IMMUNOLOGY ORIGINAL ARTICLE

Effect of standard tuberculosis treatment on naive, memory and regulatory T-cell homeostasis in tuberculosis–diabetes co-morbidity

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Summary

Perturbations in CD4$^+$ and CD8$^+$ T-cell phenotype and function are hallmarks of tuberculosis–diabetes co-morbidity. However, their contribution to the pathogenesis of this co-morbidity and the effect of anti-tuberculosis treatment on the phenotype of the T-cell subsets is poorly understood. In this study, we examined the frequency of different T-cell subsets in individuals with pulmonary tuberculosis (PTB) with diabetes mellitus (DM) or without coincident diabetes mellitus (NDM) before, during and after completion of anti-tuberculosis chemotherapy. PTB-DM is characterized by heightened frequencies of central memory CD4$^+$ and CD8$^+$ T cells and diminished frequencies of naive, effector memory and/or effector CD4$^+$ and CD8$^+$ T cells at baseline and after 2 months of treatment but not following treatment completion in comparison with PTB-NDM. Central memory CD4$^+$ and CD8$^+$ T-cell frequencies exhibited a positive correlation with fasting blood glucose and glycated haemoglobin A1c levels, whereas the frequencies of naive and effector memory or effector CD4$^+$ and CD8$^+$ T cells exhibited a negative correlation. However, the frequencies of CD4$^+$ and CD8$^+$ T-cell subsets in individuals with PTB exhibited no significant relationship with bacterial burdens. Finally, although minor alterations in the T-cell subset compartment were observed at 2 months of treatment, significantly decreased frequencies of central memory and significantly enhanced frequencies of naive CD4$^+$ and CD8$^+$ T cells were observed at the completion of treatment. Our data reveal a profound effect of coexistent diabetes on the altered frequencies of central memory, effector memory and naive T cells and its normalization following therapy.

Keywords: bacterial; diabetes; T cells.

Introduction

Among the major factors that influence the pathogenesis of pulmonary tuberculosis (PTB), type 2 diabetes mellitus (DM) is one of the foremost.[1] The global epidemic of DM has reached alarming proportions with prevalence reaching 382 million in 2013 and being predicted to increase to 592 million by 2035.[2] Interestingly, PTB and DM exhibit a geographical intersect with approximately 80% of people with diabetes living in areas where tuberculosis (TB) is also highly endemic.[2] DM is associated with an approximately threefold to fivefold increase in the risk in the development of active TB [1] and is known to impact clinical severity of TB disease and response to treatment.[3] Finally, recent estimates indicate that the population-attributable fraction of TB due to DM is now around 15%, which is higher than that of HIV.[4] Hence, it is important to understand the host–pathogen interaction of TB in the context of DM.

We and others have previously demonstrated that PTB-DM co-morbidity is characterized by elevated circulating and mycobacteria-specific levels of T helper type 1 (Th1) and Th17 cytokines,[5,6] cytokines thought to influence PTB pathogenesis. In addition, CD4$^+$ and CD8$^+$ T cells exhibit heightened activation in individuals with PTB-DM with elevated frequencies of Th1 and Th17 cytokine production.[7,8] Finally, PTB-DM co-morbidity is also characterized by alterations in the frequencies of CD4$^+$ and CD8$^+$ T-cell subsets *ex vivo*.[9] Hence, DM appears to

Table 1. Demographics of study individuals

Study demographics	Baseline (before treatment)			Post Rx 1 (2nd month)		Post Rx 2 (6th month)	
	PTB diabetes	PTB non-diabetes	P value	PTB diabetes	PTB non-diabetes	PTB diabetes	PTB non-diabetes
No. of subjects recruited	30	27	–	30	27	30	27
Gender (male/female)	23/7	24/3	–	–	–	–	–
Median age (range)	48 (25–70)	40 (25–67)	$P = 0.0581$	–	–	–	–
Median height, cm	159 (133–169)	162 (140–184)	$P = 0.2121$	–	–	–	–
Median weight, kg	52 (32–67)	46 (30–90)	$P = 0.2484$	–	–	–	–
Smear grade: 0/1+/2+/3+	0/16/9/5	0/16/6/5	–	17/11/2/0	24/3/0/0	Negative	Negative
Culture results: negative/1+/2+/3+	0/10/5/15	0/12/6/9	–	26/1/1/2	24/2/1/0	Negative	Negative

influence both the phenotype and function of T cells in PTB infection and disease.

In this study, we examined the profile of T-cell subsets at baseline and at two time-points following initiation of treatment: 2 months, which marks the end of the intensive phase, and 6 months, when treatment is completed. Our data reveal that DM differentially modulates the *ex vivo* phenotype of CD4$^+$ and CD8$^+$ T-cell subsets in individuals with PTB before, during and after the completion of treatment.

Materials and methods

Ethics statement

This study was approved by the Ethics Committee of the Prof. M. Viswanathan Diabetes Research Centre (ECR/51/INST/TN/2013/MVDRC/01). Informed consent was obtained from all participants.

Study population

We studied a group of 57 individuals with PTB: 30 individuals with DM and 27 without DM. These individuals were a subset of individuals recruited for the 'Effects of Diabetes on Tuberculosis Severity' study presently underway at the Prof. M. Viswanathan Diabetes Research Centre and the National Institute for Research in Tuberculosis.[10] Consecutively enrolled individuals were recruited for this study. The baseline demographic characteristics of the study population are shown in Table 1. PTB was diagnosed on the basis of sputum smear and culture positivity. DM was diagnosed on the basis of oral glucose tolerance test and/or glycated haemoglobin (HbA1c) levels (for known diabetics), according to the World Health Organization criteria. All the DM individuals were newly diagnosed as having type 2 diabetes and were not on any DM medication. All the individuals were HIV seronegative and anti-tuberculous treatment naive. Anthropometric measurements, haematological and biochemical parameters were obtained using standardized techniques as detailed elsewhere. All individuals had pan-sensitive *Mycobacterium tuberculosis* on sputum culture at enrolment and all received standard TB treatment (Directly Observed Treatment Short Course with isoniazid, rifampicin, pyrazinamide and ethambutol for 2 months, followed by isoniazid and rifampicin for 4 months). All individuals were smear and culture negative at the end of 6 months of therapy (Table 1). Blood samples were collected at baseline, 2 months and 6 months post-treatment initiation.

