Mesenchymal stem cells offer a drug-tolerant and immune-privileged niche to *Mycobacterium tuberculosis*

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Anti-tuberculosis (TB) drugs, while being highly potent in vitro, require prolonged treatment to control *Mycobacterium tuberculosis* (*Mtb*) infections in vivo. We report here that mesenchymal stem cells (MSCs) shelter *Mtb* to help tolerate anti-TB drugs. MSCs readily take up *Mtb* and allow unabated mycobacterial growth despite having a functional innate pathway of phagosome maturation. Unlike macrophage-resident ones, MSC-resident *Mtb* tolerates anti-TB drugs remarkably well, a phenomenon requiring proteins ABCC1, ABCG2 and vacuolar-type H⁺-ATPases. Additionally, the classic pro-inflammatory cytokines IFNγ and TNFα aid mycobacterial growth within MSCs. Mechanistically, evading drugs and inflammatory cytokines by MSC-resident *Mtb* is dependent on elevated PGE2 signaling, which we verify in vivo analyzing sorted CD45⁻Sca1⁺CD73⁺-MSCs from lungs of infected mice. Moreover, MSCs are observed in and around human tuberculosis granulomas, harboring *Mtb* bacilli. We therefore propose, targeting the unique immune-privileged niche, provided by MSCs to *Mtb*, can have a major impact on tuberculosis prevention and cure.

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**Mycobacterium tuberculosis** (Mtbc) continues to infect, cause illness (tuberculosis) and kill a large number of individuals globally. Among numerous factors that thwart any tuberculosis control program, lack of an effective vaccine and long duration of treatment are the two most critical ones. Long treatment duration is majorly attributed behind noncompliance and emergence of drug-resistant tuberculosis including multi- and extensively drug-resistant (MDR and XDRs, respectively) ones. While standard-of-care anti-TB drugs are very efficient in killing Mtbc in liquid culture and during ex vivo infection studies in macrophages, their efficacy is dramatically compromised during in vivo infection studies and in the clinical practices, requiring prolonged treatment duration. It is believed that Mtbc undergoes metabolic adaptations within host granulomas, which render these bacteria less vulnerable to the standard drugs. Driving factors, which cause such adaptations include nitric oxide (NO), redox stress (ROS), low oxygen (hypoxia), low nutrients, or altered carbon source. Curiously, whatever we know about the intracellular lifestyle of mycobacteria in the hosts is mostly through studies on macrophages. Are there additional niches of mycobacteria in vivo, which could facilitate the perceived metabolic adaptations? While there is no clear answer to the above assumption, there are certainly different other cell types which get infected inside the host including lung epithelial cells, macrophages, neutrophils, dendritic cells, adipocytes, and mesenchymal stem cells (MSCs). MSCs are peculiar among these cells since they were first reported to dampen the host immunity against tuberculosis around the granulomas. Subsequently, it was observed that these cells are the site of persistent or latent bacterial infection. Interestingly, latent bacteria are perceived to be more tolerant of anti-TB drugs. Moreover, MSCs are classically known for their immune-modulatory function. Whether MSCs provide a privileged niche to mycobacteria allowing them to withstand the drug and evade host immunity remains unclear. Potential benefits mycobacteria enjoy within these cells continue to remain obscure due to lack of systematic studies on the intracellular lifestyle of Mtbc within MSCs. MSCs can be readily isolated from bone marrow (animals) and adipose tissues (humans) thereby serving as an excellent ex vivo model to study mycobacterial lifestyle in these cells. In this study, using human adipose tissue-derived mesenchymal stem cells (ADSCs), we show that Mtbc not only escapes the effect of anti-TB drugs while residing within ADSCs but also effectively evades host immune mediators. We further establish the mechanism behind these unusual properties of ADSCs and show their relevance during in vivo infection in mice as well as studies on the human granulomas.

### Results

**Adipose-derived mesenchymal stem cells (ADSCs) support mycobacterial growth.** Human primary adipose-derived mesenchymal stem cells obtained commercially were first characterized for the expression of cell-surface markers like CD73, CD44, CD90, CD105, CD271, and the negative marker CD11b (Supplementary Fig. 1a, b). Subsequently their ability to differentiate into three different lineages i.e., adipocytes, chondrocytes, and osteocytes were also characterized (Supplementary Fig. 1c–e). Next, we infected ADSCs with **GFP-H37Rv** (MOI 1:10) with ~80 percent efficiency (see Methods, Fig. 1a). Mean fluorescence intensity (MFI) measurements at 0, 3, 6, 9, and 12 days post infection showed that Mtbc within ADSCs multiplied well (Fig. 1b), which we also confirmed by colony-forming unit (CFU) counts upon plating the bacteria released by lysing the infected ADSCs (Fig. 1c). A time-course growth analysis using CFU counts showed a massive increase in Mtbc CFU at 9 and 12 days post infection (Fig. 1c). Consistent with previous reports from several groups including ours, H37Rv survived well in human primary macrophages and THP-1 derived macrophages (Fig. 1d, e, respectively); however, its multiplication within macrophages was markedly subdued when compared with that observed within ADSCs (Fig. 1). The vaccine strain BCG showed a marked decline in survival within ADSCs by 3 days post infection (Fig. 1f), which was also true in THP-1-derived macrophages (Fig. 1g). Infection with H37Rv did not result in spontaneous differentiation of ADSCs to any of the three lineages mentioned above (Supplementary Fig. 1c–e). A microarray analysis of ADSCs infected with H37Rv for 6 days showed significant regulation of genes belonging to usual functional classes like immune regulation, inflammation, response to stress, transport pathways, and cholesterol metabolism etc. (Supplementary Fig. 2a).

ADSC-resident **Mycobacterium tuberculosis** shows drug-tolerant phenotype. Since MSCs were reported to serve as a site for bacterial persistence we were keen to understand how Mtbc residing within these cells responds to anti-TB drugs. We treated Mtbc-infected ADSCs with different doses of isoniazid (INH; 0.5, 1, and 5 μg/ml) or rifampicin (RIF; 0.1, 0.5, and 5 μg/ml) for 24 h before harvesting the cells and CFU plating on 3rd, 6th, 9th, and 12th days post infection. Even at doses as high as 5 μg/ml for INH, ~5% of Mtbc survived after drug treatment (Fig. 1h). At 0.5 μg/ml as well as at 1 μg/ml of INH nearly 10–15% of Mtbc did not get killed (Fig. 1h, Supplementary Fig. 2b). In case of RIF, almost 50% of bacteria survived at 0.1 μg/ml dose, nearly 20–25% survived at 0.5 μg/ml, which did not go down below 15% even at doses as high as 5 μg/ml (Fig. 1h, Supplementary Fig. 2b). The percentage of bacteria, which survived the drugs, was considered as percent drug-tolerant bacteria. Interestingly, drug-tolerant phenotype (INH or RIF) of ADSC-resident Mtbc was mostly independent of time spent within ADSCs as drug-tolerant H37Rv were observed as early as 3 days post infection and maintained at 6, 9, and 12 days post infection (Fig. 1i, j). At similar doses and for similar duration of treatment (i.e., 24 h) within human macrophages, there were hardly any surviving bacteria in case of INH (1 μg/ml, ~2–4%) while there were nearly 10–15% tolerant bacteria in case of RIF (0.5 μg/ml, Fig. 1i, j). These results suggest that ADSCs provide an environment, which allows Mtbc to tolerate anti-TB drugs.

