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Circulating IgA/IgG memory B cells against *Mycobacterium tuberculosis* dormancy-associated antigens Rv2659c and Rv3128c in active and latent tuberculosis

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ABSTRACT

Objective: To elucidate the antigenic potential of dormancy-associated antigens Rv2659c and Rv3128c of *Mycobacterium tuberculosis* by examining the persistence of specific IgG and IgA memory B cells (MBCs) among patients with active tuberculosis (TB), household contacts with latent tuberculosis (LTBI), and an endemic healthy control group.

Methods: Fresh peripheral blood mononuclear cells from the three study groups were used to enumerate the numbers of IgG and IgA MBCs specific to recombinant protein Rv2659c and Rv3128c by ELISpot assay. The composition of MBC subsets IgA$^+$ and IgG $^+$ was analyzed by flow cytometry.

Results: The number of IgA MBCs specific to antigen Rv2659c was significantly higher in the LTBI group than the TB group. In contrast, no significant difference was found in IgA or IgG MBCs against antigen Rv3128c. The number of IgA$^+$ MBCs was significantly higher than that of IgG$^+$ MBCs in the classical MBC subset of the LTBI group.

Conclusion: The results indicated that the dormancy-associated antigen Rv2659c induced an IgA MBCs response in individuals with latent TB, and IgA$^+$ classical MBCs formed a major portion of the MBCs subset. This new knowledge will be beneficial for the development of novel TB vaccines and their control of latent TB.

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Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb) is a major public health problem listed in Sustainable Development Goal 3. There were an estimated 10 million new cases of TB, as well as 1.2 million deaths, in 2019 (WHO, 2020). Importantly, latent infections are estimated to be present in a quarter of the global population (WHO, 2020). From this stage, approximately 5-10% of people progress to active TB during their lifetime. Thus, not only do latent TB individuals (LTBIs) become reservoirs of active TB, they also stand as a key challenge for global TB control (Raviglione and Sulis, 2016). Prevention of the reactiva-

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tion of LTBIs is the most important requirement to end TB globally (Kaufmann et al., 2017).

Mtb is an intracellular pathogen and thus most vaccine development has focused on stimulation of $CD4^+$ T cells to produce interferon-γ, interleukin 2, and tumor necrosis factor (Bozzano et al., 2014; Green et al., 2013). The role of humoral immunity against Mtb has gained renewed interest because antibodies can indirectly enhance the activity of cell-mediated immunity in control of the pathogens (de Valliere et al., 2005). Evidence of a protective role for humoral immunity, especially specific IgA and IgG antibodies, is pronounced; for example, passive transfer of monoclonal antibodies and immune sera conferred protection against Mtb infection and improved clinical outcome in mice (Buccheri et al., 2009; Guirado et al., 2006). The growth of Mtb in macrophages is more efficiently inhibited by IgG obtained from donors with LTBI than from those with active TB (Lu et al., 2016). In a mouse model, synthetic human IgA protected against Mtb infection (Balu et al., 2011). Altogether, these reports highlight the role of humoral immunity to control Mtb infections.

The persistence of Mtb within granulomas in the face of hypoxia and several stress factors – such as low pH, limited nutrients, and presence of nitric oxide – has been demonstrated. Under such conditions, Mtb undergoes reduced metabolism and may shift to a non-replicating state (Kondratieva et al., 2014). While in this state, DosR regulon-encoded and other starvation-related genes function and participate in the organism's dormancy development (Leistikow et al., 2010). Greater humoral immunoreactivity against these dormancy antigens is generally observed in latent infections (Arroyo et al., 2018; He et al., 2015; Yihao et al., 2015). For instance, the dormancy-related antigens Rv2004c and Rv2660c evoke higher IgG responses in latent than active TB (Doddam et al., 2017; He et al., 2015). Meanwhile, antigen Rv2031 induces IgA and IgG responses in both active and latent infections (Abebe et al., 2018).

Although antibodies are important for the humoral immune response, the establishment and persistence of memory B cells (MBCs) after Mtb infection is still unclear (Glatman-Freedman and Casadevall, 1998). Several studies have characterized the role of MBC responses towards Mtb infections; for example, long-lived mycobacteria-specific MBCs induced by BCG vaccination provided sustained B cell responses against mycobacterial infections (Sebina et al., 2012). In addition, the frequencies of MBCs against mycobacteria-specific antigens (ESAT-6 and CFP-10) were significantly higher in latent than in active TB, and the kinetics of circulating MBC populations were related to the stage of TB (Sebina et al., 2014). The different proportions of MBC subsets may reflect the clinical status of the Mtb-infected individuals (du Plessis et al., 2016; O'Shea et al., 2018; Sebina et al., 2014).

