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Bcells and their regulatory functions during Tuberculosis: Latency and active disease

Andre G. Loxton

DST-NRF Centre of Excellence for Biomedical Tuberculosis Research, South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 241 Cape Town, 8000, South Africa

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ABSTRACT

Tuberculosis (TB) is a global epidemic with devastating consequences. Emerging evidence suggests that B-cells have the ability to modulate the immune response and understanding these roles during *Mycobacterium tuberculosis* (*M.tb*) infection can help to find new strategies to treat TB. The immune system of individuals with pulmonary TB form granulomas in the lung which controls the infection by inhibiting the *M.tb* growth and acts as a physical barrier. Thereafter, surviving *M.tb* become dormant and in most cases the host's immunity prevents TB reactivation. B-cells execute several immunological functions and are regarded as protective regulators of immune responses by antibody and cytokine production, as well as presenting antigen. Some of these B-cells, or regulatory B-cells, have been shown to express death-inducing ligands, such as Fas ligand (FasL). This expression and binding to the Fas receptor leads to apoptosis, a major immune regulation mechanism, in addition to the ability to induce T-cell tolerance. Here, I discuss the relevance of B-cells, in particular their non-humoral functions by addressing their regulatory properties during *M.tb* infection.

1. Introduction

The TB epidemic is a global crisis and has plagued humankind throughout history (Daniel, 2006). Although the rate of TB incidence declined by 1.5% from 2014 to 2015, the World Health Organisation estimated 10.4 million new cases of TB globally in 2015, with only six countries accounting for 60% for all new cases. These included India, China, Indonesia, Nigeria, Pakistan and South Africa (WHO, 2016).

Mycobacterium tuberculosis (*M.tb*) is transmitted via contagious airborne droplets spread through the air. These droplets may be inhaled by a new host into their respiratory tract. The mycobacteria then elicit an immune response which are contained in the lungs by caseous granulomas. Approximately 10% of all infected people will develop active TB, roughly 50% in the first year and 50% at a later stage in their lifetime (Davies, 2005). This percentage increases to about 10% per year in immunocompromised individuals including those who are human immunodeficiency virus (HIV) positive (Davies, 2005). There are several exogenous factors influencing the rate of *M.tb* infection following exposure, these mainly include behavioural risk factors such as smoking, alcohol abuse, indoor air pollution, and rate of social mixing. Endogenous risk factors mainly affect the rate of the progression of TB from infection to active disease, these include circumstances which alter the immune response (Fig. 1).

M.tb's unique ability to remain dormant in the host poses a major problem for the effective control of TB (Ehlers and Schaible, 2012). The lack of medications that can eradicate latent TB infection (LTBI) leads to the potential for reactivation to active TB when the host's immune system is compromised. This presents major difficulties in countries with a high HIV prevalence. Additionally, vaccine development is based on the concept of protection post-infection. However because *M.tb* bacilli can lay dormant, a successful vaccine must have the ability to produce an immune response greater than that activated by a natural infection (Kaufmann, 2007). Approaches used to develop vaccines focus predominately on T-cell immunity, yet with increasing evidence of B-cells able to modulate the immune response, more emphasis on characterising B-cells may be of significant value (Chan et al., 2014).

The process in which B-cells and humoral immunity regulate the immune response to TB is still unclear, therefor understanding the roles of B-cells during *M.tb* infection may assist the design of new strategies to treat disease. In the following sections, the functions of B-cells, regulatory B-cells (Breg), and their cytokines are discussed, along with their roles with regards to *M.tb* infection.

2. Tuberculosis and B-cells

Immune cells are transported through the bloodstream to lymphoid and peripheral tissues where they demonstrate their functions in response to antigens. These cells are then recruited to and accumulate at

E-mail address: GL2@sun.ac.za.

