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Putting T cells to sleep: a new paradigm for immune evasion by persistent viruses

The elimination of a viral pathogen from the host is critically dependent upon a robust immune response, initiated by the humoral (employing antibodies) and cellular (employing cytotoxic T cells) arms of the immune system. Viruses that cause persistent infections in vertebrates employ various strategies to evade these host immune responses, in addition to effects on cell signalling and cell death pathways. In the last few months, a series of papers have described a new pathway of immune evasion by persistent viruses. The central theme of this strategy is to silence antigen (virus)-specific $CD4^+$ (helper) cells that promote development of a robust immune response and $CD8^+$ (cytotoxic) T cells that seek out and destroy virus-infected cells. The central player in this strategy is a protein called PD-1 and its ligands PD-L1 and PD-L2. Induction of PD-1 expression on T cells inhibits T cell activation and effector functions thereby preventing elimination of the virus. This would allow the virus to evade immune surveillance and persist in the host.

The programmed death-1 (PD-1) receptor was isolated in 1992 by subtractive hybridization as a molecule whose expression was enhanced following apoptotic stimulation of T cells (Ishida *et al* 1992). It belongs to the CD28 family of immunoreceptors and is expressed on activated B, T and myeloid cells. Till date, two PD-1 ligands have been identified; these are PD-L1 (also called B7H-1) and PD-L2 (also called B7-DC) (Khoury and Sayegh 2004). PD-L1 is expressed on T cells, B cells, macrophages and dendritic cells (DCs) and is upregulated following activation of these cells. In contrast, PD-L2 expression is only inducible on DCs and macrophages. Though the exact function of these ligands still needs to be elucidated, available data suggests that ligation of PD-1 triggers an inhibitory signalling pathway in the PD-1 expressing cells. Similar to other CD28 family members, PD-1 transduces an inhibitory signal only when engaged in combination with T cell receptor (TCR) ligation, but not when cross-linked on its own. However, the precise signalling pathways through which PD-1 transduces signals are not fully understood. The inhibitory activity of PD-1 depends upon association of the tyrosine phosphatase SHP-2 with its immunoreceptor tyrosine based switch motif (ISTM) rather than its immunoreceptor tyrosine based inhibitory motif (ITIM), more typically associated with inhibitory receptors (Latchman *et al* 2001). These features are schematically illustrated in figure 1.

The role of PD-1 in immunoregulation has been explored widely in autoimmune diseases and tumours (Dong and Chen 2003; Iwai *et al* 2002; Okazaki and Honjo 2006). PD-1 knockout mice exhibit features of autoimmunity, which led to the hypothesis that PD-1 is an important player in regulating autoreactive responses (Nishimura *et al* 1999). The expression of PD-L1 or B7-H1, on tumour cells of a variety of histologies has suggested a potential mechanism for tumour escape from immune destruction (Dong and Chen 2003; Dong *et al* 2002). Recent data has now demonstrated that the PD-1 inhibitory pathway, in addition to regulating T cell responses to self-antigens and tumours, also regulates $CD4^+$ and $CD8^+$ T cell responses to persistent viruses.

To examine the mechanisms of T cell dysfunction during persistent infection, Barber *et al* (2006) used the mouse model of infection with lymphocytic choreomeningitis virus (LCMV). An advantage of this model is the availability of LCMV strains that can cause either acute or chronic infection in adult mice. The Armstrong strain of LCMV is cleared within a week and a population of antigen-specific memory T cells is established, whereas clone 13 establishes a persistent infection that render the T cells functionally impaired or 'exhausted'. A gene expression array analysis showed that exhausted virus-specific $CD8^+$ T cells from clone 13 infection had significantly higher PD-1 expression, compared to the functional antigen-specific T cells that develop following infection with the Armstrong strain. The $CD8^+$ T cells from chronically infected mice maintained higher levels of PD-1 expression. Moreover expression of one of the