Antibody profiling by the current laboratory using high-content peptide microarray analysis demonstrated that amongst dormancy-associated proteins, antigens Rv2659c and Rv3128c were over recognized by IgA from individuals with latent TB infections (manuscript in preparation). Interestingly, antigen Rv2659c, a gene of the starvation stimulon, is reported to induce strong T cell responses in LTBI as well as IgG, both in latent and active TB infections (Bai et al., 2015; Govender et al., 2010). In contrast, antigen Rv3128c, one of the DosR antigens, appears limited in its ability to induce T cell responses, although it was associated with high IgG responses in Japanese Mtb-infected individuals (Hozumi et al., 2013). Specific MBC responses against either Rv2659c or Rv3128c antigens has not yet been determined. Thus, this study reported the first demonstration of the ability of dormancy-protein antigens Rv2659c and Rv3128c to generate IgG and IgA MBCs in LTBIs and that they induced phenotypically different MBC subsets in TB, LTBI, and endemic healthy control (eHC) groups.

Materials and Methods

Sample collection and study subjects

Heparinized blood samples of 10 ml were collected from active TB patients (N = 15), LTBI subjects (N = 25), and eHC volunteers (N = 8), aged $\geq$ 18 years and considered immunocompetent by history and physical examination, who were recruited into this study. Classification of patients with active TB, household contacts with latent TB, and eHCs followed the WHO algorithm for TB infection diagnosis (WHO, 2013). Subjects with a positive QuantiFERON-TB Gold assay (QFT) (Cellestis, Carnegie, Australia) and a normal thoracic radiograph were classified as having LTBI, whereas those with negative results of both QFT and thoracic radiograph were grouped as eHC. Diagnosis of active pulmonary TB was based on positive Mtb culture of sputum at Chiangrai Prachanukroh Hospital, Chiangrai Province, Thailand. Diagnoses were further supported by medical history, thoracic CT, and acid-fast bacilli smear staining.

Preparation of dormancy-associated Mtb proteins

Rv2659c and Rv3128c genes were PCR amplified using genomic DNA of Mtb H37Rv ATCC27294 strain as a template. The amplified PCR products of Rv2659c and Rv3128c (1128 bp and 1014 bp, respectively, in length) were later cloned with an expression plasmid, either as pET24b-Rv2659c or pET28a-Rv3128c at *NdeI* and *XhoI* cloning sites. After introduction into *Escherichia coli* (*E. coli*) BL21(DE3), the protein antigens encoded by either Rv2659c or Rv3128c genes were obtained after induction with 0.5 mM IPTG at 37°C for 3 hours (Rv2659c) or overnight (Rv3128c) in an insoluble form. The insoluble proteins were purified with 6xHis tag protein with Talon metal affinity resin (Clontech, CA, USA) in the presence of 8 M urea, as recommended by the manufacturer. Endotoxins were removed using the Toxineraser™ endotoxin removal kit (Genscript, Piscataway, USA) and endotoxin levels were checked by Toxinsensor™ chromogenic LAL endotoxin assay kit (Genscript, Piscataway, USA). The purity of proteins was determined by 15% SDS-PAGE staining with Coomassie brilliant blue and confirmed by Western blot using anti-His6 IgG (Santa Cruz Biotechnology, TX, USA). The purified Rv2659c showed the expected molecular weight of 43kDa on 15% SDS-PAGE (Figure S1A). The molecular weight of Rv3128c was about 25 kDa due to the presence of a stop codon at 700 bp (Figure S1B).

Preparation of peripheral blood mononuclear cells

Fresh peripheral blood mononuclear cells (PBMCs) were separated from 10 ml of heparinized blood using lymphocyte separation media (Biowest, Nuaillé, France) and washed twice with incomplete RPMI 1640 (Gibco, Invitrogen, Paisley, UK). The washed PBMCs were resuspended in RPMI 1640 supplemented with 10% FBS, 100 unit/ml penicillin, and 100 µg/ml streptomycin.

B cell ELISpot assay

Fresh PBMCs (1×10^6 cells/ml) were polyclonally stimulated by 1 µg/ml of R848 and 10 ng/ml rhIL-2 (Mabtech, Stockholm, Sweden), and incubated at 37°C under a 5% CO_2 atmosphere for 72 hours in 24-well cell culture plates. In this experiment, non-stimulated cells were used as negative controls under the same conditions.

Pre-activated, 96-well multiscreen filter PVDF plates (Merck, Millipore, Darmstadt, Germany) were coated with 20 µg/ml of Rv2659c purified protein, or 20 µg/ml Rv3128c purified protein, or 15 µg/ml of mAb anti-human IgG (MT91/145; Mabtech), or

