Tuberculosis 106 (2017) 25–32

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

Differences in IgG responses against infection phase related *Mycobacterium tuberculosis* (*Mtb*) specific antigens in individuals exposed or not to *Mtb* correlate with control of TB infection and progression

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ARTICLE INFO

Article history:
Received 8 February 2017
Received in revised form
2 June 2017
Accepted 4 June 2017

Keywords:
Mycobacterium tuberculosis (Mtb)
TB
IgG
Mtb DosR regulon-encoded proteins
Resuscitation-promoting factors
IVE-TB antigens

ABSTRACT

Tuberculosis (TB) occurs in only 3–10% of *Mycobacterium tuberculosis* (*Mtb*) infected individuals, suggesting that natural immunity can contain *Mtb* infection, although this remains poorly understood. Next to T-cells, a potentially protective role for B-cells and antibodies has emerged recently. However, the *Mtb* antigens involved remain ill-defined. Here, we investigated in a TB-endemic setting IgG levels against 15 *Mtb* antigens, representing various phases of *Mtb* infection and known to be potent human T-cell antigens. IgG levels against ESAT6/CFP10, Rv0440, Rv0867c, Rv1737c, Rv2029c, Rv2215, Rv2389c, Rv3616c and *Mtb* purified protein derivative (PPD) were higher in TB patients than in endemic and non-endemic controls. The only exception was Rv1733c that was preferentially recognized by antibodies from endemic controls compared to TB patients and non-endemic controls, suggesting a potential correlation with control of TB infection and progression. In patients, IgG levels against Ag85B and Rv2029c correlated with *Mtb* loads, while immunoglobulins against Rv0440 differed between genders. Our results support the potential role of certain *Mtb* antigen-(Rv1733c) specific antibodies in the control of TB infection and progression, while other *Mtb* antigen-specific antibodies correlate with TB disease activity and bacillary loads. The findings for Rv1733c agree with previous T-cell results and have implications for including antibody-mediated immunity in designing new strategies to control TB.

1. Introduction

Humoral immunity has been generally considered not to play a significant role in controlling infection by *Mycobacterium tuberculosis* (*Mtb*) [1], the facultative intracellular bacillus causing tuberculosis (TB), which causes 1.8 million deaths annually [2]. The dominant T-cell-centric paradigm in TB is in line with the classical view of a strict separation between antibody-mediated and cell-mediated immunity directed against extracellular and intracellular pathogens, respectively [3]. The elevated susceptibility to *Mtb* in individuals with primary (genetic) or acquired (HIV) cellular immunodeficiencies [4,5] and the strong correlation of polyfunctional $CD4^+$ T cells with TB vaccine-induced protection in mice [6] have contributed to that dogma. As a consequence our understanding of acquired immunity against TB and corresponding strategies for the development of new candidate TB vaccines have mainly focused on T cell responses [7]. However, it is now clear that the presence of *Mtb* specific polyfunctional T cells is not sufficient to protect the human host against TB [8]. Moreover, a recent clinical trial revealed that the frequency of polyfunctional T-cells in BCG/MVA85 vaccinated individuals did not correlate with the presence -or absence-of later development of TB disease in children [9,10].

In search of a better understanding of the complex immune response against *Mtb*, several studies have evaluated B cells [11] and immunoglobulin (Ig) responses in the context of *Mtb* infection [12]. Whereas the role of B cells is becoming increasingly

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http://dx.doi.org/10.1016/j.tube.2017.06.001

appreciated [13,14], the contribution of humoral immunity in TB host defense is under debate, although many serological studies have reported the presence of antibodies during *Mtb* infection [15]. IgG titers against *Mtb*-specific proteins are detectable in immunocompetent, latently *Mtb*-infected individuals as well as during progression of active *Mtb* infection in TB-asymptomatic smear-negative HIV-positive patients [16]. Additionally, a strong correlation has been documented between the presence of specific anti-*Mtb* lipoarabinomannan IgG responses and the absence of miliary TB in a pediatric population, suggesting the possible involvement of specific IgG in containing *Mtb* infection in infants [17]. This hypothesis has been further supported by evidence that passive immunization with serum obtained from mice immunized with RUTI, a therapeutic TB vaccine, limited the presence of the bacilli to the lungs of *Mtb*-infected severe combined immunodeficient mice [18]. Furthermore, many studies observed that several *Mtb* antigens able to confer TB protection in mice and non-human primates elicited specific mycobacterial antibodies not induced by natural *Mtb* infection [19–21]. In addition, the potential protective role of antibody responses has also been suggested by the association of Ag85A-specific IgG boosted by MVA85A vaccination in adolescents with a significantly reduced risk of developing TB [22].

