In vivo inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of Mycobacterium tuberculosis


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Mycobacterium tuberculosis continues to cause devastating levels of mortality due to tuberculosis (TB). The failure to control TB stems from an incomplete understanding of the highly specialized strategies that M. tuberculosis utilizes to modulate host immunity and thereby persist in host lungs. Here, we show that M. tuberculosis induced the expression of indoleamine 2,3-dioxygenase (IDO), an enzyme involved in tryptophan catabolism, in macrophages and in the lungs of animals (mice and macaque) with active disease. In a macaque model of inhalation TB, suppression of IDO activity reduced bacterial burden, pathology, and clinical signs of TB disease, leading to increased host survival. This increased protection was accompanied by increased lung T cell proliferation, induction of inducible bronchus-associated lymphoid tissue and correlates of bacterial killing, reduced checkpoint signaling, and the relocation of effector T cells to the center of the granuloma. The enhanced killing of M. tuberculosis in macrophages in vivo by CD4+ T cells was also replicated in vitro, in cocultures of macaque macrophages and CD4+ T cells. Collectively, these results suggest that there exists a potential for using IDO inhibition as an effective and clinically relevant host-directed therapy for TB.

Macaca | tuberculosis | granuloma | IDO | T cell

There is an urgent need to improve antitubercular treatment strategies. Tuberculosis (TB) continues to result in close to two million deaths worldwide on an annual basis, and is the single biggest killer of AIDS patients (1). Additionally, ~10% of newly diagnosed patients exhibited disease with some resistance to anti-TB drugs, ranging from multidrug-resistant to extensively drug-resistant TB (2). The failure to control TB stems from the lack of relatively poor understanding of both pathogenesis and the host factors that contribute to the susceptibility of TB disease. However, nonhuman primates (NHPs) recapitulate the complete breadth of the lung pathology and granulomatous responses that are emblematic of human disease (3). The granuloma is the site of host-Mycobacterium tuberculosis interactions, which either result in acute infection or the control of infection in a latent state (4). M. tuberculosis modulates these immune interactions to inhibit mycobacterial killing and therefore promote long-term survival of the bacillus. The expression of indoleamine 2,3-dioxygenase (IDO) is dramatically enhanced in macaque granuloma (5). IDO catabolizes Tryptophan (Trp) to kynurenine (Kyn) and other metabolites, and acts to suppress the immune response, particularly the CD4+ T cell production of IFN-γ (6). Induction of host IDO is a nascent strategy to starve pathogens of Trp, an essential amino acid (7). However, M. tuberculosis can synthesize its own Trp de novo (8), potentially an adaption for its survival during Trp catabolism by IDO in host phagocytes. Therefore, IDO production has little effect on mycobacterial metabolism and yet impacts protective host immune responses.

Here we demonstrate that increased IDO1 expression correlates with higher bacterial burden. Furthermore, IDO is particularly enriched in the macrophage-rich inner layer of the granuloma (5). This spatial expression may prevent lymphocytes, which are predominant in the external layers of the granuloma, from reaching the infected phagocytes, and this inhibition may further promote bacterial survival. We therefore hypothesize that the highly organized granulomas seen in NHPs and humans may be advantageous to M. tuberculosis due to this spatial exclusion.
of immune-protective lymphocytes. As such, the IDO pathway represents a potential target for host-directed therapy (HDT) to augment the control of TB.

Inhibitors of IDO activity [e.g., 1-methyl-tryptophan (1-MT, D-1MT)] are being evaluated as anticancer drugs. In this study, we demonstrated that D-1MT–mediated IDO inhibition resulted in somewhat increased \textit{M. tuberculosis} killing, improved clinical signs of disease, increased lymphoid follicles and proliferation of pulmonary lymphocytes, and was associated with a drastic reorganization of the granuloma that allowed lymphocyte trafficking into the macrophage-tropic internal layers. These results lend significant credence to the utilization of IDO inhibitors as an HDT strategy adjunctive to anti-\textit{M. tuberculosis} chemotherapy (9).