5 μg/ml tetanus toxoid antigen (Merck, Millipore, Darmstadt, Germany) and cultured separately at 4°C for 16 hours. After washing five times with PBS, the blocking step with 10% FBS in RPMI 1640 medium was performed for 30 minutes at RT. The stimulated and non-stimulated cells were harvested and seeded to yield 5×10^4 cells/ml in mAb anti-human IgG-coated wells, while 1×10^6 cells/ml in antigen-coated wells, and incubated at 37°C in 5% CO_2 for 16 hours. After washing with PBS, plates were further incubated with 100 μl of 1 μg/ml biotinylated detection mAb (MT78/145; Mabtech) for 2 hours at RT. Following another PBS washing, the plates were incubated with 100 μl of Streptavidin-HRP at 37°C for 1 hour and washed with PBS. To detect the reaction, 100 μl of TMB substrate for ELISpot (Mabtech) was added and incubated at RT for 15-30 minutes before stopping by rinsing with deionized water after distinct spots occurred. The antigen-specific IgA MBC detection was performed as described with slight modifications: 20 μg/ml Rv2659c purified protein, 20 μg/ml Rv3128c purified protein, 15 μg/ml of mAb anti-human IgA (MT57; Mabtech) were separately coated on the pre-activated ELISpot plates. The detected biotinylated mAb (MT20; Mabtech) was added to anti-human IgA-coated wells and biotinylated mAb (MT57; Mabtech) was used for antigen-coated wells.

Antigen-specific MBCs were counted as Spot Forming Units (SFUs) using a Bioreader 5000 Pro-F gamma ELISpot Reader (Biosys GmbH, Karben, Germany). An ELISpot with SFUs two-fold or more above the negative controls was defined as positive. The antigen-specific antibody secreting cells (ASCs) were enumerated from PBMC cultures without polyclonal activation and the frequencies of ASCs were counted as spot forming units/1 million PBMCs.

Memory B cell phenotyping

Fresh PBMCs from subjects in the active TB, LTBI, and eHC groups were used to characterize the subsets of MBCs with a panel of monoclonal antibodies conjugated with fluorochromes. Approximately 1×10^6 cells/ml of PBMCs were stained with a cocktail of CD19-FITC, CD20-Per CP, CD21-APC, and CD27-APC/fire 700 (Biolegend, San Diego, CA, USA). The B cells were phenotyped by gating as $CD19^+CD20^+CD21^+CD27^+$ activated MBCs, $CD19^+CD20^+CD21^-CD27^+$ classical MBCs, $CD19^+CD20^+CD21^-CD27^-$ atypical MBCs, and $CD19^+CD20^+CD21^+CD27^-$ naïve B cells. $CD19^+CD20^-CD21^+CD27^+$ plasmablast were excluded in the final analysis (Portugal et al., 2015; Weiss et al., 2009). Switched IgG and IgA MBCs were identified using CD19-FITC, CD20-Alexafluro 700, CD21-APC, CD27-APC/fire 700, IgG Per CP/Cy 5.5 (cloneM1310G05) (Biolegend, San Diego, CA, USA), and IgA PE (cloneB3506B4) (SouthernBiotech, Birmingham, USA). The cells were acquired by flow cytometry, FACSCanto II (BD Bioscience, Becton-Dickinson, Oxford, UK), and analyzed using FlowJo software, version 10.5.3 (Tree Star, Ashland, OR, USA).

Statistical Analysis

The non-parametric Mann-Whitney U test was used to compare differences between IgG and IgA MBCs against Rv2659c and Rv3128c, as well as to compare the differences in subsets of MBCs among active TB, LTBI, and eHC, and to determine significant differences between IgA^+ and IgG^+ MBC subsets in LTBI. Fraction of total analysis was used to analyze the fraction of MBC subsets in active TB and LTBI, and IgA^+ and IgG^+ MBC fractions in LTBI. The results with P-values < 0.05 were considered as statistically significant. Statistical analysis was performed and graphs were prepared using GradPad Prism 7 (San Diego, CA, USA).

Table 1
IgA and IgG ELISpot result of antigen Rv2659c activation.

	IgG MBCs to antigen Rv2659c		IgA MBCs to antigen Rv2659c	
	+	-	+	-
Active TB (n = 15)	5 (33.3%)	10 (66.7%)	4 (26.7%)	11 (73.3%)
LTBI (n = 15)	11 (73.3%)	4 (26.7%)	13 (86.7%)	2 (13.3%)
eHC (n = 8)	5 (62.5%)	3 (37.5%)	6 (75%)	2 (25%)

(+) = IgA/ G ELISpot positive, (-) = IgA/G ELISpot negative.
ELISpot positive: the number of SFU in antigen coated well ≥ 2-fold SFU of negative control.
ELISpot negative: the number of SFU in antigen coated well ≤ SFU of negative control.

Table 2
IgA and IgG ELISpot result of antigen Rv3128c activation.

	IgG MBCs to antigen Rv3128c		IgA MBCs to antigen Rv3128c	
	+	-	+	-
Active TB (n = 15)	4 (26.7%)	11 (73.3%)	3 (20%)	12 (80%)
LTBI (n = 15)	6 (40%)	9 (60%)	7 (46.7%)	8 (53.3%)
eHC (n = 8)	4 (50%)	4 (50%)	3 (37.5%)	5 (62.5%)

(+) = IgA/ G ELISpot positive, (-) = IgA/G ELISpot negative
ELISpot positive: the number of SFU in antigen coated well ≥ 2-fold SFU of negative control.
ELISpot negative: the number of SFU in antigen coated well ≤ SFU of negative control.

