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Human monocyte-derived macrophage responses to *M. tuberculosis* differ by the host's tuberculosis, diabetes or obesity status, and are enhanced by rapamycin

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Summary

Human macrophages play a major role in controlling tuberculosis (TB), but their anti-mycobacterial mechanisms remain unclear among individuals with metabolic alterations like obesity (TB protective) or diabetes (TB risk). To help discern this, we aimed to: i) Evaluate the impact of the host's TB status or their comorbidities on the anti-mycobacterial responses of their monocyte-derived macrophages (MDMs), and ii) determine if the autophagy inducer rapamycin, can enhance these responses. We used MDMs from newly diagnosed TB patients, their close contacts and unexposed controls. The MDMs from TB patients had a reduced capacity to activate T cells (surrogate for antigen presentation) or kill *M. tuberculosis (Mtb)* when compared to non-TB controls. The MDMs from obese participants had a higher antigen presenting capacity, whereas those from chronic diabetes patients displayed lower *Mtb* killing. The activation of MDMs with rapamycin led to an enhanced anti-mycobacterial activity irrespective of TB status but was not as effective in patients with diabetes. Further studies are warranted using MDMs from

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Conflicts of interest. All the authors declare "No conflict"

TB patients with or without metabolic comorbidities to: i) elucidate the mechanisms through which host factors affect *Mtb* responses, and ii) evaluate host directed therapy using autophagy-inducing drugs like rapamycin to enhance macrophage function.

Keywords

tuberculosis; diabetes; autophagy; host-directed therapy; macrophages; monocytes

1. Introduction

Tuberculosis (TB) occurs in 10 million people and causes 1.6 million deaths globally per year [1]. *Mycobacterium tuberculosis* (*Mtb*) is an intracellular pathogen that replicates or gets killed within macrophages. *Mtb* uses various strategies to avoid intra-cellular killing, with the most notable being the evasion of fusion of the *Mtb*-containing phagosomes with lysosomes. This reduces *Mtb* killing, lysosome-mediated degradation and mycobacterial antigen presentation to T cells [2, 3]. *Mtb* also evades targeting of its phagosome to the autophagy pathway [4, 5]. However, with appropriate macrophage activation (e.g. IFN-γ), intracellular *Mtb* can be targeted to phagolysosomes or auto-phagolysosomes, where it is killed [6]. Rapamycin induces autophagy via mTOR inactivation, and we have shown that in mice vaccinated with BCG and then infected with *Mtb*, rapamycin improves the targeting of *Mtb* to autophagolysosomes, mycobacterial digestion and antigen presentation to T cells [7, 8].

Despite the importance of macrophages in the orchestration of the innate and adaptive response to *Mtb*, macrophages display heterogeneous functional properties and ontogeny, and the anti-*Mtb* mechanisms of human macrophages are not fully understood [9, 10]. *In vitro* polarization of human monocyte-derived macrophages (MDMs) from healthy donors towards a pro-inflammatory M1 (e.g. with IFN-γ) or anti-inflammatory M2 phenotype (e.g. with IL-4) show that the former are more effective for *Mtb* killing [11]. However, human alveolar macrophages appear to express a balance of M1-like and M2-like markers [12]. This ratio may vary between healthy individuals, and can be affected by their TB infection status or host co-morbidities. Furthermore, the lung is continuously replenished by circulating monocytes that differentiate to interstitial macrophages, and this is enhanced after *Mtb* infection. These interstitial macrophages have a more effective anti-mycobacterial response when compared to alveolar macrophages [10, 12]. Thus, translation of the *in vitro* M1-M2 findings to the rational development of interventions for latent and active TB has to take into consideration the enormous heterogeneity of immune responses between humans. Such variations are influenced by the host's genetics, environment and comorbidities, with complex interactions among them. For example, among Hispanics in our US-Mexico border community, we find that type 2 diabetes (risk factor) and obesity (protective) frequently co-exist [13–16].

To understand the impact of TB status or other host comorbidities on human macrophage responses to *Mtb*, we conducted an observational study where the MDMs from TB patients, their close contacts and healthy controls were infected with *Mtb* and then evaluated for their

ability to activate T cells though antigen-presentation and killing of *Mtb in vitro*. Our goals were to (i) determine if the host's TB status or other host characteristics were associated with the capacity of MDMs for antigen presentation and *Mtb* killing, and (ii) evaluate the impact of rapamycin on these anti-*Mtb* responses. We found that in addition to TB status, obesity and diabetes were also associated with different *in vitro* MDM functions: obesity enhanced the antigen presentation capacity of MDMs, while diabetes reduced killing of *Mtb.* Furthermore, rapamycin enhanced the anti-*Mtb* functions of MDMs across individuals with or without TB, but did not appear to be as effective in patients with underlying diabetes or vascular diseases. Our findings highlight the importance of conducting studies with immune cells from well-characterized TB patients or their contacts, to elucidate relevant mechanisms that affect *Mtb* responses in-vivo and identify drugs which can be used for host directed therapy.

