Aiding and Abetting the Enemy: Nicotine Impairs the Macrophage Defense against *Mtb*

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*) that kills a large number of people in the world (1). In 2015, ~10 million new cases of TB and 2 million TB-related deaths were reported globally, earning it a place among the top 10 causes of global mortality (2). The emergence of multidrug-resistant TB is a menacing problem (2). An extensive geographical overlap exists between the areas where cigarette smoking is widespread and TB is highly prevalent. In addition, an association between cigarette smoke (CS) exposure and an increased risk of TB in children and adults has been found (3). Cigarette smoking has been shown to double the risk for the disease to develop again in individuals who have been cured of TB, an event termed "recurrent TB" (4). Thus, understanding the fundamental mechanisms by which CS exposure may suppress the host defense against *Mtb* is critical and timely as we seek to identify improved treatment and prevention strategies.

The first line of defense in eliminating *Mtb* in the lung is mediated by macrophages. Macrophages enter the site of infection, engulf *Mtb*, and may successfully eliminate it (5). However, *Mtb* has evolved numerous adaptive strategies for survival within macrophages. These include the abilities to inhibit phagosome-lysosome fusion, and to escape from the phagosome and enter the cytoplasm (5). Murine studies have shown that recruitment of significantly fewer immune cells (macrophages, dendritic cells, and T cells) in the lungs of CS-exposed mice may be responsible for an increased pulmonary *Mtb* burden (6, 7). In addition, CS exposure attenuates cytokine production and *Mtb* killing by macrophages (8, 9). Up to now, however, no studies have examined molecular mechanisms such as autophagy and the activation of immunosuppressive regulatory T (Treg) cells in the context of nicotine exposure, which suppresses anti-*Mtb* immunity and thereby allows CS to function as a major immunomodulator. In this issue of the *Journal*, Bai and colleagues (pp. 324–333) demonstrate that exposure to nicotine impairs the macrophage anti-*Mtb* defense by regulating two distinct mechanisms: first, it inhibits autophagy; second, it activates immunosuppressive Treg cells (10). Additionally, they found that the suppression of macrophage anti-*Mtb* immunity is dependent on the binding of nicotine to the nicotinic acetylcholine receptor (nAChR).

Autophagy was originally defined as a process by which cellular organelles and proteins sequestered inside a double-membrane vesicle, the autophagosome, are delivered for lysosomal degradation (11). Autophagy is known to represent a crucial anti-*Mtb* mechanism of the macrophage, and the ability to suppress autophagy is linked to *Mtb* virulence (11). Nevertheless, the effects of impairment of autophagy by CS exposure on virulence in macrophages infected with *Mtb* have not been explored. Bai and colleagues (10) demonstrate that in a human myelomonocytic cell line (THP-1), *Mtb*-induced LC3II expression (a marker of autophagic activation) was significantly reduced by pretreatment with nicotine in a dose-dependent manner. Similarly, when compared with the medium-exposed controls, nicotine-exposed murine alveolar macrophages (AMs) exhibited significantly fewer autophagosomes and a concomitant increase in the intracellular *Mtb* burden. These results collectively demonstrate that nicotine impairs macrophage anti-*Mtb* autophagy. However, the observed reduction in the number of autophagosomes in nicotine-pretreated, *Mtb*-infected macrophages is a deviation from previous non-*Mtb* studies in which CS exposure was found to decrease functional autophagy in AMs. In that regard, despite a marked increase in the accumulation of autophagosomes, the AMs of smokers exhibited an autophagic defect manifested by decreased lysosomal delivery of bacteria (11). Therefore, further studies are needed to determine whether this difference reflects a unique characteristic of anti-*Mtb* autophagy in macrophages or arises from the use of nicotine as opposed to CS extract.