**Host ABC transporters ABCC1 and ABCG2 play a key role in bacterial drug tolerance.** MSCs are known to express a high level of ABC family transporters or efflux pumps, which are often attributed to drug tolerance in case of cancer. In our microarray data, we did observe a marginal but consistent change in the expression of ABC transporters like ABCC1, also known as MRPI (Supplementary Data 1). Among many ABC family transporters tested by quantitative RT-PCR, ADSCs showed an increase in the expression of ABCC1 and ABCG2 upon Mtbc infection in MOI dependent manner (Supplementary Fig. 3a). Both intracellular as well as surface expression of ABCC1 and ABCG2 were higher in H37Rv-infected ADSCs with respect to the control cells (Fig. 1k). To test whether ABCC1 and ABCG2 were involved in imparting drug tolerance, we used known pharmacological inhibitors against them. Treatment with novobiocin (an ABCG2 inhibitor) or with MK571 (ABC1 inhibitor) led to a decline in the drug-tolerant Mtbc population (Supplementary Fig. 3b, c). Novobiocin, however, is also a well-known DNA gyrase inhibitor and it could kill Mtbc even in vitro in liquid cultures (Supplementary Fig. 3d). Unlike novobiocin, MK571 treatment did not have any effect on Mtbc growth in vitro.
Fig. 1 ADSCs support better H37Rv survival and high drug tolerance. a Representative confocal images of GFP-H37Rv-infected ADSCs at 0 h, 3rd, 6th, 9th, and 12th days post infection (dpi), Scale bars, 20 µm. b MFI of GFP-H37Rv/cell across above mentioned time points is represented in bar graph as mean ± SD, n = 3 independent experiments, each representing five fields consisting of 25–100 cells. c Growth kinetics of H37Rv within human MDMs across 7 days post infection and (e) in THP-1 macrophages across 3 days. f Growth kinetics of BCG within ADSCs and THP-1 macrophages (g) across 0, 1, 2, and 3 dpi. h H37Rv-infected ADSCs were treated with doses of isoniazid (INH), and rifampicin (RIF) for 24 h prior to 3rd, 6th, 9th, and 12th dpi, and were plated for CFU enumeration. n = 3 donors, ****p < 0.0001. i Percent drug-tolerant bacterial population to INH (1 µg/ml) within ADSCs (blue) on 3rd, 6th, 9th, 12th days and within MDMs (purple) on 3rd, 5th, and 7th dpi, respectively (see Methods). n = 3 donors (ADSC), 4 donors (MDM). j Percent drug-tolerant bacterial population to RIF (0.5 µg/ml) within ADSCs (blue) on 3rd, 6th, 9th, and 12th days and within MDMs (purple) on 3rd, 5th, and 7th dpi, respectively. n = 3 donors (ADSC), 4 donors (MDM). k Line histogram of surface and intracellular (I.C.) staining of ABCC1/MRP-1 and ABCG2/BCRP in uninfected and in H37Rv-infected ADSCs, 6 dpi. Numbers represent MFI of individual colored histogram. l Percent INH (1 µg/ml) tolerant bacterial population in ADSCs after knocking down ABCC1 (200 nM siRNA) or ABCG2 (200 nM siRNA) alone or in combination, in H37Rv-infected ADSCs, n = 4 independent experiments. m H37Rv CFU from 6th day infected ADSCs after siRNA mediated knockdown of ABCC1 and ABCG2 for 48 h prior to the time point. n = 4 independent experiments. All data were analyzed by two-tailed unpaired Student’s t test and represented as mean ± SD. Source data are included in the source data file.

( Supplementary Fig. 3e). Since pharmacological inhibitors may still have off-target effects, we knocked down these transporters using specific siRNAs. Knocking down either ABCC1 or ABCG2 led to a substantial decline in the drug-tolerant bacterial population within ADSCs (Fig. 11 and Supplementary Fig. 3f). When ABCC1 and ABCG2 both were knocked down simultaneously, the tolerant bacterial population declined further, reaching nearly 2–4 % (Fig. 11). There was no such effect on drug-
tolerant population when scrambled siRNA was used (Fig. 4f).

While the results with tolerant population did indicate the role of ABCG2 in Mtb drug tolerance within ADSCs, in parallel experimental groups where no drug was used, knocking down ABCG2 and ABCG1 led to a considerable increase in bacterial CFU (Fig. 3m). The increase in bacterial CFU was more when both ABCG1 and ABCG2 were simultaneously knocked down, whereas there was no effect when scrambled siRNA was used as a control (Fig. 3m). Similar results were also obtained when ABCG1 or ABCG2 were inhibited by corresponding pharmacological inhibitors (Supplementary Fig. 4g, h).

Role of lysosomal function in mycobacterial drug tolerance in ADSCs. While ABCG1 and ABCG2 seemed important for drug tolerance, their role in bacterial killing as noted in Fig. 4m was surprising and indicated the presence of an active bacterial mechanism in ADSCs. Interestingly inhibition of vacuolar-type H+ ATPases by bafilomycin A1 (BafA1) also dramatically reduced the drug-tolerant Mtb within ADSCs (Fig. 2a). Another lysosomal acidification inhibitor chloroquine (CQ) had a similar effect (Fig. 2a). However, conditions, which led to increased lysosomal maturation like rapamycin treatment showed glucose-tolerant population at par with the control ADSCs (Fig. 2a). Since rapamycin is a well-known inducer of autophagy, we also verified it using autophagy inhibitor 3-methyladenine (3MA), which expectedly led to a decline in the drug-tolerant population (Fig. 2a). Thus lysosomal function was probably essential to achieve drug-tolerant phenotype within ADSCs. Interestingly, in the absence of INH, conditions, which resulted in reducing the drug-tolerant population, helped bacterial survival. Thus, BafA1, 3MA, and CQ treatment resulted in increased bacterial CFU, whereas rapamycin treatment led to a decline in the CFU suggesting a role of lysosomal killing mechanism in MSCs (Fig. 2b).

The similarity in the phenotype observed by lysosomal acidification inhibitors and ABCG1/ABCG2 inhibition raises the possibility that the role of ABC proteins observed here could have more to do with the lysosomal function rather than the efflux activity at the cell surface. Before further exploring into the mechanism of drug tolerance within ADSCs, we wanted to compare this phenomenon with the reported instances of drug tolerance in macrophages.

Effect of inflammatory cytokines IFNγ and TNFα on drug tolerance within ADSCs. In macrophages, activation with inflammatory cytokines is known to induce drug-tolerant phenotype of Mtb. We reconfirmed, in THP-1 macrophages, treatment with IFNγ or TNFα led to a substantial increase in the drug-tolerant population from ~3–4% in control to 30–40% in the activated cells (Fig. 2c). At similar doses of these cytokines, in the absence of drug, nearly 50% of the bacteria got killed, in agreement with the antibacterial state these cytokines impart to the activated macrophages (Fig. 2d). In ADSCs, IFNγ treatment at 5, 12.5, and 25 ng/ml led to an increase in the INH-tolerant population (Fig. 4c). In the case of TNFα treatment, INH-tolerant Mtb population was significantly higher at 20 ng/ml (Fig. 2e). More startling observation, however, was the case where Mtb-infected ADSCs were treated with these cytokines in the absence of drugs. There was nearly dose-dependent increase in the bacterial CFU upon treatment of Mtb-infected ADSCs with IFNγ or TNFα (Fig. 2f). The pro-bacterial effect of IFNγ and TNFα on Mtb-infected ADSCs was specific to the stimulus and corresponding downstream signaling since upon neutralization with purified IFNγR1 or with anti-TNFα antibody, we could revert the pro-bacterial effect of IFNγ or TNFα stimulation on bacterial CFU (Fig. 2g). Expectedly, with a similar neutralization experiment in THP-1-derived macrophages, there was a rescue of Mtb from cytokine-mediated killing (Fig. 2h). Thus Mtb-infected macrophages and MSCs respond in a contrasting manner to IFNγ and TNFα stimulus.

Analysis of intracellular niches of Mtb shows classic phagosomal maturation dynamics in ADSCs. Results from conditions like ABCG1 or ABCG2 knockdown, BafA1 treatment or IFNγ or TNFα treatments, all of which led to an increase in the bacterial survival suggest that ADSCs, despite supporting robust growth of the bacteria, keep actively killing the bacilli. To check whether phagosomal maturation pathways as observed in macrophages are operational in a similar fashion during Mtb infection in ADSCs, we assayed for Mtb colocalization with early phagosomes (RAB5), late phagosomes (RAB7), lysosomes (LAMP1), and acidic lysosomes (LAMP1 and LysoTracker). At any given time post infection, a large number of bacteria (~40–50%) stayed within RAB5 positive early phagosomes inside the ADSCs (Supplementary Fig. 2a). While only 2–5% of bacteria were ever present in the RAB7 positive late phagosomes (Supplementary Fig. 2b). A similar distribution of Mtb is also reported within macrophages by others and also confirmed by us38 (Supplementary Fig. 3c). This reflects the phagosomal maturation arrest inflicted by Mtb in the infected macrophages.39,40

However, unlike macrophages where LAMP1-Mtb or Lysotracker-Mtb colocalization rarely crosses ~15%,27 there are more bacteria (~30–40%) present in LAMP1 or Lysotracker-LAMP1 double-positive compartments in ADSCs (Fig. 2i, j, Supplementary Fig. 4d). The matured lysosomes, i.e., LAMP1 positive acidified compartments indeed reflect the killing mechanism in ADSCs since ~80% of intracellular BCG, the strain that gets killed within ADSCs, are present in Lysotracker-LAMP1 double-positive compartments (Fig. 2k). Interestingly, treatment with IFNγ, TNFα, or MK571, in general, led to a decline in bacterial localization to LysoTracker or LAMP1 + LysoTracker compartments; however LAMP1 compartment alone showed only marginal decline (Fig. 2i, Supplementary Fig. 4d). Exclusion of Cathepsin D (CatD), the lysosomal protease, from LAMP1-Lysotracker-positive compartment strongly correlated with increased bacterial survival upon IFNγ- or MK571-treated cells (Fig. 2l, Supplementary Fig. 4d). Interestingly, ABCG1 and ABCG2 were also found to co-localize with Mtb suggesting their recruitment to the phagosomes (Supplementary Fig. 4e). At least in the case of TNFα treatment, exclusion of ABCG1 from the LAMP1-Lysotracker compartment was highly significant, whereas IFNγ or MK571 treatment showed only marginal decline (Fig. 2m) leaving an impression that ABCG1 is probably directly involved in bacterial killing. However, their direct role in Mtb killing is supported by only one set of evidences—increased bacterial survival upon their knockdown or inhibition. The strong correlation between bacterial killing and their colocalization to LAMP1-Lysotracker-CatD compartment nonetheless suggest an active role of canonical phagosomal maturation pathways in the bacterial killing within ADSCs.