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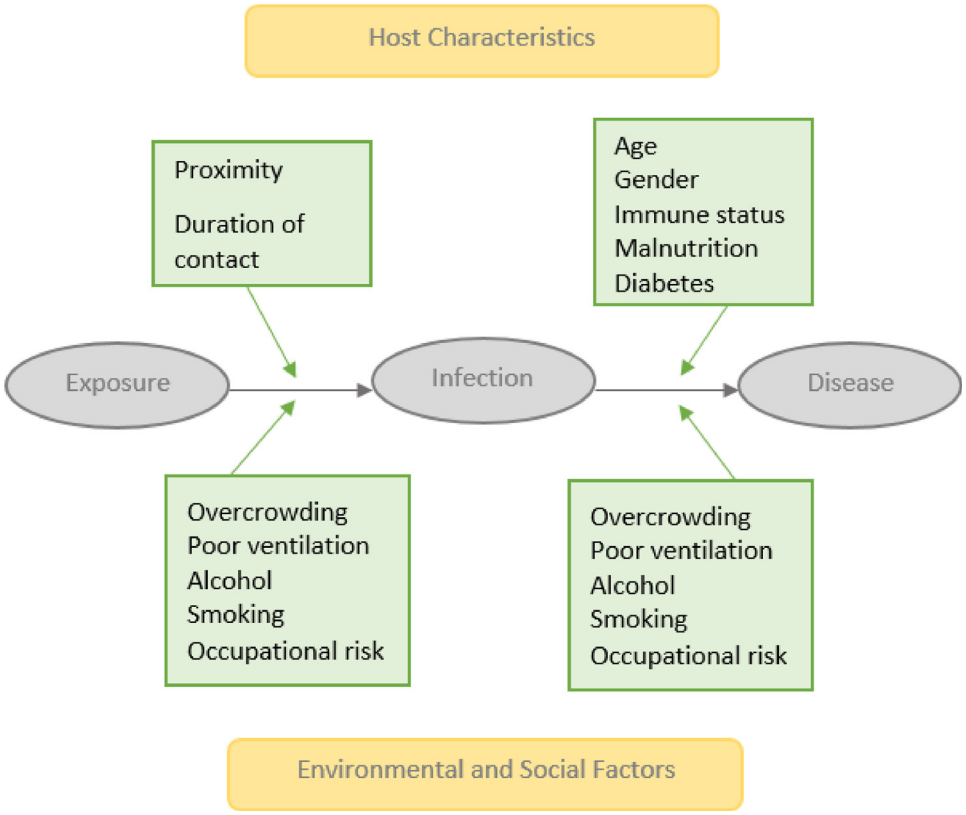

Fig. 1. The risk factors for TB infection and disease.
Adapted from Davies (2005).

the site of infection. Active recruitment and local expansion of specific immune cells results in selective accumulation at the site of infection (Brighenti and Andersson, 2012). The host immune system forms an organised conglomeration of cells known as a granuloma in response to *M.tb* infection. Granulomas are vital for the control of infection and they function as a physical, as well as an immune barrier, to prevent the spreading of the disease. They provide a micro-environment in which T-cells and macrophages can inhibit the growth of the *M.tb*. The surviving *M.tb* become dormant, unless the host immunity or the signals maintaining the granuloma diminish, which may lead to a reactivation of TB infection (Saunders and Britton, 2007). As result, the proper control of TB is essential. This is completed by the immune cells within the granuloma which activate macrophages to kill the mycobacteria and balance anti-inflammatory signals to reduce tissue damage. The most predominant lymphocyte populations include the memory $CD4^+$ Th1 cells in the granuloma and $CD8^+$ cytotoxic T-cells mainly found in the outer lymphocytic mantel around the granuloma (Brighenti and Andersson, 2012). Both these populations are the primary controllers of *M.tb* infection in humans (du Plessis et al., 2016a, 2016b). The $CD4^+$ T-cells' main function is cytokine production and immunity against mycobacteria by means of a Th1 response. $CD8^+$ T-cells also provide immunity against TB, however are more significant later in TB infection, produce IFN-γ and control the lysis of infected cells (Hernandez et al., 2010). T-cells play a vital role in activating macrophages by releasing IFN-γ and TNF (Phuah et al., 2012).

The full involvement of B-cells in the control of TB in humans is unknown, however B-cell depletion has been shown to retard the progression of mainly T-cell-mediated autoimmune diseases in humans and mice. These include type 1 diabetes and multiple sclerosis (Mariño et al., 2011; Meinl et al., 2011). A lack of cellular immune control is associated with an increased activation of humoral immune responses in *M.tb* infection in humans (Rahman et al., 2015).