2. METHODS

2.1 Participant enrollment and characterization

We conducted a cross-sectional study among HIV-negative adults 18 years or older. Newly diagnosed TB patients or their close contacts were enrolled at the Centro Regional de Tuberculosis in Reynosa, Mexico (Secretaría de Salud de Tamaulipas). Healthy controls without known history of TB exposure were enrolled in Reynosa or Nuestra Clinica del Valle in South Texas. Enrollment procedures followed guidelines from the Institutional Review Boards of the participating institutions and informed consent was signed by the participants. TB diagnosis was based on isolation of *Mtb* (confirmed) or a positive smear for acid-fast bacilli and an abnormal chest x-ray (clinical). Sociodemographic and medical risks for TB were documented as described previously [14]. Type 2 diabetes classification was based on hyperglycemia (fasting plasma glucose ≥126 mg/dL), HbA1c ≥ 6.5% or self-report, and pre-T2D as HbA1c 5.7–6.49% [17]. Macro- and micro-vascular disease were based on self-reported high blood pressure, stroke, cardiovascular diseases or peripheral neuropathies. Latent TB infection (LTBI) was based on a positive QuantiFERON-Gold In-Tube (Qiagen) or T.Spot.TB (Oxford Immunotec). Glycemic index was calculated as HbA1c * (years with diabetes). Central obesity was evaluated by waist:hip ratio (WHR).

2.2 Isolation, differentiation and pre-activation of MDMs from study participants

PBMCs were isolated from peripheral blood using density gradient centrifugation. Monocytes were purified with anti-CD14-coated magnetic beads (Miltenyi Biotech) and differentiated to MDMs in Iscove's Modified Dulbecco Medium supplemented with 10% FBS and 10 ng/mL GM-CSF for 6 days. Before the functional assays, MDMs were then plated in GM-CSF free medium for 24 hours and then incubated with or without 5 μM of rapamycin for 24h.

2.3 *Ex vivo* MDM-mediated growth containment of *Mtb.*

Frozen aliquots of log-phase *Mtb H37Rv* were thawed, sonicated and used for infection at a multiplicity of infection of one. Naive or rapamycin-treated MDMs were exposed to *Mtb* for 4 h and unbound bacteria were washed and MDMs cultured further. On day 3 lysates of

MDMs were plated on 7H11 Middlebrook agar (Difco Laboratories) for colony-forming unit (CFU) counting to evaluate MDM-mediated growth containment of *Mtb*.

2.4 *Ex vivo* MDM- mediated *Mtb* Ag85B antigen presentation to CD4 T cells

Naive or rapamycin-treated MDMs were washed after infection with *Mtb* and overlaid with the F9A6 CD4 T cell hybridoma which recognizes an Ag85B epitope on human HLA-DR1 [18]. After 24h, culture supernatants were harvested and the IL-2 secreted by the hybridoma T cells was determined by sandwich ELISA (Biolegend) [19].

2.5 Data Analysis

Data was analyzed using SAS version 9.4 (Cary, North Carolina). Chi-square or Fisher's exact tests were used to compare categorical variables. Median values were compared between study groups by the Wilcoxon rank sum test for variables with two levels, or by Kruskall-Wallis if more than two levels. For multivariable models, continuous variables were normalized by log transformation and then evaluated with generalized linear models. Paired t-tests were conducted to identify differences between +/− exposures to rapamycin within the same individual. P values were considered significant if ≤ 0.05, and borderline significant if <0.10. Graphs were plotted using GraphPad PRISM 6.0.

3. Results

3.1 Description of study participants.

We enrolled 24 TB patients (23 culture-confirmed and one clinical), 26 close contacts and 11 controls. Latent TB infection (LTBI) assays were positive for 53% of the close contacts and 30% of the controls. The latter is consistent with the background prevalence of LTBI in the Texas-Mexico border (Restrepo, unpublished). The sociodemographic characteristics of the participants are shown in Table 1. More than half were females (61.9%). The median age was 47 years, with most between 36–59 years old (71.4%), which is an age group with a high prevalence of type 2 diabetes (46%), and overweight or obesity (61.9%) in our study population [13, 14]. Table 1 also shows the characteristics of participants by TB status, our primary exposure of interest. TB patients were younger, and were more likely to have a history of smoking, low or normal weight, and less central obesity. Their low body weight is consistent with their lower serum cholesterols and triglycerides (Tables 2). TB patients also had fewer blood lymphocytes and higher monocytes and neutrophils, with higher monocyte:lymphocyte or neutrophil:lymphocyte ratios (Table 2).

3.2 *Mtb* growth containment by TB status.

