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RESEARCH ARTICLE

The distribution of B lymphocyte subpopulations in mice infected with *Mycobacterium tuberculosis* H37Ra

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ABSTRACT

Tuberculosis (TB), an infectious disease caused by the bacterium *Mycobacterium tuberculosis* (Mtb), is a major cause of morbidity and mortality worldwide. Annually, millions of new cases of tuberculosis are documented. Research in tuberculosis-immune has hitherto focused predominantly on the role of T cells in Mtb infection. Although there have been studies in progress which have supported the notion that B cells are crucial players in combating infectious diseases, the role of B cells in TB is still not fully understood. There is a paucity of in-depth analysis of various B lymphocyte subpopulations and the understanding of the immunophenotypic changes of the B-cell lineage during tuberculosis infection. Therefore, we aimed to investigate the changes in B lymphocyte subpopulations in mice infected with *M. tuberculosis* H37Ra. The percentage/frequency of peritoneal B cells ($CD45R^+$), B1b cells ($CD45R^{int}IgD^-CD5^-CD11^+$), splenic B cells ($CD45R^+$), and splenic marginal zone B (MZ B) cells ($CD45R^+CD23^-CD21^{hi}$) decreased ($P < 0.05$), while the percentage of splenic follicular B (Fo B) cells ($CD45R^+CD23^+CD21^{int}$) and lymph node B cells ($CD19^+$) increased at the 4th and 8th weeks ($P < 0.05$). It was suggested that H37Ra infection changed the distribution of B lymphocyte subpopulations. In addition, the percentage of $CD69^+$B cells and memory B cells ($CD45R^+CD27^+IgD^{+/-}$) increased in the infected mice at different infection periods ($P < 0.05$), which suggested H37Ra infection promoted B cell activation and produced a large number of memory B cells. As a conclusion, H37Ra infection can affect the distribution of B lymphocyte subpopulations, with a concomitant down-regulation of MZ B cells, which perform innate immunity, and up-regulation of adaptive immune response cells (Fo B cells and lymph node B cells). Furthermore, it has been demonstrated that H37Ra infection can promote the immune response of B lymphocytes to tuberculosis, through the stimulation of the body to produce a large number of activated and memory B cells.

Key words: B1 cell; Marginal zone B cell; Follicular B cell; Memory B cell; Immune response.

INTRODUCTION

Mycobacterium tuberculosis (Mtb) is a severe public health threat worldwide, causing 1.6 million deaths per year (Bagcchi, 2023). A plethora of studies have hitherto evaluated the roles of innate and adaptive cell-mediated immunity against tuberculosis (TB). Great progress has been made in the field of T cell-based tuberculosis research (Blanc *et al*., 2017; Kauffman *et al*., 2017; Liu & Cai, 2018). It is widely accepted that B cell and antibody-mediated responses confers protection against extracellular pathogens and that the regulation and control of intracellular organisms are through cellular immune mechanisms (Achkar *et al*., 2014, 2015; du Plessis *et al*., 2016b). However, studies in recent years have demonstrated that B cells have the capacity to protect against intracellular pathogens (including Mtb) and can have distinct roles as drivers and regulators of immunity (du Plessis *et al*., 2016b). The role of B cells in TB is complex and remains a subject of ongoing debate. Previous studies have shown that B cells can play protective (Linge *et al*., 2023; Phuah *et al*., 2016) or detrimental (Bosio *et al*., 2000) or even neutral roles (Torrado *et al*., 2013; Johnson *et al*., 1997) during Mtb infection. There is a paucity of research on the changes of B lymphocyte subpopulations in the process of *M. tuberculosis* infection. Furthermore, there is a lack of in-depth analysis of various B lymphocyte subpopulations and the understanding of the immunophenotypic changes of B-cell lineage during tuberculosis infection. Consequently, there is a necessity to study the changes of B lymphocyte subpopulations in the process of tuberculosis infection.

B cells comprise heterogeneous subsets that display distinct functional characteristics, thereby providing a broad defense spectrum against infections. The categorisation of B cell subsets can be approached through two distinct classifications: conventional and unconventional B cells, which were distinguished by their functional characteristics and immunophenotypes, respectively (Tsai *et al*., 2024). Conventional B cells, also termed follicular B (FoB) cells, represent the major B cell subset, comprising approximately 80% of B cells (Baumgarth, 2004, 2011; Palm *et al*., 2016). As the critical component for adaptive immunity, FoB cells react to infections by generating high-affinity antibodies (Baumgarth, 2004; Hoffman *et*

al., 2016). Conversely, unconventional B cells, comprising marginal zone B (MZB) cells and B1 B cells, manifest characteristics that diverge from those exhibited by FoB cells (Baumgarth, 2004, 2011; Palm *et al*., 2016; Mouat & Horwitz, 2022). These populations have different origins, surface markers, and unique anatomical locations, and they produce completely different types of antibodies. As components of the innate immune system (Baumgarth, 2011), MZB and B1 cells respond to infections more rapidly than FoB cells by primarily releasing low-affinity antibodies (Cerutti *et al*., 2013; Zouali & Richard, 2011). These cells are responsible for containing early infection, preventing relapse, and more effectively recalling a response to re-infection, respectively (Harms Pritchard & Pepper, 2018; Inoue *et al*., 2018). Memory B cells are derived from naive B cells that have been stimulated by antigen in the peripheral lymphoid organs during primary immune responses. This process is characterised by intricate interactions between B cells, antigen-presenting dendritic cells, and activated $CD4^+$ T cells. The efficacy of this process is contingent upon the synchronised expression of specific cell receptors and the requirement for co-stimulatory signals (Bergmann *et al*., 2013; Pupovac & Good-Jacobson, 2017). Following their generation, memory B cells enter the peripheral blood circulation and repopulate other lymphoid tissues, primarily bone marrow, thereby establishing a long-term memory pool. Upon antigen re-stimulation, activated memory B cells differentiate into plasma cells, thereby producing an accelerated and robust anamnestic response. This response is predominantly mediated by class-switched antibodies of higher affinity, thus enabling more efficient clearance of the antigen (Eisen, 2014; Inoue *et al*., 2018).

The utilisation of animal models facilitates a more intuitive comprehension of the pathogenesis of tuberculosis (Choreño-Parra *et al*., 2020). In this study, the mouse model of H37Ra infection was successfully established. The H37Ra strain, an avirulent strain derived from the human *M. tuberculosis* H37Rv strain, retains some of the immunogenicity of virulent strains (Castaɒo *et al*., 2014; Jena *et al*., 2014). The extremely weak virulence and relative safety for the host of H37Ra has led to its use in the study of immune response mechanisms (Castaɒo *et al*., 2014; Jena *et al*., 2014). In this study, we have analyzed the changes of B cells and their subpopulations in the process of Mtb infection by H37Ra infection model, so as to better understand the role of B cells in tuberculosis infection.

MATERIALS AND METHODS

Study mice

Female BALB/c mice of the Specific Pathogen Free type, aged 5-6 weeks and weighing 18±2 g, were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd., Beijing, China, and used for the study.

Preparation of experimental strain of *Mycobacterium tuberculosis*

The H37Ra cryopreserved strain was inoculated on modified Roche media and incubated at 37° for approximately 20 days. Well-grown dry H37Ra colonies were scraped off and mixed with normal saline (NS) to achieve a bacterial concentration of 5×10^6 colony-forming units (CFU) per 1 mL for the study.

Preparation of study infected model

A total of 24 BALB/c mice were randomly divided into two groups: a control group and an infected group (12 mice in each group). Mice in the infected group were inoculated intravenously via the tail vein with a bacterial suspension containing 10^6 CFU per 0.2 mL. The control mice were injected with 0.2 mL normal saline (NS) solution. At the 4^{th} and 8^{th} weeks post-infection, the mice were euthanised and their lungs, kidneys and livers were minced into a tissue suspension. The resulting tissue suspension was then cultured in Roche's medium, which revealed the growth of H37Ra, thus confirming the successful establishment of the H37Ra infection mouse model. Both the tissue (lungs, kidneys, liver) and immune cell (peritoneal, splenic, and lymph node cells) collections were performed simultaneously during the same procedure following the mice's sacrifice. The use of animals was approved by the local wildlife affairs authority and the Ethics Committee of Dali University (Protocol SYXK-81241133-001).