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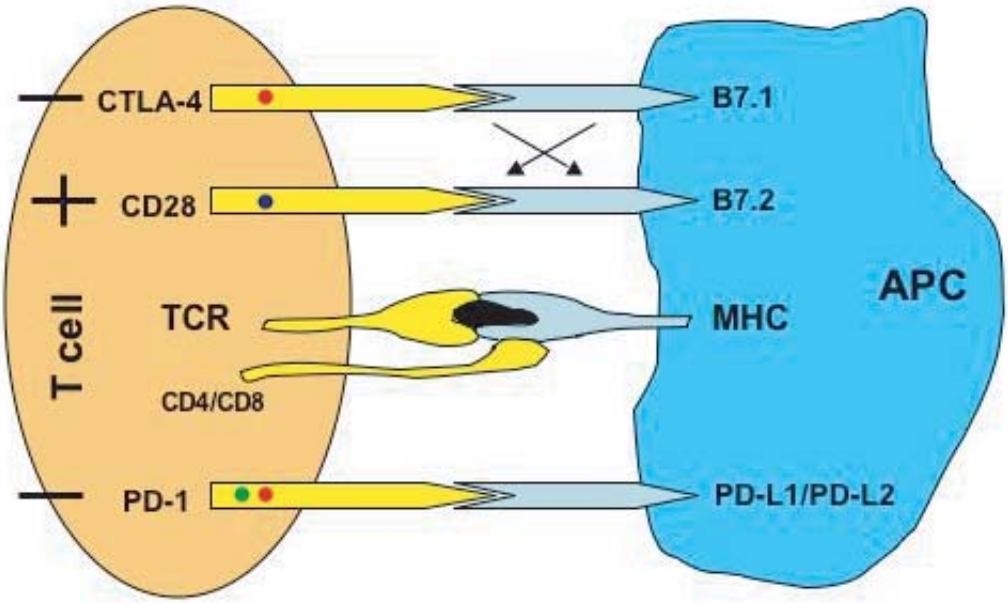

Figure 1. Schematic representation of activating (CD28) and inhibitory (PD-1 and CTLA-4) receptors on T cells and their respective ligands on APCs. The arrows indicate binding of either B7.1 or B7.2 to CD28 or CTLA-4. The plus and minus signs indicate transduction of activating and inhibitory signals respectively, after receptor ligand interaction. The red dot indicates immunoreceptor tyrosine based inhibitory motif (ITIM) in the cytoplasmic tail of CTLA-4 and PD-1 receptor, the green dot represents immunoreceptor tyrosine based switch motif (ISTM) found only in the cytoplasmic tail of PD-1 receptor and the blue dot indicates the domain in CD28 receptor which transduces activating signals.

ligands for this receptor, PD-L1, was also upregulated on mouse splenocytes. Remarkably, compared to untreated animals, chronically infected mice that were treated with a PD-L1-specific antibody displayed a marked expansion of functional virus-specific $CD8^+$ T-cells characterized by an increased ability to produce interferon-γ and tumour necrosis factor, thereby reversing the 'exhausted' phenotype. In addition, PD-L1 blockade markedly reduced the viral load and resolved infection with the persistent clone 13 strain (Barber *et al* 2006). While it was not clear whether the aberrant expression of PD-1 and PD-L1 was a cause or effect of chronic infection, an inhibition of this receptor-ligand interaction led to recovery of functional T cell responses and the clearance of an otherwise chronic virus.

Do these findings with a mouse virus have a parallel in persistent viral infections of humans? This was indeed the case for persons infected with the human immunodeficiency virus (HIV).