Interestingly, in contrast to what is known in macrophages,27 Mtb very rarely co-localized to the autophagosomes and xenophagy flux was completely absent in ADSCs (Supplementary Fig. 5a), suggesting little role if any, of autophagy in controlling Mtb within ADSCs. Curiously, ADSCs showed very high basal autophagy flux (Supplementary Fig. 5b), which is critical for maintaining the stem cell like property of these cells, highlighting the segregation of homeostatic, and antibacterial arms of autophagy as reported by us earlier.41 Moreover, treatment with IFNγ led to increased autophagy flux in ADSCs, unlike what was reported previously for macrophages (Supplementary Fig. 5e). Unlike macrophages, IFNγ treatment had no effect on cellular ROS generation in ADSCs (Supplementary Fig. 5d). Combined with the colocalization results
discussed above, these results largely explain why IFN\(\gamma\) failed to induce killing of \(Mtb\) within ADSCs.

The lipid mediator PGE2 helps MSCs exhibit pro-bacterial attributes. Results so far establish that MSCs are uncharacteristically pro-bacterial, at least during mycobacterial infections, helping them evade anti-TB drugs as well as classic host immune mediators like IFN\(\gamma\) and TNF\(\alpha\). However, there still was no clue on how MSCs execute these behaviors. To understand the mechanistic basis of the observed results, we went back to our microarray.
data to identify genes showing significant regulation upon Mtbf infection in ADSCs. The anti-inflammatory as well as immune-modulatory functions of MSCs are well known; however, in all such known cases, MSCs execute its role by modulating functions of other cells, including T cells and macrophages. Some of the key mediators that help MSCs execute these functions are PGE2, IDO1, IL6, CCL2, VEGFC, LIF etc.24,45 In our microarray data, genes from the PGE pathway like PTGS2, PTGES, and PTGR2 showed nearly eight-, four-, and fourfolds (log2) increase in expression, respectively (Supplementary Fig. 6a). Similarly, IDO1 showed sixfolds increase, whereas LIF, IL6, CCL2, and VEGF each showed more than threefold increase in expression post infection (Supplementary Fig. 6a). We first tested PGE2 levels in the culture supernatants of ADSCs that were infected with H37Rv. Consistent with the microarray data PGE2 ELISA confirmed increased synthesis and secretion of PGE2 from Mtbf-infected ADSCs (Fig. 3a). This was true for ADSCs obtained from multiple independent donors (Fig. 3a). Interestingly, treatment with IFNy or TNFa further increased PGE2 levels in the culture supernatants whereas MK571-treated cells showed almost similar level of PGE2 as infected control cells (Fig. 3a). We used celecoxib, a widely used PTGS2 (or COX2) inhibitor, which is also an FDA approved drug in the market, as a negative control. Mtbf-infected ADSCs treated with celecoxib showed negligible PGE2 levels by ELISA (Fig. 3a). Next, we treated Mtbf-infected ADSCs with celecoxib at 50, 150, and 250 μM concentrations under all the conditions tested so far in this study. Treatment with celecoxib reduced Mtbf CFU in a dose-dependent manner across the conditions including infection alone or when treated with IFNy, TNFa, or MK571 (Fig. 3b). This result was tested on ADSCs from three independent donors and each of them showed similar results (Fig. 3b). Similar results were also obtained with EP2 receptor (receptor for PGE2) antagonist PFO4418948, suggesting the involvement of signaling through the PGE2 pathway in bacterial survival (Supplementary Fig. 6b). Celecoxib was also effective in killing Mtbf within macrophages however unlike in ADSCs, it did not show any dose-dependent killing in macrophages at the tested doses (Supplementary Fig. 6c). Unlike ADSCs, there was no increase in PGE2 release by THP-1 macrophages upon infection or treatment with IFNy or TNFa (Supplementary Fig. 6d). We also verified these results by knocking down PTGS2 (COX2) using specific siRNAs (Fig. 3c and Supplementary Fig. 6e). Co-transfection with pCMV-βglucuronidase plasmid confirmed that a majority of the cells in celecoxib-treated cells were pCMV-βglucuronidase positive. Interestingly, COX2 inhibition by celecoxib also helped limit the drug-tolerant phenotype in ADSCs against INH, irrespective of treatment with IFNy or TNFa (Fig. 3e). Moreover, MK571, which itself decreases drug-tolerant Mtbf in ADSCs when combined with celecoxib treatment further reduces the drug-tolerant population of Mtbf within ADSCs (Fig. 3e). The effect of celecoxib on bacterial drug tolerance was PGE2 mediated and not due to a possible role of certain COX2 inhibitors directly on bacterial drug-resistance protein MDR1 since knocking down COX2 also led to a remarkable decline in INH tolerant as well as rifampicin-tolerant Mtbf population within ADSCs (Fig. 3f, g, respectively). Efficacy of COX2 knockdown by siRNA on bacterial drug tolerance also rule out the role of PGE2 inhibitors in directly regulating the efflux proteins as reported previously.45

MSCs serve as a niche for Mtbf during in vivo infection allowing drug tolerance in PGE2 dependent manner. While all the results so far were performed on human primary adipose tissue-derived mesenchymal stem cells, we next wanted to explore whether these cells get involved during in vivo infection in mice and humans as well as to know whether PGE2 signaling plays a similar role in vivo. We infected C57BL/6 mice with H37Rv through aerosol challenge and 4 weeks post infection, these animals were divided into four groups: control, celecoxib (50 mg/kg), INH (10 mg/kg), or INH + celecoxib (10 and 50 mg/kg, respectively); and treated for subsequent 4 and 8 weeks. We deliberately used lower INH doses (10 mg/kg instead of 25 mg/kg) in order to observe the additive effect of celecoxib during combination treatment. From the initial bacterial load of 100–150 per animal, it reached around 2 × 106 per animal by the end of 4 weeks, ~3 × 106 by the end of 8 weeks and ~5 × 106 by the end of 12 weeks in the lungs (Fig. 4a). While celecoxib treatment alone did not significantly reduce the bacterial load at 4 weeks or 8 weeks of treatment (Fig. 4a). INH treatment brought the bacterial CFU significantly down at 8 weeks post treatment (Fig. 4a). Animals, which received both celecoxib and INH showed a more significant reduction in bacterial CFU in the lungs at both 4 and 8 weeks post treatment (Fig. 4a). Similar results were also obtained in the spleen (Fig. 4b). The combination treatment was significantly more effective with respect to INH or celecoxib alone in controlling bacterial load in both lung and spleen at 8 weeks of treatment (Fig. 4b). We next sorted lung tissues from the infected animals at each time points and treatment groups into CD45+CD11b+CD11c+ (MSCs) and CD45+Ly6G−CD11b+ (macrophages) cells with a purity of more than 90% (Fig. 4c and Supplementary Fig. 7a). The sorted population was further characterized with additional cell-surface markers for macrophages and MSCs, respectively. Thus CD11b+ cells were confirmed Ly6G−, CD11c+, Ly6C+, and MHCII+ (Fig. 4d). Similarly, CD73+ cells were CD11b−, CD44+, CD90+, and CD105+ (Fig. 4d). The population of macrophages across all treatment groups declined from a
maximum at 4 weeks to 8 weeks and subsequently to 12 weeks post infection (Fig. 4e). The population of MSCs was also highest at 4 weeks post infection, which declined subsequently at 8 and 12 weeks post infection (Fig. 2f). In control uninfected animals, macrophage and MSC population was relatively lower than the infected ones at either 4 or 12 weeks post infection (Supplementary Fig. 7b). Lysing and plating these sorted cells on 7H11 media showed the presence of Mtb in both macrophages and MSCs.
Counts

Statistical analysis was done Mann–Whitney U test. Source data are included in the source data file.

(Fig. 4g, h). The number of bacilli in both cells progressively increased from week 4 to week 12, shown as the number of bacilli per 10,000 cells (Fig. 4g, h). The macrophage-resident Mtb were not affected by celecoxib at 4 weeks of treatment however showed a significant decline at 8 weeks post treatment (Fig. 4g). Similarly, the effect of INH was more pronounced in macrophages at 8 weeks post treatment (Fig. 4g). Animals which received both INH and celecoxib showed very significant decline in macrophage-resident Mtb at both 4 and 8 weeks post treatment, which was also marginally but significantly lower than INH alone group at 8 weeks post treatment (Fig. 4g). For MSC-resident Mtb, the effect of treatments was mostly similar to that observed in macrophages except for two interesting observations (Fig. 4g).

