Implications of tuberculosis reactivation after immune checkpoint inhibition

Paul T Elkington[1]*, Adrian C Bateman[1], Gareth J Thomas[2], Christian H Ottensmeier[2]

[1]NIHR Biomedical Research Centre, Clinical and Experimental Sciences Academic Unit, [2]Cancer Sciences, Faculty of Medicine, University of Southampton, UK.

* Address for correspondence:

Professor Paul Elkington

Clinical and Experimental Sciences

University of Southampton

Southampton SO16 1YD

Tel 00 44 23 8079 5928

E-mail: p.elkington@soton.ac.uk

Descriptor: 11.4 Mycobacterial Disease: Host Defenses

Funding: This work was supported by grants from the Medical Research Council MR/P023754/1 and Cancer Research UK C11512/A20256.

Word count: 995

Treatment of malignant disease with immune checkpoint inhibitors is emerging as a transformative approach. However, tuberculosis (TB) reactivation associated with these agents is being increasingly reported (Table 1). We describe a further case of TB associated with anti-programmed death-1 (PD-1) immunotherapy and perform immunohistochemical analysis of lung biopsies from TB in standard and anti-PD-1 associated TB. We discuss the potential underlying mechanisms and implications for clinical practice and research.

TB associated with pembrolizumab, an antibody to PD-1

A 62 year old lady was diagnosed with ocular melanoma, which was excised. Three years later, metastatic disease developed and immune checkpoint inhibition therapy was commenced, initially with ipilimumab, an anti–cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) antibody, and then pembrolizumab, a humanized monoclonal antibody against PD-1. The disease was stable for 2 years, but then blood liver biochemical markers became abnormal and a lung lesion was noted on computerised tomography scanning. Liver function abnormalities persisted despite immunosuppression, and so a liver biopsy was performed, which showed a single granuloma. Biopsy of the cavitating apical lung lesion showed necrotising granulomatous inflammation, and bronchial washings cultured *Mycobacterium tuberculosis* (Mtb). Anti-tuberculosis treatment was initiated, which led to clinical improvement, normalisation of liver function tests and regression of the lung lesion. Therefore, the unifying diagnosis was disseminated tuberculosis (TB) associated with immune checkpoint inhibition. However, this clinical occurrence runs counter to the current disease paradigm, which proposes that active TB results from a deficient host immune response (1).

Therefore, we performed immunohistochemical analysis of TB lung lesions in the context of a normal immune response (6 cases) and the lung biopsy of this case. Immunostaining was performed for PD-L1, CD8 and PD-1. In normal TB granulomas, PD-L1 is very highly expressed, while PD-1 co-localises with CD8, demonstrating that immune checkpoint ligands and receptors are co-expressed

(Figure 1A). In the context of anti-PD-1 therapy, a similar picture of PD-L1 and CD8 expression within granulomas is observed, while PD-1 immunoreactivity appears reduced (Figure 1B). Therefore, the immune checkpoint inhibition pathway is active within TB granulomas.

Potential mechanisms of immune checkpoint inhibition causing TB reactivation

PD-1 is a cell surface receptor that binds ligands PD-L1 and PD-L2 and has important functions in the maintenance of immune tolerance. PD-1 inhibitors are therefore used to reverse tolerance to tumours and improve immune-mediated control of malignant disease (2). The use of these checkpoint inhibitors has been transformative to the field (2). In TB, progression from latent to active infection is regarded as a failure of the immune response, as demonstrated by the increased incidence of TB in the context of HIV infection or after anti-TNF treatment for inflammatory conditions (1). Consequently, it seems highly counter-intuitive that PD-1 blockade should also cause activation of TB, as by this paradigm anti-PD-1 therapy should improve host control of TB. Indeed, PD-1 inhibition has been suggested as a host-directed therapy in TB (3), on the basis that the PD-1 pathway may inhibit an effective host response.

Mechanistically, these observations suggest that immune checkpoint signalling is important to conserve immune homeostasis within TB granulomas and prevent excessive inflammation that may lead to tissue destruction and cavitation (4). In terms of the cellular events leading to TB, depletion of Mtb-responsive T cells by anti-PD-1 treatment would be most consistent with the current paradigm whereby a greater host immune response limits Mtb growth. However, this would imply a dual effect, with immune checkpoint inhibition improving control of malignancy by immune activation, whilst concurrently suppressing anti-mycobacterial immune responses. Therefore, an alternative process seems likely.

