**Background**

CD8 T cells are critical for containment of Mycobacterium tuberculosis (Mtib) infection. IFN-γ-producing Th1 cells play a major role in protection against Mtib, but other cell types, including Th17 and CD8 T cells may also contribute to control of Mtib infection. Pecdinical data suggest that PD-1 blockade has a therapeutic potential to enhance T cell responses and control of a variety of chronic infections. However, PD-1 deficient mice are highly susceptible to Mtib infection and develop necrotic pulmonary lesions. This increased susceptibility to Mtib infection of PD-1 deficient mice is counter-intuitively due to increased IFN-γ-production by CD4 T cells. Therefore, preclinical animal model data raise the possibility that enhanced Th1 responses following PD-1 blockade may have unexpected deleterious consequences during Mtib infection. Here we report a case of a patient with Merkel cell carcinoma (MCC) that developed tuberculosis following PD-1 blockade with Pembrolizumab.

**Methods**

An 83-year old man began a clinical trial of Pembrolizumab in June 2015 for advanced MCC. The patient had no risk factors and no testing for latent TB was performed. CT scan after 11 cycles revealed a solitary lesion in the right lower lobe (1.1 x 1.6 cm). The patient underwent excision of the nodule in January 2016. Pathology revealed necrotizing granuloma and acid-fast staining of tissue sections was performed. Cryopreserved peripheral blood monocytes (PBMCs) obtained immediately prior to Pembrolizumab and at cycles 5, 8, 11, and 14 were analyzed for antigen-specific CD4 and CD8 T cell responses by intracellular cytokine staining after stimulation with purified protein derivatives (PPD). Serum samples were also analyzed for IgG responses to a panel of different Mtib antigens.

**Results**

Three previous cases of TB have been reported following PD-1 blockade. In this case, Mtib-specific Th1 responses increased after PD-1 blockade was initiated, and clinical TB arose subsequently. Importantly, this nodule was assumed to be an MCC metastasis, and would not have been recognized as due to Mtib if an excisional biopsy had not been performed. In conjunction with animal model data suggesting a plausible mechanism, and the prior reported cases, these findings suggest that Mtib is possibly a concern following PD-1 blockade.

**Conclusion**

*Figure 1.* Clinical characteristics of a patient with Merkel cell carcinoma who developed tuberculosis following Pembrolizumab treatment. (A) Chest CT image of a Merkel cell carcinoma patient before onset of tuberculosis. (B) CT image after 11 cycles of infusion. Arrow head indicates necrotizing nodule in the right lower lobe. (C) H&E staining of tissue section from the excised lung nodule. (D) Acid-fast staining of tissue section from the nodule.

*Figure 2.* M. tuberculosis-specific T cell responses in a patient with Merkel cell carcinoma following Pembrolizumab treatment. (A) Flow cytometric analysis of PD-1 expression on CD4 T cells in peripheral blood mononuclear cells (PBMCs) from a patient at different points after Pembrolizumab treatment. Dot line denotes the baseline level of PD-1 expression on CD3+CD4+ cells before treatment. (B) Fluorescence-activated cell sorting (FACS) plots of CD4 T cells showing intracellular cytokine staining for tumor necrosis factor (TNF) and interferon (IFN)-γ after 6 hour stimulation with purified protein derivatives (PPD). (C) Summary graph of PPD-specific Th1 response in PBMCs from a patient throughout disease course. (D) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin. (E) Summary graph of PPD-specific Th17 response in PBMCs from a patient throughout disease course. (F) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin. (G) Summary graph of PPD-specific Th17 response in PBMCs from a patient throughout disease course. (H) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin. (I) Summary graph of PPD-specific Th17 response in PBMCs from a patient throughout disease course. (J) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin. (K) Summary graph of PPD-specific Th17 response in PBMCs from a patient throughout disease course. (L) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin. (M) Summary graph of PPD-specific Th17 response in PBMCs from a patient throughout disease course. (N) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin. (O) Summary graph of PPD-specific Th17 response in PBMCs from a patient throughout disease course. (P) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin. (Q) Summary graph of PPD-specific Th17 response in PBMCs from a patient throughout disease course. (R) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin. (S) Summary graph of PPD-specific Th17 response in PBMCs from a patient throughout disease course. (T) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin. (U) Summary graph of PPD-specific Th17 response in PBMCs from a patient throughout disease course. (V) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin. (W) Summary graph of PPD-specific Th17 response in PBMCs from a patient throughout disease course. (X) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin. (Y) Summary graph of PPD-specific Th17 response in PBMCs from a patient throughout disease course. (Z) FACS plots of CD4 T cells showing TNF and CD107α expression after 4 hour stimulation with PPD or pokeweed mitogen (PWM) and mitomycin.

*Figure 3.* M. tuberculosis-specific antibody responses in a patient with Merkel cell carcinoma following Pembrolizumab treatment. IgG response against mycobacterial antigens was measured in sera from a patient at different points after PD-1 blockade.