**Results**

IDO1 Is Expressed in a \textit{M. tuberculosis} Burden-Dependent Manner in Infected Phagocytes and Experimental Hosts. We first studied whether IDO levels are induced in a \textit{M. tuberculosis} burden-dependent manner. We found that IDO expression is induced in \textit{M. tuberculosis}-infected murine (C3HeB/FeJ) bone marrow-derived macrophages (BMDMs) (Fig. 1A) and rhesus macaque BMDMs (Fig. 1B and Fig. S1) in vitro and in lungs of \textit{M. tuberculosis}-infected C3HeB/FeJ mice (Fig. 1C and D). Furthermore, IDO expression levels were highly correlated with lung CFUs ($P = 0.02, r^2 = 0.68$) (Fig. 1E). We have recently shown that most animals with latent TB infection (LTBI) that were subsequently coinfectected with simian immunodeficiency virus (SIV), reactivated (10). In coinfectected animals, IDO levels largely
correlated with *M. tuberculosis* burdens (Fig. 1 F and G). Animals with TB exhibited an ~20- to 40-fold IDO induction relative to baseline, whereas the expression was unperturbed in animals that did not reaggregate (Fig. 1 F and G) with high degree of concordance (*P* < 0.0001, *r*² = 0.48) (Fig. 1 H). To effectively demonstrate IDO induction as a correlate of *M. tuberculosis* burden, we measured its expression in macaques receiving chemotherapy. Daily oral administration of moxifloxacin, ethambutol, and pyrazinamide, a multidrug-resistant regimen in humans, reduced IDO1 expression relative to untreated controls (Fig. 1 F and G). Consistent with the above findings, low IDO expression was observed in animals infected with nonpathogenic *M. tuberculosis* (*Mt*) strains *Mt* ΔsigH and *Mt* ΔΔdosR (11–13) (Fig. 1 F and G). Lungs of animals infected with a high dose of *Mt* ΔsigH mutant had reduced IDO levels (Fig. 1 F and G), which again demonstrates that IDO levels were driven by uncontrolled *M. tuberculosis* replication. Unlike *Mt* ΔsigH, *Mt* ΔΔdosR exhibits only partial attenuation, and adaptive immune responses are required for its control (13), likely explaining the intermediate expression of IDO in animals infected with *Mt* ΔΔdosR (Fig. 1G). Hence, IDO is expressed in an *M. tuberculosis* burden-dependent manner. Concomitantly, the expression of the *M. tuberculosis* trpA, trpB, trpD, and trpY genes was induced in macaques with active TB (ATB) relative to LTBI (Fig. I). Thus, Trp biosynthesis is switched on in *M. tuberculosis* in vivo and correlates with host expression of IDO (Fig. I).