Day *et al* (2006) studied a cohort of HIV-infected individuals from KwaZulu Natal province in South Africa, where seroprevalence rates for HIV are in excess of 30% in certain age groups. Antigen specific $CD8^+$ T cells in 71 people who were not receiving any antiretroviral therapy exhibited significant upregulation of PD-1 expression. This correlated positively with parameters of disease progression such as plasma viral load (as measured by the amount of HIV RNA copies in blood) and loss of $CD4^+$ T cells. PD-1 expression was also analysed on cytomegalovirus (CMV)-specific, Epstein-Barr virus (EBV)-specific and vaccinia virus (VV)-specific $CD8^+$ T cells from individuals who were not infected with HIV. It was found to be intermediate on CMV-specific T cells, high for an EBV lytic epitope and low for VV-specific T cells indicating a relationship between ongoing antigen exposure and PD-1 expression (Day *et al* 2006). In this study, upregulation of PD-1 was uniquely observed on HIV-specific $CD8^+$ T cells since another inhibitory receptor, CTLA-4, was not found to be upregulated on HIV-specific T cells. This implies that chronic infection specifically upregulates the PD-1 inhibitory receptor. The expression of PD-1 on $CD4^+$ T cells also showed a positive correlation with viral loads and loss of $CD4^+$ cells. Importantly, blockade of the PD-1/PD-L1 pathway significantly restored both the number and functional competence of HIV specific $CD8^+$ T cells.

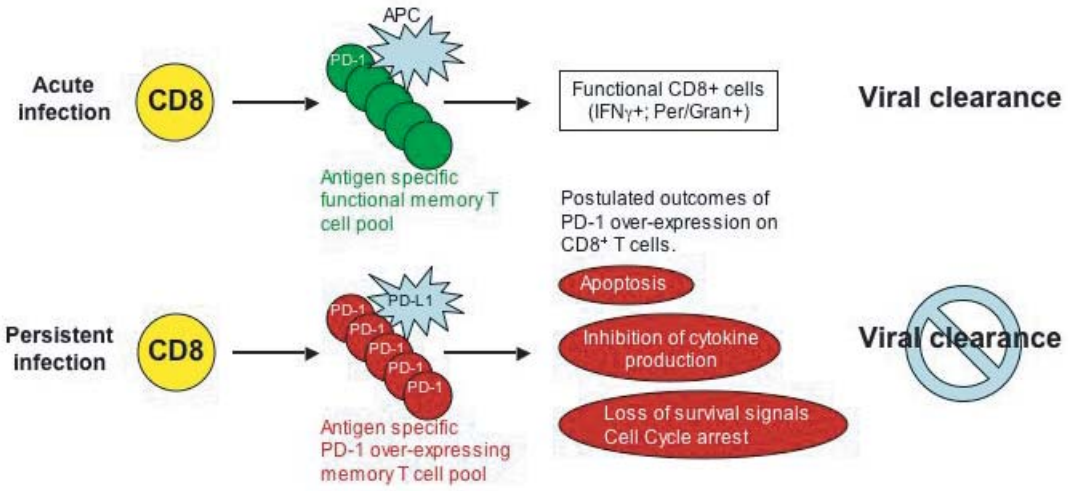

Figure 2. Schematic representation of PD-1/PD-L1 interaction in persistent infection. Antigen specific memory T cells produced after an acute infection are able to clear the virus because the cytotoxic ability of CD8 T cells is functional. Infection by a persistent virus induces expression of PD-1 and PD-L1 on T cells and APCs, respectively, which either reduces the frequency of antiviral T cells by inducing apoptosis or loss of survival signals, or lowers their cytotoxic function by inhibiting IFN-γ production.

Similar results were obtained in a related study carried out on a HIV cohort in Montreal, Canada. Trautman *et al* (2006) demonstrated that PD-1 was upregulated on HIV-specific $CD8^+$ T cells and not on CMV-specific T cells. The expression levels of PD-1 correlated with viral loads and the reduced ability of HIV-specific $CD8^+$ T cells to proliferate or produce cytokines. Blocking the interaction of PD-1 with PD-L1 enhanced the capacity of HIV-specific $CD8^+$ T cells to proliferate and produce cytokines (Trautmann *et al* 2006).

