonly co-expressed on anergic or exhausted T cells and combined blockade can cure most mice of established tumours that were largely resistant to single antibody treatment.	143,144.
T cell immunoreceptor with Ig and ITIM domains (TIGIT)	T cells, NK	CD155 on DCs	CD96 & TIGIT exert immunosuppressive effects by competing with CD226 for CD155. TIGIT-Fc fusion protein inhibits T cell activation by generating regulatory DCs. Blocking CD96 or TIGIT with mAbs improve tumor control in mice, in particular when used in combination with PD-1/PD-L1 blockade.	145–148.
CD96				
B and T lymphocyte attenuator (BTLA; CD272)	Naïve T and B cells and is further upregulated on activation.	Herpesvirus entry mediator (HVEM) expressed by most hematopoietic, endothelial & epithelial cells	Ligation of LIGHT by HVEM can be co-stimulatory, while BTLA-HVEM binding is considered co-inhibitory. BTLA has been linked to T cell dysfunction during cancer and dual blockade of BTLA and PD-1 clearly enhances anti-tumour immunity	149–152.
Tumor necrosis factor superfamily member 14 (TNFSF14; LIGHT)	Innate and adaptive immune cells including T cells			
Glucocorticoid-induced TNFR-related protein (GITR)	Regulatory T cells at high levels, resting conventional T cells at low levels but increases upon activation	GITR-ligand on APC	GITR plays a key role in dominant immunological self-tolerance maintained by $CD25^+CD4^+$ regulatory T cells. The ligand for GITR is mainly expressed on APC and antibodies to GITR have been shown to promote an anti-tumor response through loss of Treg lineage stability.	153–156.
V-domain immunoglobulin (Ig)-containing suppressor of T cell activation (VISTA)	Hematopoietic cells	unknown	Preclinical studies with VISTA blockade show promising improvement in anti-tumor T cell responses and improved survival.	157,158.

Table 2

Summary of pre-clinical or *ex vivo* studies in infectious diseases reporting benefits of targeting inhibitory immune receptors

Infection	Cell type affected	Inhibitory Receptor	Target Species	Outcomes	Refs
HIV	$CD4^+$; $CD8^+$ T cells	PD-1; CTLA4 TIGIT; LAG3	Humans/Mice	• Increased HIV-specific CD8+ T-cells (2 of 6 participants) • Current clinical trials in malignancy underway	37,38,43,64,159,160
SIV	$CD8^+$ T cells	PD-1; PD-L1; CTLA4	Macaques	PD-1 blockade expands functional virus-specific CD8 T cells and prolongs survival	38,54,59
HBV	$CD4^+$; $CD8^+$ T cells	PD-1; CTLA4; 2B4; TIM3	Humans (ex vivo)/mice/woodchuck	• PD-1, CTLA4 & TIM3 expressed on $CD4^+$ & $CD8^+$ T cells from patients with chronic HBV infection. • PD-1, CTLA4 & TIM3 blockade enhanced expansion and function of HBV-specific CD8 T cells *in vitro*.	84–86,89,97,98
HCV	$CD4^+$; $CD8^+$ T cells	PD-1; PD-L1	Human	• PD-1 is up-regulated on total and HCV-specific $CD8^+$CTLs in the peripheral blood & livers of patients with chronic infection • PD-L1 blockade restores functional competence of HCV-specific CTLs *in vitro*.	161–164
Influenza	$CD8^+$ T cells	PD-1, TIM3	Mice	• PD-1 expression on influenza virus-specific $CD8^+$ T cells is associated with a high-pathological infection. • PD-L1 blockade *in vivo* reduces virus titres and increases $CD8^+$ T cell number but T cell functionality was not restored. • TIM3 fusion protein improved immune responses.	165,166
TB	$CD4^+$; $CD8^+$ T cells	TIM3	Mice	TIM3 blockade restores T cell function and improves bacterial control, particularly in chronically infected susceptible mice	106
Listeria	$CD8^+$ T cells	PD-L1	Mice	PD-L1 plays an important costimulatory role for antigen-specific CD8 T cells	167
Malaria	$CD4^+$; $CD8^+$T cells; B cells	PD-1; PD-L1; CTLA4; LAG3;TIM3	Mice	• Accelerated parasite clearance. • Survival from lethal disease • Reduced incidence of cerebral malaria	9,26,29,31
Toxoplasma	$CD8^+$ T cells	PD-1	Mice	Blockade of the PD-1-PD-L1 reinvigorates $CD8^+$T-cell responses with prevention of mortality	168
Leishmania	$CD4^+$; $CD8^+$ T cells	PD-1, PD-L1	Mice	PD-1 blockade *in vivo* restored T cell proliferation and function with complete resolution of chronic lesions.	169–171