Collectively these results suggest a potential contribution of *Mtb* antigen specific antibodies to protection from TB disease. Various mechanisms may be involved [23] which include: opsonization, which may increase *Mtb* uptake and intracellular killing [24]; amelioration of T-cell activation, through an increased secretion of pro-inflammatory cytokines [24]; enhanced antigen presentation to CD4 T cells [25]; activation of the complement cascade (7); the formation of immuno complexes that target *Mtb* antigens and activate DCs through FcR ligation [26]; the glycosylation of the constant (Fc) domains that control the balance between pro- and anti-inflammatory antimicrobial responses [27] via up-regulated Fcγ receptor expression [28] by dendritic cells and macrophages [29]; the initiation of innate immune signaling through the activation of intracellular receptors such as TRIM21 [30].

In search of better TB diagnostics and new TB vaccine candidates, many innovative strategies have been applied to discover the *Mtb* antigenome responsible for eliciting human cellular immunity [31]. *Mtb* dormancy regulon (DosR regulon) encoded proteins as well as resuscitation promoting factor (Rpf) proteins have been associated with *Mtb* latency and found to be recognized by $CD4^+$ and $CD8^+$ T-cells in *Mtb*-infected individuals. Genome-wide unbiased antigen discovery approaches used pulmonary *in vivo Mtb* gene expression profiles in mice infected with *Mtb*, to identify IVE-TB antigens, stimulating T-cell responses in latently *Mtb*-infected individuals [32,33]. While all these groups of antigens have been extensively evaluated for their ability to induce T cell responses across many different human populations, these studies were often not complemented by serological analyses. Therefore, we decided to evaluate IgG responses against PPD and a selection of specific *Mtb* antigens, based on our previous antigen discovery work. The antigens studied here included: *Mtb* DosR regulon-encoded proteins (Rv1733c, Rv1737c and Rv2029c) [34], *Mtb* resuscitation-promoting factors (Rv0867c and Rv2389) [35], IVE-TB antigens (Rv0440, Rv0645c, Rv1980c, Rv2031, Rv2215, Rv2626c, Rv3407 and Rv3616c) [33] and *Mtb* antigens related to active growth (ESAT-6/CFP10 and Ag85B) [36,37].

2. Materials and methods

2.1. Study design and sample collection

A total of 116 stored samples collected between 2005 and 2014 were used in the present study; these were obtained from the University of Antioquia and included subjects previously recruited in Medellin, Colombia. Individuals were either smear-positive patients experiencing active pulmonary tuberculosis (TB; $n = 85$: 77 patients had no or <1 month anti-TB chemotherapy, 7 patients 1–2 months of treatment, and 1 patient > 3 months) or healthy individuals from the same endemic area with known ($n = 15$) or unknown TB ($n = 16$) exposure. Humoral responses in these two endemic groups did not differ significantly such that these were combined into a single endemic control group (EC; $n = 31$). Sera from a group of non-endemic Dutch controls (NEC; $n = 25$) were included in the study. The Dutch controls were individuals with a negative Mantoux tuberculin skin test (TST) (<15 mm) and QuantiFeron-TB Gold In–Tube test (QFT-GIT) (Cellestis, Carnegie, VIC, Australia) (≤0.3 IU/ml). None of the donors included in this study had comorbidities (HIV-infection, diabetes or cancer) at the time of recruitment.

The study protocols P207/99 and P07/048 were approved by the institutional review board of Leiden University Medical Center, The Netherlands, and by the Ethics Committee of the Instituto de Investigaciones Médicas of the Facultad de Medicina, Universidad de Antioquia. Medellín, Colombia and the local Colombian health authorities (Dirección Seccional de Salud de Antioquia and the Secretaría de Salud de Medellín), respectively.

2.2. Mycobacterial stimuli

Mtb DosR regulon-encoded proteins (Rv1733c, Rv1737c and Rv2029c), resuscitation-promoting factors (Rv0867c and Rv2389), IVE-TB antigens (Rv0440, Rv0645c, Rv1980c, Rv2031c, Rv2215, Rv2626c, Rv3407 and Rv3616c) and *Mtb* active growth phase antigens (ESAT-6/CFP10 fusion protein and Ag85B) were used in this study. HPV16E6 was included as a negative recombinant control. Recombinant production and quality control were performed as previously described [33]. *Mtb* purified protein derivative (PPD) RT-50 (Statens Serum Institut, Copenhagen, Denmark) was also included in the screening.

