DR JUHA P VÄYRYNEN (Orcid ID : 0000-0002-8683-2996)

Article type : Editorial

Regulatory mechanisms of T cell activation – from basic research discoveries to a new principle of cancer therapy and the Nobel Prize

Juha P. Väyrynen[1,2], Anne Tuomisto[1], Markus J. Mäkinen[1]

[1] Cancer and Translational Medicine Research Unit, University of Oulu, Oulu, Finland; Finland; Department of Pathology, Oulu University Hospital and Medical Research Center Oulu, Oulu, Finland
[2] Department of Oncologic Pathology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA

Emails for correspondence: juha.vayrynen@oulu.fi; anne.tuomisto@oulu.fi; markus.makinen@oulu.fi

For more than a century, scientists have been attempting to harness the immune system to fight against cancer. In the late 19th century, William Coley reported a significant number of regressions and cures after injection of streptococcal organisms into inoperable sarcoma patients to cause erysipelas.[1] In 1909, Paul Ehrlich proposed that host defence may prevent neoplastic cells from developing into tumours, which was formally introduced as the cancer immunosurveillance hypothesis nearly 50 years later by Macfarlane Burnet and Lewis Thomas. However, at those times, there was not sufficient scientific methodology and knowledge to confirm the hypotheses. "Coley's toxin" fell out of favour due to its inefficacy in many patients, its potential serious side effects, and concurrent development of other treatment modalities. A few therapy modalities based on Coley's principles have persisted, *e.g.*, intravesical administration of weakened mycobacteria against superficial bladder cancer. However, it was not until in the 1990s that substantial evidence supporting the role of the immune system in tumour control started to accumulate. One branch of this approach has resulted in the development of autologous immunotherapy modalities for few cancers, such as prostate cancer, acute lymphoblastic leukaemia, and large B cell lymphoma. Eventually, in 2010s, immune checkpoint blockade therapies were successfully developed and approved for the treatment of a variety of malignant diseases. For their fundamental work on negative T cell co-stimulation and its applications culminating in cancer therapy, the 2018 Nobel Prize in Physiology or Medicine was awarded to James P. Allison and Tasuku Honjo.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/apha.13224

During the 1990s, Allison's research group at University of California, Berkley, studied T cell surface receptor CTLA4 (cytotoxic T-lymphocyte-associated protein 4), previously discovered by Jean-François Brunet *et al*. in 1987.[2] Allison's group was among the first to unravel the role of CTLA4 as a down-regulator of immune responses (Figure 1). In their pioneering study in 1996, Allison and his co-workers reported that administration of CTLA4 antibodies to mice reduced the tumour incidence after tumour cell injection and inhibited the growth of pre-established tumours, suggesting that CTLA4 blockade enhanced antitumor responses of these mice.[3] This discovery was followed by additional promising results of the impact of CTLA4 blockade in murine tumour models from Allison's group and others,[4] and, eventually, a demonstration of the efficacy of CTLA4 inhibitor in metastatic melanoma patients in a phase III randomized controlled clinical trial in 2010.[5]

In 1992, Tasuku Honjo and his research group at Kyoto University, Japan, discovered PD-1 (Programmed cell death protein 1, PDCD1), another immune checkpoint molecule expressed on the surface of T cells (Figure 1).[6] Honjo and his co-workers continued to study this molecule, and, in 1999, they reported PD-1 to be a negative regulator of immune responses by showing that PD-1-deficient mice developed multiorgan autoimmune disease.[7] Honjo's group collaborated with researchers in Genetics Institute in Cambridge, MA and Dana-Farber Cancer Institute in Boston, MA, to identify the ligand of PD-1 (PD-L1, Programmed death-ligand 1, CD274) in 2000.[8] These findings were the foundation for subsequent clinical studies showing the efficacy of treatments targeting the PD-1/PD-L1 regulatory system in a broad range of tumour types. Notably, these immune checkpoint blockade therapies seem to be able to induce long-term durable responses in a subset of patients with metastatic cancer unresponsive to conventional chemotherapy.

The discoveries of Allison and Honjo coincide with further breakthroughs in the understanding of tumour immunology. The immunoediting theory, classifying the interactions between tumour and host into three phases — elimination, equilibrium, and escape — was formulated based on milestone murine studies describing the Darwinian selection pressure produced by the immune system trying to eliminate the tumour cells.[9] In the elimination phase, the immune system act to eradicate the tumour cells. Equilibrium describes the phase in which immune system can restrict the tumour growth but no longer eradicate it. This leads to the selection of tumour cell variants with increasing capabilities to survive from immune destruction, potentially including those with upregulated expression of T cell negative co-stimulatory ligands. In the escape phase, the tumour cells have developed traits that help them to escape from immune control. In some patients, immune checkpoint blockade can turn the direction of this process back to equilibrium or elimination.