B-cells are a subtype of lymphocytes. Naïve B-cells have not been presented with an antigen and can be divided into three groups: B-1 B-cells (which is further divided into B-1a and B-1b), follicular B-cells and marginal zone (MZ) B-cells. B-cells are divided based on their locations, migration ability and if their activation is T-dependant or T-independent (Allman and Pillai, 2008). B-cells execute many immunological functions and have been regarded mainly as protective regulators of immune responses. They produce cytokines, antibodies, and have the potential to present antigen (Mauri and Bosma, 2012). These important modulating functions affect the development of immune cells, including T-cells, macrophages, neutrophils and dendritic cells (Chan et al., 2014). B-1 cells produce low affinity antibodies and are functionally part of the innate immune response. B-1a cells are responsible for natural and autoantibody production, whereas B-1b cells produce adaptive immune responses to T-cell independent antigens (Yanaba et al., 2008). Follicular B-cells are mature B-cells that recirculate in the blood and, with the help of T-cells, can develop into antibody producing memory B-cells. They associate with follicular dendritic cells in the B-cell follicles of the lymph nodes and spleen. MZ B-cells produce antibodies responsible for protection against blood-borne pathogens, are found in the marginal zone of the spleen, and provide early protection during pathogen infections (Yanaba et al., 2008).

When naïve B-cells are activated by an *M.tb* antigen, they develop into activated plasma cells which have the ability to secrete TB-specific antibodies and produce cytokines (Rao et al., 2015). These antibodies regulate effector functions such as opsonisation of bacterial cells, antibody-dependant cellular cytotoxicity and neutralisation of secreted antigens, as seen in Fig. 2. B-cells are also effective antigen-presenting cells that respond to singular antigens or whole pathogens. These B-cells can then be presented to $CD4^+$ T-cells. As a result, B-cells contribute to the induction of $CD4^+$ T-cells responses to TB and provide early protection against infection (Abbas et al., 2014). B-cell antibodies can also promote antibody-mediated phagocytosis in which they

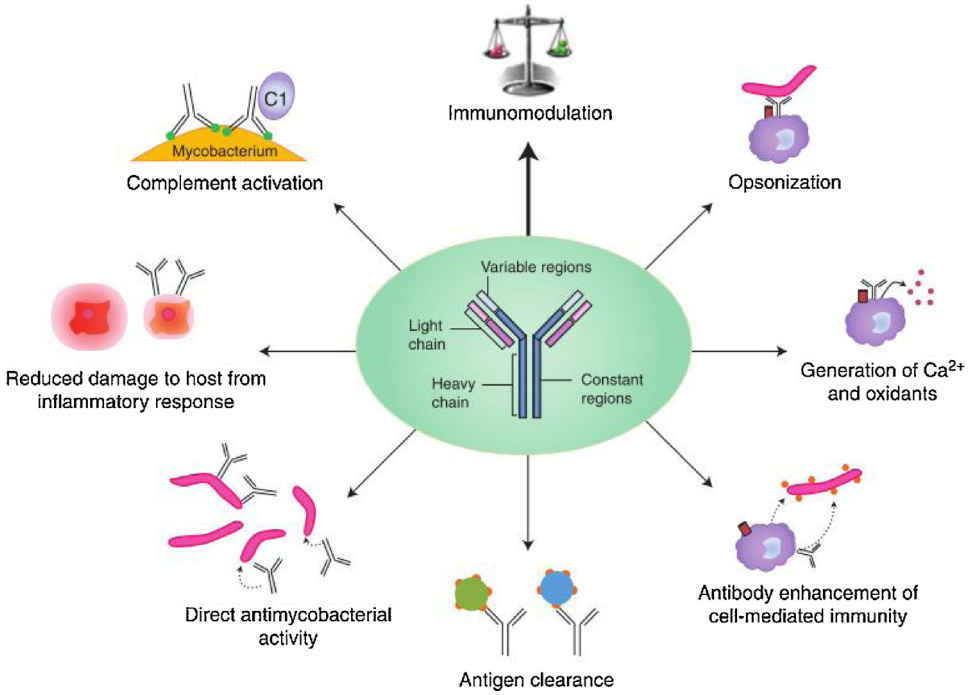

Fig. 2. A depiction of multiple mechanisms of antibody-mediated protection against TB.
Adapted from Chan et al. (2014).

modify macrophage behaviour (Phuah et al., 2012).