First, consistent with the ex vivo results, the effect of INH alone on MSC-resident Mtb was relatively less in magnitude when compared with that on macrophage-resident Mtb at 12 weeks post infection (Fig. 4h). Secondly, supplementing INH with celecoxib had a more dramatic effect on MSC-resident Mtb than macrophage-resident ones (Fig. 4g). Thus in vivo results from mouse mostly followed the observations obtained from ex vivo studies with human ADSCs.

MSCs are present in human extra-pulmonary and pulmonary tuberculosis granulomas. The results so far establish that MSCs serve as a niche for Mtb providing drug and immune-privileged niche, in PGE2 dependent manner both ex vivo and in vivo in...
animals. We next analyzed the presence and spatial localization of CD73+ cells with respect to Mtb in tissue sections from pulmonary and extra-pulmonary TB lesions from human subjects. CD73+ cells were found in and around extra-pulmonary (gut) and pulmonary tuberculosis lesions (Figs. 5a, b, respectively). Intestinal biopsy samples were taken from granuloma-positive confirmed intestinal tuberculosis (ITB) patients. In lung biopsies, in addition to CD73, we also stained for Ag85B of Mtb (Fig. 5b and Supplementary Fig. 7c). At lower magnification presence of CD73+ cells in and around granulomas was distinctly visible (Fig. 5b). At higher magnification, we could observe presence of CD73+ positive cells, which were also positive for Ag85B, suggesting presence of Mtb within these cells (Fig. 5c). We further confirmed the presence of Mtb-infected MSCs in lung granuloma sections by immunofluorescence using two different markers for MSCs-CD73 and CD105 (Fig. 5d, e and Supplementary Fig. 7d). All these experimental evidences conclusively demonstrate the presence of Mtb-infected MSCs in human tuberculosis granulomas.

Discussion

Almost everything that we know about the intracellular lifestyle of Mtb largely emerged through studies on macrophage models. The host responses and the mechanism of immune evasions are also studied keeping in mind macrophages as the primary cells where the bacteria reside. The present study was undertaken to understand how MSCs could facilitate mycobacterial persistence in the host as reported by others. This required us to explore the intracellular lifestyle of Mtb within MSCs, and not much is known about it. The immune-modulatory properties of MSCs are well known including during Mtb infection. However, in a majority of cases, the immune-modulatory effects of MSCs are studied in trans i.e., on a different cell type, which is mediated by effectors released from MSCs. Whether the innate ability of MSCs play a role in mycobacterial persistence and if these cells exhibit any cell-autonomous immune-modulatory properties, is not known. Interestingly, only virulent strain H37Rv could survive and divide well within ADSCs while BCG got killed, suggesting the presence of active innate defense mechanism in these cells. One critical aspect of mycobacterial persistence is tolerance to anti-TB drugs, which is driven by the host environment like macrophage residence, macrophage activation, low oxygen within granulomas, NO etc. Our finding that MSC-resident Mtb was tolerant to anti-TB drugs underscores the physiological advantage that these cells possess in order to harbor persistent infection as reported previously. Since adult stem cells are known to have high efflux activity via ABC transporters, which helps in drug tolerance in cases like cancer, we questioned whether these efflux proteins could also help to throw out anti-TB drugs, thereby helping in drug tolerance. Our results indeed show increased expression and involvement of ABC1C and ABCG2, in drug tolerance, both of which acted independently since their combined effects were greater than individual effects. However, inhibition of vacuolar-type H+ ATPase by BafA1 led to a more dramatic decline in drug-tolerant population, suggesting phagosomal environment to be the key factor behind drug tolerance. Interestingly, phagosomal pH is also known to alter the redox physiology of Mtb leading to drug tolerance in macrophages, suggesting some convergence in the mechanism of bacterial drug-tolerance in MSCs and macrophages. An increase in bacterial CFU upon ABC1C or ABCG2 inhibition/knockdown in the absence of INH indicated the function of these proteins other than cellular efflux of drugs. Their recruitment to bacterial phagosomes indeed points to such a possibility especially since their recruitment to the phagosomes largely correlated with bacterial killing. ABC proteins are known to have several moonlighting functions including nuclear translocation, redox balance and antigen presentation. Results suggest, at least in MSCs, they are also involved in bacterial killing in the phagolysosomal system. Whether it is associated with lysosomal acidification or transport of bactericidal effectors remains to be uncovered. It is however, possible that ABC proteins are actively excluded from getting recruited to Mtb phagosomes while being present on other endolysosomal vesicles, where through their inward transport activities sequester certain antibacterial effectors including anti-TB drugs, away from mycobacterial phagosomes in isolated vesicles. This could potentially explain why knocking down or inhibition of ABC1C or ABCG2 helps increased bacterial survival. At present we have no evidence to support whether these effectors could be H+ ions, oxidized glutathione or glutathione metal adducts, ubiquitin-derived peptides, or any other antimicrobial peptides; each of which is capable of killing the bacteria and also known targets of ABC proteins-mediated transport across biological membranes.

Mycobacterial drug tolerance can also be induced in vitro or ex vivo in macrophages. In vitro, Mtb develops drug tolerance under stress conditions like hypoxia, NO, nutrient stress etc. There are also reports, which suggest mere macrophage residence for a few hours is sufficient to induce drug tolerance in Mtb. Yet another study reported increased bacterial drug tolerance in activated macrophages. Similarly, dependence of antibiotics on pH for bacterial killing is an emerging field of investigation. More recently through a chemical screening approach, a compound was identified which help curtail bacterial tolerance to oxidative, acid and drug stress. The common thread across these studies is that when Mtb witnesses stress whether in vitro or in vivo, it activates a set of genes which inadvertently also helps them tide-over the effect of drugs. This partially explains the loss of antibacterial effects of IFNγ in MSCs and macrophages. Although, it does not explain increased bacterial survival upon inflammatory stimuli. However similar to what we noted about ABC1C or ABCG2 inhibition; pro-bacterial effects of IFNγ and TNFα had mostly to do with the lysosomal killing. Since each of these treatments was for the final 24 h before CFU plating was done, it cannot reflect increased bacterial replication rather show diminished bacterial killing. This observation however, brings an exceptionally worrisome insight on the problem of poor efficacy of every vaccine candidates tested so far. While there are several vaccine candidates at different stages of development against tuberculosis to replace or enhance BCG efficacy, the only commercially available vaccine, a closer look at each of the vaccine candidate shows that immunological parameters considered as...
Fig. 5 Mtb co-localizes with MSC in human pulmonary and extra-pulmonary granulomas. a CD73 staining of biopsies from granuloma-positive intestinal tuberculosis patient, showing polarization of CD73-positive cells around the submucosal macrogranulomas (×100 (left panel, scale bar = 100 µm), ×200 (Right panel, scale bar = 50 µm)). b CD73 and Ag85B dual staining performed on lung biopsy tissue from patients with pulmonary tuberculosis showing polarization of CD73+ cells (green arrows) toward the granulomas. Ag85B-positive organisms (black arrows) are seen inside the granulomas (×100, scale bar = 100 µm). c Representative of two independent human lung biopsies, Ag85B-positive organisms (brown color) are seen inside the histiocytes (black arrows), CD73+ cells are stained with blue chromogen (green arrows) and the cells showing both positivity for CD73+ and Ag85B+ organisms have been represented by red arrows (×400, scale bar = 20 µm). Insets below show the corresponding magnified CD73+ cells showing positivity of Ab85B staining. d Immunofluorescence staining performed on formalin-fixed paraffin-embedded (FFPE) tissue of human lung biopsies from patients with known tuberculosis show green fluorescence for Ag85B+ (green arrows), red fluorescence for CD73+ cells (red arrows) and colocalization signals are marked with white arrows. The strong colocalization area is shown in the yellow inset and magnified in the panel at the right. In the further right panel, corresponding green and red channel fluorescence is shown. Scale bar is 10 µm. e FFPE tissue processed for dual IF staining show Ag85B+ only (green arrows), CD105+ MSCs (red arrow) and cells positive for both CD105 and Ag85B (white arrow). The strong colocalization area is shown in the yellow inset and magnified in the panel at the right. In the further right panel, corresponding green and red channel fluorescence is shown. Scale bar is 10 µm. Data shown in this figure are representative of seven independent experiments.
the correlates of protection are common across them. Thus, whether it is MTB-HVAC or TB/FLU-04L, Ad5Ag85A, MVAg85A, or others, they all rely on generating strong INFγ producing CD4+ and/or CD8+ T cells. However given the unconventional pro-bacterial effects of IFNγ on MSCs, these vaccines can only generate an immune response that kills bacterial population in macrophages but not in MSCs thereby blunting the efficacy.