2.3. Quantitation of serum IgG antibody responses

Total IgG responses were measured by ELISA [21]. Microlon® high binding 96-well plates were coated for 2 h at 37 °C with PPD, recombinant proteins (5 μg/ml) or PBS 0.4% Bovine Serum Albumin (BSA) used as blank. After removing unbound proteins using washing buffer (PBS containing 0.05% Tween-20), plates were blocked for an hour at 37 °C with blocking buffer (PBS containing 1% BSA and 1% Tween-20). After washing plates three times, diluted samples (1:400) were added to the wells and incubated overnight at 4 °C. Then, plates were rinsed six times with washing buffer and incubated with polyclonal rabbit anti-human total IgG/HRP (P0214 Dako, Glostrup, Denmark). After 2 h at 37 °C, plates were washed four times, and tetramethylbenzidine substrate (TMB; Sigma) was added and incubated for 15 min in the dark at room temperature. By adding H_2SO_4, the reaction was stopped, and absorbance was measured using a BioRad Microplate Reader 680 (BioRad Laboratories, Veenendaal, The Netherlands) at 450 nm. The blank median optical density (OD_{450}), which ranged 0.05–0.11 in TB, 0.05–0.07 in the EC and 0.05–0.08 in the NEC, was subtracted for each sample to avoid background influence.

2.4. Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 6.0). The Mann–Whitney test was used to compare the difference between groups of donors based on the IgG antibody levels measured as OD_{450} induced by stimulation with the antigens

Table 1
Characteristics of tuberculosis (TB) patients included in the study.

Characteristics	TB Patients ($n = 85$)
Age, y, median (range)	34 (16–73)
Male sex	44/85
Presence of BCG scar	64/85
Sputum smear results	
1–5 bacilli	28
5–10 bacilli	23
>10 bacilli	34
Days of treatment, median (range)	9 (0–155)
TB recurrences	14/85

BCG: Bacillus Calmette Guérin.

Table 2
Characteristics of Healthy Controls (HC) Included in The Study.

Characteristics	HC	
	EC ($n = 31$)	NEC ($n = 25$)
Age, y, median (range)	29 (19–65)	38 (27–62)
Male sex	12/31	8/25
Presence of BCG scar	12/14	2/25
TST positive*	8/8	0/16

EC: endemic controls; NEC: non-endemic controls; BCG: Bacillus Calmette Guérin; TST: tuberculin skin test. HC: healthy controls; TB: tuberculosis patients.
*: induration of 15 or more millimeters.

or PPD. A p value less than 0.05 was considered statistically significant.

3. Results

3.1. Differences in Mtb specific IgG responses to infection phase related Mtb specific protein antigens in subjects exposed or not to Mtb

IgG levels against 15 *Mtb* recombinant protein antigens, previously shown to induce significant T cell responses in latently TB infected individuals (LTBI) were measured in 141 sera by ELISA. *Mtb* PPD was used as a positive control antigen. Sera were obtained from Colombian patients with active (pulmonary) tuberculosis (TB) ($n = 85$), Colombian endemic controls (EC) ($n = 31$) and Dutch non-endemic controls (NEC) ($n = 25$) (Tables 1 and 2). Differences among donor groups were evaluated by the Mann–Whitney test.

Combining the IgG levels against the 15 phase related *Mtb* specific protein antigens, TB patients showed a higher humoral response than EC and NEC ($p = 0.0378$ and < 0.0001, respectively). A significant difference ($p = 0.0015$) was also observed between the total levels of antibodies produced by EC and NEC (Fig. 1A). A highly significant difference was observed in anti-tuberculin IgG antibody levels between TB patients and EC ($p < 0.0001$), between TB patients and NEC ($p < 0.0001$), and between EC and NEC ($p < 0.0017$) (Fig. 1B). Similarly, high serum levels of anti-ESAT6/CFP10 IgG antibodies were found in TB patients compared to EC ($p < 0.0001$) and NEC ($p < 0.0001$) (Fig. 2A). However, no differences were found between EC and NEC, suggesting that the observed differences in PPD specific IgG between EC and NEC might be due to exposure to non-tuberculous, cross-reactive mycobacterial antigens, present in PPD but absent from ESAT6/CFP10 protein. Although less significant, the IgG responses detected against Rv3616c resembled the trend described for ESAT6/CFP10 (TB vs. EC: $p = 0.0243$; TB vs. NEC: $p = 0.0340$) (Fig. 2B). In addition, serum IgG levels against other *Mtb* antigens were found to be higher in TB patients than in EC (Rv0867c) and NEC (Rv0440, Rv1737c, Rv2029c, Rv2215, Rv2389c, Rv3616c) and were also higher in EC than in NEC (Rv0440, Rv1733c, Rv1737c, Rv2389c) (Table 3). Notably, IgG antibody levels against one latency antigen, Rv1733c, were highest in the EC compared to the TB group and NEC ($p < 0.0001$) (Fig. 2C). This is interesting as T cell responses against Rv1733c are normally higher in LTBI than TB patients, in contrast to ESAT6/CFP10. No differences between the groups were observed in the total IgG levels against HPV16E6, used as unrelated control antigen (Fig. 2D), or against the other *Mtb* antigens tested (Ag85B, Rv0645c, Rv2031c, Rv2626c and Rv3407) (Table 3).