**D-1MT Treatment Improves the Clinical Outcomes and Reduces Lung Tissue Pathology.** Macaques recapitulate several aspects of human TB including ATB and LTBI (5, 11–26), HIV coinfection-mediated reactivation TB (10, 15), as well as immune protection (5, 21). We tested the importance of IDO signaling in vivo in acutely infected macaques by treating the animals with D-1MT, a specific inhibitor of IDO activity. Treatment was initiated 1 wk after *M. tuberculosis* infection (Fig. S2). The progression of TB was significantly altered in treated macaques, as reflected by clinical outcomes (Fig. 2). Whereas all of the control animals had to be killed within 5 wk of *M. tuberculosis* infection, D-1MT–treated animals survived until 8 wk, exhibited significantly delayed kinetics, and lower levels of serum C reactive protein (CRP) compared with controls (Fig. 2A). Similarly, treated animals exhibited limited weight loss over time compared with control animals (Fig. 2B). This finding was consistent with the low bacterial burdens detected in treated animals (Fig. 2 C–E). CFUs were determined from bronchoalveolar lavage (BAL) in two groups each at week 1 (the time when treatment was initiated), week 3 (i.e., 2 wk after treatment), and at the end point, at which time *M. tuberculosis* CFUs were also assessed in the lung tissues. The control animals exhibited significantly higher *M. tuberculosis* burdens (*P* < 0.05) in BAL at week 3 and in the terminal lung samples compared with the D-1MT–treated animals. BAL data are shown for at least three animals (Fig. 2C). However, the CFU levels in the BAL did not differ between the two groups at week 1, before initiating treatment, indicating that the initial infections were similar (Fig. 2C). The total bacterial burdens in terminal lungs (Fig. 2D) and bronchial lymph node (BLN) (Fig. 2E) were significantly lower in D-1MT–treated animals than in the control animals (1.0 log, *P* < 0.05). The bacterial burdens were also lower in liver, kidney, spleen in D-1MT–treated animals than controls (Fig. 2E). D-1MT–treated animals presented with fewer granulomas (Fig. 2F) relative to control animals (Fig. 2G) and exhibited significantly lower (*P* < 0.005) lung pathology (Fig. 2H). Hence, animals treated with D-1MT not only had better clinical outcomes (Fig. 2A and B) and reduced pathology (Fig. 2 F and H), but also exhibited reduced bacterial burdens (Fig. 2 C–E). Together, these results underscore our contention that, although active TB developed in all animals, the disease in D-1MT–treated animals progressed more slowly and to a lesser extent.

**D-1MT Treatment Reduces the IDO Enzymatic Activity.** The central area of the BAL cytosin that contained regular, monolayer-distributed cells, as confirmed by H&E staining (Fig. S2), was used for Kyn staining on samples obtained from D-1MT–treated and control animals at week 3. Kyn is one of the end products of IDO enzymatic activity. Numerous studies have implicated it in the immunosuppressive function of this signaling pathway (27). It is also known to be a ligand for the a9 aryl hydrocarbon receptor signaling pathway (28). Confocal microscopy revealed greater levels of Kyn accumulation in controls, relative to D-1MT–treated animals (Fig. 3A). Furthermore, quantification revealed a highly significant difference in Kyn accumulation between D-1MT–treated animals and controls (Fig. 3B). Kyn levels were also significantly higher in D-1MT–treated animals (Fig. 3A). Kyn is one of the end products of IDO enzymatic activity could also be observed in the treated group of animals at later stages (up to week 8) during the infection (Fig. S2F). These results establish that the changes in disease progression in D-1MT–treated animals were correlated with inhibition of IDO.

**Effect of the in Vivo Modulation of IDO Signaling on T Cell Phenotype.** We phenotyped mononuclear cells isolated from dematricized lung and BAL from the end point. Using these samples, we assessed temporal changes in both T cell numbers and T cell phenotypes in the lungs. BAL data at the end point was compared between two groups (Fig. 3 D–H), as previously described (10–13, 16, 20–23). The quantification of memory subsets was established for T cell populations based on CD28 and CD95 coexpression for which a representative flow cytometry plot and
Fig. 3. Assessing the IDO enzymatic activity in M. tuberculosis-infected macaques in vivo and its impacts on T and B cell phenotypes. BAL staining 3 wk after M. tuberculosis-infection; red, Kyn; blue, nuclei; gray, DIC marked with white arrowheads pointing toward the lining that appears a cell membrane indicates Kyn deposition within a cell. [Scale bars, 100 μm (Upper); 20 μm (Lower).] (A) Kyn quantification (B) and Kyn/Trp ratio by ELISA (C). In A, images are shown at different scale with more number of cells in a field from D-1MT–treated animals. Phenotype of memory T cells in BAL and lung samples at necropsy with respect to proliferation as measured by Ki67 positivity in D-1MT–treated (orange) and controlanimals (gray) (D–H). A representative flow-density plot from lungs of memory T cells expressing Ki67 (H). Costaining with CD20 and CD3 exhibits iBALT in D-1MT–treated (L), control animals (L); red, CD20+ B cells; green, CD3+ T cells; blue, macrophages. [Scale bars, 20 μm (L, Left and Right, and J, Right); 40 μm (L, Middle and J, Left).] White box indicates CD3+ T cells (L) and CD20+ B cells (L) found in iBALT follicle. Quantification of B cells in the multiple lesions from both groups: D-1MT–treated (orange circle) and control (gray circle) (K) (means ± SEM). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.001 using a (B and K) Student’s t test or (C) two-way ANOVA or (D–G) repeated-measures t test.