How Mtb enjoys such a privileged lifestyle within ADSCs became finally apparent through the microarray analysis revealing a massive increase in the synthesis and secretion of PGE2 by infected ADSCs. PGE2 is a multifunctional effector, with diverse roles in immune regulation. PGE2-mediated immunomodulation of other cells by MSCs has also been extensively reported. Here we report a unique autocrine immune-modulatory function of PGE2 in MSCs. Inhibiting PGE2 signaling was able to reverts the pro-bacterial effects of IFNγ, TNFα, or MIF on, suggesting PGE2 as the converging factor, which helps better bacterial survival within ADSCs. In contrast to the pro-bacterial role of PGE2 observed by us, several studies in the past report protective role of PGE2 against tuberculosis. Similarly, EP2 receptor knockout mice also show increased bacterial burden in the lungs. Interestingly, PGE2 treatment is more effective in controlling lung CFU only in hyper-susceptible animals lacking IL1R1, with absolutely no effect in WT animals. On the similar line, WT animals and ptgs2 animals (lacking the enzymatic activity) did not have any difference in bacterial survival. On the other hand, during the late phase of mycobacterial infection and not during the early phase of infection, COX2 inhibition has protective effects in vivo. PGE2 is also known to inhibit antibacterial effector functions of phagocytes including phagocytosis, NO production, lysosomal killing and antigen presentation. Incidentally, aspirin is currently in the clinical trial as adjunct therapy against tuberculosis meningitis in adults. Most COX2 inhibitors, specially nonsteroid anti-inflammatory drugs like aspirin, ibuprofen, rofecoxib, and celecoxib etc. are routinely used for controlling diverse inflammatory states. Results from our experiments suggest smart inclusion of COX2 inhibitors in standard tuberculosis treatment/prevention regimens could enhance the efficacy of treatment. Presence of MSCs in human granulomas, in both pulmonary and extra-pulmonary tuberculosis cases, provides a valid basis to test such combinations for clinically favorable outcomes.

The two major hurdles in the tuberculosis control program are (a) lack of effective vaccine and (b) highly diminished efficacy of anti-TB drugs in vivo with respect to in vitro. This study shows MSCs contribute to both these crucial aspects of tuberculosis control. The remodeling of lung granulomas during tuberculosis has been explored previously. However, we show that recruitment and infection of MSCs in the granulomas could be critical events during remodeling considering the lifestyle of Mtb in MSCs is radically different than those in macrophages. The study therefore also highlights the limitations of reliance on ex vivo data generated through macrophage infection experiments in the past. We believe targeting the immune-privileged environment of MSCs will help develop alternative strategies to enhance both treatment and vaccine efficacy.

Animal experiments were approved by Institutional Animal Ethics Committee, ICGB (ICGB/IAEC/280718/CL--14).

Reagents, antibodies, and plasmids. Phthorbol 12-myristate 13-acetate (PMA), bafilomycin A1, rapamycin, 3MA, COX, PKH, Mtb drugs (rifampicin, and pyrazinamide), chemical inhibitors (MK371, novobiocin, cebicox, DMSO, BSA, MTT (1–4-dimethylthiazole-2-y)-3,5-diphenylformazan), and paraformaldehyde were obtained from Sigma Aldrich Co (St Louis, MO, USA). All IR conjugated secondary antibodies were procured from Invitrogen Molecular Probes, Carlsbad, CA, USA. PGE2 ELISA kit, istorizid, propidium iodide (PI) were from Camyan Chemical, USA. Lysotracker red, JC-1, and cellrox green were from Life Technologies, USA. Human IFN-γ and human TNF-α were purchased from ebioscience, sIKRAs (ABCI,CABC2, CACON). C2C12 myocytes from GE DHARMACON. Safranin O, Oil Red O and Alizarin Red S were purchased from SRL chemicals. All antibodies and their corresponding details are provided in the table below (Table 1).

Cell culture. ADSCs were purchased from life technology (cat no.—R7878115) and maintained in memsPRO RS media (cat no. 12746012) supplemented with growth factors at 37°C, 5% CO2, humidified incubator as per the manufacturer instructions and guidelines. For all in vitro experiments, ADSC were seeded at the required density, allowed to adhere to the surface for 24–36 h before proceeding with the experiment.

Human MDMs were isolated from human PBMCs, which in turn were separated from blood of healthy human donors using ficoll-layering and centrifugation. Briefly, heparinized blood was diluted in 1:1 ratio by volume with DPBS. Diluted blood was layered on Ficoll-paque (Himedia) and centrifuged at 2000 rpm for 45 min. Interface containing PBMC was isolated carefully and washed twice with DPBS. Cells were diluted in RPMI 1640 media containing 10% fetal bovine serum (FBS) to a concentration of 1 × 106 cells/ml. Cells were put in a six well tissue culture plate and incubated for 3 h in a humidified 5% CO2 chamber at 37 °C. Non adherent cells were removed followed by two washes with RPMI. Complete media containing 50 ng/ml recombinant human M-CSF (R&Dsystems, 216-MC/CF) was added and cells were allowed to differentiate for 7 days into macrophages in a humidified 5% CO2 chamber at 37°C. Human monocyteic cell line THP-1 were obtained from American Type Culture Collection (ATCC) and cultured in RPMI 1640 medium along with 10% FBS at 37°C, 5% CO2 humidified incubator. THP-1 derived macrophages (dTHP-1) were obtained by treating THP-1 cells with 20 ng/ml phorbol myristate acetate (PMA, sigma) for 24 h followed by washing and maintenance in complete media.

Mycobacteria. M. tuberculosis, M. bovis, and M. avium were cultured and maintained in 7H9 medium supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC). M. smegmatis was cultured in L-72 media. All bacterial cultures were harvested in mid-log phase (OD600~0.8) and used for infection.

Mycobacterial infection and detection. Freshly made and passaged M. bovis BCG cultures (strain M. bovis BCG C7) were used for infection. M. bovis BCG C7 strain was cultured in 7H9 broth containing Tween 80 and supplemented with 10% ADC. M. avium was cultured in 7H9 broth with Tween 80 and OADC. M. tuberculosis H37Rv was cultured in 7H9 broth containing Tween 80 and OADC. All cultures were routinely counted using a Thoma chamber and fresh cultures were used for infection.

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Methods

Ethical clearance. Studies on human samples were approved by IEC of AIIMS (Ref no. IEC-304-02-06-2017) and ICGB (Ref no. ICGB/IEC/2017-06-ver1 and ICGEB/IEC/2016/03). ITB biopsy samples were obtained after getting the informed consent from the patients. Human lung biopsy samples and control sections were obtained from Department of Pathology, AIIMS, New Delhi. These biopsy samples were taken for diagnostic purpose in these patients with written informed consent. Use of the archived leftover biopsy samples were approved by the institute’s EC, as detailed above. Access to these materials is subject to the institutional guidelines.

Animal experiments were approved by Institutional Animal Ethics Committee, ICGB (ICGB/IAEC/280718/CL--14).

Bacterial cultures and in vitro infection experiments. Bacterial strains: H37Rv seed stock was received from Colorado State University, BCG (Danish strain) was obtained from University of Delhi, South Campus. For in vitro experiments, virulent laboratory strain H37Rv, BCG, and GFP-H37Rv bacterial cultures were
grown in 7H9 media (BD Difco) supplemented with 10% Albumin–Dextrose–Catalase (ADC, BD, Difco) and incubated in an orbital shaker at 100 rpm, 37 °C until the mid-log phase. GFP-H37Rv was prepared by electroporating virulent H37Rv strain with pMN437-GFPm2 vector (Addgene, 32362) and was maintained at 50 μg/ml hygromycin 7H9-ADC media. Single cell suspension required for carrying out infection experiments were prepared by passing bacterial cultures through a series of different gauge needles: 4 samples of bacterial suspensions were prepared by resuspending into fresh media and incubated with cells for infection. To visualize infected cells, MTT solution was added to each well of 96 well plates and incubated for appropriate time in cell incubator. Thereafter were quantified by measuring their absorbance at 560 nm in the plate reader.

Immunoblotting. For western blotting experiments, ADSCs were washed with ice cold PBS before their incubation with Buffer A solution (20 mM HEPES, 10 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, and 0.5%v/v Trition-X-100) with 1X Protease Arrest (G’ Biosciences) for 15 min on ice for lysis. Cell lysate was centrifuged at 4 °C at 6000 g for 10 min and supernatant was collected. Protein quantification was done using BSA as standard in Bradford assay. Protein sample was mixed with 6x loading dye and subjected to SDS PAGE and transferred to nitrocellulose membrane for immunoblotting. Blots were immunoblotted with primary (1:1000) and then with secondary antibody (1:15000) made in blocking buffer. Blots were imaged with Odyssey Infra Red Imaging system (LI-COR Biosciences).