Ex vivo analysis

All antibodies used in the study were from BD Biosciences, BD Pharmingen, eBioscience, Biolegend and R&D Systems. Whole blood was used for *ex vivo* phenotyping, which was performed on all 57 individuals. Briefly, 250-µl aliquots of whole blood were used, and a cocktail of monoclonal antibodies specific for various immune cell types was added. T-cell phenotyping was performed using antibodies directed against CD45 PerCP (clone 2D1; BD, Franklin Lakes, NJ), CD3 AmCyan (Clone SK7; BD), CD4 PeCy7 (clone SK3; BD), CD8 APCH7 (clone SK1; BD), CD45RA Pacific Blue (clone H1100; Biolegend, San Diego, CA), CD25 (clone M-A251; BD), CD127 (clone eBioRDRS; eBioscience, San Diego, CA), Foxp3 (clone 236A/E7, eBioscience) and CCR7 FITC (clone 3D12, eBioscience). Naive cells were classified as CD45RA$^+$ CCR7$^+$, central memory cells as CD45RA$^-$ CCR7$^+$, effector memory cells as CD45RA$^-$ CCR7$^-$, effector cells as CD45RA$^+$ CCR7$^-$ and regulatory T cells as CD4$^+$ CD25$^+$ Foxp3$^+$ CD127^{dim}.[11] Following 30 min of incubation at room temperature erythrocytes were lysed using 2 ml of FACS lysing solution (Becton Dickinson Biosciences Pharmingen, San Diego, CA), and cells were washed twice with 2 ml of 1× PBS and suspended in 200 µl of PBS (Lonza, Walkersville, MD). Surface antibodies were then used followed by washing and permeabilization with BD Perm/Wash buffer (BD

Biosciences) and stained with Foxp3 for an additional 30 min before washing and acquisition. Eight-colour flow cytometry was performed on a FACSCanto II flow cytometer with FACSDIVA software, version 6 (Becton Dickinson, Franklin Lakes, NJ). The gating was set by forward and side scatter, and 100 000 gated events were acquired. Data were collected and analysed using FLOW JO software (TreeStar, Ashland, OR). The gating strategy for T-cell subsets from whole blood is shown in the Supplementary material, Fig. S1.

Statistical analysis

Geometric means (GM) were used for measurements of central tendency. Comparisons were made using the Mann–Whitney U-test or Wilcoxon signed rank test and correlations with the Spearman Rank test. Analyses were performed using GRAPHPAD PRISM Version 6 (GraphPad, San Diego, CA).

Results

Biochemical and haematological features of the study population

No significant differences in age or gender were observed between the respective groups. PTB-DM individuals exhibited significantly higher levels of fasting and post-prandial glucose, HbA1c, serum triglycerides, total

cholesterol, low-density lipoprotein and very-low-density lipoprotein cholesterol (Table 2). They also exhibited significantly higher levels of total bilirubin but not other biochemical parameters (Table 2). Finally, PTB-DM individuals exhibited no significant difference in haematological parameters, with the exception of absolute neutrophil counts, which were significantly higher (Table 3). This is in confirmation of our previous data on PTB-DM individuals exhibiting higher levels of neutrophilia.[12]

PTB-DM is associated with heightened percentages of central memory CD4^{+} T cells and diminished percentages of naive, effector memory, effector and regulatory CD4^{+} T cells

To study the influence of DM on CD4^{+} T-cell subset homeostasis in PTB, we examined the percentages of five different CD4^{+} T-cell subsets (naive, central memory, effector memory, effector and regulatory) in PTB-DM and PTB-NDM individuals at baseline and at 2 and 6 months following anti-TB therapy. As shown in Fig. 1(a), PTB-DM is characterized by increased percentages of central memory CD4^{+} T cells and decreased percentages of naive, effector memory, effector and regulatory CD4^{+} T cells before treatment in comparison with PTB-NDM individuals. Similarly, at 2 months following initiation of treatment, PTB-DM is characterized by increased percentages of central memory subsets and decreased percentages of naive, effector and regulatory subsets (Fig. 1b). In contrast,

Table 2. Baseline biochemical parameters of study individuals

Study demographics	Baseline (before treatment)			Post treatment (6th month)		
	PTB diabetes	PTB non-diabetes	P value	PTB diabetes	PTB non-diabetes	P value
Fasting blood glucose, mg/dl	120 (98–293)	90 (68–101)	**P < 0·0001**			
Post prandial glucose, mg/dl	257 (203–448)	119 (76–137)	**P < 0·0001**			
Glycated haemoglobin level, %	9·3 (6·6 –14·6)	5·6 (5·0 –5·9)	**P < 0·0001**	8·8 (5·2–17·7)	5·4 (4·6–6·1)	**P < 0·0001**
Serum triglycerides, mg/dl	107 (66–178)	76 (39–113)	**P < 0·0001**			
Total cholesterol, mg/dl	182 (110–294)	162 (86–182)	**P = 0·0298**			
HDL cholesterol, mg/dl	37 (22–58)	35 (19–69)	P = 0·6957			
LDL cholesterol, mg/dl	95 (51–162)	83 (49–107)	**P = 0·0204**			
VLDL cholesterol, mg/dl	44 (18–76)	36 (15–48)	**P = 0·0039**			
Urea, mg/dl	18 (7–30)	16 (9–25)	P = 0·8982			
Creatinine, mg/dl	0·85 (0·6–1·0)	0·8 (0·6–1·2)	P = 0·2241			
Total bilirubin, mg/dl	0·5 (0·3–1·2)	0·3 (0·1–0·7)	**P = 0·0282**			
Total protein, g/dl	8·2 (6·3–9·0)	8·2 (7·1–9·7)	P = 0·8132			
Serum albumin, g/dl	4·1 (2·5–4·6)	4·1 (3·1–5·1)	P = 0·5735			
Serum globulins, g/dl	4 (3·2–5·1)	4·3 (3·2–5·0)	P = 0·4264			
AST, U/l	15 (6–31)	18 (10–48)	P = 0·2059			
ALT, U/l	17 (6–57)	14 (8–43)	P = 0·5636			
Alkaline phosphatase, U/l	278 (162–499)	235 (159–624)	P = 0·0562			
Vitamin D3, ng/ml	18 (3·1–48)	16 (3–49)	P = 0·2241			

ALT, alanine transaminase; AST, aspartate transaminase.
The values represent geometric means and range.
Bold values indicate statistically significant differences.