Cell harvesting

Four weeks after infection, 6 mice in the infected group and 6 mice in the control group were euthanised, and the following cells were obtained: peritoneal cells, splenic cells and lymph node cells. This procedure was repeated at the 8^{th} week after infection.

A small incision was made in the abdominal skin of the mouse. Subsequently, 5 mL of 3% FBS-PBS and 2-3 mL of air were injected into the abdominal cavity of the mouse via a 10mL-syringe. The body of the mouse was then gently shaken to wash peritoneal cells. The fluid in the peritoneal cavity was subsequently collected, and the peritoneal exudate cells (PECs) were washed by centrifugation at 1200 r/min (radius: 15cm) for 5 min. The cell concentrate was then adjusted to a concentration of 10^7 cells/mL, after which it was stored for subsequent use.

The spleen of each mouse was ground aseptically and filtered with a cell strainer with a pore size of 70 μm (BD Falcon) in order to remove the envelope and connective tissue, thus producing a single cell suspension. The erythrocytes in cell suspension were lysed with Gey's solution (0.15 M NH_4Cl, 1 mM $KHCO_3$, 0.1 mM Na_2EDTA) and the cell suspension was washed with a 3% FBS-blood dilution solution. The cell concentrate was then adjusted to a concentration of 10^7/mL and stored for subsequent use. A portion of the splenic cells, devoid of erythrocytes, was cultivated in a 10% FBS-RPMI-1640 medium (GIBCO) containing L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin, utilising a 12-well U-shaped bottom culture plate at a concentration of 10^6 cells/mL. The cells were then added to 0.1 IU purified protein derivative of tuberculin (TB-PPD) (Beijing Xiangrui Biological Products Co., Ltd., China) at a concentration of 10^6 cells/mL, following which the plate was incubated at 37° and 5% CO_2 for 24 h. The cells were then stained as described below. TB-PPD was not added as control.

The cervical and axillary lymph nodes were collected, ground, and filtered with a cell strainer to remove the capsule and connective tissue, thus obtaining a single cell suspension. The lymphocytes were then obtained by centrifuging the cell suspension in 3% foetal bovine serum (FBS) at 1200 r/min (radius: 15cm). The cell concentrate was then adjusted to 10^7 cells/mL, after which it was stored for subsequent use.

Cell staining

All above cells, including peritoneal cells, splenic cells and lymph node cells from the healthy and infected mice, were stained. The peritoneal cells were stained with CD45R-PE, IgD-FITC, Mac1(CD11b)-APC, and CD5-PERCP. The splenic cells were stained with CD45R-PE, CD23-FITC, CD21-CD35-APC, CD69-FITC, CD27-APC, and IgD-FITC. The cultured splenic cells were stained with CD45R-PE and CD69-FITC. The lymphocyte obtained from the lymph nodes were stained with CD19-PE and CD92.0-FITC. Above all anti-mouse fluorescent antibodies were purchased from BD Biosciences (USA). A total of 50,000 cells/sample were acquired on a FACScalibur (BD Biosciences, USA) and analyzed with Cell Quest software (BD Biosciences, USA). The dead cells and cell debris were removed and all samples/cells had >95 % viable cells.

Statistical analysis

All data were evaluated with Statistical Product and Service Solutions 26.0 (SPSS26.0). Experimental data were expressed by mean ± standard deviation, and the frequency of B lymphocytes between the two groups were compared with independent *t*-test. A *P*-value less than 0.05 was considered to be statistically significant.

RESULTS

Basic analysis data from the all animal samples

The samples from infected and healthy control animals were analyzed with the above described methodologies. The results show in the Table 1.

Distribution of peritoneal B cells

The frequency of peritoneal B cells ($CD45R^+$) (4W: (46.62±7.05) %; 8W: (54.90±8.20) %) and B1b cells ($CD45R^{Int}IgD^-CD5^-CD11^+$) (4W: (5.14±1.74) %; 8W: (5.91±1.62) %) decreased in H37Ra-infected mice compared to the healthy control mice at both the 4th and 8th weeks ($P < 0.05$) (Table 1, Figure 1). The H37Ra-infected mice exhibited an increase in the frequency of B1a cells ($CD45R^{Int}IgD^-CD5^+CD11^+$) ($P$=0.013) at the 4th week in comparison to the control mice. However, there was no significant difference observed in the frequency of B1 cells ($CD45R^{Int}IgD^-$) between the 4th and 8th weeks of H37Ra infection (Table 1, Figure 1).

Distribution of splenic Fo B cells and MZ B cells in mice

Splenic B cells ($CD45R^+$) (4W: (7.45 ± 4.37) %; 8W: (12.82 ± 4.41) %) and marginal zone (MZ) B cells ($CD45R^+CD23^-CD21^{hi}$) (4W: (17.70 ± 6.73) %; 8W: (16.98 ± 6.81) %) from H37Ra-infected mice decreased compared to the healthy controls at the 4th and 8th weeks ($P < 0.05$) (Table 1, Figure 2). Furthermore, a significant increase in the frequency of splenic follicular (Fo) B cells ($CD45R^+CD23^+CD21^{int}$) was observed in comparison to the control mice at the 8th week ($P < 0.01$) (Table 1, Figure 2). Furthermore, the frequency of Fo B cells was found to be significantly higher in H37Ra mice at the 8th week than in those infected at the 4th week (P<0.001) (Table 1).

Distribution of B cells from lymph nodes in mice

The frequency of $CD19^+$ B cells (4W: (45.68 ± 8.16) %; 8W: (46.93 ± 5.19) %) in the lymph nodes of mice infected with H37Ra was found to be significantly elevated in comparison to healthy controls at both the 4th and 8th weeks post-infection ($P < 0.01$). The magnitude of this increase was more pronounced at the 4th week of H37Ra infection (Table 1, Figure 3).

Distribution of splenic activated B cells in mice

Irrespective of presence or absence of TB-PPD in the culture medium, H37Ra-infected mice exhibited elevated levels of activated CD69 molecules in comparison to healthy controls at the 4th and 8th weeks ($P < 0.05$) (Table 1, Figure 4). B cells were fully activated at the 4th week after H37Ra infection, presenting similar percentage of activated B cells at the 4th and 8th weeks (Table 1). In addition, there was no significant increase in the frequency of activated B cells at the 4th week after stimulation with the specific antigen TB-PPD when compared with the non-stimulated cells in H37Ra-infected mice (Table 1, Figure 4).

Distribution of splenic memory B cell in mice

The splenic memory B cells ($CD45R^+CD27^+IgD^{+/-}$) and isotype-switched memory B cells ($CD45R^+CD27^+IgD^-$) from H37Ra-infected mice were increased when compared to the control mice at the 4th and 8th weeks ($P < 0.05$). However, there was no significant difference in the frequency of non-isotype-switched memory B cells ($CD45R^+CD27^+IgD^+$) (Table 1, Figure 5).