Confocal microscopy. For confocal microscopy experiments, bacteria were stained with PKH67, a green lipophilic dye, according to the manufactures protocol and resuspended in final media and incubated with cells for infection. To visualize acidified compartments, LysoTracker red dye (LysoTracker Red DND-99; Life Technologies) was added to the sample wells at a concentration of 500 nM for 30 min prior to fixation. Cells were fixed in 4% (w/v) PFA for 15–20 min, followed by permeabilization with 0.1% (w/v) Triton X-100 in PBS and then incubation with blocking buffer. The plates were incubated at 37 °C for 1 hour to allow bacterial growth, and counts were performed after 21 days. Drug-tolerant population was calculated as percent CFU with INH treatment is X and INH + IFNγ treatment is Y, % tolerant population would be Y/X × 100.

Table 1: Details of antibodies used.

| Serial No. | Antibody | Cat. no.       | Company                  | Application (dilution) |
|------------|----------|----------------|--------------------------|------------------------|
|            |          |                | IFA/FACS | Confocal | WB | IHC |
| 1          | MAP1LC3B | NB100-2220     | NovusBiological          | 1:200 | 1:1000 |
| 2          | GAPDH    | sc-48167       | Santa Cruz Biotechnology | 1:5000                |    |
| 3          | COX2     | ab62331        | Abcam                    | 1:100                 |    |
| 4          | Rab5     | sc-309         | Santa Cruz Biotechnology | 1:200                 |    |
| 5          | Rab7     | sc-6563        | Santa Cruz Biotechnology | 1:200                 |    |
| 6          | ABCC1    | sc-18835       | Santa Cruz Biotechnology | 1:100                 |    |
| 7          | LAMP1    | sc-20011       | Santa Cruz Biotechnology | 1:200                 |    |
| 8          | ABCG2    | sc-58222       | Santa Cruz Biotechnology | 1:100                 |    |
| 9          | Cathepsin D | ab19555     | Abcam                    | 1:200                 |    |
| 10         | CD73-FITC (clone AD2) | 561254 | BD Bioscience          | 1:100                 |    |
| 11         | CD11b-PE | 557321         | BD Bioscience            | 1:100                 |    |
| 12         | CD271    | ab8874         | Abcam                    | 1:100                 |    |
| 13         | CD44     | M5668P         | Thermo Scientific        | 1:100                 |    |
| 14         | CD90     | ab181469       | Abcam                    | 1:100                 |    |
| 15         | CD105    | ab114052       | Abcam                    | 1:100                 |    |
| 16         | CD45-APC (clone 30-F11) | 559864 | BD Bioscience          | 1:100                 |    |
| 17         | CD73-BV450 (clone TY/23) | 561544 | BD Bioscience          | 1:50                  |    |
| 18         | CD11b-APC-Cy7 (clone M1/70) | 557657 | BD Bioscience          | 1:100                 |    |
| 19         | Ly6G-PE (clone 1A8) | 551461 | BD Bioscience          | 1:100                 |    |
| 20         | Sca-1-PE-CFS94 (clone D7) | 562730 | BD Bioscience          | 1:100                 |    |
| 21         | CD90-BBS35 (clone OX7) | 564607 | BD Bioscience          | 1:100                 |    |
| 22         | CD44-BV605 (Clone IM7) | 563058 | BD Bioscience          | 1:100                 |    |
| 23         | CD105-PE (Clone M37/18) | 562759 | BD Bioscience          | 1:100                 |    |
| 24         | Ly6c-BV605 (Clone AL-21) | 563011 | BD Bioscience          | 1:100                 |    |
| 25         | I-A/I-E-B8700 (clone 2G9) | 746086 | BD Bioscience          | 1:100                 |    |
| 26         | CD11c-PE-Cy7 (clone HL3) | 558079 | BD Bioscience          | 1:100                 |    |
| 27         | Rabbit anti-Ag85B | ab43019 | Abcam                    | 1:2000                |    |
| 28         | Mouse anti-CD73 (ID7) | ab91086 | Abcam                    | 1:1000                |    |
| 29         | Mouse anti-CD44 (SN6) | ab11414 | Abcam                    | 1:500                 |    |
| 30         | Rabbit IgG1 polyclonal isotype control | ab37415 | Abcam                    | 1:2000                |    |
| 31         | Mouse IgG1 kappa monoclonal isotype control | ab81032 | Abcam                    | 1:1500;1:1500        |    |
| 32         | Goat anti-rabbit Alexa fluoro 405,488, 568, 647 | A31556 | Thermo Scientific       | 1:200                 |    |
|            |          | A11034         | Thermo Scientific       | 1:400                 |    |
|            |          | A11011         | Thermo Scientific       | 1:200                 |    |
|            |          | A21245         | Thermo Scientific       | 1:200                 |    |
| 33         | Goat anti-mouse Alexa fluoro 405,488, 568, 647 | A31553 | Thermo Scientific       | 1:200                 |    |
|            |          | A28175         | Thermo Scientific       | 1:400                 |    |
|            |          | A11031         | Thermo Scientific       | 1:200                 |    |
|            |          | A21235         | Thermo Scientific       | 1:200                 |    |
by PBS twice. For antibody staining, cells were treated with ammonium chloride for 15 min. Cells were again washed with PBS and incubated with 0.2% Triton X-100 in 1× PBS for 20 min to permeabilize. Hybridization was carried out by blocking solution (3% BSA in 1× PBS) for 1 h. Cells were then incubated with primary antibody (1:200) for 2–3 h at RT, followed by PBS wash and conjugated secondary antibody (1:400) for an hour. Cells were then washed with 1× PBS and coverslips were mounted in ProLong Gold antifade reagent (Life Technologies). Images were acquired by NIS-Elements software using the Nikon A1R laser scanning confocal microscope equipped with a Nikon Plan Apo VC x20, NA 0.75, and Plan Apo VC x100 oil. NA 1.40 objectives were used. Serial confocal sections, 0.5 μm thick, were acquired with a z-stack spanning 10–15 μm to form a composite image. Images were analysed using Imaris, NIS-Elements and image J software.

Flow cytometry. Surface and intracellular protein staining in ADSCs were carried out using flow cytometry. At required time points, cells were scrapped off, pelleted, and washed. Cells were pelleted down at 1000 rpm and blocked with 3% BSA in 1× PBS and incubated with primary antibody (1:100) for 3 h in blocking buffer followed by incubation with Alexa Fluor 488 conjugated secondary antibody (1:200) for 2 h (surface expression). Intracellular expression was assessed after permeabilizing cells with 0.5% saponin, followed by blocking, primary (1:100) and secondary antibody (1:200) incubation. After incubations, cells were washed with 1× PBS and reseeded in 1× PBS and samples were acquired in BD FACs Canto II by using FACS Diva acquisition software. For measurement of cellular ROS, cells were scrapped at required time point and stained with CellROX Green before acquisition on BD FACs Canto II. Staining of the dyes were performed as per the manufacturer’s directions. The data was analyzed using Flow Jo V10.5.3 software.

Real time PCR and microarray. Total RNA from ADSCs was isolated using mDI RNA isolation kit. cDNA was synthesized from 500 ng of total RNA by reverse transcriptase PCR using Bio-RAD Script cDNA synthesis kit according to the manufacturer’s protocol. The cDNA samples were run in triplicate using β-tubulin and actin as normalizing control, respectively, using SYBR green dye for real time fluorescence acquisition on the Bio-Cfx 96 Real time PCR system. Primers were custom synthesized by Sigma Aldrich Chemicals Ltd. Primer sequences were: ACTB (F:CGAGAAACCAGAGGCTTATTAC, R:ACAGACGAGCAGAAAACAGA) AAG2 (F:CTCTGGCTGCAAGCAACACTATG, R:CGCAGACACAGGATGATA), Tubulin (F:TTGTCGAGATCTTAGGACAGACAACR: CGTATACCCATGGGACCAGAAC) AATC (F:ACCTCTGATCGATGCTGGG, R: CCTGGTAGACAGGCTAAGT)

For microarray, total RNA from ADSC uninfected or infected H37Rv was extracted using mDI RNA isolation kit. Samples were sent to BiomiTivid Technologies, Bangalore for cDNA synthesis and hybridization to 25 “Illumina human WholeGenome-6 version 2 BeadChips” using standard illumina protocols. Six samples (three replicates each for uninfected and infected cells) were used, with 1× PBS and resuspended in 1× PBS and acquired in BD FACs Canto II by using FACS Diva acquisition software. For measurement of cellular ROS, cells were scrapped at required time point and stained with CellROX Green before acquisition on BD FACs Canto II. Staining of the dyes were performed as per the manufacturer’s directions. The data was analyzed using Flow Jo V10.5.3 software.