Results

Rv2659c-specific IgA memory B cell frequencies were higher in LTBI subjects than in active TB patients

Persistence of antigen-specific circulating MBCs against dormancy-associated antigens coded by Rv2659c and Rv3128c genes was studied in three groups (active TB, LTBI, and eHC). The antigen-specific MBCs having two-fold or higher SFU than that of total SFU in the negative control were defined as positive ELISpot. Most of the LTBI group had MBCs positive by ELISpot specific to antigen Rv2659c, especially IgA MBCs that were detected in approximately 88% of the LTBI group (Table 1). Meanwhile, MBCs against antigen Rv3128c purified protein were found in approximately 50% of the group (Table 2). In contrast, 20-30% of subjects with active TB had positive IgA and IgG MBC responses to antigens Rv2659c and Rv3128c (Tables 1 and 2). The mean SFUs of IgA and IgG MBCs against antigens Rv2659c and Rv3128c purified protein in active TB, LTBI, and eHC were compared. The mean SFU of IgA MBCs specific to antigen Rv2659c (per 1 million cells) in LTBI was significantly greater than that of active TB patients ($P = 0.001$) (Figure 1A), but there was no significant difference in IgG MBCs (Figure 1B). In the meantime, no differences were observed for the SFUs of anti-Rv3128c IgG and IgA MBCs (Figures 2A and 2B).

Enumeration of circulating ASCs against antigen Rv2659c purified protein in PBMCs without prior polyclonal activation was performed because short-lived ASCs were detected in the peripheral circulation during early or clinically active infections (Portugal et al., 2017). In this study, both IgG and IgA ASCs against antigen Rv2659c purified protein showed no significant difference among the three study groups (Figures S2A and S2B). This suggested that antigen Rv2659c triggered antigen-specific MBCs as well as antigen-specific ASCs.