Granuloma-associated B-cells are present in clusters and keep in close proximity with CXCR5$^+$ T-cell subsets and TB-infected macrophages (Chan et al., 2014). There is a significant up-regulation of IL-10 and IL-21 in TB granulomas which may assist regulatory and humoral immune responses at the site of infection and these B-cells may be involved in this mediation (Rahman et al., 2015). In non-human primates, granuloma B-cells express CXCR5$^+$, may be positive for Ki-67 and have upregulated HLA-DR expression (Phuah et al., 2012). These activated B-cells, plasma cells and antibodies are found in high concentrations within the granuloma and have similar characteristics to those of germinal centres. Phuah et al. (2012) demonstrated that plasma cells, within the granuloma produced mycobacteria-specific antibodies and infected tissues, contained higher levels of TB-specific IgG. These findings illustrate that B-cells are present and actively secreting antibodies specific for *M.tb* antigens at the site of infection in non-human primates (Phuah et al., 2012). Additionally, B-cells can be antigen-presenting cells that can engulf antigens or whole *M.tb* bacilli and present them to T-cells in the granuloma (Zhu et al., 2010). Collectively, these findings illustrate that B-cells regulate host-pathogen interactions at the site of infection and there may be a significant benefit to further study these interactions in the granuloma as immune cells are recruited to and accumulate there.

3. Non-humoral B-cells and their regulatory cytokines

Inflammation is an effective immune response that is controlled by the release of cytokines and anti-inflammatory mediators (Rosser and Mauri, 2015). Cytokines are important regulators for the development of naïve CD4$^+$ T-cells once presented with antigen (Zhu et al., 2010). It is well known that CD4$^+$ T-cells play a vital role in determining B-cell responses, however the role of B-cells modulating T-cells is less well characterised.

B-cells have the ability to produce pro- and anti-inflammatory cytokines when stimulated whole organisms (such as *M.tb*) or Toll-like receptor (TLR) antigens, these include TNF-α, IL-10, IL-1β, IL-17 and IL-21 (du Plessis et al., 2016a, 2016b). This illustrates that B-cells respond to stimulations in a non-humoral manner, with the subset plasma (memory) B-cells (CD19$^+$CD27^{+-}CD138$^+$) contributing the highest production of cytokines. The IL-10 production is remarkably higher in plasma (memory) B-cells (CD19$^+$CD27^{+-}CD138$^+$) than in plasma B-cells (CD19$^+$CD138$^+$) and the greatest cytokine-production was attained by TLR4 and TLR9 based stimulations. BCG stimulation induced IL-1β production the strongest (du Plessis et al., 2016a, 2016b)

Du Plessis et al. (2016a, 2016b) also illustrated that plasma (memory) B-cells (CD19$^+$CD27^{+-}CD138$^+$) drive IL-21 and TNF-α production. IL-21, a major growth factor for naïve B-cells, is responsible for the differentiation of naïve and memory B-cells into plasma cells, and has the ability to induce the maturation of CD8$^+$ T-cells (Good et al., 2006). TNF-α has many functions and has a cytotoxic synergy with human interferon. TNF-α is necessary for the formation as well as the maintenance of granulomas. They may also effect chemokine production and extend the lifetime of plasmablasts (Cassese et al., 2003).