IDO Inhibition in Vivo Causes Broad-Spectrum Improvement in Granuloma Function. We assessed if IDO inhibition improved the function of granulomas in effectively controlling M. tuberculosis infection directly correlates with protection from M. tuberculosis infection (13). Thus, the presence of granuloma-associated iBALT is correlated with protection from M. tuberculosis infection (13). The presence of B cells and their follicular organization were greater in D-1MT–treated animals (Fig. 3I) relative to control animals (Fig. 3I). The total number of B cells enumerated in multiple lesion sections of lung was significantly higher (P = 0.0013) for D-1MT–treated animals compared with controls (Fig. 3K). These results further support our previous observations that protection from M. tuberculosis infection directly correlates with the presence of granuloma-associated iBALT (10, 13, 19, 22).

the primary mechanisms of Trp catabolism-mediated immune dysfunction in the context of M. tuberculosis infection.

As it is well established that Trp catabolism orchestrated by IDO1 inhibits T cell proliferation (33, 34), mediated directly by end products of this pathway (34), we propose that inhibition of T cell proliferation by IDO1 is one of the key mechanisms of granuloma function in vivo.
(Fig. 4 and Figs. S6 A–D and S7 A and B). Host genes involved in the proinflammatory cytokine storm and especially the NF-κB network were induced to higher levels in controls relative to D-1MT–treated animals, highlighting the acute nature of infection in that group (Fig. 4A, two left-most heat maps). The expression of a majority of type I IFN signaling/neutrophil response genes, which are well-characterized biomarkers of active TB (35), was also higher in controls (Fig. 4A). Supervised analyses further revealed signatures of elite granuloma performance in D-1MT–treated animals. These responses were associated with a reduction in T cell inhibitory signaling, including the immune checkpoint inhibitors LG3, CTLA4, IDO1, CD27, and CD244, and so forth, all of which exhibited lower expression in D-1MT–treated animals (Fig. 4A). LG3 expression was detected in CD3+ as well as CD3− T cells (Fig. S7A and B). LG3 is expressed in populations of activated T cells, such as T helper cells and NK cells (23, 36), and in the lymphocyte-rich outer layer of the granulomata during ATB (23), and contributes to the reprogramming of the Th1 response (37). Such an environment is likely to be supportive of M. tuberculosis persistence rather than its clearance. Concomitantly, the lungs of treated animals presented with a signature of enhanced T cell stimulation and function: for example, increased CCR5 [involved in cross-talk between T cells and macrophages via its ligands CCL16 and CCL8 (38, 39)], CCL25 [involved in T cell development (40)] and NFATC [involved in the main long-lived T cell responses (41)] expression. This was accompanied by increased growth receptor and calcium signaling, signifying a rapidly proliferating thymocyte population (Fig. 4A). In contrast, the lungs of control animals exhibited correlates of T cell dysfunction and exhaustion (Fig. 4A), in agreement with other recent work (42). These results indicate that T cell dysfunction and exhaustion in control animals leads to better M. tuberculosis survival than in treated animals (P < 0.05) (Fig. 2E).