A rapid T cell-driven immune activation could lead to greater recruitment of permissive monocytes or neutrophils to TB granulomas, which are thought to be deleterious in TB (1). Alternatively, this augmented immunity may result in increased cytotoxicity or matrix-metalloproteinase-driven extracellular matrix destruction, which favours Mtb growth and leads to transmission of infection (4). An unorthodox recent proposal is that active TB can result from an auto-inflammatory or autoimmune process, a hypothesis supported by diverse clinical and experimental observations (5). Analysis of gene expression profiles of patients with TB, infection and autoimmune disease also suggests a common underlying mechanism between TB and autoimmunity (6). Intriguingly, the most common adverse events from immune checkpoint inhibitors are autoimmune in nature (2), consistent with this hypothesis. Along similar conceptual lines, Divangahi and Behr have recently proposed that T cell mediated immune tolerance may be equally important in host control of TB as effector functions (7).

Implications for clinical practice and research

The clinical implications of this phenomenon are wide. New lesions in a patient with known cancer are likely to be diagnosed as malignant progression, leading to significant under-diagnosis. We suggest that biopsy of progressive lesions in this context is indicated to exclude TB, as it is treatable with antibiotics. Furthermore, patients with cancer should be screened by Mantoux test or interferon-γ release assay prior to receiving anti-PD-1 treatment, in the same way that screening is routine prior to anti-TNF treatment. If previous Mtb exposure is diagnosed, chemoprophylaxis may be indicated to prevent active TB.

Regarding the research implications, the host-pathogen interaction is finely balanced, with only a small subset of Mtb exposed individuals developing pulmonary disease to continue transmission (1, 4). The clinical observations that are emerging from the biologic treatment era provide entirely novel and highly relevant insights into human immune function and host-pathogen interactions. The

increase in active TB after immune checkpoint inhibition suggests that excessive immunity may be just has harmful as insufficient immunity. Supporting this concept, Comstock demonstrated in a study of 82,000 individuals that a strong response to TB antigens, which should be considered protective, actually associates with progression to active TB (8). Infection of mice deficient in PD-1 results in rapidly lethal inflammation (9, 10), consistent with a protective role in TB. Perhaps the most sobering implication is that it reinforces the finely balance knife-edge of the human-Mtb interaction. Simply driving an exaggerated immune response, without first defining determinants of progression versus protection in TB, risks inadvertently accelerating transmission and worsening the pandemic in the longer term (4).

Acknowledgements

We thank Monette Lopez for excellent technical assistance.

References

1. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP. The immune response in tuberculosis. *Annu Rev Immunol* 2013; 31: 475-527.

2. Boussiotis VA. Molecular and Biochemical Aspects of the PD-1 Checkpoint Pathway. *N Engl J Med* 2016; 375: 1767-1778.

3. Zumla A, Rao M, Wallis RS, Kaufmann SH, Rustomjee R, Mwaba P, Vilaplana C, Yeboah-Manu D, Chakaya J, Ippolito G, Azhar E, Hoelscher M, Maeurer M, Host-Directed Therapies Network c. Host-directed therapies for infectious diseases: current status, recent progress, and future prospects. *Lancet Infect Dis* 2016; 16: e47-63.

4. Elkington PT, Friedland JS. Permutations of time and place in tuberculosis. *Lancet Infect Dis* 2015; 15: 1357-1360.

5. Elkington P, Tebruegge M, Mansour S. Tuberculosis: An Infection-Initiated Autoimmune Disease? *Trends Immunol* 2016; 37: 815-818.

6. Clayton K, Polak ME, Woelk CH, Elkington P. Gene Expression Signatures in Tuberculosis Have Greater Overlap with Autoimmune Diseases Than with Infectious Diseases. *Am J Respir Crit Care Med* 2017; 196: 655-656.

7. Divangahi M, Behr MA. Cracking the Vaccine Code in Tuberculosis. *Am J Respir Crit Care Med* 2018; 197: 427-432.

8. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *American journal of epidemiology* 1974; 99: 131-138.

9. Lazar-Molnar E, Chen B, Sweeney KA, Wang EJ, Liu W, Lin J, Porcelli SA, Almo SC, Nathenson SG, Jacobs WR, Jr. Programmed death-1 (PD-1)-deficient mice are extraordinarily sensitive to tuberculosis. *Proc Natl Acad Sci U S A* 2010; 107: 13402-13407.