IL-21, a T-cell-derived cytokine, is notably higher in TB lesions than when compared to other tissues in the distal lung parenchyma. IL-21 is a B-cell stimulatory cytokine that controls the function of germinal centre B-cells and induces IgG-secreting plasma cells (Rahman et al., 2015). A supporting study demonstrated that IL-21R-deficient mice showed a significant inefficiency in IgG1 production after antigen priming. This illustrated the crucial role of IL-21 in plasma B-cell differentiation (Ozaki et al., 2002). Additionally, it was more recently observed that the in vivo production of IL-10 is dependent on the production of IL-21 (Yoshizaki et al., 2012). Thus, one may reason that IL-10 is mainly produced by regulatory B-cells (Bregs) cells found in TB lesions, despite the number of other cell types that could potentially produce IL-10 (Rahman et al., 2015). IL-21 may assist the increase of IgG-secreting B-cells and as a result, contributes to TB infection persistance. This could be due to the result of an impaired cellular immune response (Rahman et al., 2015). Furthermore, increased IL-21 levels promote the suppression of perforin expression in T-cells and promote viral replication in the spleen of mice infected with the Coxsackievirus (Xie et al., 2011).

Inflammatory effector B-cell subsets may enhance the development of Th1 responses by producing IL-12, IFN-γ and TNF-α. This results in

an immune response and an early control of the TB infection (Chan et al., 2014). On the other hand, anti-inflammatory B-cells have the ability to secrete IL-4, IL-33 and TGF-β, which disrupt the Th1 inflammatory response and reduce tissue damage (Zhang et al., 2012).

4. Regulatory B-cells

B-cells are broadly known to be positive regulators of the immune response as they produce antigen-specific antibodies. But, Breg cells have the ability to also negatively regulate the immune response by producing regulatory cytokines, as well as by interacting directly with T-cells (Yoshizaki et al., 2012). Shimamura et al. (1982) demonstrated that injecting C57BL/6 mice with sheep erythrocytes resulted in the production of suppressor B lymphocytes which subsequently induced antigen-specific suppressor T lymphocytes (Shimamura et al., 1982a, 1982b). Wolf et al. (1996) later showed that mice deficient in B-cells rarely recovered from experimental autoimmune encephalomyelitis (EAE), whereas wild-type (WT) mice did. This illustrated that B-cells have the immunomodulatory function of supressing the severity of the inflammatory disease (Wolf et al., 1996).

IL-10 producing B-cells- functionally named, B10, in humans and mice have an additional suppressive ability compared to other B-cells. IL-10 is an immune regulatory cytokine that allows Breg cells to inhibit tissue-specific inflammation (Mauri and Bosma, 2012). B10 cells therefor have the capability to suppress T-cell functions such as the differentiation of Th1 cells that produce IFN-γ and TNF-α, as well as Th17 cells that produce IL-17 (Flores-Borja et al., 2013). B10 cells have suppressive functions in healthy patients and stimulate the expansion of $FoxP3^+$ regulatory T-cells (Treg), whilst a loss of suppressive ability is observed, including the ability to maintain $FoxP3^+$ Treg cells, in patients with autoimmune disorders. This immune suppression is mediated by IL-10 (Flores-Borja et al., 2013). Additionally, CD19-deficient mice lack B10 regulatory cells, which leads to worsened disease symptoms and exacerbated inflammation during contact sensitivity, as well as in the EAE model for multiple sclerosis (Matsushita et al., 2008; Yanaba et al., 2008).

Patients infected with *M.tb* have higher levels of $CD19^+CD1d^+CD5^+$ Breg cells in the peripheral blood which supresses Th17 responses and inhibits the production of IL-22 (Zhang et al., 2014). However, $CD19^+CD1d^+CD5^+$ Breg cells are also found in peripheral blood of healthy patients but with a lower inhibitory activity. This suggests that this Breg subset plays a role in maintaining immune tolerance, like Tregs, in healthy patients (Zhang et al., 2012). Zhang et al. (2014) illustrated that individuals with cavitary TB had higher levels of $CD19^+CD1d^+CD5^+$ Breg cells compared to TB patients without cavity. However, because cavitary TB is a severe form of the disease, this may illustrate that $CD19^+CD1d^+CD5^+$ Breg cells impair protective immunity and result in a more severe disease (Zhang et al., 2014). Similarly, they further observed that an increase of TB antigen-specific IL-22 response during anti-TB treatment correlates with a decrease in $CD19^+CD1d^+CD5^+$ Breg cells (Zhang et al., 2014).