The lungs of D-1MT–treated animals concomitantly also expressed more intense antimicrobial responses, characterized by induction of certain CD8+ T cell genes: for example, CTSL (involved in the degradation of pathogens) and other related genes (13, 48) or the proinflammatory cytokine CXCL9 (49). Additionally, expression of MMPs, IL-8, PAK3, and TIAM (13, 48) (Fig. 4A). IL-8 not only augments the ability of leukocytes to phagocytose and kill bacilli, but it also governs T cell recruitment, thus potentially enhancing immunity to M. tuberculosis infection (49). Transcriptomics suggested greater T cell apoptosis in D-1MT–treated animals, and so to verify this we immunostained for the T cell receptor CD3 and performed a TUNEL assay. Immunostaining revealed that animals treated with D-1MT exhibited greater T cell apoptosis (Fig. 4D and E), explaining why there was a comparable number of T cells in both groups (Figs. S4 and S5), despite the higher T cell proliferation in treated animals (Fig. 3 and Fig. S4).

Inhibition of IDO Permits the Reorganization of the TB Granuloma and Allows CD4+ T Cells Access to the Lesion Core. The expression of IDO in M. tuberculosis–infected macaques primarily occurred in the myeloid (inner half) ring of the granuloma and colocalized with the CD68+/CD163+ signal. This result reinforced our belief that antigen-presenting cells (APCs) in the lung express IDO in response to acute M. tuberculosis replication. We hypothesized that this may lead to the reorganization of the tuberculous, such that the CD4+ T cells in the lymphoid (outer half) ring are excluded from the pathogen-rich regions, thereby facilitating greater survival and persistence of M. tuberculosis.

Next, since the expression of IDO specifically occurs in the inner myeloid ring of the tuberculous, we tested the translocation of T cells to the center of the lesion (necrotic center) in lung granuloma of D-1MT–treated and control animals (Fig. 5). Toward this end, we studied if the demarcation between the necrotic center and the lymphocytic layer was more disrupted in the lesions derived from IDO-inhibitor–treated animals relative to untreated animals where it was well defined (Fig. 5 C and D). We found drastic and significant differences (P < 0.05) in the number of CD4+ T cells present in the inner half of the center of the lesions derived from D-1MT–treated relative to untreated animals (Fig. 5 E and F). This result reinforces the role of control animals in the recruitment of CD3+ T cells to the lesion and their migration from the lymphocytic layer to the necrotic center of granuloma were higher in D-1MT–treated animals (Fig. 5E). These results clearly indicate that IDO signaling affects T cell infiltration into the necrotic centers of granulomas. However, quantification of cells staining positive for IDO indicated that the numbers in the macrophage layer were indistinguishable between the groups (Fig. S7). Additionally, anti-M. tuberculosis staining revealed a higher number of bacilli in the lung lesions derived from control- in comparison with D-1MT–treated animals (Fig. 6). Greater bacillary signal was present in the necrotic center of the treated animals compared with the macrophage-rich layer in control animals (Fig. 6). The effector role of CD8+ T cells in the control of M. tuberculosis infection has recently been described (10). In the present study, we determined that the proliferation of central and effector memory CD8+ T cells was significantly enhanced in D-1MT–treated animals than controls (Figs. 3 and 6). Therefore, we sought to better characterize the phenotype of the lymphocytes migrating toward necrotic lesions (Fig. 5E). Approximately 60% of these cells in lesions from D-1MT–treated animals were found to be positive for granzyme B, which was significantly greater than in controls (Fig. 6). A correlation between the frequency of granzyme-expressing T cells and control of human pulmonary TB has been previously described (50). Granzyme B expression on lymphocytes migrating to the lesion core upon IDO inhibition suggests that the latter controls tissue remodeling events. We speculate that IDO
inhibition permits lymphocytes with cytotoxic phenotype to migrate to the center of the tuberculoma and assist in the control of \( M. \) tuberculosis replication. It is possible that some of these cells may be classic CD8\(^{+}\), but the role of other CD3\(^{+}\) populations, such as NKT cells and mucosal-associated invariant T cells, cannot be ruled out. These results indicate that intragranulomatous T cell function is radically altered by D-1MT–mediated inhibition of IDO activity.