3.2. IgG responses to specific Mtb antigens differ between TB patients by gender and clinical characteristics

The IgG responses detected within the TB patient group were analyzed in more detail with respect to age, gender, evidence of BCG scar and clinical characteristics reported at recruitment (Table 2). Interestingly, IgG levels against Rv0440 were higher in males than in females ($p = 0.0193$), suggesting a significant influence of gender on the humoral immune response against this antigen (Fig. 3A). In addition, individuals with a high number of acid-

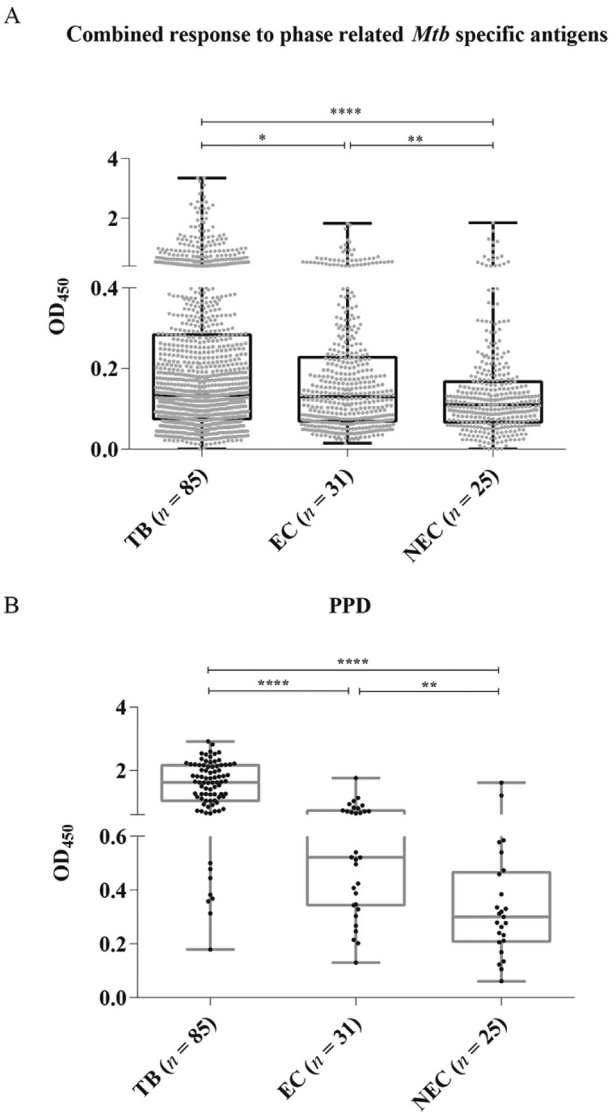

Fig. 1. Cumulative IgG responses to *Mycobacterium tuberculosis* (*Mtb*) antigens. Box-and-whisker plots feature individual dots that correspond to the optical density (OD) values measured in response to all 15 *Mtb* antigens included in this study (A) and PPD (B). Values are shown for sera from 85 TB patients (TB), 31 endemic controls (EC) and 25 non-endemic controls (NEC). The background was subtracted from all samples. A p-value of ≤0.05 was considered significant. Significance levels are indicated as follows: *: $p = 0.01–0.05$; **: $p = 0.001–0.01$; ***: $p = 0.0001–0.001$; ****: $p \leq 0.0001$.