We examined if the anti-*Mtb* responses of MDMs differed by TB status. For evaluation of *Mtb* growth containment, we increased our sample power by merging the TB contact and healthy control groups into a "no TB" group, given their similar outcomes (Table S1). We found that the MDMs from TB patients had a lower capacity to contain *Mtb* growth (higher CFUs) when compared to the TB contacts or controls (Fig 1A). Among the non-TB groups the TB contacts and healthy controls had lower CFUs when compared to TB patients, regardless of their LTBI status (Table S1). Therefore, the TB contacts and healthy controls were analyzed jointly for CFU analysis to increase sample power, and are referred to as "No

TB". The reduced *Mtb* growth containment remained significant in the TB patients (versus no TB), after controlling for each of the host characteristics that differ between these two study groups in Table 1 (Table S2). In summary, the TB status of the host is independently associated with *Mtb* growth containment by MDMs.

3.3 Antigen presentation by TB status.

To assess antigen-presentation, MDMs were exposed to *Mtb* H37Rv to allow for phagocytosis and the follow-up processes that precede antigen presentation (e.g. phago-lysosome or auto-phagolysosome fusion, generation and loading of digested peptides to MHC-II). The infected MDMs then were exposed to a CD4 T-cell clone specific for the mycobacterial antigen 85B, and the antigen presentation-induced secretion of IL-2 was measured as a marker of T cell activation. Since this assay detects only HLA-DR1 subjects, four HLA-DR1-negative participants were excluded from analysis. We found that the MDMs from the TB contacts induced higher IL-2 levels when compared to TB patients or controls (Fig 1B; Table S1). After additional analysis of the non-TB study groups (TB contacts, healthy controls or by LTBI status; Table S1), we selected the TB patients and their close contacts for final data analysis of IL-2 secretion (the healthy controls were excluded due to small size and difference with contacts). TB status remained independently associated with lower T cell activation after controlling for the variables that differed between the TB and TB contacts in Table 1 (age, smoking, BMI, central obesity, diabetes, complete blood counts, platelets and lipids; Table S2). These data confirmed that there are differences between the MDMs of TB patients versus TB contacts in their capacity to activate T cells in response to Antigen 85B which is an immunodominant and widely recognized antigen by TB patients.

3.4 Host comorbidities associated with *Mtb* growth containment and antigen presentation.

We evaluated if host characteristics other than TB status were associated with the functional responses of MDMs. Table 3 (graphical summary) and Table S3A (detailed statistics) show the results for age, sex and host factors that were significantly associated. To simplify the interpretation, we grouped the variables into categories (e.g. sociodemographics, diabetes diagnosis, history and complications, obesity and dyslipidemias, platelet and white blood cell counts). For CFU analysis, *Mtb* growth was inversely correlated with obesity measures when all participants were combined, but not among TB or non-TB participants separately. Among TB contacts only, *Mtb* growth was positively correlated with higher platelet counts, monocyte counts (borderline), and with measures of a chronic history of diabetes (diabetes awareness, glycemic index and self-reported years with diabetes). The CFU counts in the TB group alone was not associated with host characteristics. In summary, *Mtb* CFUs were associated with host factors in non-TB participants, and correlated with monocyte counts, and a chronic history of diabetes.

For antigen presentation, higher IL-2 was associated with measures of obesity (BMI or waist-hip ratio) and cholesterols (HDL and total cholesterol) among the TB patients, contacts or both combined. In contrast, triglycerides were negatively correlated with IL-2 secretion in TB patients only (Tables 3 and Table S3A). The contrasting relationships for

BMI and triglycerides by TB status is shown by regression analysis (Fig 2). In summary, IL-2 secretion is associated with different host factors in TB versus TB contacts, but an association with central obesity was observed in both TB states.

3.5 Impact of rapamycin on *Mtb* growth containment and antigen presentation by TB status or other host characteristics.

Our findings indicate that MDMs retain unique properties after *in vitro* culture that reflect the in-vivo environment of their monocyte precursors. To evaluate if these MDMs could be modified *in vitro* to improve their functional responses to *Mtb*, parallel experiments to the ones just described were carried out with MDMs pre-incubated with rapamycin, an agent that blocks mTOR and an inducer of autophagy [20]. Regardless of the participant's TB status, the paired analysis of the MDMs from the same participant, with or without rapamycin, consistently showed that rapamycin reduced *Mtb* CFUs and enhanced the expression of IL-2 by T lymphocytes (Fig 3). At a group level, the CFU values were now similar between TB, TB contacts or healthy controls ($p = 0.146$), but the MDMs from TB patients had borderline lower IL-2 than TB contacts ($p = 0.095$; Table S1).

We evaluated if the treatment with rapamycin also eliminated differences in the MDM responses to *Mtb* by host characteristics other than TB status. This was not the case for CFUs, particularly for the non-TB group. In these participants there was a persistence in the associations with features of a chronic diabetes as observed with MDMs alone, plus additional associations were now detected with diabetes diagnosis or its frequent complications (macro- or micro-vascular diseases) (Fig 2B; Tables 3 and S3A). Conversely, in the presence of rapamycin, few host categories were now associated with IL-2 outcomes (Tables 3 and S3A). In summary, rapamycin can modify the already established phenotype of MDMs for improved responsiveness to *Mtb* or its antigens. However, certain host characteristics, including diabetes and vascular diseases, may be less responsive to these rapamycin-induced changes.