We conclude that blockade of IDO signaling leads to significantly better control of \( M. \) tuberculosis infection and reduces the signs of TB disease by promoting the proliferation of memory T cell subtypes and by enhancing the ability of granulomas to kill \( M. \) tuberculosis. Because of the possibility that disruption of granuloma following D-1MT treatment might lead to increased dissemination of \( M. \) tuberculosis to extrapulmonary tissues, we performed CFU assays in BLN, liver, kidney, and spleen at the time of necropsy. The CFU counts in these organs revealed lesser bacterial burdens than controls; however, these numbers were statistically insignificant in liver and spleen but drastically reduced in BLN in

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**Fig. 4** Impact of inhibiting IDO on lung gene expression. Selected modules derived from significantly enriched pathways based on the method described elsewhere (70) in BAL microarray datasets are shown. The horizontal colored bar on top of each module for a category represents a range in gene-expression magnitude in logarithmic base2. Heat-map clusters: “black” to “yellow” to “red,” lower (fold-change –1.5 cut-off) to higher expression (4). The change in gene expression (2\(^{-\Delta\Delta Ct}\) by RT-PCR (B), cytokine assay (C) in lung homogenates of D-1MT–treated (orange) and control animals (gray) relative to uninfected lung samples as base line. GAPDH was used as an internal reference. Bars with no statistics shown are nonsignificant between two groups (e.g., IFN-\( \gamma \)) \( (P = 0.3381) \) (C). Immunofluorescence-based detection of T cell apoptosis by TUNEL assay. The arrowheads (white) in each magnified image indicate apoptotic cells [scale bars, 20 \( \mu \)m (Right); magnification, 20x (Left)] (D). Data obtained by counting multiple fields with T cells positive for TUNEL staining using a Leica confocal microscope (Leica Microsystems) (E). The data (means ± SEM) from animals from both groups were used for analysis; *\( P < 0.05 \), ****\( P < 0.001 \) using a Student’s t-test.
comparison with controls (Fig. 3F). Thus, CFU measurements ruled out that disruption of granuloma by D-1MT treatment does not cause an increase in bacterial dissemination to extrapulmonary tissues, but indeed, these animals have overall lesser bacterial burdens.

**Inhibition of IDO Signaling in Macrophage: CD4+ T Cell Cocultures Restricts Mycobacterial Growth.** We cocultured *M. tuberculosis*-infected rhesus macrophages where IDO expression had been silenced, with *M. tuberculosis*-specific CD4+ T cells in vitro, and measured bacterial burden (Fig. S1) used were from rhesus macaques with acute TB infection. The siRNA specifically affected IDO1 (Fig. S1 F and J) and not IDO2 (Fig. S1 F and K) expression. IDO1 silencing resulted in a greater control of *M. tuberculosis* replication when macrophages were cocultured with CD4+ T cells but not in macrophages alone (Fig. S1E). The silencing of IDO1 resulted in increased levels of IFN-β (Fig. S1 F and K).
and L), while the expression of IFN-γ (Fig. S1 F and M) and the internal control GAPDH (Fig. S1F) was not perturbed.

**Discussion**

*M. tuberculosis* utilizes the host granulomatous response to persist in the face of strong immunity (51), by modulating both innate (52) and adaptive (53) immune responses. The fate of *M. tuberculosis* infection is decided in the granuloma; some lesions affect elite control of *M. tuberculosis* replication via enhanced killing, but others fail, resulting in uncontrolled replication and spread. Therefore, the potential to modulate granulomatous responses in favor of bacterial killing by enhancing natural immunity using HDT exists (54, 55). These HDT approaches can channel the chronic immune dysregulation displayed by granulomas that fail, resulting in counterproductive lung pathology, into productive responses characterized by sterilization of granulomas.