DISCUSSION

B cells and humoral immunity have been demonstrated to modulate the immune response to various intracellular pathogens (including Mtb) (Achkar *et al*., 2015; Chan *et al*., 2014). In murine TB, evidence has demonstrated that B cells can modulate the level of granulomatous reaction, cytokine production, and the T cell response (Chan *et al*., 2014; Kozakiewicz *et al*., 2013). In this study, a decrease in peritoneal B cells and splenic B cells, and an increase in B cells from lymph nodes, was observed in H37Ra-infected mice at the 4th and 8th weeks in comparison to healthy controls. This finding is consistent with previous studies that reported a redistribution of B cells in the peripheral immune organs during H37Rv infection,

Table 1. Descriptive statistics of frequency of B lymphocyte subpopulations in different samples from infected H37Ra and healthy control mice

Population	The 4th week		*P*-value	The 8th week		*P*-value	Week 4 vs week 8 of infected groups (*P*-value)
	Control (%)	H37Ra (%)		Control (%)	H37Ra (%)		
$CD45R^+$ (Peritoneal B cell)	73.41±7.99	46.62±7.05	<0.001**	75.83±5.26	54.90±8.20	<0.001**	0.090
$CD45R^{Int}IgD^-$ (B1 cell)	43.67±6.30	38.03±5.34	0.125	42.78±5.66	36.32±5.47	0.072	0.596
$CD45R^{Int}IgD^-CD5^+CD11^+$ (B1a cell)	25.68±4.69	33.91±4.71	0.013*	29.08±4.86	31.81±5.46	0.382	0.491
$CD45R^{Int}IgD^-CD5^-CD11^+$ (B1b cell)	8.05±1.67	5.14±1.74	0.014*	14.54±3.31	5.91±1.62	<0.001**	0.443
$CD45R^+$ (Splenic B cell)	20.77±6.04	7.45±4.37	0.001**	20.04±4.31	12.82±4.41	0.017*	0.060
$CD45R^+CD23^+CD21^{int}$ (Fo B cell)	9.54±3.50	11.47±3.67	0.360	25.43±6.85	41.93±6.53	0.002**	<0.001**
$CD45R^+CD23^-CD21^{hi}$ (MZ B cell)	29.89±8.55	17.70±6.73	0.021*	33.27±5.81	16.98±6.81	0.001**	0.857
$CD19^+$ (B cell from lymph nodes)	28.83±7.21	45.68±8.16	0.004**	32.44±4.50	46.93±5.19	<0.001**	0.758
$CD69^+$ activated B cell (No TB-PPD)	10.79±2.30	16.30±3.04	0.005**	11.30±2.63	14.54±2.08	0.039*	0.270
$CD69^+$ activated B cell (Add TB-PPD)	11.65±2.29	19.73±5.40	0.007**	14.54±2.08	16.81±2.31	0.009**	0.252
$CD45R^+CD27^+IgD^{+/-}$ (Memory B cell)	6.53±2.81	9.90±2.20	0.043*	8.95±3.15	13.61±3.99	0.049*	0.074
$CD45R^+CD27^+IgD^-$ (Isotype-switched memory B cell)	5.61±2.65	9.98±3.02	0.024*	6.74±2.62	11.55±4.02	-2.455	0.460
$CD45R^+CD27^+IgD^+$ (Non-isotype-switched memory B cell)	0.66±0.19	0.52±0.29	0.364	0.79±0.24	0.64±0.18	2.277	0.414

Annotation: The symbol "*" represents P<0.05; The symbol "**" represents P<0.01.

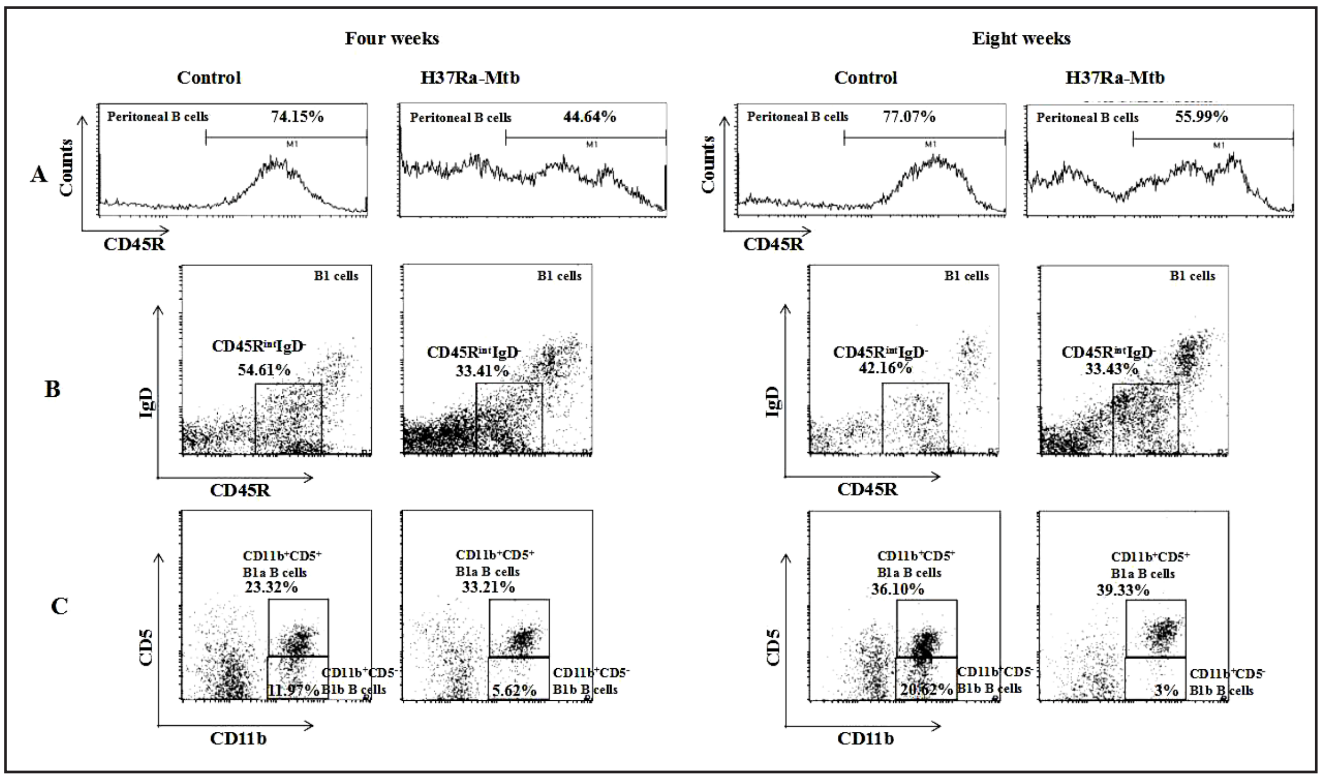

Figure 1. Distribution of peritoneal B cells at the 4th and 8th weeks. Annotation: The above showed a typical individual in the control group and the infected group. A: Peritoneal B cells; B: B1 cells; C: B1a and B1b cells.