Table 3. Baseline haematological parameters of study individuals

Haematology profile	PTB-DM	PTB-NDM	P value
Red blood cell count, $\times 10^6$ cells/µl	4·9 (3·7–6·2)	4·6 (3–6·2)	NS
White blood cell count, $\times 10^3$ cells/µl	9800 (6300–14 700)	8300 (5200–18 700)	NS
Lymphocyte count, cells/ml	1775 (1008–2940)	1820 (884–3071)	NS
Neutrophil count, cells/ml	6525 (3484–10 496)	5698 (3348–14 586)	**P = 0·0408**
Monocyte count, cells/ml	870 (252–1414)	803 (102–1870)	NS
Eosinophil count, cells/ml	189 (87–819)	296 (73–913)	NS
Platelet count, $\times 10^3$ platelets/µl	342 (215–591)	378 (131–676)	NS

The values represent geometric means and range.
Bold values indicate statistically significant differences.
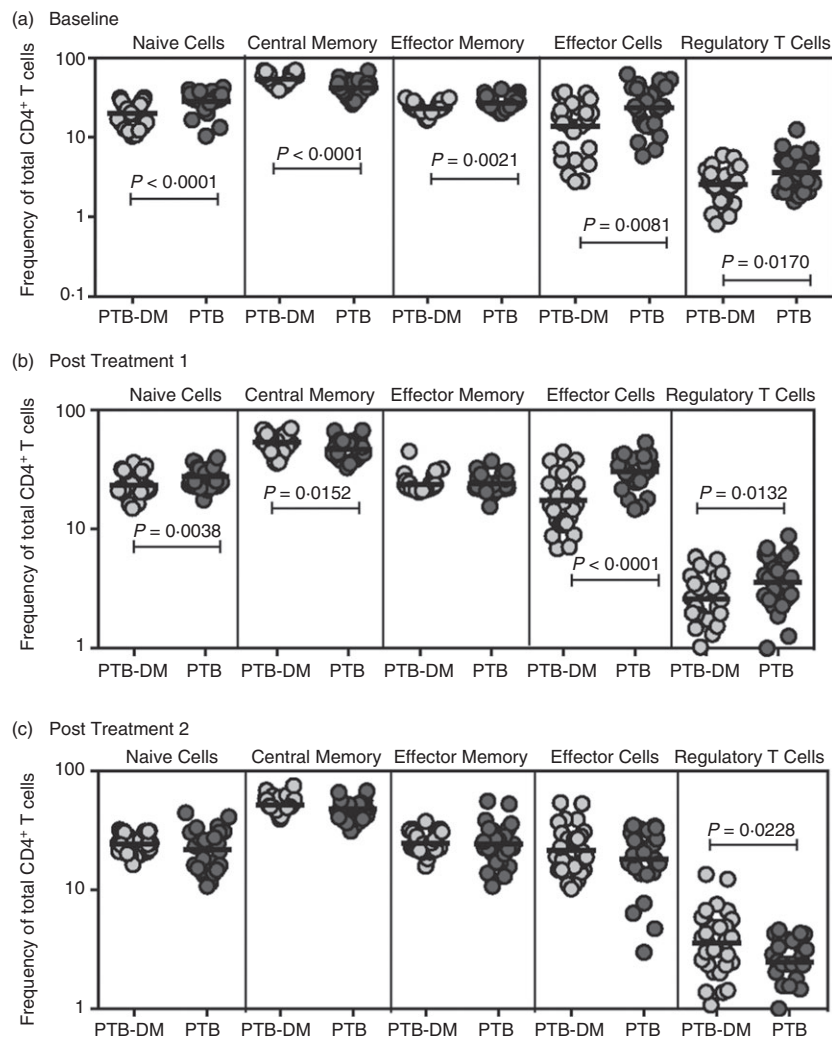
Figure 1. Pulmonary tuberculosis–diabetes mellitus co-morbidity (PTB-DM) is associated with altered frequencies of CD4^{+} T-cell subsets at baseline and following treatment. The frequencies of CD4^{+} T-cell subsets in PTB-DM ($n = 30$) and PTB ($n = 27$) individuals at baseline (a) and at 2 months (b) and 6 months (c) following treatment. The data are represented as scatter plots with each circle representing a single individual. P-values were calculated using the Mann–Whitney test.

as shown in Fig. 1(c), there were no significant differences in the percentages of naive, central memory, effector memory or effector CD4^{+} T cells between PTB-DM and PTB-NDM individuals and significantly increased percentages of regulatory CD4^{+} T cells in PTB-DM at the completion of 6 months of treatment. Therefore, DM is associated with alterations in the subset distribution of CD4^{+} T cells in PTB before, during and after treatment.

