Research Highlights

Commentaries on role of PD-1 molecules in bacterial and viral infections

'Exhaustive' look at PD-1/PDL-1 blockade *in vivo*

Evaluation of: Velu V, Titanji K, Zhu B *et al.*: Enhancing SIV-specific immunity *in vivo* by PD-1 blockade. *Nature* 458(7235), 206–210 (2009).

Since the initial description of programmed death (PD)-1 in the early 1990s, there has been a plethora of papers further investigating this CD28-family member, which have revealed complex and intriguing functions for PD-1 that far exceed its involvement with programmed cell death [1]. Knockout studies have implicated a potential role for PD-1 in peripheral tolerance and autoimmunity due to the development of a lupus-like disease in mouse models [2]. Most notably in chronic viral infections such as lymphocytic choriomeningitis virus, antigen-specific T cells acquire an 'exhausted' phenotype characterized by a reduction in proliferative and cytotoxic ability as well as the expression of high levels of PD-1 [3]. This observation of a correlation between T-cell exhaustion and PD-1 surface expression has also been observed clinically in patients suffering from chronic viral infections such as HIV, hepatitis C virus and hepatitis B virus, which suggest PD-1 as a possible target for immunotherapy.

“...these studies suggest that PD-1 blockade could be a potential immunotherapy applied to HIV infections in humans.”

In their recent article, Velu *et al.* provided a comprehensive assessment of the effects of PD-1 blockade *in vivo* while demonstrating some of the potential benefits [4]. In their primate model of acute and chronic SIV infection, rhesus macaques were given an antihuman PD-1 monoclonal antibody early (10 weeks)

and late (~90 weeks) in disease progression. PD-1 blockade induced an expansion of SIV-specific T cells in the blood and gut and increased T-cell functionality, as shown by increased numbers of triple-cytokine producers (IL-2, IFN- γ and TNF- α) as well as CD8 T cells expressing perforin and granzyme cytotoxic markers. In addition to enhancing cellular immunity to SIV infection, PD-1 blockade increased the SIV-specific humoral immunity as determined by increased Env-specific antibodies in the plasma. The combined cellular and humoral effects of PD-1 blockade lead to a decrease in plasma viral loads and increased survival of the macaques. An investigation of the safety of repeated doses of PD-1 blockade was conducted by an extensive analysis of various markers of autoimmunity and no significant changes in any of the tested parameters were observed. Taken together, these studies suggest that PD-1 blockade could be a potential immunotherapy applied to HIV infections in humans.

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PD-1 blockade during prophylactic vaccination

Evaluation of: Finnefrock A, Tang A, Li F *et al.*: PD-1 blockade in rhesus macaques: impact on chronic infection and prophylactic vaccination. *J. Immuno.* 182, 980–987 (2009).

The PD-1/PDL-1 interaction serves as a negative regulator of immune responses to pathogens [1]. Initially discovered as a molecule that was expressed on the surface of cells undergoing programmed cell death, subsequent research has implicated a pivotal role for PD-1 signaling in peripheral tolerance, autoimmunity, cancer immune evasion and T-cell exhaustion in chronic viral infections [2–4]. Blocking the inhibitory signaling of PD-1 has been investigated as a potential immunotherapy for chronic viral infections.

In their recent article, Finnefrock *et al.* investigated the possible therapeutic potential of blocking PD-1/PDL-1 signaling using monoclonal antibodies (mAbs) specific for human PD-1 in a chronic viral infection model [5]. After selecting the mAb with the highest binding affinity and ability to block the PD-1/

PDL-1 ligand interaction *in vitro*, it was then tested *in vivo* to see if PD-1 blockade could rescue exhausted T cells in chronically SIV-infected (for at least 18 months) rhesus macaques. A transient increase in viral loads was seen prior to returning to or dropping below baseline levels when PD-1 blockade was used in combination with integrase inhibitor antiretroviral therapy; however, there was no improvement in the functionality of the T cells. In a different model, the authors were able to demonstrate that prophylactic vaccination of naive rhesus macaques with an adenovirus vector encoding SIV-gag was more effective when the anti-PD-1 mAb was given at the time of the vaccination. It appeared that the PD-1 blockade served as an adjuvant leading to an increase in epitope-specific T cells.

These results suggest that PD-1 blockade therapy could be used to expand epitope-specific T cells in a prophylactic vaccination model when used in combination with other antiviral therapies. In addition, PD-1 blockade could potentially be effective in controlling viral loads during

chronic viral infections due to viral loads returning to or falling below baseline levels in this model.

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PD-1 on macrophages and bacterial infection

Evaluation of: Huang X, Venet F, Wang Y *et al.*: PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis. *Proc. Natl Acad. Sci.* 106, 6303–6308 (2009).

The balance between the positive and negative signaling delivered by various immune cells through immunostimulatory molecules dictates the destiny of the immune response towards the pathogens. Recently, PD-1, a CD28-superfamily negative signaling molecule [1] was discovered, which is expressed on various

cell types such as T cells, B cells, natural killer cells and monocytes [2]. The PD-1/PDL-1 signaling pathway plays a significant role in autoimmune disorders, cancer, viral infections, transplantation and allergy [3]. However, the role of PD-1 molecules during a bacterial infection is poorly understood.

Recently, Haug *et al.* provided insight into the role of PD-1 during bacterial sepsis using a PD-1 knockout (*PD-1^{-/-}*) mouse model and suggested that PD-1 was a novel therapeutic target for sepsis treatment [4]. Sepsis by intestinal bacteria was induced using a standard cecal ligation and puncture method. The deleterious effect of sepsis is multisystem

organ failure that results in death [5]. The authors have shown that when compared to wild-type (WT) mice, the *PD-1^{-/-}* mice had milder inflammatory responses and reduced bacterial load, and consequently no sepsis-induced death. Macrophages play a critical role in bacterial clearance [6]. The results showed that there was a rapid upregulation of PD-1 both at the genomic and protein level in sepsis-induced WT mouse macrophages resulting in macrophage dysfunction as characterized by lower bactericidal activity and decreased inflammatory cytokine production. The authors have also shown that the depletion of peritoneal macrophages in *PD-1^{-/-}* mice results in increased

sepsis and death of mice suggesting that peritoneal macrophages are important to maintain the protective effect induced by the *PD-1* gene deficiency during sepsis. They observed a similar pattern of PD-1 upregulation in monocytes from patients with septic shock suggesting that PD-1 may serve as a potential marker for monocyte/macrophage dysfunction during the development of sepsis. The results from Haung *et al.* suggest that the regulation of macrophage PD-1 would be a good immunotherapeutic strategy for the treatment of sepsis. Unlike viral infections and cancer where PD-1 upregulation in T cells plays a major role in attenuating immune responses, this study demonstrates that the upregulation of PD-1 plays a critical role in the reduced ability of macrophages to clear bacterial infections.

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