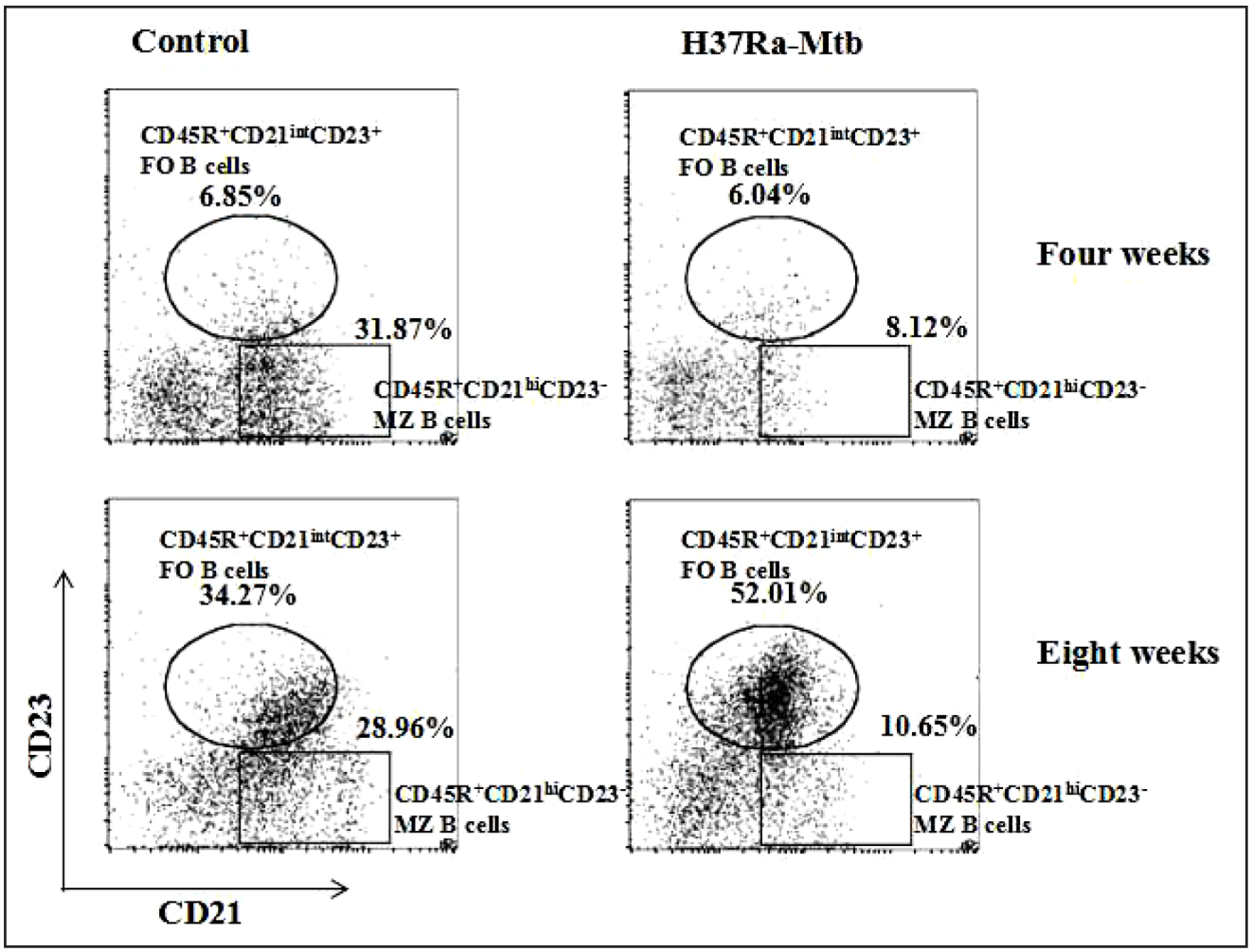

Figure 2. The distribution of Fo B cells and MZ B cells at the 4th (upper panel) and 8th weeks (low panel) in different groups by FACS. Annotation: The above showed a typical individual in the control group and the infected group.

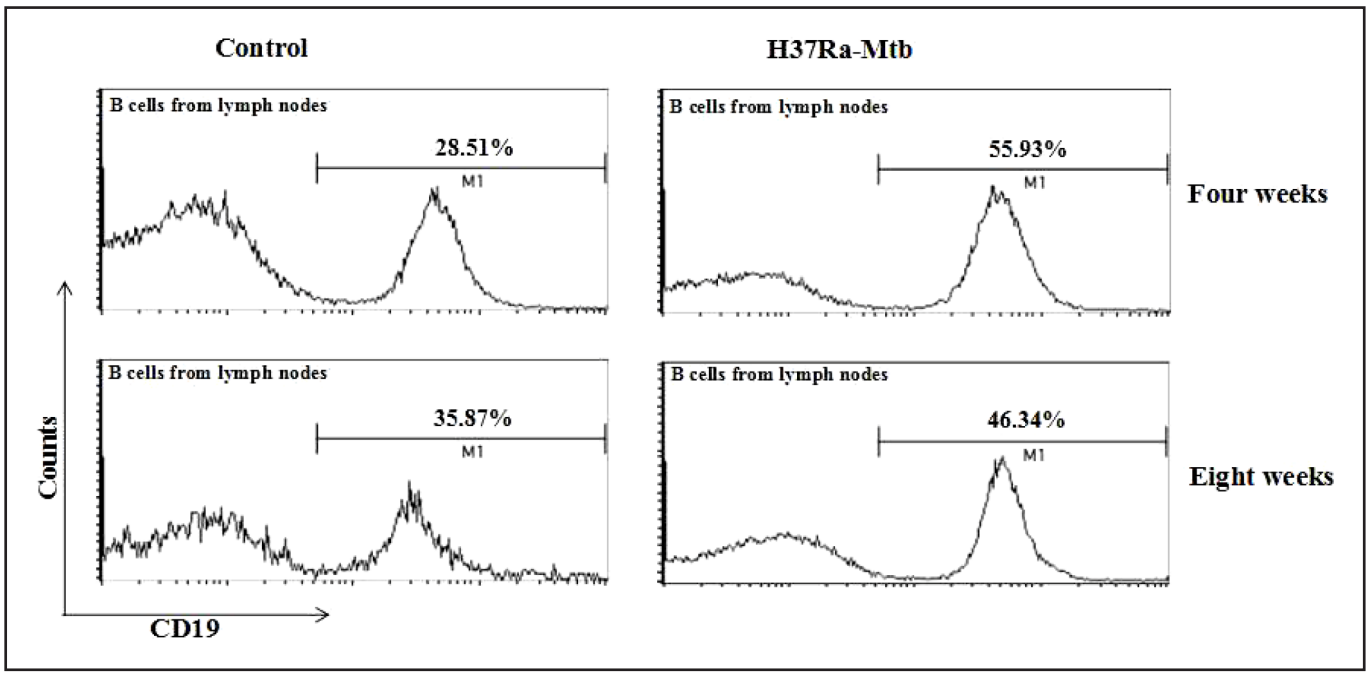

Figure 3. The distribution of total B cells ($CD19^+$) from lymph nodes at the 4^{th} (upper panel) and 8^{th} weeks (low panel) in different groups by FACS. Annotation: The above showed a typical individual in the control group and the infected group.

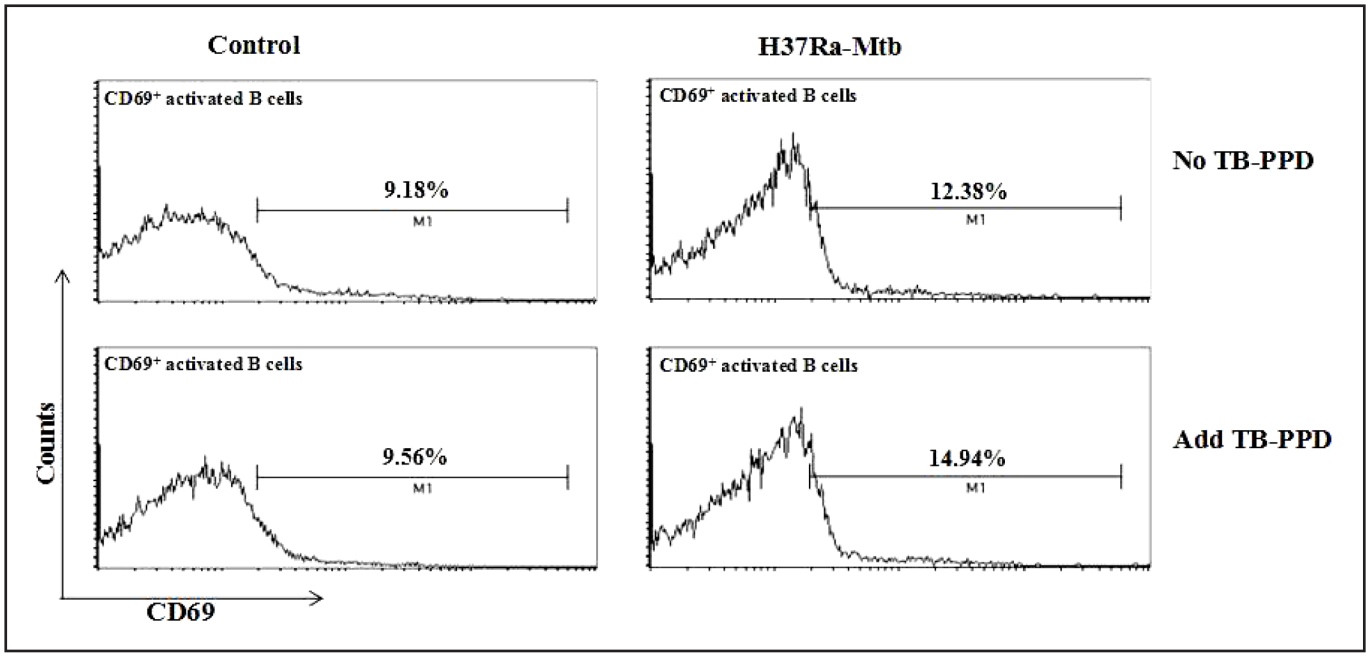

Figure 4. The frequency of activated B cells from mice cultured without TB-PPD (upper panel) and with TB-PPD (low panel) at the 4^{th} week by FACS. Annotation: The above showed a typical individual in the control group and the infected group.