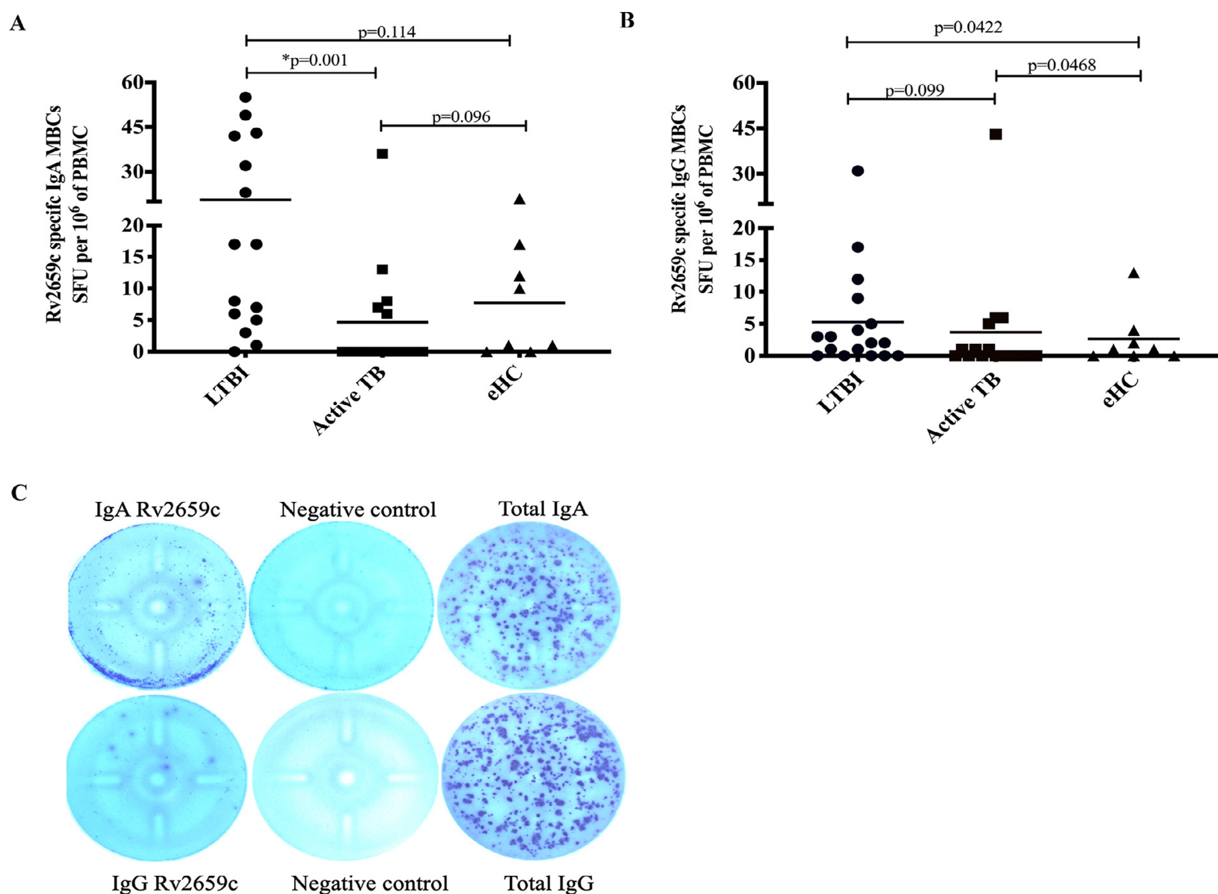

Figure 1. Rv2659c-specific IgA MBCs were higher in the LTBI group.
Specific MBC subclasses detected by B cell ELISpot among the three groups [LTBI (n = 15), active TB (n = 15), and eHC (n = 8)] are shown as Rv2659c-specific IgA B cells (A) and IgG B cells (B). The representative image (C) demonstrates an ELISpot readout image of Rv2659c-specific IgA B cells (upper panel) and Rv2659c-specific IgG B cells (lower panel) from representative subjects of the LTBI group. The negative control was unstimulated PBMCs; the positive control was 1 μg/ml of R848 and 10 ng/ml rhIL-2 stimulated PBMCs. The data represent the number of spot forming units (SFUs); statistical analysis used a non-parametric Mann-Whitney U test
*P-value < 0.05

Classical memory B cells were significantly expanded in LTBI

Evaluation of MBC subsets was performed based on the combined expression patterns of CD19, CD20, CD21, and CD27 (Joosten et al., 2016) (Figure 3A). This analysis showed a significant increase in frequencies of double-negative atypical MBCs in active TB patients compared with LTBI subjects ($P < 0.0001$) and eHCs ($P < 0.0001$) (Figure 3B). Interestingly, double-positive classical MBCs were present in significantly higher percentages in LTBI subjects than active TB patients ($P = 0.002$) (Figure 3C). However, the frequencies of naïve B cells and activated MBCs were not significantly different among the three groups (Figures 3D and 3E). The proportions of different MBC subsets in LTBI and active TB were analyzed. A significant expansion of classical MBCs was observed in LTBI subjects, and double-negative atypical MBCs were significantly increased in patients with active TB disease. There was no difference in the proportion of naïve B cells between the LTBI and active TB groups (Figures S3A and S3B).

The highest frequency IgA^{+} memory B cells arose from classical MBC subsets of LTBI

Unlike classical MBCs, atypical MBCs (or exhausted memory B cells) express high levels of inhibitory receptors and are very hyporesponsive to B cell receptor stimulation (Joosten et al., 2016). Classical MBCs are able to persist long term and respond upon re-challenge with the same antigens (Oliviero et al., 2020). In this study, the highest frequencies of classical MBCs and anti-Rv2659c IgA MBCs were detected in the LTBI group. Thus, antigen-specific IgG and IgA classical MBCs may confer immune responses in LTBI which are both prompt and long lasting. To determine the principal MBC subset in which IgG and IgA MBCs are found, IgA^{+} and IgG^{+} MBCs were enumerated in subjects with LTBI who exhibited positive ELISpots to Rv2659c by flowcytometry. The surface expression of IgA and IgG was observed within different MBC subsets (Figure 4A). The frequency of IgA^{+} MBCs was significantly higher than that of IgG^{+} MBCs in classical memory B cells ($P = 0.0002$) (Figure 4B). Moreover, IgG^{+} MBCs were significantly more frequent than IgA^{+} MBCs in atypical MBCs ($P = 0.011$) (Figure 4C). In addition, the proportion of IgA^{+} MBCs was highest in classical MBCs (Figure 4D). This suggests that most IgA MBCs in subjects with LTBI arose from the classical MBC subset.

Discussion

Rv2659c-specific B cell responses were demonstrated from LTBI subjects in this study. The antigen Rv2659c had greater ability in triggering MBCs development than antigen Rv3128c, indicating that the MBCs response can vary among dormancy-related antigens. This might be due to phase-dependent antigenicity of

Figure 2. Rv3128c-specific IgA and IgG MBCs demonstrated no difference.
Specific MBC subclasses detected by B cell ELISpot among the three groups [LTBI group (n = 15), active TB patients (n = 15), and eHC group (n = 8)] are shown as Rv3128c-specific IgA B cells (A) and IgG B cells (B). The representative image (C) demonstrates an ELISpot readout image of Rv3128c-specific IgA MBCs (upper panel) and Rv3128c-specific IgG MBCs (lower panel) from representative subjects of the LTBI group. Unstimulated PBMCs and 1 μg/ml of R848 and 10 ng/ml rhIL-2 stimulated PBMCs were used as negative and positive controls, respectively. The data represent the number of spot forming units (SFUs); statistical analysis used a non-parametric Mann-Whitney U test *P-value < 0.05.