Tissue Immunohistochemistry. Tissue Immunohistochemistry studies were carried out using formalin-fixed paraffin-embedded tissues. Four-micron sections of formalin-fixed paraffin-embedded tissues were taken on the coated slide. Deparaffinization was done by dipping the slides in xylene for 5 min (two changes), acetone for 2–3 min, alcohol for 2–3 min and then under running/tap water. Antigen retrieval was performed with citrate buffer (pH 6) in microwave oven, at 100 °C, 900 MW for 30 min. Samples were allowed to cool down, washed thrice with Tris buffer (pH 7.5). Endogenous peroxidase blocking was done with 4% Hydrogen peroxide in methanol for 20 min. Rabbit anti-Ag85B (ab43019) primary antibody (pH 6) was added (1:2000) and incubated for 2 h at the room temperature. Thereafter, three washings were given with Tris buffer (pH 7.5). Universal polymer-base secondary antibody (Skytek Laboratories, USA) was incubated at the room temperature for 30 min, and the reaction product was developed with 3,3’-diaminobenzidine chromogen. Skin biopsy from patients with cutaneous tuberculosis was used as positive control. Three subsequent washings with Tris buffer were given at 5 min interval. After that the Mouse anti-CD73 antibody (ab91086, pH 6, dilution: 1:500) was added and incubated in the room temperature for overnight. Three washings were given with Tris buffer for 5 min each. Rabbit anti-Ag85B (ab43019) primary antibody (pH 6) was added (1:2000) and incubated for 2 h at the room temperature. Three washings were given in Tris buffer. A VECTA® Blue Alkaline Phosphatase chromogen was used to develop the color of the reaction (Blue AP), prepared in Tris HCl (pH 8.2) and the color development was monitored under microscope. Skin biopsy from patients with cutaneous tuberculosis was used as positive control. Three subsequent washings with Tris buffer were given at 5 min interval. After that the Mouse anti-CD73 antibody, Mouse IgG1, kappa monocular (NCG01) isotype control was (ab81032) used at 1:500 dilution. Rest of the steps followed are same as above.

Tissue Immunofluorescence staining. Dual immunofluorescence study was performed on formalin-fixed paraffin-embedded (FFPE) lung biopsies. The sections cut were deparaffinized in xylene for 5 min (two changes), acetone for 2–3 min, alcohol for 2–3 min and then under running/tap water. Thereafter the slides were washed in RO water for three times and antigen retrieval was done in pH 9 for 40 min at the room temperature. Thereafter the sections were treated with Protease K (Sigma, dilution: 1:25) for 30 min. The slides were then put in Tris EDTA buffer at pH 9 at 4 °C for 40 min, followed by three washings in Tris buffer for 5 min each. Rabbit anti-Ag85B (ab43019) primary antibody (pH 6) was added (1:2000) and incubated for 2 h at the room temperature. Thereafter Goat anti-Rabbit IgG (H+L) highly cross absorbed secondary antibody conjugated with Alexa Fluor 488 (Thermofisher, dilution: 1:200) was incubated at room temperature for 15 min and observed under fluorescent microscope green channel. The slides were washed in Tris buffer for three times 5 min each. Subsequently the Mouse anti-CD73 antibody (ab91086, dilution: 1:1600) or anti-CD105 antibody (SN6, ab11414, dilution: 1:500) was added and incubated in the room temperature for about 2.5 h. Thereafter Goat anti-mouse IgG (H+L) highly cross absorbed secondary antibody conjugated with Alexa Fluor 568 (Thermofisher, dilution: 1:200) was incubated at the room temperature for 1 h and washed with RO water for 1 min. The slides were washed with RO water followed by mounting with glycerin and stored in 4 °C. All steps were performed in dark room. After satisfying regarding the staining the colocalization analysis was done with confocal microscope as described earlier. Skin biopsy from patients infected with Mtb was used as positive control. Along with the positive controls isotype controls were used as follows: Rabbit IgG polyclonal isotype control (ab 37415) was added in 1:2000 dilutions in the place of anti-Ag85B and incubated for 2 h at the room temperature. Rest of the steps was same as described above including the secondary antibody. In the place of anti-CD73 antibody, Mouse IgG1, kappa monocular (NCG01) isotype control was (ab81032) used at 1:500 dilution. Rest of the steps followed are same as above.

Statistical analysis. Data collection was done using Microsoft Excel (2011). Statistical significance for comparisons between two sets of the experiments was done using unpaired two-tailed Student’s t-test. For multiple treatment experiments one-way ANOVA followed by multiple comparison analysis was performed in GraphPad PRISM 8. For animal experiments, nonparametric Mann–Whitney U test was performed.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.
Data availability

The raw files for the microarray experiment are available at GEO database “GSE133803 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133803]”. For all the plots in Figs. 1, 2, 3, 4 and Supplementary Figs. 1, 2, 3, 4, 5, 6, 7b, corresponding raw data are provided in the Source Data File. Any additional relevant data are available on request. Source data are provided with this paper.