In another study, the number of circulating $CD19^+CD27^{hi}CD38^{hi}$ plasmablasts in the peripheral blood expressing cell-surface IgG was notably higher in individuals with active TB compared to the healthy individuals (Ashenafi et al., 2013). $CD19^+CD24^{hi}CD38^{hi}$ Breg subset has been reported to regulate inflammation and autoimmunity in humans and mice (Yanaba et al., 2008; Iwata et al., 2011). Similarly, Breg cells may enhance pulmonary infiltration of $FoxP3^+$ Treg cells which inhibit allergic inflammation in mice infected with helminth (Amu et al., 2010). However, Breg cells have also been discovered to inhibit macrophages from producing TNF-α (Iwata et al., 2011).

In contrast to the Iwata et al. (2011) who reported the suppressive ability of Bregs depends on IL-10, another study illustrated that IL-10 plays no role in the Breg inhibition of T-cells (Zhang et al., 2012). This was seen previously by Tretter et al. (2008), in which human $CD25^+$ Breg cells exhibited suppressive activity independent of IL-10 production. Zhang et al. (2012) also observed that co-culture of $CD4^+$ T-cells and $CD19^+$ B-cells caused a reduction in IL-10 production. Similarly, this was also observed in the murine model where B-cell-deficient mice had an increased production of IL-10 than the WT mice (Maglione et al., 2007). Zhang et al. (2012) further illustrated that B-cells can suppress the actvity of $CD4^+$ T-cells without the induction of Tregs. Additionally, effective antituberculosis treatment was observed to restore the Th1 response, reduce the amount of Breg cells in the peripheral blood and enhance the production of IL-22, which is in agreement with previous studies which report that IL-22 plays a role in preventing the replication of intracellular *M.tb* (Zeng et al., 2011; Zhang et al., 2014). This illustrates that low frequencies of Breg cells may be insufficient to prevent inflated inflammatory responses in individuals with autoimmune diseases and that high Breg cell frequencies may prevent antimicrobial effector responses necessary for TB control (Rao et al., 2015).

Fas ligand (FasL) expression was mainly reported on activated NK cells, T-cells, tumour cells and at sites of immune privilege, however studies have shown that Breg cells can also express FasL, as well as other death-inducing ligands (Hahne et al., 1996). Though the cytokine-based regulatory functions of B-cells have come into view, the expression of death-inducing ligands and their ability to control Th cell reactions by B-cells have been overlooked. FasL, similarly to IL-10, is highly expressed in the $CD5^+$ B-cell population, illustrating that these cells may have a specialised regulatory function (Yang et al., 2013). FasL is a member of the tumour necrosis factor protein family and binds to its receptor, Fas, which triggers apoptosis (Hahne et al., 1996). Apoptosis, or activation-induced cell death, is a major mechanism of immune regulation mediated by death-inducing ligands (Lundy, 2009). FasL expression can be induced under many circumstances which are summarised in Table 1 (below). In a recent study, van Rensburg et al. (2016, 2016b) showed that successful TB treatment induces B-cells with a regulatory phenotype, including the ability to express FasL. The study illustrates that there is an increased FasL and IL5RA expression from B-cells in individuals with TB compared to healthy controls. Similarly, the expression of FasL and IL5RA increases during TB treatment. These results suggest that higher FasL and IL5RA expression is associated with a healthy individual and can be an indication of regression from active TB to LTBI or health (van Rensburg et al., 2016a, 2016b).

Maglione et al. (2007) infected B-cell deficient mice with *M.tb* and found this led to aggravated lung granuloma formation, as well as increased T-cells and neutrophils when compared to WT mice. Remarkably, this increased imflammatory response was not as a result of the absence of IL-10 production, as IL-10 was upregulated in the infected B-cell deficient mice (Maglione et al., 2007). Another study proposed that B-cells produce IL-10 not at the local site of inflammation but rather in the spleen (Yanaba et al., 2008). Still, the expression of death ligands may be induced in B-cells after activation by *M.tb* (Kemp et al., 2004).

Additionally, emerging data suggest that B-cells participate in the control and prevention of many T-cell mediated diseases such as,

Table 1
Stimulators of FasL expression on B-cells, adapted from Lundy (2009).