PTB-DM is associated with heightened percentages of central memory CD8$^+$ T cells and diminished percentages of naive and effector memory CD8$^+$ T cells

To study the influence of DM on CD8$^+$ T-cell subset homeostasis in PTB, we examined the percentages of four different CD8$^+$ T-cell subsets (naive, central memory, effector memory and effector) in PTB-DM and PTB-NDM individuals at baseline and at 2 and 6 months following anti-TB therapy. As shown in Fig. 2(a), PTB-DM is characterized by increased percentages of central memory CD8$^+$ T cells and decreased percentages of naive and effector memory CD8$^+$ T cells before treatment in comparison with PTB-NDM individuals. Similarly, at 2 months following initiation of treatment, PTB-DM is characterized by increased percentages of central memory CD8$^+$ T cells and decreased percentages of naive CD8$^+$ T cells (Fig. 2b). In

contrast, as shown in Fig. 2(c), there were no significant differences in the percentages of naive, central memory, effector memory or effector CD8$^+$ T cells between PTB-DM and PTB-NDM individuals at the completion of 6 months of treatment. Hence, DM is associated with alterations in the subset distribution of CD8$^+$ T cells in PTB before, during and after treatment, albeit with some differences in comparison to changes in CD4$^+$ T-cell subsets.

CD4$^+$ T-cell subsets exhibit a relationship with hyperglycaemia but not with bacterial loads in PTB

To determine the influence of hyperglycaemia on CD4$^+$ T-cell subset distribution in PTB, we examined the correlation between CD4$^+$ T-cell subsets and fasting blood glucose or HbA1c levels in all individuals with PTB (with or
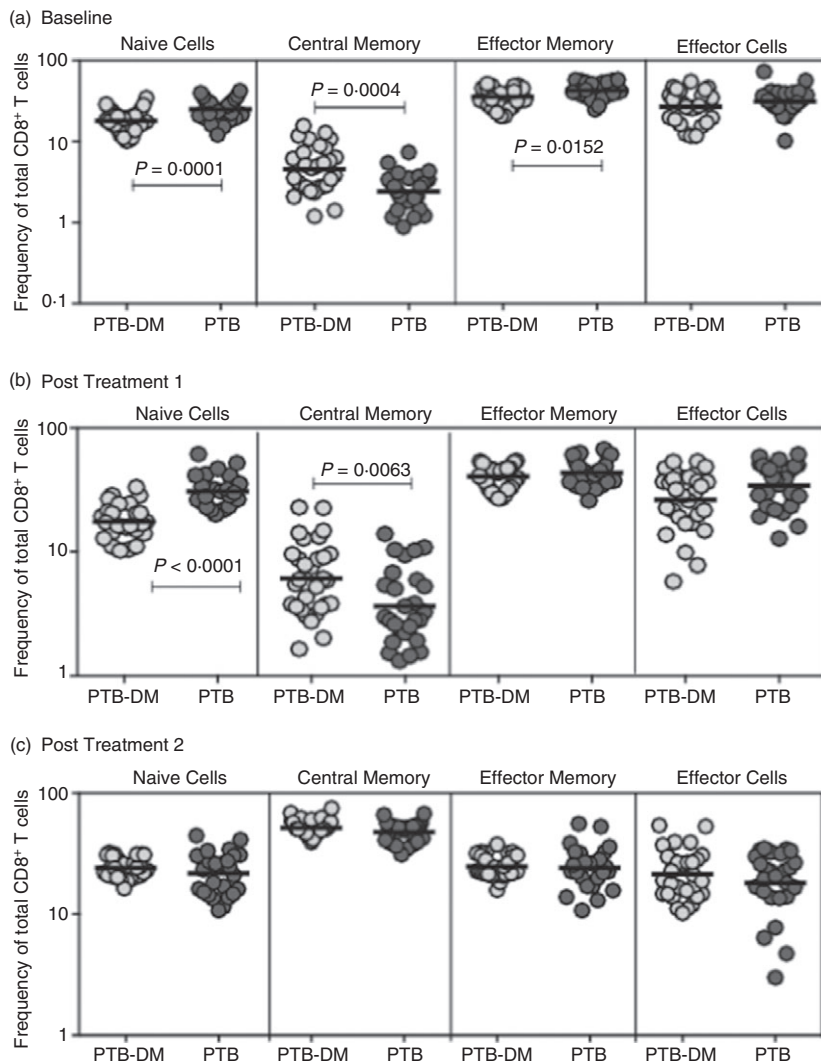
Figure 2. Pulmonary tuberculosis–diabetes mellitus co-morbidity (PTB-DM) is associated with altered frequencies of CD8$^+$ T-cell subsets at baseline and following treatment. The frequencies of CD8$^+$ T-cell subsets in PTB-DM ($n = 30$) and PTB ($n = 27$) individuals at baseline (a) and at 2 months (b) and 6 months (c) following treatment. The data are represented as scatter plots with each circle representing a single individual. P-values were calculated using the Mann–Whitney test.

without DM). As shown in Fig. 3(a), central memory CD4^{+} T cells exhibited a significant positive correlation with fasting blood glucose levels, whereas naive and effector memory CD4^{+} T cells exhibited a significant negative relationship. Similarly, as shown in Fig. 3(b), CD4^{+} T-cell subsets exhibited a similar pattern of relationship with HbA1c.

To determine the influence of bacterial burdens on CD4^{+} T-cell subset distribution in PTB, we also examined the relationship between CD4^{+} T-cell subsets and bacterial smear grades, classified as 1^{+}, 2^{+} and 3^{+}. However, as shown in Fig. 3(c), the percentages of CD4^{+} T cell subsets exhibited no significant correlation with bacterial smear grades in individuals with PTB with or without DM.