4. Discussion

Our findings show that the host's TB status is a major determinant of the differential response of MDMs to *Mtb* in vitro. The MDMs from TB patients (versus non-TB controls) were less effective for T cell activation and control of intracellular *Mtb* growth (Fig 1; Table 3). Other host characteristics were also associated with the functional responses of MDMs to mycobacteria. Notably, obesity or cholesterols correlated with T cell stimulation, while a chronic history of diabetes was associated with lower containment of intracellular *Mtb* growth. The compromised responses of TB patients to *Mtb in vitro* may be a consequence of having this disease. However, in the non-TB group, the *in vitro* findings mimicked the epidemiology of TB, with the MDMs from chronic diabetes patients showing a compromise in *Mtb* containment, while those from the obese showing higher antigen presentation. Our findings with patient's monocytes highlight the importance of studying macrophage-*Mtb* interactions in the context of the host's TB status or other characteristics like obesity and diabetes.

We have previously shown a beneficial effect of an autophagy inducer, rapamycin, on the protective effect of BCG vaccination in mice. Rapamycin enhanced the targeting of *Mtb* to autophagolysosomes, that resulted in improved *Mtb* killing, antigen presentation of mycobacterial peptides and T cell activation [7, 8]. Likewise, in the present study, the anti-*Mtb* responses of human MDMs were enhanced by rapamycin across all study groups. However, rapamycin accentuated the lower *Mtb* growth containment in participants with diabetes or vascular diseases (Table 3; Fig. 4), suggesting that in certain comorbidities, the response to rapamycin may be reduced. Nevertheless, and even though we did not directly evaluate autophagy, our observations provide support for the use of autophagy enhancers to redirect macrophage responses against *Mtb* among patients with comorbidities. Interestingly, metformin is another autophagy inducer that also appears to have beneficial effects against *Mtb* but our sample size was insufficient to evaluate its impact [21–24].

The beneficial effects of rapamycin on TB response in mice prompted us to evaluate its impact on human macrophages, and as a proof-of-principle for evaluating macrophage plasticity in TB [8]. One of its purified GMP -grade derivatives (e.g. Everolimus) has been proposed as a host-directed therapy for TB [20, 25]. Even though studies are required to evaluate its risks vs beneficial effects on host immunity to *Mtb*, it is encouraging to see that in clinical trials among elderly (e.g. influenza vaccination, anti-aging), low dose rapamycin has been shown to be safe and effective in boosting immune responses [26, 27].

Active TB (versus no TB) was associated with compromised MDM responses to *Mtb*, which is consistent with their higher bacterial burden in-vivo. Several underlying differences in the MDMs from TB patients (versus no TB) may contribute to their more compromised response. These include: i) a higher proportion of M2-like vs M1 macrophages [28], ii) a predominance of oxidative phosphorylation (more permissive) versus glycolysis [10], and iii) a lower capacity for *Mtb* autophagy [29]. Some of these features are related. For example, M1 macrophages (versus M2) have a predominant glycolytic metabolism and a higher capacity for *Mtb* autophagy and killing [10]. The beneficial effects of rapamycin on the anti-*Mtb* responses of MDMs may be partly attributed to enhanced autophagy, but further research is needed to understand these relationships given that rapamycin inhibits mTOR, and hence glycolysis [30].

The host characteristics associated with MDM function provided insights into the paradoxical relationship between obesity and diabetes in TB pathogenesis. First, our observed correlation between obesity and IL-2 secretion provided support for the epidemiological observation of a higher protection from TB in obese individuals [15, 16]. We posit that the chronic inflammation induced by the obese state leads to mononuclear cells that are “primed” to respond quickly and more intensely to antigenic stimuli, favoring *Mtb* elimination. This is analogous to the ‘trained immunity phenotype’ induced by obesity [31]. Second, the correlation between a chronic history of diabetes and lower *Mtb* growth containment by MDMs is consistent with studies that have shown defective responses to *Mtb* by diabetic mononuclear cells [32–37]. Third, obesity and diabetes were associated with different MDM functions: obesity enhanced the capacity of MDMs to stimulate T cells while diabetes compromised intracellular *Mtb* killing. These *in vitro* findings provide support for the hypothesis that low BMI combined with chronic hyperglycemia leads to a

synergistic compromise in macrophage function. We did not have the sample power to divide the study groups by diabetes and BMI status, but our hypothesis is consistent with a recent population-based cohort where BMI and diabetes were independent determinants of TB risk (diabetes hazards ratio (HR) 2.31, 95% CI 1.93, 2.78; underweight HR 2.87, 95% CI 2.15, 3.82), but the hazard ratio for active TB among underweight individuals was 8.30 (95% CI 4.43, 15.54) [38]. However, the results from other studies suggest that the interaction between diabetes, obesity and TB risk is more complex [16, 39, 40].