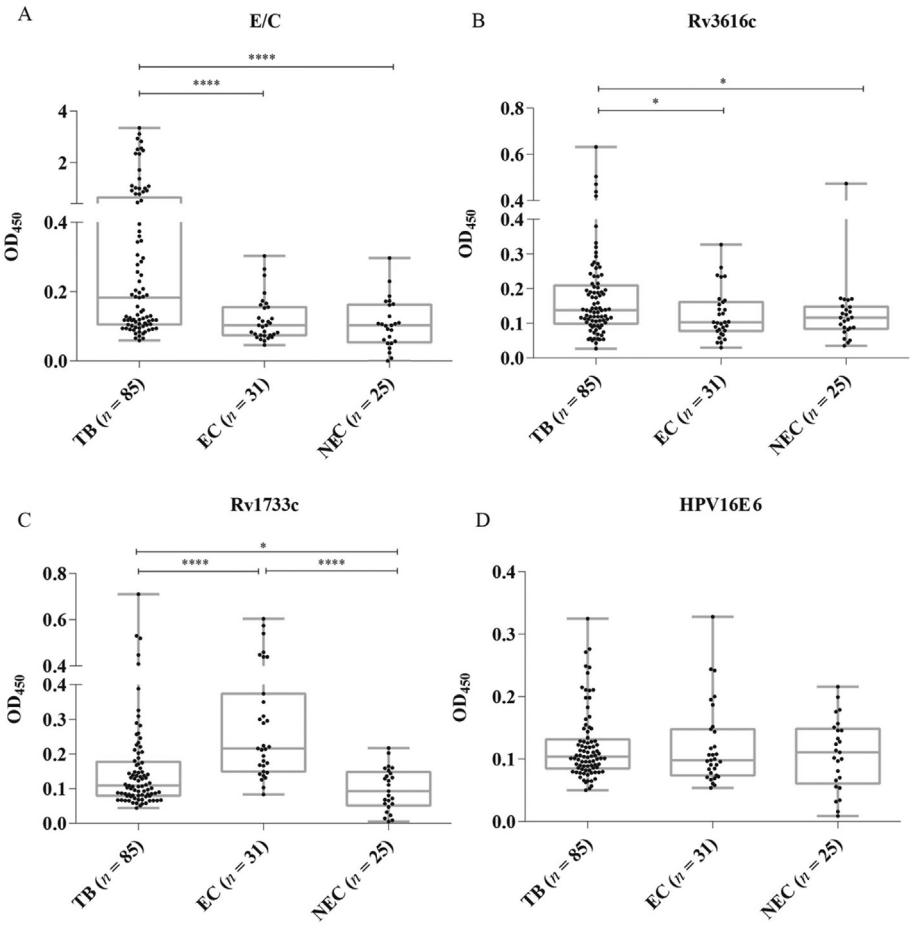

Fig. 2. Antibody responses to specific *Mtb* antigens and PPD. Box-and-whisker plots include individual dots that correspond to the optical density (OD) values measured in response to the following antigens: (A) ESAT6/CFP10 (E/C), (B) Rv3616c, (C) Rv1733c, (D) HPV16E6. (A), (B), (C), and (D) show values with background subtracted. Significance differences were evaluated between 85 TB patients (TB), 31 endemic controls (EC) and 25 non-endemic controls (NEC). Significance levels are indicated as follows: *: p = 0.01–0.05; **: p = 0.001–0.01; ***: p = 0.0001–0.001; ****: p ≤ 0.0001.

Table 3
Comparison of specific IgG antibody levels between TB patients, Endemic Controls (EC) and Non-Endemic Controls (NEC).

Mtb Stimuli	Between-groups comparison		
	TB vs. NEC	TB vs. EC	EC vs. NEC
PPD			
E/C			
Ag85B			
Rv0440			
Rv0645c			
Rv0867c			
Rv1733c			
Rv1737c			
Rv1980c			
Rv2029c			
Rv2031c			
Rv2215			
Rv2389c			
Rv2626c			
Rv3407			
Rv3616c			

p value	
	≤ 0.0001
	≤ 0.001
	≤ 0.01
	≤ 0.05
	> 0.05

TB: TB patients; EC: endemic controls; NEC: non-endemic controls. E/C: ESAT6/CFP10.

