Pneumocystis Pneumonia: Checkpoint Inhibition to the Rescue?

The fungus *Pneumocystis jiroveci* causes pneumonia only in immunosuppressed patients, such as in advanced human immunodeficiency virus (HIV) infection or after organ transplantation. Extrapulmonary infections are rare. With the increase in use of immunosuppressive medications, the incidence of pneumonia caused by *P. jiroveci* (PCP) in patients without HIV is increasing (1). Despite a decreased pathogen burden in patients without HIV, morbidity and mortality are significantly higher in this patient population (2). Although adjunctive corticosteroid therapy is a mainstay in the treatment of PCP in patients with HIV (3), its benefit in patients without HIV has not been demonstrated (2). Thus, new pathophysiological insights might help to improve therapy in PCP, particularly in patients without HIV. PD-1 (programmed cell death 1) is an inhibitory receptor expressed on a variety of immune cells, and its ligand PD-L1 is widely expressed on hematopoietic and nonhematopoietic cells. PD-1 prevents autoimmunity by promoting T-cell tolerance and regulating T-cell exhaustion. Monoclonal antibodies targeting PD-1, its ligand PD-L1, or CTLA-4 (cytotoxic T-lymphocyte antigen 4) are so-called checkpoint inhibitors (CPIs), which prolong survival of patients with various malignancies by increasing antitumor T-cell activity (4). Autoimmunological side effects are common and may involve a variety of organs (5). CPIs might constitute a treatment option for certain infections (6), and research in this field is needed.

In this issue of the *Journal*, Zhang and colleagues (pp. 767–782) convincingly demonstrate that the PD1-PD1L axis is involved in the immune response against *P. jiroveci* in humans and *P. murina* in mice (7). Analysis of peripheral blood of human patients under immunosuppressive therapy showed a marked upregulation of PD-1 and its ligand PD-L1 on $CD4^+$ and $CD8^+$ T cells during the course of PCP. In wild-type mice, *P. murina* infection led to a strong upregulation of PD-1, particularly on $CD4^+$ and $CD8^+$ cells isolated from the lungs, whereas lymphocytes in the peripheral blood showed almost no differences in PD-1 or PD-L1 expression over time. Next, the authors compared wild-type with PD-1 knockout mice; here, infection with *P. murina* in PD-$1^{-/-}$ animals accelerated pathogen clearance, enhanced production of various cytokines, and increased cell influx, resulting in faster weight gain 3 weeks after infection. In lung macrophages, PCP was shown to upregulate PD-1 and PD-L1 (8), but this work further demonstrates that PD-1–deficient alveolar macrophages (AM) possess an enhanced capacity to clear zymosan. In line with that, PD-$1^{-/-}$ AM exhibited an increased transcription of Clec7a, which encodes for dectin-1, an important recognition receptor involved in the clearance of *Pneumocystis* (9). AM harvested during the first weeks of infection displayed increased gene expression of a variety of M1 and M2 markers in PD-1 knockout AM, with a strong upregulation of iNOS (inducible nitric oxide synthase) and enhanced nitrite levels. Pharmacological inhibition of nitric oxide (NO) production at least partially reversed the improved clearance of *P. murina in vivo*. These data match with a previous report on the importance of NO in the clearance of *Pneumocystis* (10).

Finally, the authors corroborated the findings of improved pathogen clearance and weight gain in the knockout animals by pharmacological inhibition of PD-1 using anti–PD-1 antibodies. To mimic an immunosuppressive state, one group of animals also received dexamethasone. Unfortunately, PD-1 antibodies were administered right from the beginning and not sequentially during the course of infection, in order to test for a potential therapeutic application. Surprisingly, in dexamethasone-treated animals, the deletion or blockade of PD-1 did not markedly alter pathogen burden, whereas weight gain did improve significantly in this setting. The degree of lung injury and the percentage of neutrophils in the BAL rather than the pathogen burden correlates with disease severity in patients (with and without HIV) with PCP (11). PD-1 deletion or blockade reduced neutrophil numbers in dexamethasone-treated and untreated mice, which aligns nicely with reduced morbidity in both groups. Thus, assessing pathogen clearance and morbidity is a strength of this study and should always be performed in the context of PCP. Although the work by Zhang and colleagues provides evidence on the role of the PD-1/PD-L1 axis in PCP, the mechanism and the precise contribution of various cell types and mediators remains elusive (7). Deletion of PD-1 led to an upregulation of factors known to be involved in *Pneumocystis* clearance, such as IL-12, IL-21/22, and GM-CSF (12), which matches with increased pathogen clearance. In contrast, a plethora of cytokines and mediators with partially opposing functions, such as IL-4, IL-10, IL-12, IL-17, or IFN-γ, were concomitantly upregulated on a transcriptional level in the whole lung. Similarly, M1 and M2 markers such as Arginase 1 (Arg-1) and iNOS on F4/80-positive cells were simultaneously increased in AM. Thus, more refined experiments delineating the specific contributions of various cell types in the lung—as for example by distinguishing resident and recruited macrophages—are required. Also, the significance of an increase in Th1 and Th17 cells 3 to 4 weeks after infection in PD-$1^{-/-}$ animals is unclear. Previous work demonstrated no role for IL-17 in pathogen clearance in PCP in mice (11), although contradictory data exist (13). Thus, a mechanistic function of these $CD4^+$ subsets in the context of PD-1 inhibition remains to be elucidated.

In general, CPIs might have the potential to booster the immune system in infectious diseases. In this context, treatment of immunosuppressed patients seems to be an attractive option. Recently, PD-1 blockade was reported to improve the clinical outcome of some patients with fatal JC-virus encephalitis (14); however, unmasking immune reconstitution inflammatory

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syndrome and the occurrence of JC encephalitis under treatment with nivolumab diminishes optimism (15). Preclinical studies in sepsis have shown that CPI targeting CTLA-4 improved survival when a low dose was administered, but a high dose increased mortality (16). This emphasizes the complexity of immunological interventions in infectious diseases and the need to precisely understand the contribution of various cell types over time *in vivo*. Thus, future studies should focus on cell-specific contributions of PD-1/PD-L1 in PCP using various models of immunodeficiency, with an emphasis on lung injury and morbidity. ■

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