CD8^{+} T-cell subsets exhibit a relationship with hyperglycaemia but not with bacterial loads in PTB

To determine the influence of hyperglycaemia on CD8^{+} T-cell subset distribution in PTB, we examined the correlation between CD8^{+} T-cell subsets and fasting blood glucose or HbA1c levels in all individuals with PTB (with or without DM). As shown in Fig. 4(a), central

memory CD8^{+} T cells exhibited a significant positive correlation with fasting blood glucose levels, whereas naive CD8^{+} T cells exhibited a significant negative relationship. Similarly, as shown in Fig. 4(b), central memory CD8^{+} T cells exhibited a significant positive correlation with HbA1c levels, whereas naive and effector CD8^{+} T cells exhibited a significant negative relationship.
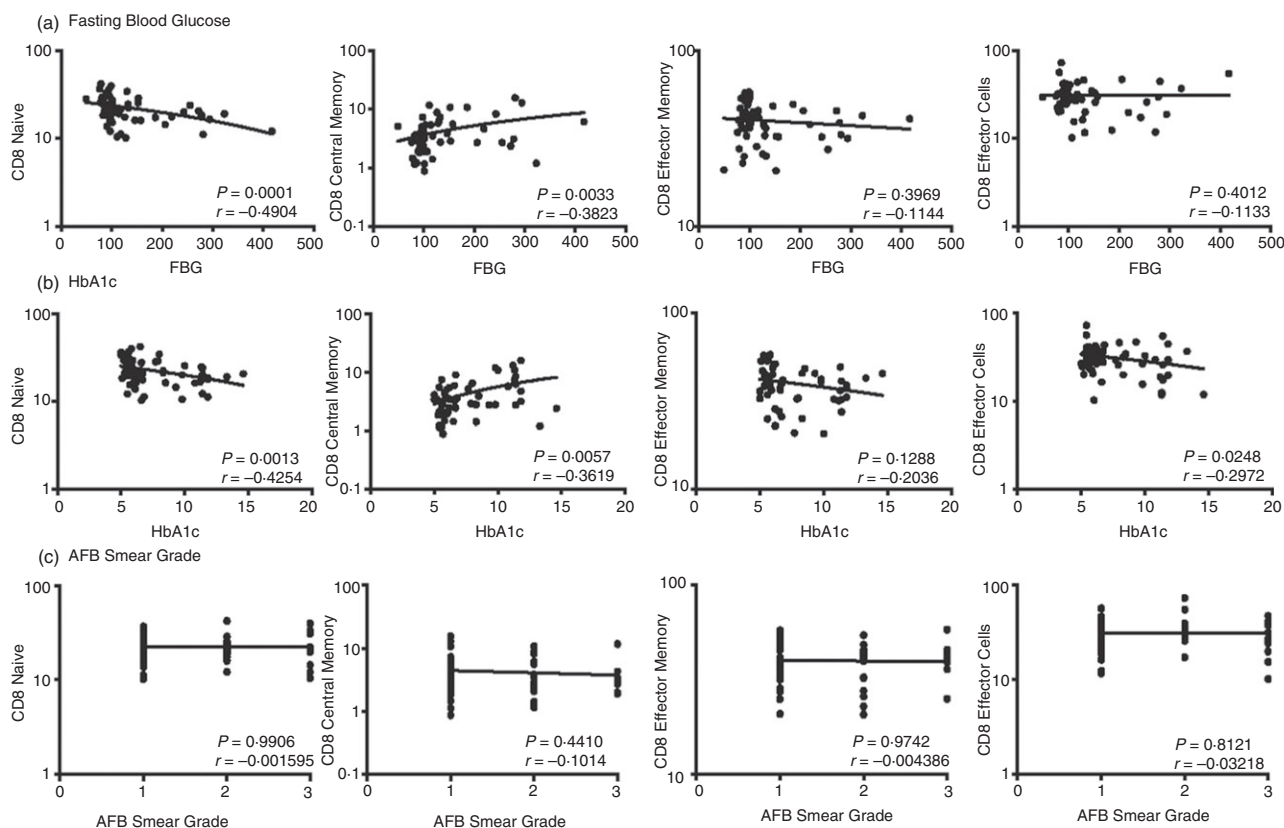
To determine the influence of bacterial burdens on CD8^{+} T-cell subset distribution in PTB, we also examined the relationship between CD8^{+} T-cell subsets and bacterial smear grades, classified as 1^{+}, 2^{+} and 3^{+}. However, as shown in Fig. 4(c), the percentages of CD8^{+} T-cell subsets exhibited no significant correlation with bacterial smear grades in individuals with PTB with or without DM.

Treatment-induced changes in CD4^{+} T-cell subsets in individuals with PTB

Alterations in T-cell subsets following treatment in individuals with PTB has not been well described. Therefore, to determine the impact of treatment on the *ex vivo* phenotype of CD4^{+} T-cell subsets across the entire cohort of
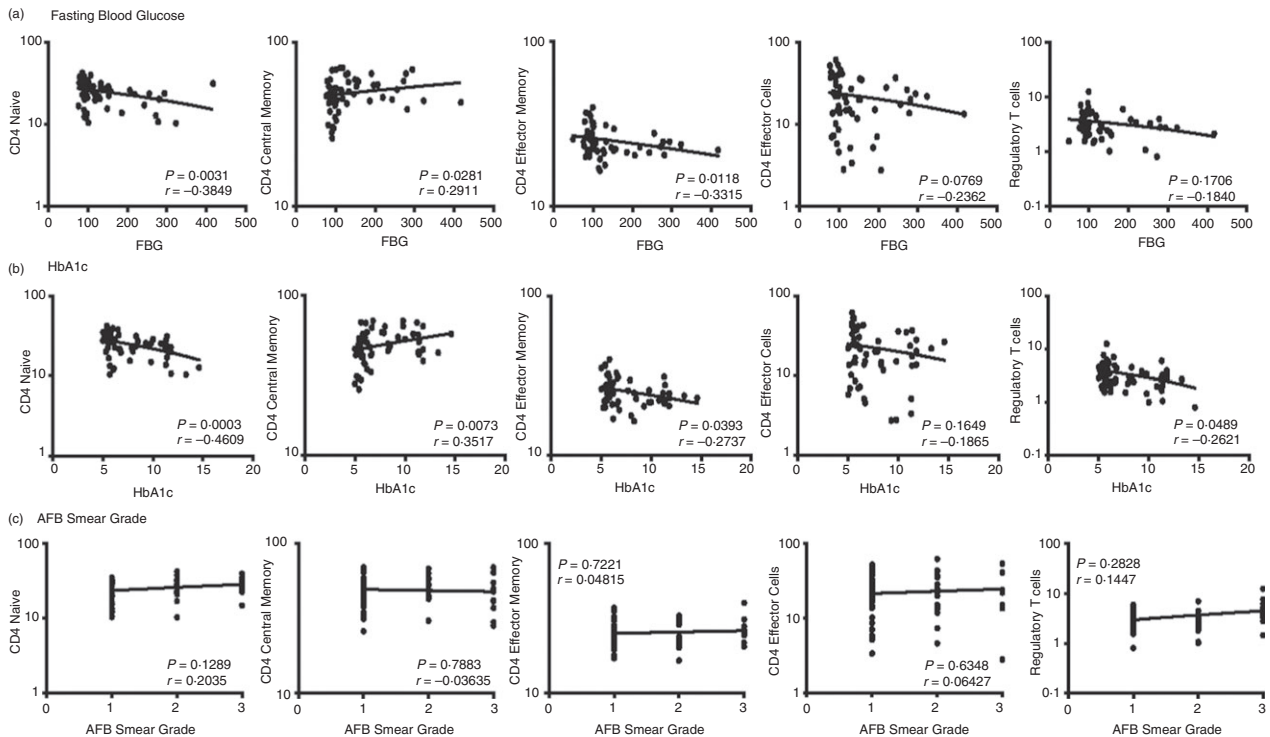
Figure 3. Relationship between CD4^{+} T-cell subsets and hyperglycaemia or bacterial burdens in individuals with pulmonary tuberculosis–diabetes mellitus co-morbidity (PTB-DM) and with PTB only. (a) The correlation between the frequencies of CD4^{+} T-cell subsets and fasting blood glucose levels at baseline in all individuals. (b) The correlation between the frequencies of CD4^{+} T-cell subsets and HbA1c levels at baseline in all individuals. (c) The correlation between the frequencies of CD4^{+} T-cell subsets and bacterial burdens as determined by smear grades (1^{+}, 2^{+} or 3^{+}) in all individuals. The data are represented as scatter plots with each circle representing a single individual. *P*-values were calculated using the Spearman Rank correlation.