with a decreased frequency of B cells in the spleen and an increased frequency in lymph nodes (Lv *et al*., 2017a, 2017b). The lymph nodes are the main site of immune response of B cells, and B cells from lymph nodes are generally more mature than those in the spleen (Lv *et al*., 2017b). The present findings were consistent with those of Borrero *et al*. (2013), who demonstrated that H37Ra infection can promote the development of B cells towards maturity and increase B cells from lymph nodes (Borrero *et al*., 2013). Mice deficient in B cells resulted in enhanced tuberculous lung inflammation, tissue neutrophilia, and increased local production of IL-10, providing further evidence that B lymphocytes were essential for the immune response to Mtb (Maglione *et al*., 2007). Previous studies have found that the B cell repertoire was observed unique variations in during active tuberculosis infection when compared to healthy controls, other lung-based diseases and over the course of TB treatment (du Plessis *et al*., 2016a).

B1 cells, constituting for 5% of the total B cell population in mice, are found in the abdominal cavity, the intestine, and pleural cavity, with a lesser prevalence in the spleen and lymph nodes (Dorshkind & Montecino-Rodriguez, 2007). B1 cells are distinguished by their prolonged lifespan, self-renewal capacity, spontaneous secretion of IgM, and multi-reactivity to a broad spectrum of pathogens

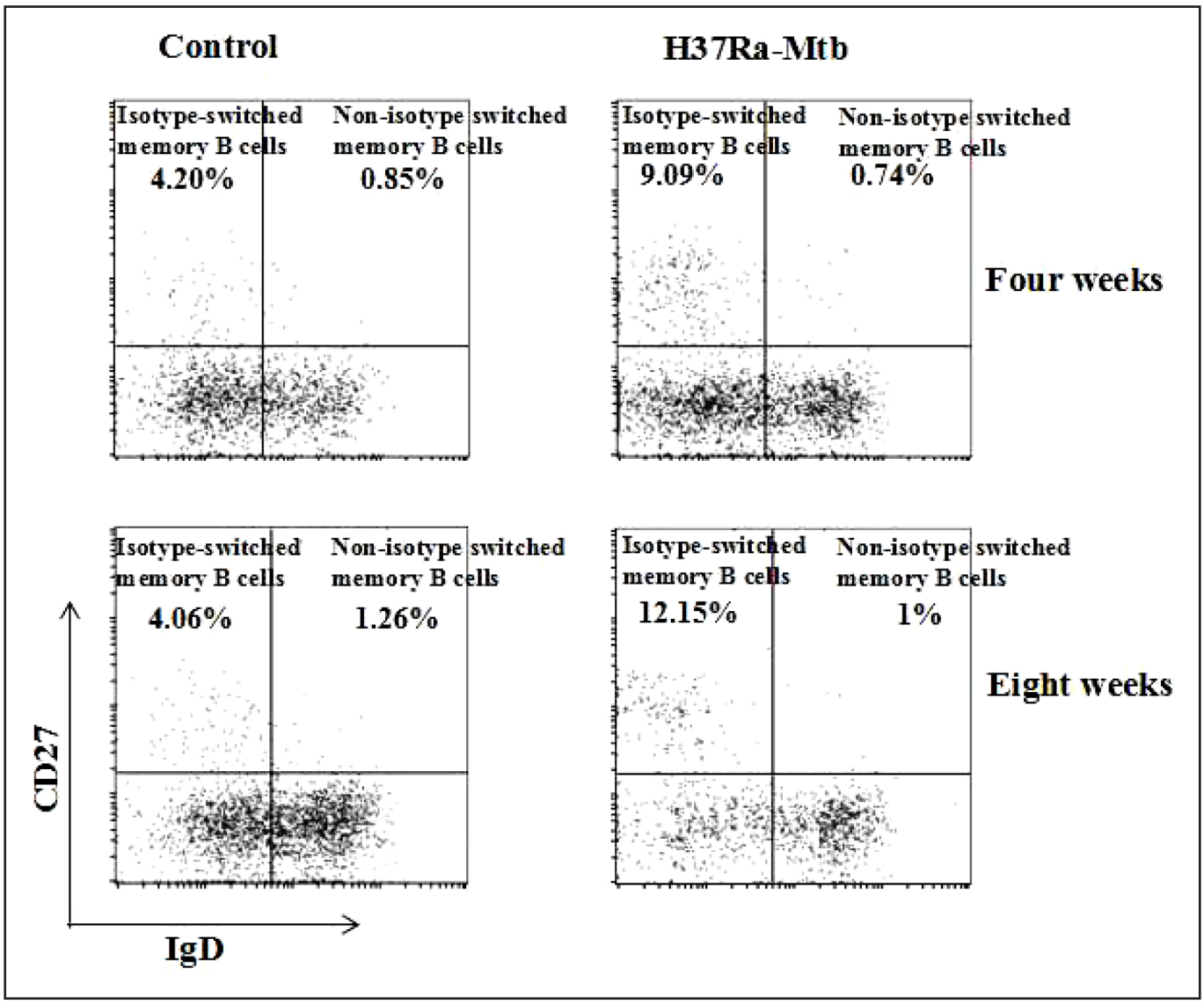

Figure 5. The distribution of splenic isotype-switched memory B cells and non-isotype-switched memory B cells at the 4th (upper panel) and 8th weeks (low panel) in different groups by FACS. Annotation: The above showed a typical individual in the control group and the infected group.

(Baumgarth, 2011). Peritoneal B1 cells represent the primary line of defence against gastrointestinal pathogens. They have the capacity to rapidly produce specific antibodies against thymus-independent antigens (TI antigens), thereby playing a pivotal role in the early stage of infection through the execution of humoral immune function. In addition to their direct role in immune function, B1 cells also act as antigen- presenting cells (APCs), effectively presenting antigens to T cells (Haas *et al*., 2005). B-1 cell subsets, principally B-1a, have been shown to be major responders to in vitro stimulation with various phospholipids, including those from *Mycobacterium* (Ordoɒez *et al*., 2018). However, further research is required to fully elucidate the complex and multifaceted role of B1 cells in immune system function (Kreslavsky *et al*., 2018). The results of this study demonstrated that B1 cells remained unchanged in H37Ra-infected mice at the 4th and 8th weeks post-infection. However, B1b cells ($CD45^{int}$ $IgD^{-}CD5^{-}CD11^{+}$) decreased and B1a cells ($CD45^{int}$ $IgD^{-}CD5^{+}CD11^{+}$) increased in the early infection. It suggested that B1a cells might play a significant role in the early stage of H37Ra infection and contribute to the anti-tuberculosis immune response. As the infection progresses to its later stage (week 8), there was no significant difference in the frequency of B1a cells between infected mice and healthy ones, but B1b cells decreased significantly. This decline may be attributed to the activation of body cavity B1 cells in response to Mtb infection, which subsequently migrate to lymph tissues and differentiate into cytokine and antibody-secreting cells (Waffarn *et al*., 2015; Smith & Baumgarth, 2019). These cells then undergo a rapid loss of CD11b expression (Waffarn *et al*., 2015; Smith & Baumgarth, 2019). Concomitantly, analogous outcomes were previously observed in H37Rv-infected mice (Lv *et al*., 2017a). Ordo ɒez *et al*. (2018) demonstrated that a higher proportion of B-1a cells exhibited differentiation, manifesting the plasma cell marker $CD138^{+}$ in comparison to B-1b cells (Ordoɒez *et al*., 2018). Furthermore, both B-1a and B-1b cells demonstrated a response to *M. tuberculosis* lipids, characterised by enhanced IgM secretion; however, B-1a cells exhibited a more pronounced response. Moreover, an elevated rate of B-1a cell differentiation into total IgM-secreting plasma cells was observed following stimulation with *M. tuberculosis* H37Rv lipids (Ordoɒez *et al*., 2018).