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References

1. Russell, D. G., Barry, C. E. 3rd & Flynn, J. L. Tuberculosis: what we don’t know can, and does, hurt us. Science 328, 852–856 (2010).
2. Voss, G. et al. Progress and challenges in TB vaccine development. FEMS Microbiol. Rev. 39, 199–219 (2015).
3. Streicher, E. M. et al. Emergence and treatment of multidrug resistant (MDR) and extensively drug-resistant (XDR) tuberculosis in South Africa. Infect., Genet. Evolution 12, 686–694 (2012).
4. Ehlers, S. & Schaible, U. E. The granuloma in tuberculosis: dynamics of a host-pathogen collision. Front. Immunol. 3, 411–411 (2013).
5. Bushoff, H. I., 8th & Barry, C. E. 3rd Tuberculosis—resistance and respiration in the absence of growth. Nat. Rev. Microbiol. 3, 70–80 (2005).
6. Cunningham-Bussel, A., Zhang, T. & Nathan, C. F. Nitrite produced by Mycobacterium tuberculosis in human macrophages in polysaccharide oxygen impacts bacterial ATP consumption and gene expression. Proc. Natl Acad. Sci. USA 110, E256–E263 (2013).
7. Waynick, L. G. & Hayes, L. G. An in vitro model for sequential study of shiftdown of Mycobacterium tuberculosis through two stages of nonreplicating persist. Infect. Immun. 64, 2062–2069 (1996).
8. Yang, C.-S., Yuki, J.-M. & Jo, E.-K. The role of nitric oxide in mycobacterial infections. Immun. Netw. 9, 46–52 (2009).
9. Fleitner, K., Garnier, A. L. & Stallings, C. L. Mycobacterium tuberculosis transcription machinery: ready to respond to host attacks. J. Bacteriol. 198, 1360–1373 (2016).
10. Mehta, M., Rajmani, R. S. & Singh, A. Mycobacterium tuberculosis WhiB3 responds to vacuolar pH-induced changes in mycolothiol redox potential to modulate phagosomal maturation and virulence. J. Biol. Chem. 291, 58 (2016).
11. Mehta, M. & Singh, A. Mycobacterium tuberculosis WhiB3 maintains redox homeostasis and survival in response to reactive oxygen and nitrogen species. Free Radic. Biol. Med. 131, 50–58 (2018).
12. Cosma, C. L., Sherman, D. R. & Ramakrishnan, L. The secret lives of the pathogenic mycobacteria. Annu. Rev. Microbiol. 57, 641–676 (2003).
13. Guirado, E., Schlesinger, L. S. & Kaplan, G. Macrophages in tuberculosis: friend or foe. Semin. Immunopathol. 35, 563–583 (2013).
14. Beiger-Bompadre, M. et al. Mycobacterium tuberculosis infection modulates adipose tissue biology. PLOS Pathog. 13, e1006676 (2017).
15. Lyalova, I. V. Neutrophils in tuberculosis: heterogeneity shapes the way? Molecules Infection 5, 49 (2018).
16. Russell, D. G., Cardona, P.-J., Kim, M.-J., Allain, S. & Altare, F. Foamy macrophages and the progression of the human TB granula. Nat. Immunol. 10, 943–948 (2009).
17. Scordio, J. M., Knoll, D. L. & Torrello, J. B. Alveolar epithelial cells in Mycobacterium tuberculosis infection: active players or innocent bystanders? J. innate Immun. 8, 3–14 (2016).
18. Khan, A. et al. Mesenchymal stem cells internalize Mycobacterium tuberculosis through scavenger receptors and restrict bacterial growth through autophagy. Sci. Rep. 7, 15010 (2017).
19. Raghuvanshi, S., Sharma, P., Singh, S., Van Kaer, L. & Das, G. Mycobacterium tuberculosis evades host immunity by recruiting mesenchymal stem cells. Proc. Natl Acad. Sci. USA 107, 21653–21658 (2010).
20. Das, B. et al. CD271(+) bone marrow mesenchymal stem cells may provide a niche for dormant Mycobacterium tuberculosis. Sci. Transl. Med. 5, 170ra113–170ra113 (2013).
21. Zhang, Y., Yew, W. W. & Barer, M. R. Targeting persisters for tuberculosis control. Antimicrob. Agents Chemother. 56, 2223–2230 (2012).
22. Gomez, J. E. & McKinney, J. D. M. Tuberculosis persistence, latency, and drug tolerance. Tuberculosis 84, 29–44 (2004).
23. Wakamoto, Y. et al. Dynamic persistence of antibiotic-stressed mycobacteria. Science 339, 91–95 (2013).
24. Bernardo, M. E. & Fibbe, W. E. Mesenchymal stromal cells: sensors and switchers of inflammatory metabolism. Cell Stem Cell 10, 457–463 (2012).
25. Singer, N. G. & Caplan, A. I. Mesenchymal stem cells: mechanisms of inflammation. Annu. Rev. Pathol. 6, 457–478 (2011).
26. Zhao, Q., Ren, H. & Han, Z. Mesenchymal stem cells: immunomodulatory capability and clinical potential in immune diseases. J. Cell. Immunother. 2, 1–20 (2016).
27. Chandra, P. et al. Mycobacterium tuberculosis inhibits RAB7 recruitment to selectively modulate autophagy flux in macrophages. Sci. Rep. 5, 16320 https://www.nature.com/articles/srep16320 (2015).
28. Karim, A. et al. Express path analysis identifies a tyrosine kinase Src-centric network regulating divergent host responses to Mycobacterium tuberculosis infection. J. Biol. Chem. 286, 40307–40319 (2011).
29. Kumar, D. et al. Genome-wide analysis of the host intracellular network that regulates survival of Mycobacterium tuberculosis. Cell 140, 731–743 (2010).
30. Gottesman, M. M., Fojo, T. & Bates, S. E. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat. Rev. Cancer 2, 48–58 (2002).
31. Szczesny, C. et al. Evaluation of gyrase B as a drug target in Mycobacterium tuberculosis. J. Antimicrob. Chemother. 67, 415–421 (2012).
32. Zaman, G. J. et al. The human multidrug resistance-associated protein MRP is a plasma membrane drug-efflux pump. Proc. Natl Acad. Sci. USA 91, 8826–8826 (1994).
33. Deretic, V. Autophagy, an immunologic magic bullet: Mycobacterium tuberculosis phage mutation block and how to bypass it. Future Microbiol. 3, 517–524 (2008).
34. Shrama, R., Madhusudhan, K. S. & Ahuja, V. Intestinal tuberculosis versus HDAC inhibition: switchers of inflammation. J. Cell. Immunother. 26, 161–172 (2016).
54. Adams, K. N. et al. Drug tolerance in replicating mycobacteria mediated by a macrophage-induced efflux mechanism. Cell 145, 39–53 (2011).
55. Beamer, G., Major, S., Das, R. & Campos-Neto, A. Bone marrow mesenchymal stem cells provide an antibiotic-protective niche for persistent viable *Mycobacterium tuberculosis* that survive antibiotic treatment. Am. J. Pathol. 184, 3170–3175 (2014).
56. Sharom, F. J. ABC multidrug transporters: structure, function and role in chemoresistance. Prasunical disorders 9, 105–127 (2007).
57. Singh, A. et al. Expression of ABCG2 (BCRP), a marker of stem cells, is regulated by NrF2 in cancer cells that confers side population and chemoresistance phenotype. Mol. Cancer Ther. 9, 2365–2376 (2010).
58. Mishra, R. et al. Targeting redox heterogeneity to counteract drug tolerance in replicating *Mycobacterium tuberculosis*. Sci Transl Med 11, https://doi.org/10.1126/scitranslmed.aaw635 (2019).
59. Cole, S. P. C. & Deeley, R. G. Transport of glutathione and glutathione conjugates by MRPs. Trends Pharmacol. Sci. 27, 438–446 (2006).
60. Liang, S.-C. et al. ABCG2 localizes to the nucleus and modulates CDH1 expression in lung cancer cells (J). Neoplasia 17, 265–278 (2015).
61. Seyffer, F. & Tanpe, R. ABC transporters in adaptive immunity. Biochim. et. Biophys. Acta 1850, 449–460 (2015).
62. Chandra, S. et al. Mrp1 is involved in lipid presentation and iNKT cell activation by *Streptococcus pneumoniae*. Nat. Commun. 9, 4279 (2018).
63. Bryan, J. et al. ABCC8 and ABCC9: ABC transporters that regulate K+ channels. Pflug. Arch. 453, 703–718 (2007).
64. Hampshire, T. et al. Stationary phase gene expression of *Mycobacterium tuberculosis* following a progressive nutrient depletion: a model for persistent organisms? *Tuberculosis* 84, 228–234 (2004).
65. Barte, I. L. et al. Antibiotic bactericidal activity is countered by maintaining pH homeostasis in *Mycobacterium smegmatis*. mSphere 1, https://doi.org/10.1128/mSphere.00176-16 (2016).
66. Flentie, K. et al. Chemical disarming of isoniazid resistance in *Mycobacterium tuberculosis* using an epigenetic approach. Antimicrob. Agents Chemother. 58, 841–848 (2014).
67. Broekman, W. et al. TNF-alpha and IL-1beta-activated human mesenchymal stromal cells increase airway epithelial wound healing in vitro via activation of the epidermal growth factor receptor. Respir. Res. 15, 3 (2016).
68. Ryan, J. M., Barry, F., Murphy, J. M. & Mahon, B. P. Interferon-gamma does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. Clin. Exp. Immunol. 149, 353–363 (2007).
69. Matta, S. K. & Kumar, D. Hypoxia and classical activation limits *Mycobacterium tuberculosis* survival by Akt-dependent glycolytic shift in macrophages. Cell Death Discov. 2, 16022, http://www.nature.com/cddiscovery (2016).
70. Atmakuri, K., Penn-Nicholson, A., Tanner, R. & Dockrell, H. M. Meeting report: 5th global forum on TB vaccines, 20–23 February 2018, New Delhi India. *Tuberculosis* 113, 55–64 (2018).
71. Bertholet, S. et al. Identification of human T cell antigens for the development of vaccines against *Mycobacterium tuberculosis*. J. Immunol. (Baltim., Md.: 1950) 181, 7948–7957 (2008).
72. Lewinsohn, D. A., Lewinsohn, D. M. & Scriba, T. J. Polyfunctional CD4+ T cells as targets for Tuberculosis Vaccination. Front. Immunol. 8, 1262–1262 (2017).
73. Ritz, N., Hanekom, W. A., Robins-Browne, R., Britton, W. J. & Curtis, N. Influence of BCG vaccine strain on the immune response and protection against tuberculosis. FEMS Microbiol. Rev. 32, 821–841 (2008).
74. Kalinski, P. Regulation of immune responses by prostaglandin E2. J. Immunol. 188, 21–28 (2012).
75. Mayer-Barber, K. D. et al. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. Nature 511, 99–103 (2014).
76. Divangahi, M. et al. *Mycobacterium tuberculosis* evades macrophage defenses by inhibiting plasma membrane repair. Nat. Immunol. 10, 899–906 (2009).
77. Kaup, V. et al. An important role of prostanooid receptor EP2 in host resistance to *Mycobacterium tuberculosis* infection in mice. *J. Infect. Dis.* 206, 1816–1825 (2012).
78. Rangel Moreno, J. et al. The role of prostaglandin E2 in the immunopathogenesis of experimental pulmonary tuberculosis. *Immunology 106*, 257–266 (2002).
79. Mai, N. T. et al. A randomised double blind placebo controlled phase 2 trial of adjunctive aspirin for tuberculous meningitis in HIV-uninfected adults. *elife 7*, https://doi.org/10.7554/elife.33478 (2018).
80. Crofford, L. J. Use of NSAIDs in treating patients with arthritis. *Arthritis Res. Ther.* 15(Suppl 3), S2–S2 (2013).
81. Dheda, K. et al. Lung remodeling in pulmonary tuberculosis. *J. Infect. Dis.* 192, 1201–1209 (2005).

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**Author contributions**

Concept and experiment design: N.J., D.K.; experiments: N.J.; animal experiments: N.J., L.S., V.S.; data analysis: N.J., H.K., P.D., V.A., S.K., D.K.; IHC and IFA experiments on human samples: P.D., S.K., V.A., N.J.; manuscript writing: N.J., P.D., D.K.; funding: D.K.; overall supervision: D.K. All authors reviewed and commented on the manuscript and agreed to the final version.

**Competing interests**

The authors declare no competing interest.

**Additional information**

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