Apart from increased expression of PD-1 on T cells, expression of one of its ligands, PD-L1, was shown to be upregulated on monocytes from HIV patients (Trabattoni *et al* 2003). The PD-L1 expressing $CD14^+$ cells also produced more IL-10 in these patients and upregulation of PD-L1 correlated with plasma viral loads (Trabattoni *et al* 2003).

Petrovas *et al* (2006) have revealed another interesting aspect of PD-1 upregulation on HIV-specific T cells. According to this study, PD-1 expression is crucial for regulating the survival of virus-specific $CD8^+$ T cells during HIV infection. Expression of PD-1 was found to be higher on memory $CD8^+$ T cells according to antigen specificity. For instance, memory $CD8^+$ T cells specific for a poorly controlled persistent infection like HIV showed higher expression of PD-1 compared to memory T cells-specific for a well-controlled persistent virus (cytomegalovirus) or a virus causing acute infection (vaccinia virus). Interestingly, PD-1 expression was the sole determinant of sensitivity of virus-specific T cells to apoptosis and was not directly associated with the inability of $CD8^+$ T cells to produce cytokines. In other words, enhanced expression of PD-1 on $CD8^+$ T cells in HIV infection is proposed to predispose these cells to apoptosis, resulting in decreased frequency of antiviral T cells. Manipulation of PD-1 expression could influence the ability of these cells to survive and expand which in turn led to increased numbers of cells becoming functional in producing cytokines (Petrovas *et al* 2006). A model for PD-1 mediated T cell exhaustion is presented in figure 2.

Similar $CD8^+$ T cell exhaustion has also been reported for infection with another successful persistent human viral pathogen, the hepatitis C virus (HCV). High levels of PD-1 expression were observed on HCV-specific $CD8^+$ T cells in persons with persistent HCV infection (Urbani *et al* 2006). The expression of PD-L1 on hepatocytes has been shown to be strongly enhanced by activated T cells and viral infection, and markedly augmented by type I or type II interferons. Moreover, PD-L1 expression on hepatocytes induced apoptosis in T cells (Muhlbauer *et al* 2006). A role for epithelial PD-L1 and PD-L2 in

virus-initiated immune responsiveness in the airways has also been suggested in the context of a viral infection (Tsuda *et al* 2005) .

Recently, an immunoregulatory role has been proposed for the PD-1/PD-L1 interaction in liver. Naïve self-reactive T cells in the liver may get activated due to their cross-reactivity to some viral antigens. The regulation by PD-1 prevents undesirable or excessive activation of effector T cells in peripheral organs such as liver (Iwai *et al* 2003). Although these safeguards are critical for the avoidance of autoimmunity, they may restrict the host's ability to counteract infectious agents. Some pathogens can therefore escape from surveillance of the host immune system and cause chronic infection. The fact that the absence of a PD-1 inhibitory signal can augment antiviral immunity has significant implications for not only an understanding of the pathogenic mechanism but also for the treatment of persistent infection of the liver.

It will be worth investigating why of the roughly 30 receptors known to exert inhibitory effects on lymphocytes and other cells of the immune system, PD-1 is a central regulator of T cell exhaustion during persistent infection. Characterization of the structure, function and expression of the PD-1 receptor will be important for further understanding its role in regulating immune responses and designing more directed therapeutic strategies.

These studies offer possible new candidates that may be used for therapeutic intervention in HIV, HCV and other persistent infections. Although the number of inhibitory candidates that can be targeted in persistent viral infection is relatively small, these results should also be viewed with caution when considering their therapeutic importance given that the PD-1/PD-L1 pathway is important in preventing activation of autoreactive T cells.

Nevertheless, the discovery of PD-1/PD-L1 effects in persistent viral infections offers new insight into immune evasion by viruses. Further investigations of this signalling pathway will be of immense importance for understanding the mechanistic complexities involved in the interplay between viruses and their hosts.

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