A limitation of our study was the small sample size. For CFU analysis we merged the healthy and TB contact groups given their similar results, but this was not possible for the IL-2 assay. Despite this limitation, we identified significant differences by TB status, observed consistency across other host characteristics, and controlled for confounders when evaluating differences between TB study groups. A larger study is warranted to allow further break down of the contacts and controls by LTBI status, identify the phenotype of the monocytes and MDMs *ex vivo* (M1- vs M2-like) and elucidate the molecular mechanisms that underlie low antigen presentation an *Mtb* growth-containment in TB.

In summary, our results indicate the persistence of unique MDM phenotypes *in vitro* that are associated with host's characteristics, most notably TB status, obesity and diabetes. Rapamycin improved the *Mtb* responses of MDMs in individuals with or without TB, but hosts with some comorbidities such as diabetes and vascular diseases may be less responsive. While *in vitro* studies with healthy monocytes are important to identify the mechanisms by which human macrophages can kill *Mtb*, our findings confirm the importance of complementing with experiments using cells from TB patients and their close contacts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations1

TB	[1] tuberculosis
MDMs	monocyte-derived macrophages
Mtb	Mycobacterium tuberculosis
LTBI	Latent TB nfection

CFU	colony-forming unit
BMI	body-mass index
HDL	high-density cholesterol
HR	hazards ratio

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Highlights:

- TB patient's macrophages have lower antigen-presentation and *Mtb* killing capacity.
- Obesity enhances but diabetes diminishes macrophage antigen-presenting function
- Diabetic macrophages have lower *Mtb*-killing capacity
- Rapamycin improves anti-*Mtb* responses, but diabetes patients are less responsive.

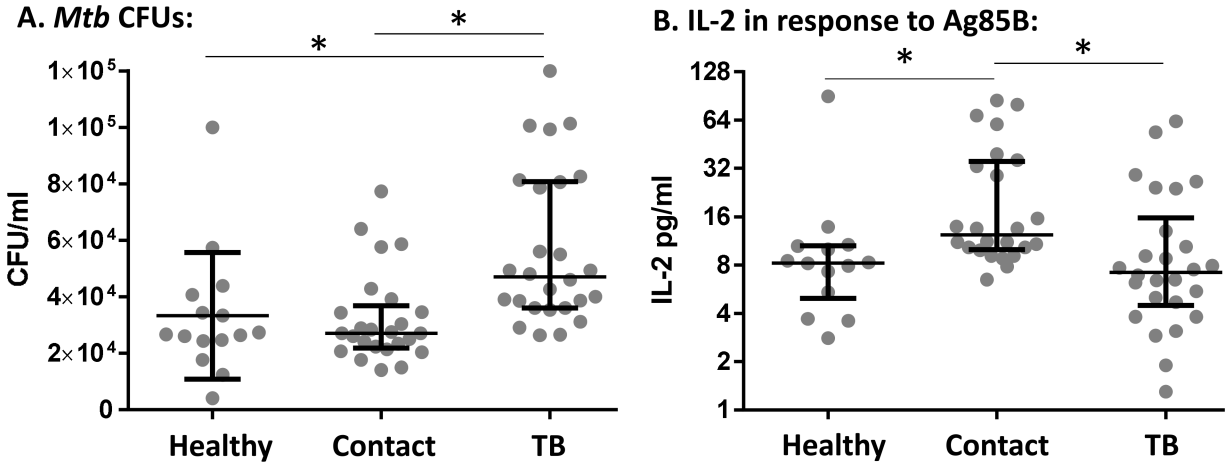

Figure 1. MDMs from patients with TB, their household contacts or healthy donors show a differential ability to restrict the growth of *Mtb* and present antigen to T cells.

Monocytes were differentiated into MDMs in vitro and used for functional assays. **A**. *Mtb* infected MDMs were incubated for 3 days and their cell lysates were then plated for CFUs on 7H11 agar plates. **B**. The MDMs were infected using *Mtb* for 4 hr, washed and overlaid with the F9A6 CD4 T cell clone specific for the mycobacterial antigen Ag85B. After 24 hr the supernatants were evaluated for IL-2 using sandwich ELISA. Each dot represents an individual participant; horizontal lines indicate the median and 25% and 75% interquartile ranges. * Differences between TB groups were identified by analysis of covariance and the Scheffé post hoc test ($p < 0.05$).