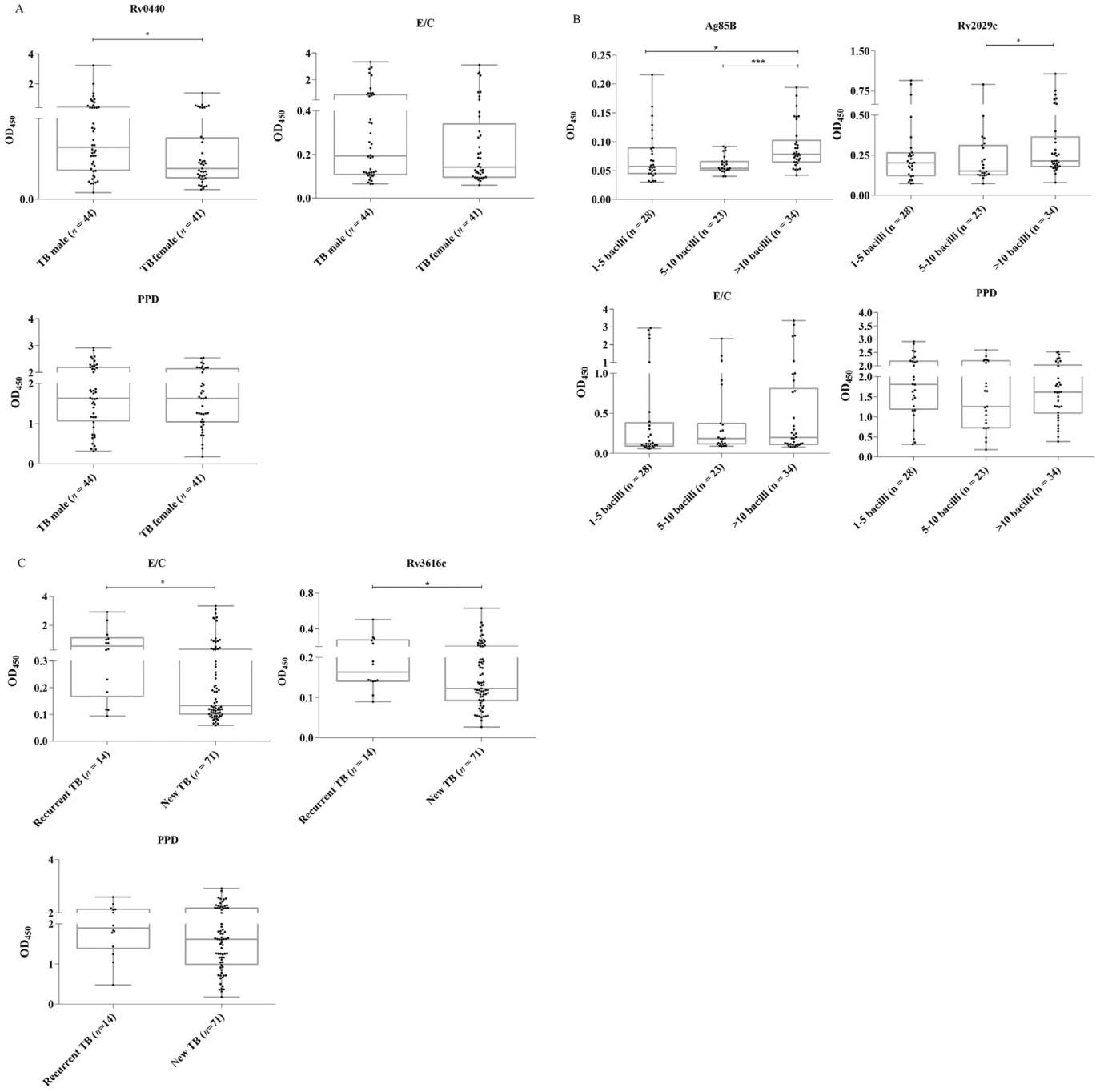

Fig. 3. Antibody responses to specific *Mtb* antigens in TB patients with diverse demographic and clinical characteristics. Box-and-whisker plots include individual dots that correspond to the optical density (OD) values measured in response to the following antigens: Rv0440, Ag85B, Rv2029c, ESAT6/CFP10 (E/C), Rv3616c and PPD. Different demographic and clinical characteristics were compared in TB patients: (A) gender, (B) bacterial load and (C) TB cases with recurrent (TB recurrent) or new diagnosed disease (new TB). Significance levels were indicated as follows: *: $p = 0.01–0.05$; **: $p = 0.001–0.01$; ***: $p = 0.0001–0.001$; ****: $p \leq 0.0001$.

fast bacilli detected in sputum by smear microscopy (bacilli > 10) tended to have increased levels of IgG antibodies against Rv2029c and Ag85B compared to patients with lower numbers of detectable bacilli (>10 vs. 5–10 bacilli; Rv2029: $p = 0.0194$; >10 vs. 5–10 bacilli; Ag85B: $p = 0.0186$; >10 vs 1–5 bacilli; Ag85B: $p = 0.0002$) (Fig. 3B). This was not the case for ESAT6/CFP10 or PPD (Fig. 3). Moreover, TB cases with an actual recurrence episode had higher levels of IgG to ESAT6/CFP10 and Rv3616c than the cases experiencing the first episode of pulmonary TB (ESAT6/CFP10: $p = 0.0177$; Rv3616c: $p = 0.0481$) (Fig. 3C). Age, days of treatment and presence of BCG scar did not appear to have any influence on specific IgG levels.