Marginal zone (MZ) B-cells and B1 B-cells are classified as "innate B lymphocytes" due to unique developmental and functional characteristics (Zouali & Richard, 2011). In rodents, these B-cell subsets exhibit an activated phenotype that allows their rapid proliferation and differentiation into Ab-secreting cells upon stimulation with thymus-independent (TI) antigens (Ags). These cells have been observed to secrete antibodies of the IgM and IgG3 isotypes with a relative preference (Won & Kearney, 2002).

MZ B-cells comprise an innate subset localized to the marginal sinuses of the spleen. In comparison with FO B-cells, they have the capacity to respond to specific foreign antigens more rapidly (Pillai *et al*., 2005). This ability to respond more quickly enables them to provide early immune responses to blood-borne particulate antigens. In addition to their role as an early source of pathogen-specific, T-cell-independent IgM, MZ B-cells serve to bridge the innate and adaptive immune systems. For instance, studies of the spirochete *Borrelia hermsii* demonstrated that MZ B-cells directly interact with blood-borne *Borrelia spirochetes* and are activated in vivo to produce pathogen-specific IgM within 72 hours of infection (Belperron *et al*., 2005). MZB cells could utilize IgM to regulate immune cells by engaging Fc receptors. For instance, the Fc receptor, an IgM receptor, has been demonstrated to regulate the functions of monocytes, macrophages, granulocytes, T cells, and systemic cytokine patterns (Lang *et al*., 2013; Liu *et al*., 2018a; Yu *et al*., 2018). Moreover, it has been shown to contribute to protection against various infections, including *Listeria monocytogenes*, *Citrobacter rodentium*, and influenza virus (Lang *et al*., 2013; Liu *et al*., 2018a; Yu *et al*., 2018). MZB cells exhibit diverse roles in different infectious diseases, including protection (Belperron *et al*., 2007; Lo *et al*., 2021) and exacerbation (Bankoti *et al*., 2012; Lee & Kung, 2012). Despite the absence of a comprehensive understanding of the direct antimicrobial effects of IgM on Mtb, it has been hypothesised that MZB cells may regulate cell-mediated immunity and cytokine patterns against TB through IgM-mediated mechanisms (Tsai *et al*., 2024). Furthermore, the enhancement of MZB cell responses during BCG vaccination has the potential to enhance vaccine efficacy by leveraging their regulatory functions to optimise immune responses (Tsai *et al*., 2024). The targeting of regulatory functions of B cells can be a valuable strategy for the development of TB vaccines. Fo B cells are important cells for specific immune response (Schneider *et al*., 2001). It is the most abundant type of B cell, responsible for specific humoral immunity and the production of an immune response to protein antigens (Pillai *et al*., 2005). The results of this study demonstrated that Fo B cells increased and MZ B cells decreased at 8 weeks after H37Ra infection, thereby indicating that H37Ra infection affected the distribution of Fo B cells, and Fo B cells played a major role in the immune response to H37Ra infection. This alteration was conducive to the body's specific immune response to Mtb infection. A parallel observation was made in our previous study, which revealed that *M. tuberculosis* H37Rv infection led to an increase in the frequency of increased Fo B cells and a decrease in the frequency of MZ B cells (Lv *et al*., 2017a). This finding closely aligns with the present study. Some scholars reported that marginal zone (MZ) B cells were significantly lower during active TB disease when compared to other-lung based diseases and over the course of TB treatment, and it was suggested that (MZ) B cells could be used as biomarker for treatment response (du Plessis *et al*., 2016a).

Activation of B cells leads to the surface expression of the c-type lectin CD69 (Abreu *et al*., 2014). In the present study, the frequency of $CD69^+$ B cells was found to be elevated in infected mice compared to healthy mice at the 4^{th} week, in addition to after stimulation with antigen (TB-PPD). This suggested that infection could promote the increase of activated B cells. Consistent with these observations, our earlier studies demonstrated that the frequency of CD69-expressing B cells increased in mice infected with H37Rv (Liu *et al*., 2018b). It has been reported that the multidrug-resistant TB (MDR-TB) patients had a significantly increased frequency of CD69-expressing B cells than the healthy controls (Abreu *et al*., 2014). The activation of B cells has been demonstrated to initiate the humoral immune response and to play a role in the body's anti-infection immunity.

Memory B cells produce primarily IgA and IgG, and the interaction of CD27 with its ligand CD70 on helper T cell surface leads to an up-regulation of immunoglobulin production and subsequently to memory B cell differentiation into antibody-secreting plasma cells (Agematsu *et al*., 1997). The results showed that memory B cells ($CD45R^+CD27^+IgD^{+/-}$) and isotype-switched memory B cells ($CD45R^+CD27^+IgD^-$) increased in H37Ra mice at the 4^{th} and 8^{th} weeks, which was consistent with the previous results of H37Rv-infected mice (Liu *et al*., 2018b; Lv *et al*., 2017b). Isoptype-switched memory B cells were considered to be a category of post-germinal centre B cells, which have been shown to present hypermutated and class-switched immunoglobulin genes (Llinɴs *et al*., 2011). However, Abreu's study demonstrated there was a significant reduction in unswitched memory B cells in MDR-TB patients, while no significant difference was observed in the frequency of post-switch memory B cell subpopulations between MDR-TB patients and healthy donors (Abreu *et al*., 2014). The underlying causes of this discrepancy require further investigation.

CONCLUSION

The results demonstrated that H37Ra infection affected the distribution of B lymphocyte subpopulations, leading to a redistributed of B cell populations in peripheral immune organs. H37Ra infection down-regulated MZ B cells, which contribute to innate immunity, while up-regulating Fo B cells and total lymph node B cells, which are central to adaptive immune responses. This infection also enhanced the activation of B cells and promote the production of memory B cells, which promoted the process of B cell immune response against tuberculosis. In this study, although the frequency of B1 cells did not show abnormalities, the frequency of B1b cells decreased and that of B1a cells increased. Further research is required to determine the functional significance of these B cell subpopulation changes during H37Ra infection.

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Conflict of interest

The author declares that they have no conflict of interests.

REFERENCES

Abreu, M.T., Carvalheiro, H., Rodrigues-Sousa, T., Domingos, A., Segorbe-Luis, A., Rodrigues-Santos, P. & Souto-Carneiro, M.M. (2014). Alterations in the peripheral blood B cell subpopulations of multidrug-resistant tuberculosis patients. *Clinical and Experimental Medicine* **14**: 423-429. https://doi.org/10.1007/s10238-013-0258-1

Achkar, J.M., Chan, J. & Casadevall, A. (2014). Role of B cells and antibodies in acquired immunity against *Mycobacterium tuberculosis*. *Cold Spring Harbor perspectives in medicine* **5**: a18432. https://doi.org/10.1101/cshperspect.a018432

Achkar, J.M., Chan, J. & Casadevall, A. (2015). B cells and antibodies in the defense against *Mycobacterium tuberculosis* infection. *Immunological Reviews* **264**: 167-181. https://doi.org/10.1111/imr.12276

Agematsu, K., Nagumo, H., Yang, F.C., Nakazawa, T., Fukushima, K., Ito, S., Sugita, K., Mori, T., Kobata, T., Morimoto, C. *et al*. (1997). B cell subpopulations separated by CD27 and crucial collaboration of $CD27^+$ B cells and helper T cells in immunoglobulin production. *European Journal of Immunology* **27**: 2073-2079. https://doi.org/10.1002/eji.1830270835