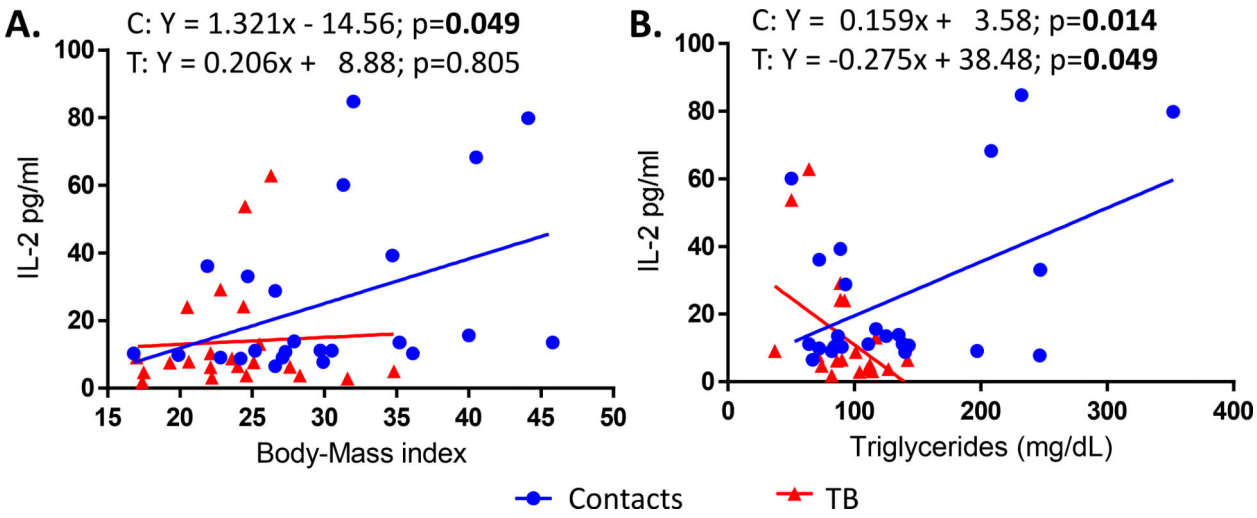

Figure 2. Contrasting differences between participants with TB patients or their contacts, in the relationships between T cell activation (IL-2 secretion) by *Mtb* infected MDMs and either BMI or triglycerides.

MDMs from TB patients or contacts were infected with *Mtb* and co-incubated with a T cell clone specific for Ag85B. After 24h, the activation of T cells was measured by the secretion of IL-2. Regression analysis shows a positive correlation between IL-2 and BMI or triglycerides among TB contacts, but no relationship (for BMI) or an inverse relationship (for triglycerides) among TB patients. Each dot represents a participant.

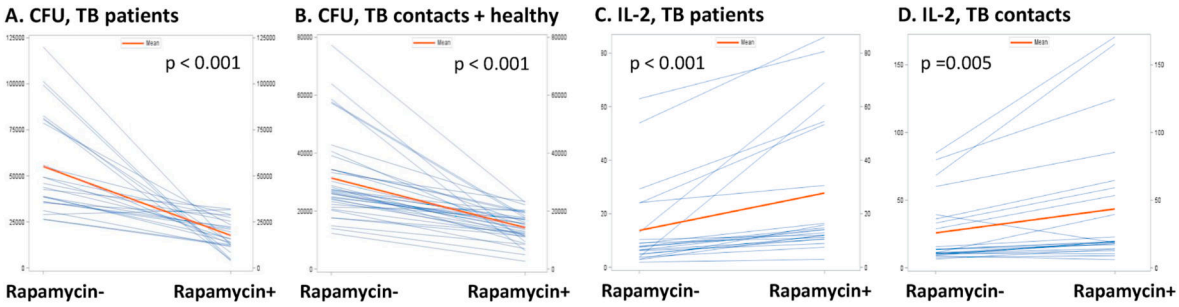

Figure 3. Impact of rapamycin on MDM function.

Paired profiles for the MDMs of a given participant exposed to +/− rapamycin, prior to evaluation of *Mtb* growth containment measured by CFUs (A, B) or of their induction of IL-2 expression by T cells (C, D). **No TB** = TB contacts + healthy controls. **Thin lines** = represent a given individual; **Bold red line** = mean of all participants.

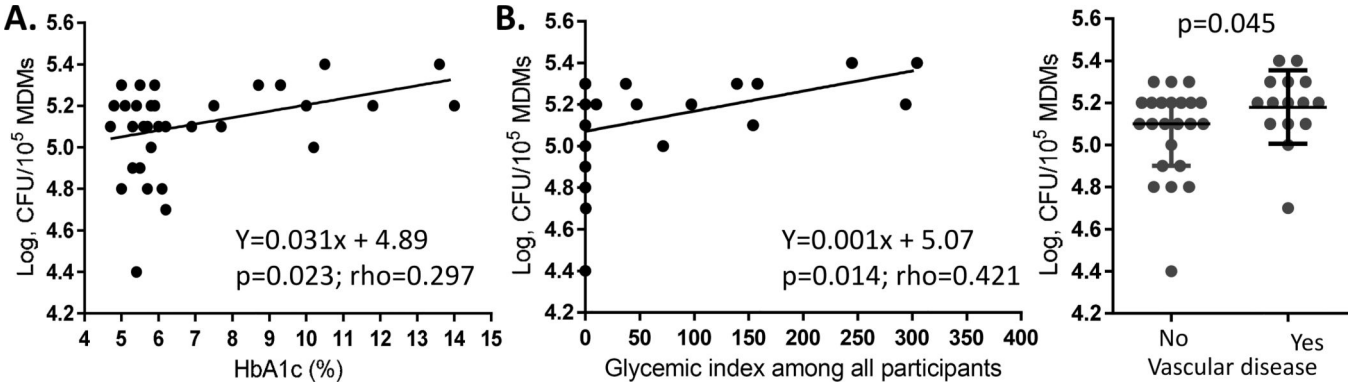

Figure 4. Diabetes-related host factors are associated with higher CFU counts in MDMs treated with rapamycin.