4. Discussion

Although the role of humoral immunity in tuberculosis (TB) is widely debated, an increasing number of studies has proposed B cells and antibodies as significant components of the immune response against *Mycobacterium tuberculosis* (*Mtb*) [11,23]. The improved containment of TB through passive transfer of immune

polyclonal sera in mice [18], the treatment of TB in a mouse model by monoclonal immunoglobulins [38], the increased TB susceptibility in human hosts with antibody deficits [17], the association of *Mtb* antigen directed antibody titres with reduced *Mtb* loads in animals [19–21] and with reduced TB risk in BCG/MVA85A vaccinated infants [22], and the expansion of *in vitro* studies suggesting a microbicidal role of certain PPD directed antibodies [24,27] all tend to lend support to the hypothesis that humoral-mediated immunity might contribute to an overall protective response to *Mtb*.

As mentioned above, many *Mtb* antigens have been discovered by different strategies [39] focusing on analysing T cell responses, but these studies rarely included antigen recognition by antibodies. We therefore decided to investigate the presence of IgG antibodies against 15 *Mtb* antigens representing different stages of *Mtb* infection: ESAT-6/CFP10 and Ag85B generally representing the active *Mtb* replication stage [36,37]; Rv1733c, Rv1737c and Rv2029c, representing *Mtb* DosR regulon-encoded proteins [34]; Rv0867c and Rv2389, two resuscitation-promoting factors, expressed during *Mtb* reactivation [35]; Rv0440, Rv0645c, Rv1980c, Rv2031, Rv2215, Rv2626c, Rv3407 and Rv3616c which genes are up-regulated during *in vivo Mtb* pulmonary infection in mice [33].

As described in other studies [40,41], *Mtb* specific antibody levels were significantly higher in TB patients than in EC and NEC. The IgG response to each *Mtb* antigen mostly followed the trend of higher IgG levels correlating with higher *Mtb* exposure. To our knowledge, five antigens (Rv0645, Rv2215, Rv2626c, Rv3407 and Rv3616c) were studied here for the first time in the context of humoral TB immunity. The other *Mtb* stimuli (PPD, ESAT6/CFP10, Ag85B, Rv0440, Rv0867c, Rv1733c, Rv1737c, Rv1980c, Rv2029c, Rv2031c and Rv2389c) have been evaluated in other serological studies [40–46], which also showed higher IgG antibody levels against specific antigens in TB patients than in the control groups. Out of the 15 *Mtb* phase related antigens screened, nine showed a different humoral profile between the groups analyzed. Except for one (Rv2215), all the antigens recognized by antibodies are known to be secreted proteins or to have predicted transmembrane regions (according to *Tuberculist:* http://tuberculist.epfl.ch/). IgGs against two (Rv2215 and Rv3616c) out of the five antigens that were originally included here were also increased in TB patients compared to controls. Our results for ESAT6/CFP10 and Rv0867c are in agreement with previous reports [40,42,44,46]. Although for Rv2029c, Rv1737c and Rv2389c we could not confirm a significant difference between the antibody levels in TB patients and EC, as recently observed in a Brazilian cohort [46], we nevertheless found increased IgG antibody levels in TB compared with NEC. While controversial results have already been reported on the humoral response against Ag85B and Rv1980c [15,40–43], IgG antibodies against Rv2031c have been mostly described to be increased in TB patients [15,42,44,45]. In line with some of these studies but in contrast to others, Rv1980c-, Rv2031c- and Ag85B-specific IgG levels did not show any differences between the groups in our cohort. The slightly different serological antigen recognition profile we find compared to other studies is not surprising. Variation in antibody responses has already been extensively discussed previously, and has been ascribed to multiple factors, including the different immunogenetic backgrounds of infected hosts; the expression and production of diverse mycobacterial antigens during multiple stages of *Mtb* infection; different antigen recognition patterns in different individuals by genetically restricted T helper cells, which contribute to the induction of antibodies [42], as well as microbiota- and NTM-exposure related factors. Moreover, polymorphisms that might occur in B cell epitopes regions [23] could also contribute to the wide variability found here and in other reports. This has been suggested for Rv1980 by two studies [47,48], which showed a high rate of mutations affecting the B cell epitopes repertoire of that antigen in clinical isolates.