Figure 4. Relationship between CD8^{+} T-cell subsets and hyperglycaemia or bacterial burdens in individuals with pulmonary tuberculosis–diabetes mellitus co-morbidity (PTB-DM) and with PTB only. (a) The correlation between the frequencies of CD8^{+} T-cell subsets and fasting blood glucose levels at baseline in all individuals. (b) The correlation between the frequencies of CD8^{+} T-cell subsets and HbA1c levels at baseline in all individuals. (c) The correlation between the frequencies of CD8^{+} T-cell subsets and bacterial burdens as determined by smear grades (1^{+}, 2^{+} or 3^{+}) in all individuals. The data are represented as scatter plots with each circle representing a single individual. P-values were calculated using the Spearman Rank correlation.

PTB patients (irrespective of DM co-morbidity), we examined the different subsets at 2 months and 6 months following initiation of treatment. As shown in Fig. 5(a), we found no significant differences in the percentages of CD4^{+} T-cell subsets, except effector memory CD4^{+} T cells, which showed a significant increase at 2 months following treatment. However, as shown in Fig. 5(b), we observed a significant increase in the percentages of naive CD4^{+} T cells and a significant decrease in the percentages of central memory CD4^{+} T cells at the completion of treatment (6 months). Hence, the alterations in the percentages of CD4^{+} T-cell subsets in PTB are reversed by anti-TB treatment.
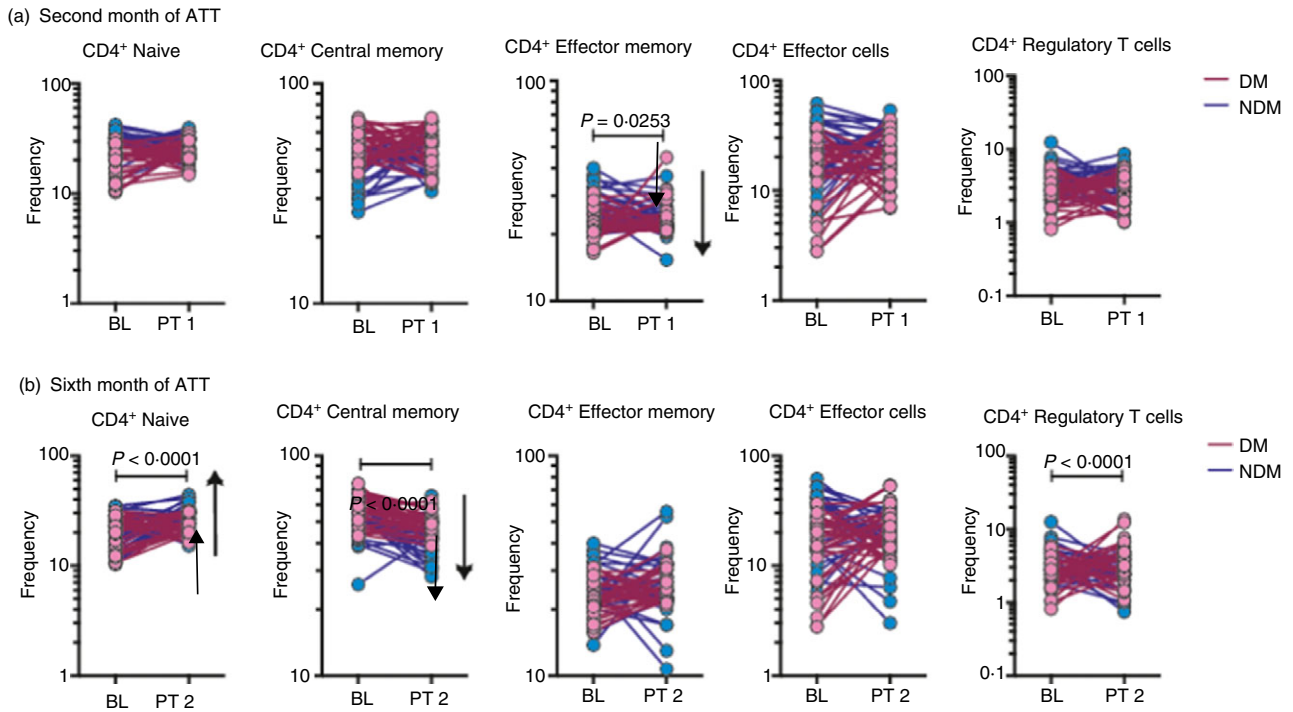
Treatment induced changes in CD8^{+} T-cell subsets in individuals with PTB