The MDMs from non-TB participants (TB contacts and healthy controls) were incubated with rapamycin and Mtb as described in the Methods. After 72h of infection with *Mtb* H37Rv, the *Mtb* growth-containing capacity of MDMs was evaluated based on *Mtb* CFUs counts. **A-B**. Correlation coefficients (rho) and simple logistic regression results (equation and p values) are shown. **C**. Median and IQR (horizontal lines) from participants with or without vascular disease. Each dot represents a participant.

Table 1.

Characteristics of participants by TB status [a]

	All n=63	No TB [b] n=39	Contacts n=26	TB n=24	TB vs no TB [b] p value [c]	TB vs Contacts p value [c]
Age	47 (16)	47 (16)	47 (17)	44.5 (23)	0.307	0.247
Age groups					**0.022**	**0.044**
18 to 35 y	12 (19)	5 (12.8)	4 (15.4)	7 (29.2)		
36 to 59 y	45 (71.4)	28 (71.8)	17 (65.4)	17 (70.8)		
60+ y	6 (9.5)	6 (15.4)	5 (19.2)	0 (0.0)		
Sex					0.132	0.173
Female	39 (61.9)	27 (69.2)	18 (69.2)	12 (50)		
Male	24 (38.1)	12 (30.8)	8 (30.8)	12 (50)		
Smoking pack-year	0 (0.0)	0 (0.0)	0 (0.2)	0.3 (5.1)	**0.002**	**0.022**
BCG vaccine	56 (88.9)	35 (89.7)	24 (92.3)	21 (87.5)	0.795	0.558
Obesity, BMI	29 (9.6)	29 (9.6)	28.8 (9.5)	23.8 (5)	**<0.001**	**<0.001**
Central obesity, Waist-hip ratio					**0.006**	**0.026**
Excellent+Good+Avge	21 (33.3)	8 (20.5)	6 (23.1)	13 (54.2)		
At risk	42 (66.7)	31 (79.5)	20 (76.9)	11 (45.8)		
Diabetes					0.629	0.281
No	34 (54)	22 (56.4)	17 (65.4)	12 (50)		
Yes	29 (46)	17 (43.6)	9 (34.6)	12 (50)		
HbA1c (%)	5.9 (3.3)	5.9 (3.3)	5.8 (0.8)	6.1 (2.8)	0.173	**0.038**
Micro- or macro-vascular disease					0.377	0.173
No	36 (57.1)	24 (61.5)	18 (69.2)	12 (50)		
Yes	27 (42.9)	15 (38.5)	8 (30.8)	12 (50)		
Diabetes awareness, in DM [d]					**0.046**	**0.014**
Self-reported	24 (82.7)	12 (70.5)	5 (55.5)	12 (100)		
New diagnosis	5 (17.3)	5 (29.5)	4 (44.4)	0 (0.0)		
Glycemic index, in DM [d]	71.4 (153.3)	71.4 (153.3)	10 (138.6)	41.2 (120.4)	0.706	0.594
Years with diabetes, in DM [d]	7 (16.9)	7 (16.9)	1 (15.9)	5 (10.6)	0.578	0.616

[a] Discrete variables expressed as n(column %) and continuous variables as median (IQR);

[b] "No TB" includes TB contacts and healthy controls;

[c] Statistical differences established by Chi-square of Fisher's exact for categorical or Wilcoxon rank sum test for continuous variables, with P values in bold highlight significant and borderline significant results;

[d] n=29 for "DM only" groups; Glycemic index = HbA1c x Years with DM; BMI, Body-mass index; Note: Healthy controls alone are not shown given no significant differences with TB contacts

Table 2.