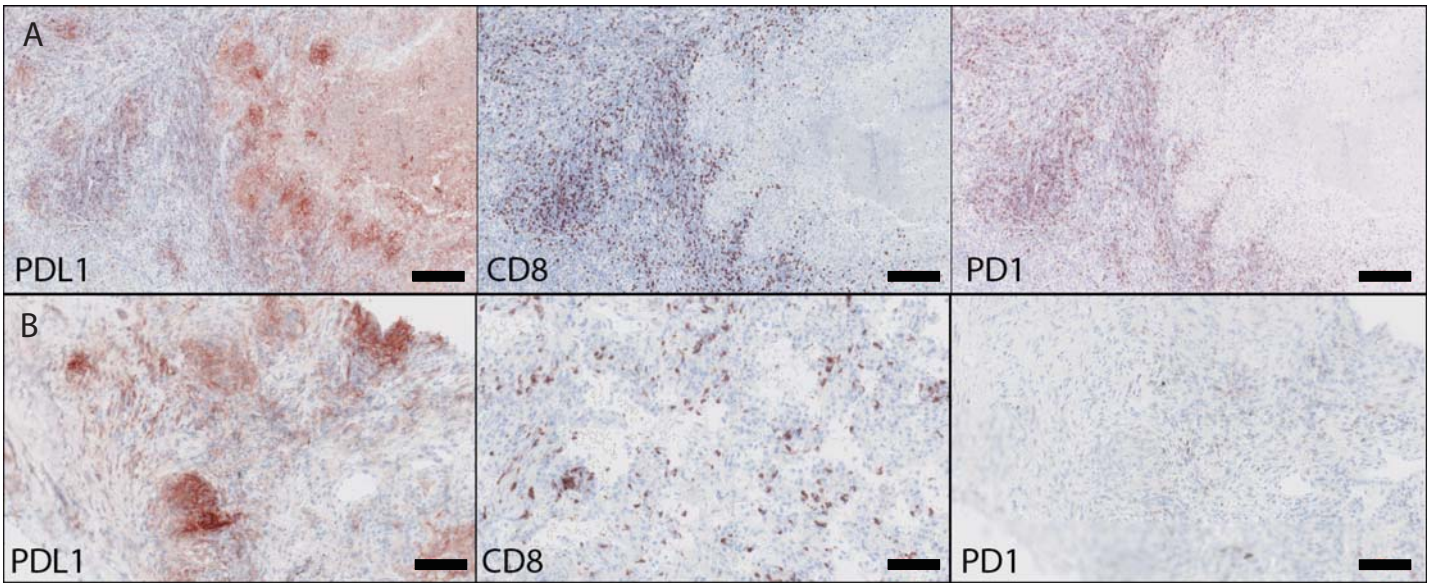
10. Barber DL, Mayer-Barber KD, Feng CG, Sharpe AH, Sher A. CD4 T cells promote rather than control tuberculosis in the absence of PD-1-mediated inhibition. *J Immunol* 2011; 186: 1598-1607.

First author	Year	Malignancy	Inhibitor	Site of TB	DOI
Fujita K	2016	Non small cell lung cancer	Nivolumab	Pulmonary	10.1016/j.jtho.2016.07.006
Lee JJ	2016	Hodgkin's lymphoma	Nivolumab	Pulmonary	10.3109/0284186X.2015.1125017
Chu YC	2017	Non small cell lung cancer	Nivolumab	Pericardial	10.1016/j.jtho.2017.03.012
Picchi H	2018	Melanoma	Pembrolizumab	Pleural	10.1016/j.cmi.2017.12.003
Picchi H	2018	Non small cell lung cancer	Nivolumab	Spinal	10.1016/j.cmi.2017.12.003
Jensen K	2018	Non small cell lung cancer	Nivolumab	Pulmonary	10.1080/0284186X.2018.1433877
Elkington P	2018	Melanoma	Pembrolizumab	Pulmonary and hepatic	

Table 1: Summary of accumulating evidence of TB reactivation with anti-PD-1 immune checkpoint inhibition.

Figure legend

Figure 1: PD-L1 and PD-1 are expressed in human lung TB granulomas. Six lung biopsies of patients with a final diagnosis of TB were immunostained for PD-L1, CD8 and PD-1. (A) PD-L1 is highly expressed by macrophages within the granuloma, and PD-1 is expressed by CD8 positive T cells. (B) In the pembrolizumab-treated patient, strong PD-L1 and CD8 immunoreactivity was observed, while PD-1 staining appeared less strong. Scale bars A: 200µm, B: 100µm.


A
PDL1
CD8
PD1
B
PDL1
CD8
PD1