the dormancy-related antigens during the course of Mtb infection (Mattos et al., 2016). Between the LTBI and active TB groups, IgA MBCs specific to antigen Rv2659c purified protein were significantly greater in the LTBI group, whereas the frequencies of IgG MBCs did not significantly differ. Similarly, from a previous study, anti-Rv2659c IgG levels in the serum were not significantly different between LTBI individuals and active TB patients (Bai et al., 2015). One explanation of the IgA predominance might be its involvement in mucosal immunity against airborne mycobacterial infection (Balu et al., 2011). This concept is supported by Zimmermann's finding that IgA monoclonal antibody specific to Mtb can inhibit infection in epithelial and macrophage-derived cell lines (Zimmermann et al., 2016). Thus, it could be that IgA MBCs directed against antigen Rv2659c play a crucial role in preventing the reactivation of LTBIs. This would be due to the rapid and specific response of MBCs differentiating into new ASC populations when re-stimulated by antigens (Amanna IJ, 2007; Pinna et al., 2009).

Short-lived antibody secreting cells can be readily detected in peripheral circulation during early or clinically active infections, but are rarely present in peripheral blood in the absence of antigens or viable pathogens (Odendahl et al., 2005). In contrast, MBCs are long-lived and may persist in infected people throughout their lives (Blanchard-Rohner et al., 2009; Kelly DF, 2005; Odendahl et al., 2005; Rosado et al., 2011; Tarlinton et al., 2008). The presence of antigen-specific ASCs in the absence of MBCs in peripheral circulation can also indicate active Mtb infection (Sebina et al., 2014). Moreover, a previous study demonstrated that antigen-specific circulating ASCs were mostly detected in active TB patients (Renier et al., 2018). However, both circulating IgG and IgA ASCs specific to antigen Rv2659c were not significant between the active TB and LTBI groups in this study, which might be due to the small number of patients analyzed.

Different compositions of MBC subsets can be observed across the spectrum of Mtb infections (O'Shea et al., 2018). Under chronic antigenic exposure, MBCs can differentiate into atypical MBCs, which are also known as hypo-responsive cells, and might be generated from dysfunctional germinal centers or extant classical MBCs (Moir et al., 2008; Portugal et al., 2015). According to the current results, the fraction of atypical MBCs was significantly increased in active TB patients compared with subjects with LTBI. Consistent with these findings, a recent study conducted in the United Kingdom reported that active TB patients had a significant expansion of atypical MBCs together with a decrease in classical MBCs (O'Shea et al., 2018). Similarly, circulating B cells were also decreased in active TB patients, but an atypical phenotype was relatively increased in a separate study (Joosten et al., 2016). Expansion of atypical MBCs might be associated with failure of B cell responses in active TB because atypical MBCs have impaired functions such as proliferation, and immunoglobulin and cytokine production (Joosten et al., 2016; O'Shea et al., 2018). As previously reported, classical MBCs have greater antibody production, as do atypical MBCs (Portugal et al., 2017). The current study observed that classical MBCs were significantly more frequent in LTBI subjects than in active TB patients. T cells play important roles in MBCs development through several ways: during T cell-dependent MBC development, CD4^{+}