Stimulators of FasL on B-cells	References
Schistosome egg antigen	Lundy and Boros (2002)
Lipopolysaccharide	Hahne et al. (1996)
Trypanosoma cruzi infection	Zuñiga et al. (2002)
HIV or SIV infection	Samuelsson et al. (1997)
MuLV infection	Rich et al. (2006)
Epstein-Barr virus infection	Tanner and Alfieri (1999)
Phorbol myristate acetate-ionomycin	Hahne et al. (1996)
Ligation of CD54 (Burkitt lymphoma)	Kim et al. (2007)
B7-H4 ligation (EBV lines)	Song et al. (2008)
Cockroach allergen	Lundy et al. (2005)
Toxic mistletoe lectin	Büssing et al. (1999)

autoimmunity and airway inflammation. Some of these studies suggest that the expression of FasL and cell death induced by B-cells could have a central role in inducing and maintaining tolerance. Minagawa et al. (2004) studied this role of FasL in transplantation mice models in which H-Y, a male histocompatibility antigen, containing male skin grafts were rejected by syngeneic female mice. Though, when the female mice were injected with male spleen cells prior to the skin graft, it was accepted. The study further illustrated with the use of WT and FasL-deficient, lupus-prone (*lpr*), female mice as well as, WT, *lpr*, and FasL-deficient, *gld*, male mice that H-Y tolerance is regulated by $FasL^+$ splenocytes (Minagawa et al., 2004). They then demonstrated that purified splenic $FasL^+$ B-cells were sufficient to induce tolerance and acceptance of skin grafts, suggesting that naïve, splenic B-cells in mice can induce T-cell tolerance. These results are similar to those found in the schistosome mouse model conducted by Lundy and Boros (2002).

5. B-cells during latent and active Tuberculosis

There is a limited understanding to the role of B-cells in TB infection as immunoglobulins derived from B-cells were not able to play a significant role in infections with intracellular pathogens (Achkar et al., 2015). However, recent studies, although conflicting, have shown otherwise. In individuals with active TB, the rate of B-cells were described as unchanged (Barcelos et al., 2006), increased (Wu et al., 2009), or decreased (Corominas et al., 2004) compared to those of healthy individuals. Additionally, individuals with LTBI were described to have lower B-cell rates when compared to healthy individuals (Corominas et al., 2004), whilst individuals post successful TB treatment were described to have higher rates of B-cells (Barcelos et al., 2006; Joosten et al., 2016).

In a murine model in which the mice were B-cell deficient and infected with *M.tb*, the *M.tb* was reported to be more virulent and the bacterial load was significantly higher than that of WT mice (Maglione et al., 2007). Additionally, the pulmonary granuloma formation of these mice were less severe and they had a delayed dissemination of bacteria. This illustrates that B-cells are necessary for the mediation of granuloma formation (Bosio et al., 2000; Maglione et al., 2007). Furthermore, these mice were then reconstituted with naïve B-cells and developed pulmonary granulomas and dissemination patterns similar to those of WT mice (Bosio et al., 2000).

In a B-cell-deficient cynomolgus macaque model of TB infection during the acute phase, no difference in the overall disease profession, pathology or clinical outcome was observed compared to WT models. However, when analysing the individual granulomas, B-cell depletion resulted in altered cytokine and T-cell responses, decreased levels of inflammation and an increased bacterial load (Phuah et al., 2016). Additionally, there were higher rates of T-cells producing IL-10, IL-17 and IL-2, whereas an overall lower rate of IL-10 and IL-6 in the granulomas. This could be attributed to the B-cell depletion (Phuah et al., 2016). These findings illustrate that B-cells are able to regulate the granuloma response to TB infection in macaques during acute infection.

During active TB disease, the production of several immuno-regulatory and inflammatory cytokines may affect the outcome of the disease at the site of infection. Th1 (IFN-γ and TNF-α) and Th17 (IL-17) cytokines control granuloma formation and stimulate the bactericidal functions of T-cells and macrophages, whilst anti-inflammatory (IL-10 and TGF-β) and Th2 (IL-4 and IL-13) cytokines neutralise over exaggerated Th1 responses and promote humoral responses (Rahman et al., 2015).