Bagcchi, S. (2023). WHO's global tuberculosis report 2022. *Lancet Microbe* **4**: e20. https://doi.org/10.1016/S2666-5247(22)00359-7

Bankoti, R., Gupta, K., Levchenko, A. & Stäger, S. (2012). Marginal zone B cells regulate antigen-specific T cell responses during infection. *Journal of Immunology* **188**: 3961-3971. https://doi.org/10.4049/jimmunol.1102880

Baumgarth, N. (2004). B-cell immunophenotyping. *Methods in Cell Biology* **75**: 643-662. https://doi.org/10.1016/s0091-679x(04)75027-x

Baumgarth, N. (2011). The double life of a B-1 cell: self-reactivity selects for protective effector functions. *Nature Reviews Immunology* **11**: 34-36. https://doi.org/10.1038/nri2901

Belperron, A.A., Dailey, C.M. & Bockenstedt, L.K. (2005). Infection-induced marginal zone B cell production of *Borrelia hermsii*-specific antibody is impaired in the absence of CD1d. *Journal of Immunology* **174**: 5681-5686. https://doi.org/10.4049/jimmunol.174.9.5681

Belperron, A.A., Dailey, C.M., Booth, C.J. & Bockenstedt, L.K. (2007). Marginal zone B-cell depletion impairs murine host defense against *Borrelia burgdorferi* infection. *Infection and Immunity* **75**: 3354-3360. https://doi.org/10.1128/IAI.00422-07

Bergmann, B., Grimsholm, O., Thorarinsdottir, K., Ren, W., Jirholt, P., Gjertsson, I. & Mårtensson, I.L. (2013). Memory B cells in mouse models. *Scandinavian Journal of Immunology* **78**: 149-156. https://doi.org/10.1111/sji.12073

Blanc, L., Gilleron, M., Prandi, J., Song, O.R., Jang, M.S., Gicquel, B., Drocourt, D., Neyrolles, O., Brodin, P., Tiraby, G. *et al*. (2017). *Mycobacterium tuberculosis* inhibits human innate immune responses via the production of TLR2 antagonist glycolipids. *Proceedings of the National Academy of Sciences of the United States of America* **114**: 11205- 11210. https://doi.org/10.1073/pnas.1707840114

Borrero, R., García, M.L., Canet, L., Zayas, C., Reyes, F., Prieto, J.L., Infante, J.F., Lanio, M.E., Kadir, R., López, Y. *et al*. (2013). Evaluation of the humoral immune response and cross reactivity against *Mycobacterium tuberculosis* of mice immunized with liposomes containing glycolipids of *Mycobacterium* smegmatis. *BMC Immunology* **14**: S13. https://doi.org/10.1186/1471-2172-14-S1-S13

Bosio, C.M., Gardner, D. & Elkins, K.L. (2000). Infection of B cell-deficient mice with CDC 1551, a clinical isolate of *Mycobacterium tuberculosis*: delay in dissemination and development of lung pathology. *Journal of Immunology* **164**: 6417-6425. https://doi.org/10.4049/jimmunol.164.12.6417

Castaño, D., García, L.F. & Rojas, M. (2014). Differentiation of human mononuclear phagocytes increases their innate response to *Mycobacterium tuberculosis* infection. *Tuberculosis* **94**: 207-218. https://doi.org/10.1016/j.tube.2014.01.001

Cerutti, A., Cols, M. & Puga, I. (2013). Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. *Nature Reviews Immunology* **13**: 118-132. https://doi.org/10.1038/nri3383

Chan, J., Mehta, S., Bharrhan, S., Chen, Y., Achkar, J.M., Casadevall, A. & Flynn, J. (2014). The role of B cells and humoral immunity in *Mycobacterium tuberculosis* infection. *Seminars in Immunology* **26**: 588-600. https://doi.org/10.1016/j.smim.2014.10.005

Choreño-Parra, J.A., Bobba, S., Rangel-Moreno, J., Ahmed, M., Mehra, S., Rosa, B., Martin, J., Mitreva, M., Kaushal, D., Zúñiga, J. *et al*. (2020). *Mycobacterium tuberculosis* HN878 infection induces human-like B-Cell Follicles in mice. *The Journal of Infectious Diseases* **221**: 1636-1646. https://doi.org/10.1093/infdis/jiz663

Dorshkind, K. & Montecino-Rodriguez, E. (2007). Fetal B-cell lymphopoiesis and the emergence of B-1-cell potential. *Nature Reviews Immunology* **7**: 213-219. https://doi.org/10.1038/nri2019

du Plessis, W.J., Keyser, A., Walzl, G. & Loxton, A.G. (2016a). Phenotypic analysis of peripheral B cell populations during *Mycobacterium tuberculosis* infection and disease. *Journal of Inflammation* **13**: 23. https://doi.org/10.1186/s12950-016-0133-4

du Plessis, W.J., Walzl, G. & Loxton, A.G. (2016b). B cells as multi-functional players during *Mycobacterium tuberculosis* infection and disease. *Tuberculosis* **97**: 118-125. https://doi.org/10.1016/j.tube.2015.10.007

Eisen, H.N. (2014). Affinity enhancement of antibodies: how low-affinity antibodies produced early in immune responses are followed by high-affinity antibodies later and in memory B-cell responses. *Cancer Immunology Research* **2**: 381-392. https://doi.org/10.1158/2326-6066.CIR-14-0029

Haas, K.M., Poe, J.C., Steeber, D.A. & Tedder, T.F. (2005). B-1a and B-1b cells exhibit distinct developmental requirements and have unique functional roles in innate and adaptive immunity to *S. pneumoniae*. *Immunity* **23**: 7-18. https://doi.org/10.1016/j.immuni.2005.04.011

Harms Pritchard, G. & Pepper, M. (2018). Memory B cell heterogeneity: remembrance of things past. *Journal of Leukocyte Biology* **103**: 269-274. https://doi.org/10.1002/JLB.4MR0517-215R

Hoffman, W., Lakkis, F.G. & Chalasani, G. (2016). B cells, antibodies, and more. *Clinical Journal of the American Society of Nephrology* **11**: 137-154. https://doi.org/10.2215/CJN.09430915

Inoue, T., Moran, I., Shinnakasu, R., Phan, T.G. & Kurosaki, T. (2018). Generation of memory B cells and their reactivation. *Immunological Reviews* **283**: 138-149. https://doi.org/10.1111/imr.12640

Jena, L., Kashikar, S., Kumar, S. & Harinath, B.C. (2014). Effect of single amino acid mutations on function of *Mycobacterium tuberculosis* H37Rv and H37Ra by computational approaches. *The Indian journal of tuberculosis* **61**: 200-206.

Johnson, C.M., Cooper, A.M., Frank, A.A., Bonorino, C., Wysoki, L.J. & Orme, I.M. (1997). *Mycobacterium tuberculosis* aerogenic rechallenge infections in B cell-deficient mice. *Tuberculosis and Lung Diseases* **78**: 257-261. https://doi.org/10.1016/s0962-8479(97)90006-x

Kauffman, K.D., Sallin, M.A., Sakai, S., Kamenyeva, O., Kabat, J., Weiner, D., Sutphin, M., Schimel D., Via, L., Barry III, C.E. *et al*. (2017). Defective positioning in granulomas but not lung-homing limits CD4 T-cell interactions with *Mycobacterium tuberculosis*-infected macrophages in rhesus macaques. *Mucosal Immunology* **11**: 462-473. https://doi.org/10.1038/mi.2017.60

Kozakiewicz, L., Phuah, J., Flynn, J. & Chan, J. (2013). The Role of B cells and humoral immunity in *Mycobacterium tuberculosis* infection. *Advances in Experimental Medicine and Biology* **783**: 225-250. https://doi.org/10.1007/978-1-4614-6111-1_12

Kreslavsky, T., Wong, J.B., Fischer, M., Skok, J.A. & Busslinger, M. (2018). Control of B-1a cell development by instructive BCR signaling. *Current Opinion in Immunology* **51**: 24-31. https://doi.org/10.1016/j.coi.2018.01.001