Laboratory tests by TB status[a]

	N	All	No TB [b]	TB	Contacts	TB vs no TB [b] p value [c]	TB vs Contacts p value [c]
Lipids (mg/dL)	60						
Total Cholesterol (200)	60	174.5 (42)	174.5 (42)	132.5 (26.5)	171 (40)	**<0.001**	**<0.001**
HDL (40 M, 50 F)	62	43 (12)	43 (12)	35 (13.5)	43 (13)	**0.001**	**0.002**
LDL (100)	60	109.5 (50.6)	109.5 (50.6)	75.2 (26.3)	97.5 (47.1)	**<0.001**	**0.001**
Triglycerides (150)	63	111 (75)	111 (75)	89.5 (34.5)	114 (105)	**0.017**	**0.036**
Complete blood counts (x10e3/μl) **Platelets** (146–388)	46	253 (81.5)	253 (81.5)	441 (238)	252 (82)	**<0.001**	**<0.001**
White blood cells (4.8–10.9)	46	6.7 (2.4)	6.7 (2.4)	8.8 (2.2)	6.5 (2.7)	**0.007**	**0.009**
Neutrophils (2.3–7.7)	46	3.9 (1.6)	3.9 (1.6)	5.7 (3.1)	3.7 (1.6)	**0.001**	**0.001**
Lymphocytes (0.8–3.3)	46	1.8 (1)	1.8 (1)	1.2 (0.9)	1.8 (1.1)	**0.008**	**0.018**
Monocytes (0.2–1.0)	46	0.5 (0.2)	0.5 (0.2)	0.6 (0.3)	0.5 (0.2)	**0.009**	**0.015**
Monocyte:lymphocyte ratio	46	0.2 (0.2)	0.2 (0.2)	0.4 (0.4)	0.2 (0.1)	**<0.001**	**<0.001**
Neutrophil:lymphocyte ratio	46	2.2 (1.3)	2.2 (1.3)	5 (5.1)	1.8 (1.3)	**<0.001**	**<0.001**

[a] Data expressed as median (interquartile range);

[b] No TB = Contacts + healthy;

[c] Non-parametric measures used to compare median values between study groups for variables with two levels (Wilcoxon rank sum test) or more than two levels (Kruskall-Wallis), with P values in bold highlight significant and borderline significant results; HDL = High density cholesterol; LDL= Low density cholesterol; Note: Healthy controls alone are not shown given no significant differences with TB contacts; Normal cut-offs values or ranges are indicated in parenthesis, in mg/dL units for lipids and x10e3/μl for complete blood counts; M=males; F=females;

Table 3.

Summary of host factors associated with MDM antigen presentation and *Mtb* growth in-vitro, with and without rapamycin [a]

	Functional outcomes: CFU (*Mtb* growth)						IL-2 (Antigen presentation)					
		A. MDM alone			B. MDM + Rapamycin			C. MDM alone			D. MDM + Rapamycin		
	Participants groups by TB status:	All	C+H	TB	All	C+H	TB	C+TB	C	TB	C+TB	C	TB
Socio-demographics	Age (yrs)						↗						
	Sex (Male/Female)						↑ male						
	Smoking, pack-yr					↘ (bor)	↗		↗ (bor)		↘ (bor)		↘
DM diagnosis, history and complications	DM (Yes/No)					↑ (bor)							
	Fasting blood glucose				↗ (bor)	↗							
	HbA1c					↗							
	DM awareness (Aware Yes/No; DM only)	↑	↑		↑	↑							
	Glycemic index (DM only)		↗		↗ (bor)	↗							↗ (bor)
	Years with DM		↗			↗							↗
	Macro- or microvascular disease (Yes/No)				↑	↑							
Obesity and plasma lipids	Obesity (BMI)	↘						↗	↗		↗ (bor)		
	Central obesity (At risk/Normal WHR)								↑	↑			
	Total Cholesterol	↘						↗					
	HDL cholesterol	↘						↗		↗ (bor)			↗
	LDL cholesterol	↘											
	Triglycerides	↘								↘			
Platelets and white blood cell counts	Platelets	↗	↗					↘ (bor)					↗
	Lymphocytes							↗	↗ (bor)				
	Monocytes	↗	↗ (bor)							↗			
	Neutrophils	↗					↘ (bor)						↗ (bor)
	Monocyte:lymphocyte ratio	↗								↗			↗
	Neutrophil:lymphocyte ratio						↘ (bor)	↘			↘ (bor)		

[a] Sample sizes, measurement units and detailed statistics shown in Tables S3A-B; **Green background** = beneficial response vs Mtb (e.g. ↑ IL-2 or ↓ CFUs); **Red background** = Compromised response to *Mtb* (e.g. ↓ IL-2 or ↑ CFUs); **No background** = No association; **Vertial arrows (↑, ↓)** indicate the direction of associations for categorical variables with referent category indicated next to each variable; **Slanted arrows (↗, ↘)** indicated the direction of correlations for continuous variables; **Arrows alone**, significant associations ($p \leq 0.05$); **Arrows with "(bor)"**, borderline significant ($0.051 \geq p \leq 0.09$);

Definitions and abbreviations: All = All study participants (contacts, healthy and TB); **BMI** = Body-mass index; **C** = TB contacts; **CFU** = *Mtb* colony-forming units; **DM** = Diabetes; Glycemic index = **HbA_{1c}** x years with DM; **H**= healthy controls; **MDM** = Monocyte-derived macrophage; **TB** = Tuberculosis patients; **WHR** = Waist:Hip ratio; **Vacular diseases** are self-reported;