Bai and colleagues (10) also report that nicotine stimulates FoxP3 (Treg) cells. They detected increased production of the immunosuppressive cytokines transforming growth factor β (TGF-β) and IL-10, and arginase activity (a marker for deactivated M2 phenotype macrophages) in cocultures of nicotine-exposed Treg cells with bone marrow–derived macrophages (BMDMs). In addition, a significantly higher number of intracellular *Mtb* colony-forming units were recovered from naive (unexposed) BMDMs cocultured with nicotine-exposed Treg cells than from BMDMs cocultured with control (unexposed) Treg cells or from BMDMs alone. Bai and colleagues (10) also found that *Mtb* induces Treg cells, which helps to establish infection by inhibiting the proliferation of T helper 1 (Th1), Th2, and Th17 cells, and skewing macrophage differentiation toward a deactivated M2 phenotype. Nicotine also enhances the ability of Treg cells to suppress the anti-*Mtb* defense of macrophages via upregulation of TGF-β and IL-10, as well as by promoting the differentiation of macrophages toward the M2 phenotype. CS exposure has been shown to increase production of the immunosuppressive cytokines TGF-β and IL-10 by Treg cells (11), and the current study shows that nicotine induces a similar response. Nevertheless, future investigations are needed to further define the molecular and cellular mechanisms by which nicotine-exposed Tregs increase the *Mtb* burden in macrophages through M2 polarization.

In place of commercially available nicotine, Bai and colleagues (10) used CS extract on macrophages treated with a nonselective chemical antagonist (mecamylamine) of nAChR signaling, or on macrophages isolated from nAChR knockout mice ($\alpha7^{-/-}$, $\beta2^{-/-}$, and $\beta4^{-/-}$). Nicotine is an agonist for nAChR, an integral membrane-bound homo-/heteropentameric receptor comprised of α (α1–α10) and/or β (β1–β4) subunits. The nAChRs are the receptors for the cholinergic antiinflammatory pathway (CAP), which maintains immune homeostasis with the help of effectors from the nervous system (e.g., the efferent vagus nerve and acetylcholine) and immune cells. Inhibition of nAChR signaling by mecamylamine treatment or the use of gene-deficient mice partially abrogates the ability of CS extract to suppress the anti-*Mtb* defense

of macrophages. Moreover, the absence of the β4 subunit of nAChR results in a maximum reversal of CS-mediated impairment of the macrophage anti-*Mtb* defense. These results suggest a critical role for nAChR signaling in CS-mediated suppression of the macrophage defense against *Mtb*. However, a comprehensive analysis of possible ramifications associated with the involvement of the nAChR-CAP axis in CS-mediated suppression of the macrophage anti-*Mtb* defense is needed.

Previous studies have shown that CS, a complex mixture of toxic and bioactive chemicals, abets respiratory pathogens by suppressing immune defenses and by augmenting bacterial virulence (12, 13). Although much is still to be revealed about the complex interplay between immune effectors and bacterial virulence factors in the presence of CS, with every new discovery we identify novel cellular and molecular effectors that potentially can be harnessed therapeutically to alleviate respiratory infections like TB in individuals exposed to CS. However, additional studies are required to assess the effects of CS exposure on autophagy and Treg cell functions in smokers infected with *Mtb*.

In conclusion, the current article shows that nicotine interacts with nAChR on the surface of macrophages and impairs their anti-*Mtb* defense by direct (suppression of autophagosomal killing) as well as indirect (induction of immunosuppressive FoxP3 [Treg] cells) mechanisms. Establishing nicotine as an important immunomodulatory component of CS is highly relevant from a public health viewpoint because nicotine is the principal component of the e-liquid contained in the cartridges of various electronic nicotine delivery systems that are gaining in popularity among the youth in the United States (14). Nevertheless, a limitation of Bai and colleagues' study is their exclusive use of nicotine. Further studies are required to demonstrate whether other key components of CS, including acrolein and reactive oxygen intermediates, suppress anti-*Mtb* immunity in macrophages through similar mechanisms. Another limitation is their use of human (THP-1) and mouse leukaemic monocyte-macrophage (RAW 264.7) cell lines. Thus, the data need to be validated in human and mouse primary macrophages. Future studies are also needed to determine whether nicotine regulates inhibition of phagosome-lysosome fusion or escape from the phagosome to the cytoplasm by *Mtb*. Although macrophages are critical for the immune defense against *Mtb*, the role of the nAChR-CAP axis in the anti-*Mtb* defense cannot be mediated solely by macrophages, as other myeloid and stromal cells reside in the lungs. Finally, the *in vitro* findings need be replicated in an *in vivo* model using CS (firsthand or secondhand) to demonstrate their true clinical relevance. ■

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