that, during chronic infection, microorganisms exploit these pathways in their own favor to establish an infection. By inducing overexpression of programmed death 1 (PD-1) and its ligands (PD-L1 and PD-L2) on various immune cells, M. tuberculosis effectively suppresses the T-helper type 1 (Th1) immune response. Recently, we [1, 2] and others [3–5] demonstrated that M. tuberculosis inhibits T-cell, natural killer cell, and natural killer T-cell effector functions (ie, cytokine release, proliferation, apoptosis, and cytotoxic T-lymphocyte activity) by exploiting the PD-1–PD-L1/PD-L2 pathways.

In their correspondence, Stephen-Victor et al examined the expression of PD-L1 on CD4+ T cells and dendritic cells (DCs) with different stimuli (polyclonal stimulation by anti-CD3 anti-CD28, M. tuberculosis, and bacillus Calmette-Guérin). Under such stimulatory conditions, they did not see significant changes in the frequencies of PD-L1+ CD4 T cells. Furthermore, they did not see the rise in frequencies of interferon γ (IFN-γ)–producing CD4+ T cells upon PD-L1 blockade. However, they did see an increase in soluble IFN-γ expression upon blocking of PD-L1 on DCs. Thus, even though their conclusions are in agreement with ours—the PD-1 pathway suppresses the IFN-γ response during tuberculosis—there are certain divergences from the results of our previous study of patients with tuberculosis [1].

We studied PD-L1 expression on CD3+ T cells and monocytes from patients with pulmonary tuberculosis. Stephan-Victor et al showed that PD-L1 was not overexpressed on CD4+ T cells during in vitro infection of enriched healthy T cells. Their conclusion that our observed overexpression of PD-L1 among patient-derived T cells may have been due to higher PD-L1 expression on CD8+ T cells is valid. We are happy to note that their results involving induction of PD-L1 on in vitro–differentiated healthy DCs after infection further substantiates our similar observation for monocytes. Therefore, both studies demonstrate that
the PD-1–PD-L1 pathway is involved in dampening the IFN-γ response during tuberculosis and that blocking this pathway provides a rationale to target the pathway for immune restoration.

Their results differ from ours in the following respects: (1) there was a marginal increase in PD-L1 expression on CD4⁺ T cells, and (2) there was a lack of modulation in the frequency and quantity of IFN-γ expression following blockade of PD-L1. The differences possibly stem from the marked difference in their experimental conditions. They followed a sophisticated but completely in vitro approach, whereas we performed our experiments with cells isolated from a near-natural milieu of peripheral blood mononuclear cells from patients with pulmonary tuberculosis. This may underlie the divergences in the results.

Upon activation, T cells (either CD4⁺ or CD8⁺) are reported to express PD-L1 or PD-L2; however, the activation-induced expression of PD-L1 or PD-L2 depends on several factors, such as the presence of cytokines (Th1 or Th2) in the milieu and the type and nature of other inflammatory immune cells, particularly inflammatory macrophages. The presence of IFN-γ, STAT4 signaling, and antigens presented by inflammatory macrophages are critical for the upregulation of PD-L1 on CD4⁺ T cells [3, 6]. Because the findings in Figure 1A from the letter by Stephen-Victor et al were obtained using sorted CD4⁺ T cells, the absence of a competent immune environment may have been a limiting factor contributing to the marginal change in the frequencies of PD-L1–expressing CD4⁺ T cells. It would have been interesting if they had performed this experiment with sorted CD8⁺ T cells, as well. We feel that it is critically important to provide a nearly complete immune environment that mimics the real in vivo situation for the expression of PD-L1 on T cells.

As shown in Figure 1B of their correspondence, Stephen-Victor et al did not observe significant changes in either the frequencies of IFN-γ⁺CD4⁺ T cells or the quantity of soluble IFN-γ secreted by these cells. In this experiment, CD4⁺ T cells were first stimulated with anti-CD3 anti-CD28 (control), M. tuberculosis, and bacillus Calmette-Guérin, with or without blocking PD-L1, and the cells were further activated with PMA plus ionomycin. We have 2 serious concerns in this respect. First, contrary to our study, they provided polyclonal stimulation in vitro to the sorted cells, which possibly masked the M. tuberculosis–specific T-cell response, both in terms of IFN-γ production and their PD-1–PD-L1–mediated apoptosis. PD-1 signaling suppresses the effector T-cell functions (eg, cytokine secretion and proliferation) by phosphorylation of PD-1 cytoplasmic tyrosines and SHP-2 association with the immunoreceptor tyrosine-based switch motif of PD-1. Recruitment of SHP-2 dephosphorylates signaling through the PI3K pathway and downstream signals through Akt. PD-1 ultimately decreases the induction of cytokines, such as IFN-γ and interleukin 2, and cell survival proteins, such as Bcl-xL and Bcl-2. When signaling through CD28 is delivered at the same time as PD-1 and T-cell receptor (TCR) ligation, inhibitory effects can be overcome, and cytokine production and cell survival is enhanced [7, 8]. Therefore, any strong stimulation can neutralize the inhibitory effect of PD-1 signaling. Since Stephen-Victor et al provided strong polyclonal stimulation (initially through the TCR and then with PMA plus ionomycin), it is quite possible that the effect of the PD-1 blockade was masked and that the M. tuberculosis–specific readout was diluted by the polyclonal nature of their stimulation. Such a high percentage of IFN-γ⁺ cells are usually not observed with any antigen-specific assay system. We performed >50 PD-1–PD-L1 blocking experiments but never observed such high levels of IFN-γ–producing CD4⁺ T cells. Therefore, the divergence of their results from our observations involving patients with pulmonary tuberculosis may be due to their experimental approach and use of sorted T cells from healthy donors in an in vitro setting. We should be cautious in interpreting and comparing data obtained from such different systems.

We are thankful to Stephan-Victor et al for their comments about our methods of capturing intracellular cytokines with brefeldin A for flow cytometry–based assays. We understand their concerns and would like to clarify that we did not perform blocking experiments by exposing the cells to brefeldin A for 48 hours or 72 hours. We cultured the cells for 48 hours or 72 hours, but brefeldin A was present only during the last 12 hours. We also excluded dead cells from our analysis by use of ethidium monoazide bromide staining, which is capable of excluding dead cells during intracellular cytokine staining and analysis. Recently, we reported our methods with more clarity [2]. We regret the lack of this clarity in our earlier article [1]. Thus, the error they pointed out is not applicable to our current study.

Overall, both studies highlight the critical role of the PD-1–PD-L1 pathway in host immunosuppression during tuberculosis.

Notes

Potential conflicts of interest. All authors: No reported conflicts.

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