Du Plessis et al. (2016a, 2016b) discovered distinctive B-cell variations during active TB infection, other lung-based diseases, as well as after treatment. Memory B-cell ($CD19^+IgM^+CD138^+CD27^+$) frequencies were notably higher at diagnosis compared to post treatment (6 months) in both class switched and non-class switched phenotypes. Circulating MZ B-cells could differentiate between TB diagnosis and the end of treatment, as well as when compared to other-lung based diseases. These results warrant further research as potential biomarkers for TB treatment (du Plessis et al., 2016a, 2016b).

Interestingly, during active TB, a decreased expression of mRNA of B-cell associated genes was described and returned to normal again following treatment. Additionally, between the time of diagnosis and the completion of treatment, these genes were the most differentially regulated (Cliff et al., 2013). In another study, IL-13 mRNA expression was described 190–250 days prior to TB diagnosis in high risk individuals for TB infection (Sloot et al., 2015).

FASLG, IL-4, IL5RA, APRIL and CD38 expression were found to be at lower levels in individuals with TB compared to healthy controls. However, after 6 months, FASLG and IL5RA expression were increased in individuals with TB again. This illustrates that the activity of B-cells reduces during *M.tb* infection and is restored post successful TB treatment and suggessts B-cells play a protective role in the immune system against *M.tb* infection (van Rensburg et al., 2016a, 2016b).

Circulating B-cell frequencies are lower in individuals with active TB. Additionally, B-cells obtained from individuals with both active disease or LTBI are functionally impaired, yet found to be normal in individuals post successful TB treatment (Joosten et al., 2016). These impairments were more severe in individuals with active TB compared to those with LTBI. However, within the LTBI group, considerable variation was observed. This variation may be a reflection of the spectral nature of LTBI (Lawn and Zumla, 2011). These findings suggest that these impairments occur during active *M.tb* replication. Moreover, the B-cell depletion notably influenced the T-cell populations which supports the notion that B-cells are functionally involved in the activation of T-cells (Joosten et al., 2016). These findings cannot demonstrate whether inherent defects in B-cells allow for the progression from exposure to TB disease in the individuals or if the B-cell defect is attained as a result of *M.tb* infection. Overall, this data demonstrates that B-cells are functionally impaired during active TB which has significant consequences for the activation of T-cells and could affect the outcome of TB infection.

6. Conclusion

Although our knowledge of the role of B-cells and specifically Breg cells during *M.tb* infection and TB disease is still limited, studies have shown B-cells to play an active role in protection against *M.tb*. B-cell mediated immune responses against *M.tb* in mice and non-human primates suggests a critical role of B-cells in the early stages of infection. Additionally, the regulatory functions of B-cells and their association with T-cells may play a vital role in determining the outcome of TB infection in humans. Low levels of Breg cells may fail to prevent inflated inflammatory responses, whilst high levels prevent antimicrobial effector responses required to control infections. From recent studies, we deduce that FasL expression by B-cells is of significant value in viral, bacterial and parasitic infections, as well as in immune-mediated diseases. This expression by B-cells also plays a central role in the induction of T-cell tolerance, resulting in either a protective or pathogenic role depending on the disease under question. In conclusion, further characterising the role of B-cells, as well as regulatory B cells, during *M.tb* infection and disease can aid in the discovery of new approaches to the prevention and treatment of *M.tb* infection and TB disease.

7. Future applications

Lundy and Boros (2002) illustrated that splenic $CD5^+$ B-cells express FasL and produce IL-10 at higher frequencies than splenic $CD5^-$ B-cells. This, combined with studies illustrating increased T-cell activation in $CD5^+$ B-cell-deficient mice, may suggest that these B-cell populations can regulate T-cell activation (Lundy et al., 2005). It would be interesting to study these cells further as they could potentially be a target for producing cell-based, antigen-specific immunotherapy strategies by regulating T-cell mediated inflammation.

Additionally, van Rensburg et al. (2016, 2016b) demonstrated that IL5RA and FASLG were decreased in individuals with TB compared to healthy controls, however these genes were upregulated again 6 months' post treatment. This suggests that these genes may be used as a bio-signature to observe the outcome of the treatment.

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