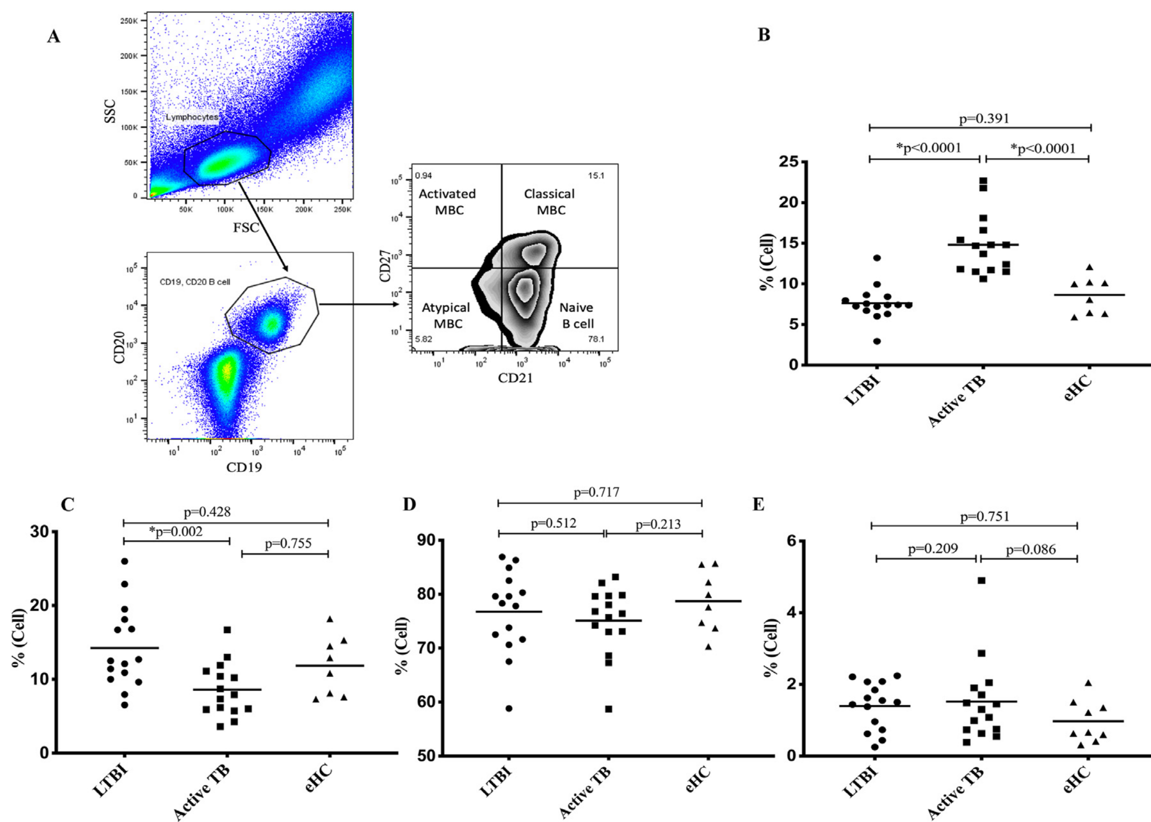

Figure 3. Classical MBCs were more frequent in LTBI subjects, while atypical MBCs were in active TB patients.
Phenotypic analysis of different MBC subsets was performed among the three groups [LTBI (n = 15), active TB (n = 15), and eHC (n = 8)]. $CD19^+$ $CD20^+$ B cells were gated by a CD10-PE Cy7 marker to classify $CD10^-CD19^+CD20^+$ mature B cells. Based on this population, CD21-APC and CD27-APC/Cy7 were used to identify different subsets of memory B cells (A), atypical MBCs (B), classical MBCs (C), naïve MBCs (D), and activated MBCs (E). The experiments were evaluated by flow cytometry and analyzed by FlowJo, version 10.5.3. The statistical analysis was performed by non-parametric Mann-Whitney U test
$^*P < 0.05$ was statistically significant

T helper cells interact with the antigen-activated B cells, leading to germinal center formation (Kurosaki et al., 2015). In the germinal center, B cells receive a different array of cues from germinal center follicular $CD4^+$ helper T cells such as cytokines and other co-stimulatory molecules (CD40L and ICOS) (Vinuesa et al., 2016). Consequently, germinal center B cells undergo proliferation, somatic hypermutation, class switching, and differentiation into MBCs (Good-Jacobson, 2018). Since the route by which an antigen enters the body is one of the important factors determining antibody class switching (Palm and Henry, 2019), mucosal pathogens primarily induce IgA class-switching (Cerutti et al., 2011). This is a plausible explanation for the significant increase in IgA^+ MBCs compared with IgG^+ MBCs in classical MBCs of LTBI subjects found in this study. Hence, it is suggested that both significant IgA^+ classical MBCs expansion and IgA MBCs specific to Rv2659c purified protein in LTBI subjects might play crucial roles in the maintenance of latent infections.

The major limitation of this study was the small number of sample sizes in each study group: 15 active TB, 25 LTBI, and eight eHC. Thus, these observations might be tentative findings to demonstrate the immunogenic potential of antigen Rv2659c and Rv3128c in triggering IgG/ IgA MBCs response in Mtb-infected subjects. A larger sample size study would help to confirm the results of IgA and IgG MBCs responses to these Mtb dormancy-associated antigens.

In conclusion, this study highlighted the induction of IgA MBC responses by antigen Rv2659c purified protein along with phenotypical differences of the MBCs in various parts of the spectrum of Mtb infection. The authors are aware that the study did not evaluate the longevity of anti-Rv2659c IgA antibody, although it did demonstrate that antigen Rv2659c purified protein can trigger MBC responses in LTBI subjects and, remarkably, the differentiation of IgA MBCs into ASCs. Moreover, significant expansions of classical MBCs (largely IgA^+ MBCs) were found to be associated with latent TB infections. This new insight may be beneficial in the development of novel antibody-stimulating TB vaccines in the future.

Authors contributions

PTS performed the experiments, statistical analysis and interpretation, and wrote the manuscript. CS edited the manuscript. JH and SW expressed the proteins. SPK performed the experiments. SM, NS, and HY provided select samples. PC and PK reviewed the data and interpreted the results. CL conceived and designed the experiments, reviewed and analyzed the data, and finalized the manuscript. All authors read and approved the manuscript.

Declaration of interests

The authors have no conflicts of interest to declare.