To determine the impact of treatment on the *ex vivo* phenotype of CD8^{+} T-cell subsets across the entire cohort of individuals with PTB, we examined the different subsets at 2 months and 6 months following initiation of

treatment. As shown in Fig. 6(a), we found no significant differences in the percentages of CD8^{+} T-cell subsets, except effector memory CD8^{+} T cells, which showed a significant increase at 2 months following treatment. However, as shown in Fig. 6(b), we observed a significant increase in the percentages of naive and effector CD8^{+} T cells and a significant decrease in the percentages of central memory CD8^{+} T cells at the completion of treatment (6 months). Hence, the alterations in the percentages of CD8^{+} T-cell subsets in PTB are reversed by anti-TB treatment.

Discussion

Both CD4^{+} and CD8^{+} T cells are important in protective immunity against TB. These cell types can be further classified into four major subsets based on their expression of activation markers and chemokine receptors. Using CD45 and CCR7 expression, these cells can be subdivided into naive, central memory, effector memory and effector T-cell compartments in circulation.[11] CCR7^{+}

Figure 5. Treatment-induced alterations in CD4$^+$ T-cell subsets in individuals with pulmonary tuberculosis–diabetes mellitus co-morbidity (PTB-DM) and with PTB only. (a) The frequencies of CD4$^+$ T-cell subsets in all individuals before and 2 months following anti-TB treatment. (b). The frequencies of CD4$^+$ T-cell subsets in all individuals before and 6 months following anti-TB treatment. The data are represented as line diagrams with each line representing a single individual. The pink lines represent PTB-DM individuals and the blue lines represent the PTB individuals. *P*-values were calculated using the Wilcoxon signed rank test.

memory T cells are termed central memory T cells and are able to home to secondary lymphoid organs and produce high levels of IL-2 but low levels of other cytokines, whereas CCR7$^-$ memory T cells are termed effector memory T cells and are able to produce high levels of effector cytokines, exert rapid effector functions and are able to home to peripheral tissues. Both central memory and effector memory T cells have been shown to play important roles in protective immune responses in animal models of vaccination or protection with central memory T cells dominating the antigen-specific immune response in vaccination experiments.[13] Moreover, while latent TB is thought to be associated with expansion of effector and central memory T cells, active TB disease is associated with expansion of only central memory T cells.[14]

CD4$^+$ and CD8$^+$ T-cell responses are known to be altered in DM, with hyperactivation of T cells being a major feature.[7,8] Impaired glucose tolerance and diabetes are typically associated with poor control of infection,[15,16] impaired vaccination responses,[17] elevated inflammatory activity[5,6,18] and shorter leucocyte telomere length.[19] In addition, elevated HbA1c levels are associated with the accumulation of differentiated T cells in cytomegalovirus-positive individuals.[20] These observations raise the question of whether the immune effects of hyperglycaemia

could involve effects on memory T-cell subsets. We have previously demonstrated that PTB profoundly alters the memory repertoire of CD8$^+$ T cells but not CD4$^+$ T cells, whereas latent TB has only a minimal effect on both compartments and DM alone primarily alters the memory repertoire of CD4$^+$ T cells but not CD8$^+$ T cells.[9] We had also previously demonstrated that latent and active TB cannot be differentiated based on the CD4$^+$ T-cell memory subsets alone as very few changes in these subsets were observed.[9] However, no longitudinal studies on the evolution of the memory response in TB-DM have been reported.