*M. tuberculosis* can overcome the restriction imposed by IDO and the resulting Trp insufficiency, by biosynthesizing this amino acid (8). IDO potently suppresses CD4+ T cells via a variety of mechanisms, including limiting their proliferation (56), induction of immunoregulatory APCs, and by promoting the differentiation of Th0 cells into Tregs (57, 58). Thus, high IDO activity has been correlated with pathogen burden and sepsis during infection (59), especially with intracellular pathogens (60), including *M. tuberculosis* (61). Here, inhibition of IDO activity by a potent yet safe inhibitor in macaques led to a slightly better control of *M. tuberculosis* replication, and somewhat reduced pathology and disease severity, accompanied by increased proliferation of CD4+ and CD8+ memory and effector populations, and the inhibition of lung marker T cell exhaustion and dysfunction. This was associated with reorganization of the granuloma, with T cells otherwise present in the peripheral region of lesions being able to gain greater access to the core region.

It has recently been shown that mycobacterial infection results in the reprogramming of macrophages in the granuloma to a flattened, epithelial phenotype (62). These results, taken together with our study, suggest that the ability of pathogenic mycobacteria to replicate within host lungs is intricately linked to lesion organization, and disruption of this process represents an attractive future strategy for the control of TB.

Our results have implications both for the fundamental understanding of why granulomas are unable to achieve their full potential during *M. tuberculosis* infection and for providing clues to
likely targets of productive HDT against TB, including IDO. These results suggest that the complex and highly ordered architecture of the primate (and human) lung tuberculosis may in fact be beneficial to the pathogen by preventing contact between T cells and pathogen-containing APCs. It may, however, be possible to alter granuloma architecture by inhibiting IDO signaling, and thereby allowing T cells access to the lesion core while also fostering the development of the follicular organization of B cell-containing iBALT. Such lesions appeared to overcome checkpoint inhibition and T cell dysfunction, greatly promoting bacterial killing.

Inhibition of IDO signaling in vivo, as well as in vitro, enhanced the expression of the type I IFNs, although the expression of all type I downstream genes (e.g., IFI1, IFIT2, IFIT3, and OAS1) was not induced. These results are not surprising, given that IDO can be induced by both IFN-γ as well as type I IFN. This regulation of IDO by type I vs. type II interferons is context- and cell-type–dependent (63). It appears that type I IFN plays a major role in triggering IDO expression in primate alveolar macrophages in the context of *M. tuberculosis* infection, and therefore, inhibition of IDO enzymatic activity likely causes induction of type I IFN gene expression via feedback (63, 64). Whereas type I IFN is an important antiviral mechanism (65), its induction correlates with increased lung pathology and exacerbated disease upon *M. tuberculosis* infection (66). As such, approaches targeting type I IFN signaling have been successfully attempted in experimental models of TB (66). Our data suggest that the concurrent silencing of IDO signaling and type I IFN signaling could lead to a more profound control of TB in macaque (and human) lungs. Furthermore, testing the potential of such HDT alone, as well as concurrently with anti-TB chemotherapy, could pave the way for future clinical applications. Our results suggest that therapeutic strategies aimed at eliminating or reducing the levels of cells with IDO induction following *M. tuberculosis* infection, such as myeloid-derived suppressor cells in the lung, may also result in reduction of TB. Finally, we have not discussed as part of this report the conundrum that IDO expression on nonhematopoetic cells following *M. tuberculosis* infection may indeed have a protective effect for the host, as has been shown in the murine model (67). Moreover, several novel IDO inhibitors are being generated (e.g., Indoximod), and it may be possible in future studies to test if they are preclinically superior to D-1MT in suppressing IDO activity.

Materials and Methods

**In Vivo.** Ten rhesus macaques were infected with a high dose of *M. tuberculosis* CDC1551 (~200 CFU) via the aerosol route, as described previously (10–16, 23). Five animals were randomly chosen to be in the treatment group and were D-1MT–treated daily, via the oral route with an IDO enzymatic activity inhibitor, D-1MT (45 mg/kg body weight) 1 wk after *M. tuberculosis* infection. The remaining animals served as controls.