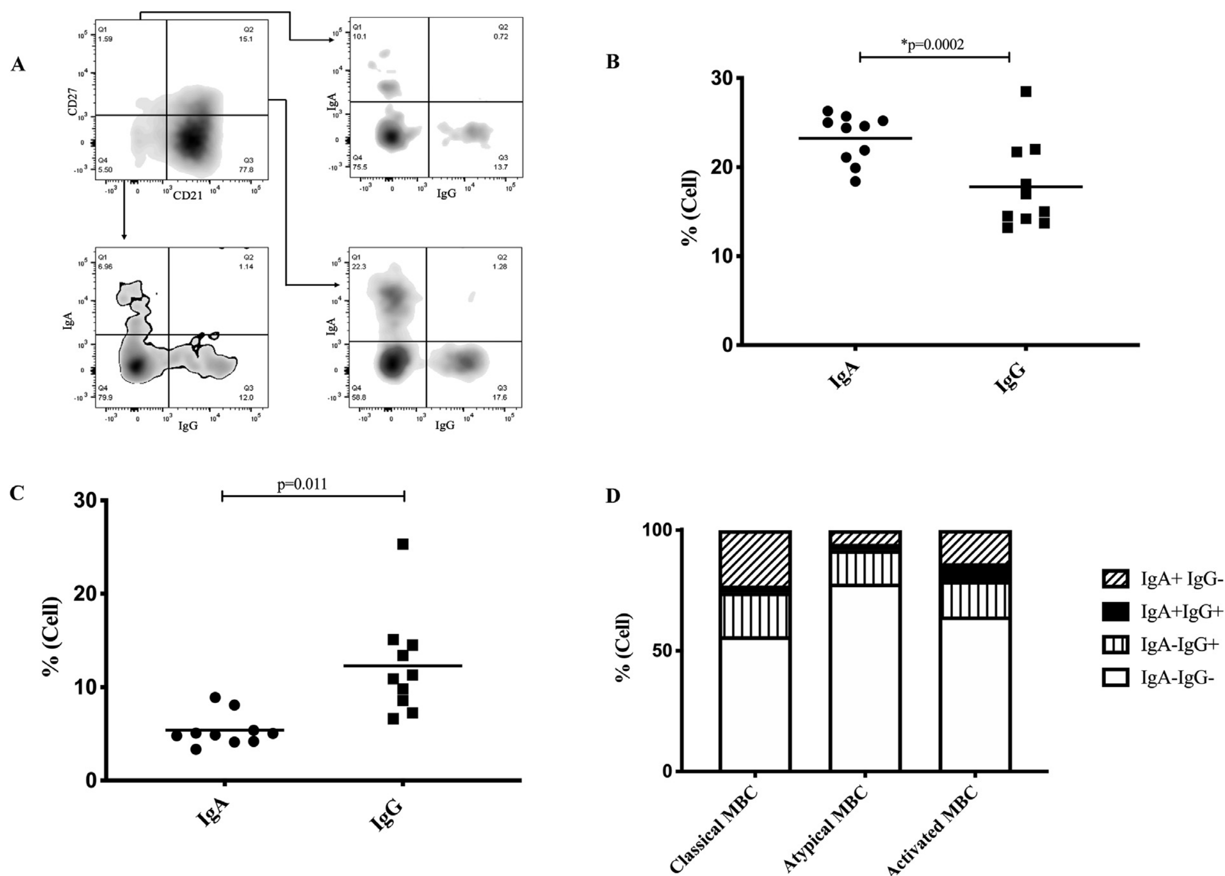

Figure 4. IgA$^+$ MBCs were more frequent in classical MBCs, while IgG$^+$ MBCs were in atypical MBCs.
The distribution of IgA+ and IgG+ MBCs among different MBC subsets are shown in LTBI individuals (n = 10). Based on a B cell population, these cells were gated by CD21-APC and CD27-APC/Cy7 to identify different subsets of memory B cells. Then, IgA$^+$ and IgG$^+$ MBC cells were identified by gating IgA PE and IgG Per CP/Cy 5.5 (A). Data were analyzed as IgA$^+$ and IgG$^+$ cells in classical MBC subsets (B); IgA$^+$ and IgG$^+$ cells in atypical MBC subsets (C). Proportional distribution of IgG+ and IgA+ in classical, atypical, and activated MBC subsets are demonstrated (D). Data were retrieved by flow cytometry and analyzed by FlowJo, version 10.5.3. The statistical analysis was performed using non-parametric Mann-Whitney U test and Fraction of total analysis. *P-value < 0.05.

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Ethical Approval

Written informed consent was given by each subject or his/her proxy to the Department of Medical Sciences, Ministry of Public Health (Thailand). The consent form and process, specimen collection, and the experimental protocol were reviewed and approved by the Central Institutional Review Board, Mahidol University, Thailand (No. MU-CIRB 2016/041.2803). All experiments were conducted in accordance with relevant guidelines and regulations.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2021.07.033.

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