Co-stimulatory and co-inhibitory signalling have critical roles in T cell biology beyond cancer immunology; T cell activation requires both antigen presentation by major histocompatibility complex (MHC) and co-stimulation, while co-inhibitory receptors attenuate T cell activity, maintaining immune responses within a desired physiologic range (Figure 1). Based on current understanding, CTLA4 primarily acts in the areas of T cell priming (*i.e*., secondary or tertiary

lymphoid tissue), where its expression in effector T cells is upregulated quickly, within one hour, following T cell activation, and it is trafficked from T cell intracellular vesicles to cell surface. It then competes with the T cell surface costimulatory receptor CD28 for its ligands CD80 and CD86, expressed by antigen presenting cells. In addition, regulatory T cells constitutively express CTLA-4 at high levels in order to restrain the immune response and maintain self-tolerance. Instead, PD-1/PD-L1 axis appears to mainly function in inflamed peripheral tissues, where PD-1 is expressed in activated lymphocytes and PD-L1 expression is induced by inflammatory cytokines in a variety of cell types including antigen presenting cells, vascular endothelial cells, and tumour cells (Figure 1).

Vast amount of investigation is currently directed into developing immune checkpoint inhibition into more effective treatment in a wider range of tumour types. Anti-CTLA-4 plus anti-PD-1 combination therapy was recently approved in the treatment of metastatic melanoma, with favourable outcomes compared with either monotherapy.[10] It is not clear, why some patients respond to the treatment while others do not. Tumour PD-L1 expression has been found to predict the efficacy of treatment targeting PD-1/PD-L1 regulatory system in some tumour types, including non-small-cell lung cancer. However, many patients whose tumours do not express PD-L1 have also responded to the treatment. Also, high mutation burden in tumours has been associated with improved responses to checkpoint inhibition but does not completely differentiate responders from non-responders. The co-regulation of T cell activation has been proven to be extremely complex, involving many additional co-regulatory molecules, such as LAG3, TIM3, VISTA, ICOS, OX40, and GITR. The development of anti-CTLA4 and anti-PD-1/PD-L1 therapy was preceded by discoveries on their basic biology by Allison, Honjo, and others. Now, deeper insight of the physiology of the regulatory network of T cell activation is required to rationally develop immune checkpoint blockade therapy further, while avoiding potential serious side effects, such as cytokine release syndrome and autoimmune reactions caused by overactive immune response.

An analogy has been drawn between cancer therapy and three-legged stool, sitting on a base of surgery, radiotherapy, and chemotherapy.[11] In the past few decades, cancer immunotherapy has emerged as the fourth leg of this stool, changing the outcome of some of the patients not responsive to other present treatment modalities. Future research is required to enlighten the optimal combinations of these treatments. Nobel prizes have previously been awarded for discoveries in several cancer treatment strategies, including hormonal treatment of prostate cancer (Charles Brenton Huggins 1966), chemotherapy (Gertrude B. Elion and George H. Hitchings 1988), and bone marrow transplantation for leukaemia (E. Donnall Thomas 1990). The attribution of the 2018 Nobel Prize in Physiology or Medicine to James P. Allison and Tasuku Honjo honours their landmark basic research discoveries on the physiological regulatory mechanisms of T cell activation, which have served as a foundation for a new principle for cancer therapy.

Conflict of interest: The authors declare that there is no conflict of interest.

References

1. Coley WB. The treatment of inoperable sarcoma with the 'mixed toxins of erysipelas and bacillus prodigiosus: Immediate and final results in one hundred and forty cases. *JAMA* 1898;XXXI:456–65.
2. Brunet JF, Denizot F, Luciani MF, Roux-Dosseto M, Suzan M, Mattei MG, Golstein P. A new member of the immunoglobulin superfamily--CTLA-4. *Nature* 1987;328:267–70.
3. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* 1996;271:1734–6.
4. Kwon ED, Hurwitz AA, Foster BA, Madias C, Feldhaus AL, Greenberg NM, Burg MB, Allison JP. Manipulation of T cell costimulatory and inhibitory signals for immunotherapy of prostate cancer. *Proc Natl Acad Sci U S A* 1997;94:8099–103.
5. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJM, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010;363:711–23.
6. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* 1992;11:3887–95.
7. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 1999;11:141–51.
8. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000;192:1027–34.
9. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, Schreiber RD. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 2001;410:1107–11.
10. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, Linette GP, Meyer N, Giguere JK, Agarwala SS, Shaheen M, Ernstoff MS, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med* 2015;372:2006–17.
11. DeVita VT, Rosenberg SA. Two hundred years of cancer research. *N Engl J Med* 2012;366:2207–14.

Figure legends

Fig. 1. T cell activation, positive and negative co-stimulation, and cancer therapy. Antigen presenting cells can present antigens by major histocompatibility complex (MHC) class II and other cells, including tumour cells, by MHC class I. T cell priming requires co-stimulatory signal, the best characterized of which is the binding of CD28 to its ligand (CD80 or CD86). Naïve T cells express high levels of surface CD28, whereas CTLA4 is mainly stored in intracellular vesicles. After antigen presentation, CTLA4 is transported to the cell surface and its expression is upregulated. CTLA4 is then able to bind to CD80 or CD86, for which is has higher affinity than CD28, limiting excessive the T cell activation. Activated T cells upregulate PD-1 expression, whereas inflammatory cytokines induce PD-L1 expression in a variety of cell types including antigen presenting cells, vascular endothelial cells, and tumour cells. PD-1/PD-L1 system thus forms a negative feedback loop, preventing excessive peripheral T cell responses. Antibodies against these negative co-stimulatory molecules have been shown to enhance anti-tumour immunity in murine tumour models and several types of human cancer.