**NHPs, Infection, Sampling, Killing, and Clinical Pathology.** All 10 animals were negative for tuberculin skin test (TST) before infection, but tested positive 3 wk after *M. tuberculosis* infection. Blood and BAL were collected before and after *M. tuberculosis* infection and during the time-course of D-1MT treatment till necropsy. CFUs were measured in BAL 1 wk after *M. tuberculosis* infection and every 2 wk thereafter until the end point. Lung pathology was determined as described previously (11, 15). CFUs were also measured in lung and lymph node tissues derived at necropsy, as described previously (10–16, 23). Human end points were predefined in the animal-use protocol and applied as a measure of reduction of discomfort as described earlier to kill animals as necessary (10, 11, 13). The Tulane National Primate Research Center Institutional Animal Care and Use Committee and the Tulane Institutional Biosafety Committee approved all procedures. All animal procedures were performed in strict accordance with NIH guidelines.

**Immunostaining and Confocal Microscopy.** H&E histology, immunostaining, and confocal microscopy were performed on formalin-fixed, paraffin-embedded tissues, as previously described (5, 10–13, 15, 19, 22, 23, 68–71). Staining for Kyn was performed on fixed (4% formaldehyde) BAL cytospin frozen slides using a Kyn–specific polyclonal antibody (ImmunoSmol). For preparation of frozen BAL slides, 0.1–0.5 million BAL cells were cytospun on a positively charged glass slides (Inform; PerkinElmer) at 700 × g for 7 min at room temperature, followed by washing two times with PBS (1× solution; Thermo Fisher Scientific). Slides were kept for evaporation of residual liquid for 10 min followed by addition of 4% PFA (room temperature) and incubation for overnight and stored in −80°C.

**ELISA.** Trp and Kyn were quantified in blood plasma samples from all animals before infection (baseline) and every week thereafter until necropsy, using a Trp and Kyn ELISA kit strictly as per the manufacturer’s instructions (ImmunoSmol).

**Flow Cytometry.** Flow cytometry was performed on whole blood, BAL, and lung samples from all animals, as previously described (10–13, 16, 20, 21, 23, 26). Briefly, memory subsets were established for T cell populations based on CD28 and CD95 coexpression (72), as previously described (11), and subdivided based on CD3+, CD28+, CD95− subsets being defined as central memory, and CD3+, CD28−, CD95+ being defined as effector memory. Various antibodies and their amount used for staining are described in Table S2.

**Cytokine Assay.** Cytokine assays were performed in lung samples derived at time of necropsy from D-1MT–treated and control animals, as well as from naïve (not infected with *M. tuberculosis* and untreated) lungs (as baseline) following the procedures described earlier (11, 23, 68–71).

**Quantitative Real-Time RT-PCR and Transcriptomics.** Total RNA from BAL obtained at baseline and 3 wk after *M. tuberculosis* infection of two representative animals from each group, was amplified using MessageAmp II aRNA Amplification Kit (Thermo Fisher Scientific) and processed for microarray and qRT-PCR, as described previously (11, 13).

**In Vitro Culturing.** Monocyte derived macrophages (MDMs) were generated from macaque blood and cultured with CD4+’s, as described previously (73). A subset of MDMs was treated with IDO1-specific siRNA 24 h before *M. tuberculosis* infection (multiplicity of infection = 10:1) (Table S1) to inhibit IDO expression, as described previously (71). Samples were collected at 0 and 24 h post-infection (71) and used for CFU assay, qRT-PCR, and immunocytochemistry.

**Statistics.** Unless otherwise stated, statistical analyses were performed with Prism 7 (GraphPad). For statistics, either Mantel–Cox (log-rank) survival analysis, Student’s *t* test with repeated measures, or one-way ANOVA with Bonferroni multiple comparisons was performed. When required, a goodness-of-fit in linear regression was performed for the statistical analysis between two groups.

More details can be found in SI Materials and Methods.

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