Lang, K.S., Lang, P.A., Meryk, A., Pandyra, A.A., Boucher, L.M., Pozdeev, V.I., Tusche, M.W., Göthert, J.R., Haight, J., Wakeham, A. *et al*. (2013). Involvement of Toso in activation of monocytes, macrophages, and granulocytes. *Proceedings of The National Academy of Sciences of The United States of America* **110**: 2593-2598. https://doi.org/10.1073/pnas.1222264110

Lee, C.C. & Kung, J.T. (2012). Marginal zone B cell is a major source of Il-10 in *Listeria monocytogenes* susceptibility. *Journal of Immunology* **189**: 3319-3327. https://doi.org/10.4049/jimmunol.1201247

Linge, I., Kondratieva, E. & Apt, A. (2023). Prolonged B-lymphocyte-mediated immune and inflammatory responses to tuberculosis infection in the lungs of TB-resistant mice. *International Journal of Molecular Sciences* **24**: 1140. https://doi.org/10.3390/ijms24021140

Liu, J., Zhu, H., Qian, J., Xiong, E., Zhang, L., Wang, Y.Q., Chu, Y., Kubagawa, H., Tsubata, T. & Wang, J.Y. (2018a). Fcm receptor promotes the survival and activation of marginal zone B cells and protects mice against bacterial sepsis. *Frontiers in Immunology* **9**: 160. https://doi.org/10.3389/fimmu.2018.00160

Liu, W., Lv, Y., Wang, C., Zhao, W., Wang, T. & Bai, L. (2018b). Influencing on development and differentiation of B cells in *Mycobacterium tuberculosis* infected BALB/c mice. *Chinese Journal of Zoonoses* **34**: 703-707.

Liu, Y. & Cai, H. (2018). The Lrp of *Mycobacterium tuberculosis* regulates the innate immune response of macrophages. *Cellular & Molecular Immunology* **15**: 934-936. https://doi.org/10.1038/cmi.2018.6

Llinàs, L., Lázaro, A., Salort, J., Matesanz-Isabel, J., Sintes, J. & Engel, P. (2011). Expression profiles of novel cell surface molecules on B-cell subsets and plasma cells as analyzed by flow cytometry. *Immunology Letters* **134**: 113-121. https://doi.org/10.1016/j.imlet.2010.10.009

Lo, L.W., Chang, C.W., Chiang, M.F., Lin, I.Y. & Lin, K.I. (2021). Marginal zone B cells assist with neutrophil accumulation to fight against systemic *Staphylococcus aureus* infection. *Frontiers in Immunology* **12**: 636818. https://doi.org/10.3389/fimmu.2021.636818

Lv, Y., Bai L., Liu, W.W., Wang, C. & Zhao, W. (2017a). Distribution of B lymphocyte subsets in BALB/c mice infected with *Mycobacterium tuberculosis*. *Journal of Pathogen Biology* **12**: 25-28. https://doi.org/10.13350/j.cjpb.170106

Lv, Y., Bai, L., Liu, W.W., Wang, C. & Zhao, W. (2017b). Study on lymphocyte subset and level of antibody in BALB/c mice infected with *Mycobacterium tuberculosis*. *Journal of Tropical Medicine* **17**: 583-587.

Maglione, P.J., Xu, J. & Chan, J. (2007). B cells moderate inflammatory progression and enhance bacterial containment upon pulmonary challenge with *Mycobacterium tuberculosis*. *Journal of Immunology* **178**: 7222-7234. https://doi.org/10.4049/jimmunol.178.11.7222

Mouat, I.C. & Horwitz, M.S. (2022). Age-associated B cells in viral infection. *PLoS Pathogens* **18**: e1010297. https://doi.org/10.1371/journal.ppat.1010297

Ordoñez, C., Savage, H.P., Tarajia, M., Rivera, R., Weeks-Galindo, C., Sambrano, D., Riley, L., Fernandez, P.L., Baumgarth, N. & Goodridge, A. (2018). Both B-1a and B-1b cells exposed to *Mycobacterium tuberculosis* lipids differentiate into IgM antibody-secreting cells. *Immunology* **154**: 613-623. https://doi.org/10.1111/imm.12909

Palm, A.K.E., Friedrich, H.C. & Kleinau, S. (2016). Nodal marginal zone B cells in mice: a novel subset with dormant self-reactivity. *Scientific Reports* **6**: 27687. https://doi.org/10.1038/srep27687

Phuah, J., Wong, E.A., Gideon, H.P., Maiello, P., Coleman, M.T., Hendricks, M.R., Ruden, R., Cirrincione, L.R., Chan, J., Lin, P.L. *et al*. (2016). Effects of B cell depletion on early *Mycobacterium tuberculosis* infection in cynomolgus macaques. *Infection and Immunity* **84**: 1301-1311. https://doi.org/10.1128/IAI.00083-16

Pillai, S., Cariappa, A. & Moran, S.T. (2005). Marginal zone B cells. *Annual Review of Immunology* **23**: 161-196. https://doi.org/10.1146/annurev.immunol.23.021704.115728

Pupovac, A. & Good-Jacobson, K.L. (2017). An antigen to remember: regulation of B cell memory in health and disease. *Current Opinion in Immunology* **45**: 89-96. https://doi.org/10.1016/j.coi.2017.03.004

Schneider, P., Takatsuka, H., Wilson, A., Mackay, F., Tardivel, A., Lens, S., Cachero, T.G., Finke, D., Beermann, F. & Tschopp, J. (2001). Maturation of marginal zone and follicular B cells requires B cell activating factor of the tumor necrosis factor family and is independent of B cell maturation antigen. *The Journal of Experimental Medicine* **194**: 1691-1697. https://doi.org/10.1084/jem.194.11.1691

Smith, F.L. & Baumgarth, N. (2019). B-1 cell responses to infections. *Current Opinion in Immunology* **57**: 23-31. https://doi.org/10.1016/j.coi.2018.12.001

Torrado, E., Fountain, J.J., Robinson, R.T., Martino, C.A., Pearl, J.E., Rangel-Moreno, J., Tighe, M., Dunn, R. & Cooper, A.M. (2013). Differential and site specific impact of B cells in the protective immune response to *Mycobacterium tuberculosis* in the mouse. *PloS ONE* **8**: e61681. https://doi.org/10.1371/journal.pone.0061681

Tsai, C.Y., Oo, M., Peh, J.H., Yeo, B.C.M., Aptekmann, A., Lee, B., Liu, J.J.J., Tsao, W.S., Dick, T., Fink, K. *et al.* (2024). Splenic marginal zone B cells restrict *Mycobacterium tuberculosis* infection by shaping the cytokine pattern and cell-mediated immunity. *Cell Reports* **43**: 114426. https://doi.org/10.1016/j.celrep.2024.114426

Waffarn, E.E., Hastey, C.J., Dixit, N., Soo Choi, Y., Cherry, S., Kalinke, U., Simon, S.I. & Baumgarth, N. (2015). Infection-induced type I interferons activate CD11b on B-1 cells for subsequent lymph node accumulation. *Nature Communications* **6**: 8991. https://doi.org/10.1038/ncomms9991

Won, W.J. & Kearney, J.F. (2002). CD9 is a unique marker for marginal zone B cells, B1 cells, and plasma cells in mice. *Journal of Immunology* **168**: 5605-5611. https://doi.org/10.4049/jimmunol.168.11.5605

Yu, J., Duong, V.H.H., Westphal, K., Westphal, A., Suwandi, A., Grassl, G.A., Brand, K., Chan, A.C., Föger, N. & Lee, K.H. (2018). Surface receptor Toso controls B cell-mediated regulation of T cell immunity. *Journal of Clinical Investigation* **128**: 1820-1836. https://doi.org/10.1172/JCI97280

Zouali, M. & Richard, Y. (2011). Marginal zone B-cells, a gatekeeper of innate immunity. *Frontiers in Immunology* **2**: 63. https://doi.org/10.3389/fimmu.2011.00063