Interestingly, the only exception was antigen Rv1733c to which specific IgG antibody levels were found to be significantly higher in EC than in both TB patients and NEC. The EC group is likely to have been exposed to *Mtb* in the past [36], and the pattern of IgG levels agrees well with the T cell signature that has repeatedly been observed against this latency antigen: Rv1733c is preferentially recognized by T cells from LTBI individuals compared to TB patients in both EU, African, South American populations as well as in India (unpublished) [34,35]. In other work we have also shown that NTM can induce higher IFN-γ response against Rv1733c in NTM-exposed donors than in unexposed controls [49]. Therefore, it was not unexpected to find increased anti-Rv1733 IgGs in the EC group considering the endemic setting of the study population where both Mtb and NTM are present. In any case, the higher levels of Rv1733c antibodies and the difference found between the EC and the other groups are relevant as this data suggest Rv1733c specific antibodies levels are associated with control of TB infection and progression. We have also found high titers of antibodies in HLA-DR3 transgenic mice after immunisation with a multistage-polyepitope vaccine containing a Rv1733c epitope, or with a long synthetic peptide both derived from Rv1733c [20,21]. To our knowledge, only one other study has evaluated the anti-Rv1733 IgG in another endemic cohort [46], however, this did not reveal the same trend as we describe here. The different outcome could be attributed to the different IgG isotypes analyzed (IgG1 vs. total IgG), the different readout used (ELISA index vs. OD_{450}), differences in study populations and differences in infection levels. Our observations provide a lead towards further studies addressing the precise role of Rv1733c specific antibodies in TB infection.

Of note, the levels of antibodies measured were higher for PPD and ESAT6/CFP10 than for any other *Mtb* antigen. As discussed for other pathogens [50], however, more might not be necessarily better in qualitative terms: higher levels of antibodies could be paradoxically less protective than lower levels, contributing to the so-called 'prozone-like effect.' For TB, the high levels of anti-tuberculin IgG found not only in TB patients but also in high-risk TB contacts is in line with such a theory [51,52]: in contrast to donors with low tuberculin antibody titers, sera from individuals with high PPD-IgG titers were found to modulate the proliferative activity of the PBMCs of autologous donors either by increasing or blocking PBMC stimulation. Additionally, it has been found previously that in active TB disease the expression of the high-affinity IgG receptor (FCGR1A) is higher than in LTBI, which could suggest a significant contribution of antibodies to TB pathogenesis [28].

Although the size of the groups compared in the present study was relatively small, cases with recurrent TB had higher levels of antibodies against ESAT6/CFP10 and Rv3616c, while antibody levels against Ag85B and Rv2029c were associated with bacterial load at the time of recruitment, confirming a trend reported previously [44]. Examining the correlation of clinical information with the antibody production in the TB group, we also found that anti-Rv0440 IgG levels differed according to gender, confirming the trend in a previous study in which the levels of antibodies against a set of *Mtb*-specific antigens were higher in males than in females [53]. This result could be influenced also by other factors, such as an increased antigen load due to a more severe disease in one of the two groups. This effect could not be assessed due to a lack of information regarding the disease severity of the patients enrolled. Antibodies against Rv0440 have also been observed in other pathological conditions such as in the progression of inflammatory [54] and autoimmune diseases [55]. For instance, IgG against Rv0440 has been suggested to be protective against adjuvant-induced arthritis, whose related human pathology is more frequent in females than males [56]. Underlying gender-dependent

pathogenic and pathophysiological processes could be of broader relevance to other diseases as well.

Our study had several limitations. First of all, the small size of the groups limits the impact of our conclusions, especially on the demographic parameters. We could not evaluate the impact of helminth infection on the measured IgG levels, although that condition is recognized to affect either humoral or cellular immunity to *Mtb* [57]. Moreover, we investigated only the total amount of IgG antibodies against specific *Mtb* antigens but not the contribution of each IgG isotype, the antibody avidity or the different glycosylation patterns present in the Fc portion of the immunoglobulins detected [12,27], which could have given more insights into the antibodies functionality.

In conclusion, our collective findings support the need to further investigate and understand the pleiotropic effect of antibodies in the immune response to *Mtb*, their influence on TB disease outcome and their possible contribution to control *Mtb* infection.

Conflict of interests

The authors report no conflict of interests.

Acknowledgement

This work was financially supported by EC HORIZON2020 TBVAC2020 (Grant Agreement No. 643381); EC ITN FP7 VACTRAIN project, and by Colciencias, Bogotá, Colombia (1115-459-21461), Comité para el Desarrollo de la Investigacion (CODI) (01554), Programa Sostenibilidad Universidad de Antioquia.

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