Our data reveal four major features in terms of CD4$^+$ and CD8$^+$ T-cell phenotypes in the peripheral blood of PTB-DM patients. First, central memory CD4$^+$ and CD8$^+$ T cells are the predominant cell type in terms of percentages in PTB-DM compared with PTB-NDM individuals and this is accompanied by a concomitant decrease in the percentages of other T-cell subsets. Second, the heightened frequencies of central memory T cells are directly associated with hyperglycaemia in the presence of active TB but not with bacterial burden, as estimated by sputum smear grade. Third, the elevated frequencies of central memory T cells and depressed frequencies of other T-cell subsets are at least partially reversible upon standard TB treatment, indicating that
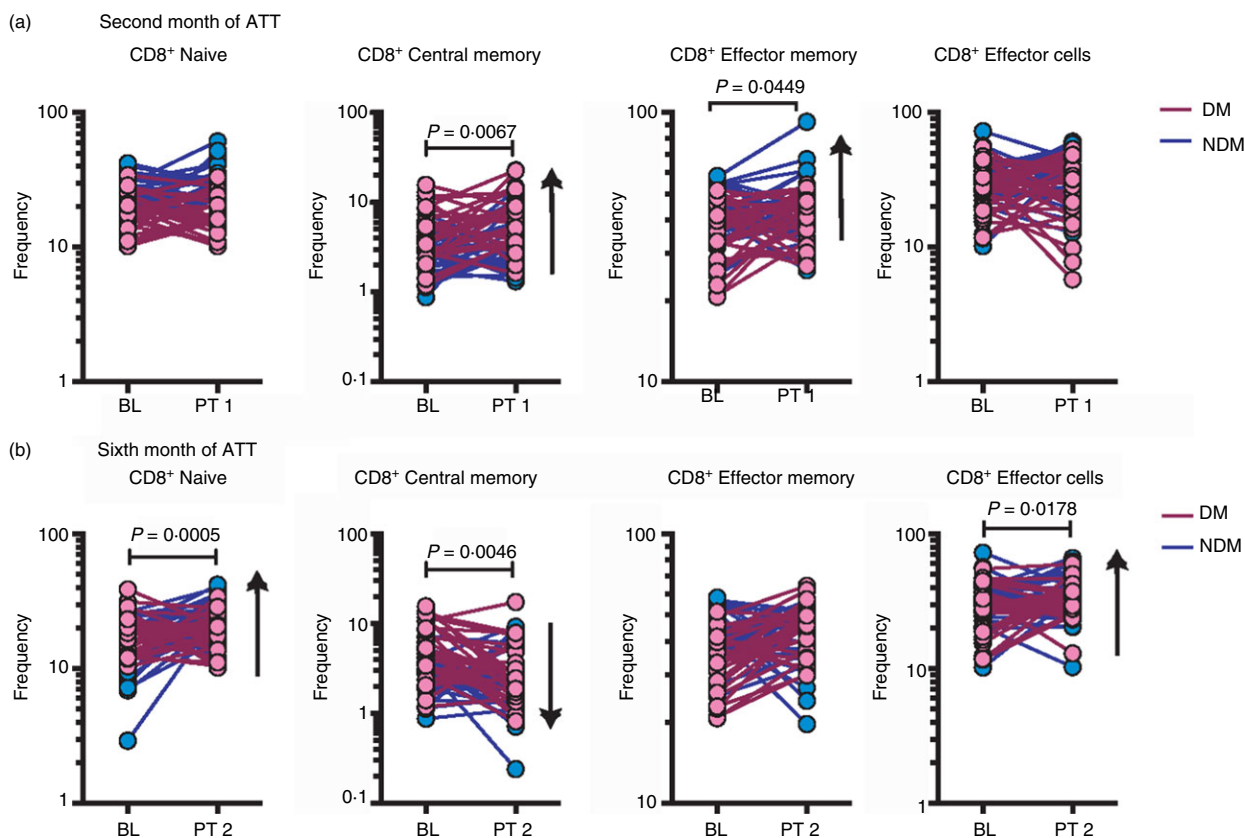
Figure 6. Treatment-induced alterations in CD8^{+} T-cell subsets in individuals with pulmonary tuberculosis–diabetes mellitus co-morbidity (PTB-DM) and with PTB only. (a) The frequencies of CD8^{+} T-cell subsets in all individuals before and 2 months following anti-TB treatment. (b) The frequencies of CD8^{+} T-cell subsets in all individuals before and 6 months following anti-TB treatment. The data are represented as line diagrams with each line representing a single individual. The pink lines represent PTB-DM individuals and the blue lines represent the PTB individuals. P-values were calculated using the Wilcoxon signed rank test.

the presence of TB bacilli does influence the memory T-cell phenotype in these individuals. Finally, the percentages of natural regulatory T cells (Treg cells) are significantly diminished in PTB-DM at baseline and early following treatment but gets completely reversed upon completion of treatment. However, since sputum smear grade is not the most accurate measure of bacterial burdens, it might still be possible that bacterial antigens could influence T-cell subset distribution.

We postulate that the elevated percentages of central memory T cells and decreased percentages of effector memory T cells could reflect T-cell migration patterns to the site of infection. Hence, higher influx of effector and effector memory T cells to the lungs could result in the peripheral distribution of increased proportions of central memory T cells. This model would be consistent with a greater level of immune pathology associated with PTB-DM co-morbidity.[21] The expanded percentages of central memory T cells in circulation could also contribute to the exaggerated T-cell responses commonly found in individuals with PTB-DM.[22] Future studies examining the cytokine profile of central memory versus effector memory T cells should offer

further insight into this process. The clear associations between either fasting blood glucose or HbA1c and proportions of memory T cells indicate a positive relationship between hyperglycaemia and T-cell subset distribution in TB-DM co-morbidity. Evidence from *in vitro* studies suggest that strong, repetitive antigen receptor stimulation by infectious agents can lead to the up-regulation of the GLUT1 receptor on T cells and to enhanced glucose uptake.[23] Previous studies have shown that this elevated glucose uptake is associated with increased T-cell activation, pro-inflammatory cytokine production and an elevated threshold for apoptosis.[24,25] Hence, our data add to the growing body of evidence indicating an effect on hyperglycaemia on the homeostatic or infection-driven function of T cells in DM. Another intriguing observation in our study was the influence of DM on the phenotype of T-cell subsets in all PTB individuals.

Data from our study also show differences in the proportion of Treg cells in peripheral blood of PTB-DM versus PTB-NDM patients with reciprocal variation from baseline to TB treatment completion. Treg cells are known to play a vital role in protection against metabolic

diseases and a unique population of Treg cells residing in the visceral fat are thought to be the primary drivers of this protective effect.[26,27] However, deficiencies in Treg frequencies or numbers in the periphery have also been implicated in increased susceptibility to insulin resistance and pathogenesis of DM.[28] Our data indicate that TB disease is associated with altered frequencies of peripheral Treg cells, which are further influenced by successful treatment. It has been reported that while *ex vivo* Treg cells are highly glycolytic, memory T cells predominantly depend on fatty acid oxidation.[29] Interestingly, upon *in vitro* culture, Treg cells engage both glycolysis and fatty acid oxidation to proliferate while memory T cells mainly rely on glucose metabolism.[30] This provides a potential mechanism for the differential effect of DM on memory versus regulatory T cells in PTB.

Our study suffers from the limitations of being a descriptive study, having a limited sample size and measuring only percentages of T-cell subsets and not absolute numbers. Nevertheless, our results clearly delineate a profound impact of diabetes on the homeostatic T-cell profiles in individuals with active TB. It would be interesting to study the impact of PTB-DM on the immune responses to other pathogens. Being longitudinal in design, our study also clearly defines the evolution of this phenotype with progression of treatment. Our study also implies that alterations in T-cell subsets potentially contribute to the immune responses observed in PTB-DM comorbidity and suggests that this complication of diabetes is driven by chronic hyperglycaemia. Future studies elaborating on the mechanism of this T-cell subset's role in PTB-DM could help to define key checkpoints to be targeted for immune intervention in this co-morbid condition.

Acknowledgements

Conflict of interest

None reported.

Disclosure

The authors have no competing interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Gating strategy for estimating frequencies of CD4$^+$ and CD8$^+$ T cell subsets.