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Review

B lymphocytes in anti-mycobacterial immune responses: Pathogenesis or protection?

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ABSTRACT

The role of B cells and antibodies in tuberculosis (TB) immunity, protection and pathogenesis remain contradictory. The presence of organized B cell follicles close to active TB lesions in the lung tissue raises the question about the role of these cells in local host-pathogen interactions. In this short review, we summarize the state of our knowledge concerning phenotypes of B cells populating tuberculous lungs, their secretory activity, interactions with other immune cells and possible involvement in protective vs. pathogenic TB immunity.

1. Introduction

Tuberculosis (TB) remains a major global health problem, and drug-resistant TB is a continuing threat. In 2016, there were 600,000 new TB cases, representing different types of antibiotic resistance, including MDR- and XDR-TB, and 1.3 million HIV-negative and 0.37 million HIV-positive TB patients died [1].

$CD4^+$ T lymphocytes, recognizing antigens of *M. tuberculosis* in the context of the MHC Class II molecules on the surface of infected macrophages and dendritic cells and activating these cells by IFN-γ, are thought to offer the most prominent protective mechanism of adaptive immunity against *M. tuberculosis* infection (reviewed in Refs. [2,3]). Although the dogma of a protective role of $CD4^+$ T cell-derived IFN-γ in tuberculous lung tissue was recently seriously challenged [4], detailed analysis of slowly developing experimental TB in mice confirmed that this pathway really protects the host, at least when lung granulomata are formed rapidly enough and massive consolidated pneumonia is not developed [5]. These recent observations in the mouse TB model are in line with the concept of the protective role of early granuloma formation proposed on the basis of the results obtained in guinea pigs [6] and macaques [7].

Formation of lung granulomata is the hallmark of TB pathogenesis, and this process was characterized in considerable detail [8,9]. The central area of a TB granuloma contains mycobacteria-infected macrophages and is surrounded by layers and conglomerates of various immune cells, including B lymphocytes [10–13]. The role of B cells and antibodies in TB immunity, protection and pathogenesis is not completely defined. Meanwhile, B lymphocytes actively migrate to TB-infected lung tissue and, in the vicinity of granulomata, form structures resembling B cell follicles (BCF) of secondary lymphoid organs (SLO), which are often called tertiary lymphoid organs (TLO) or ectopic lymphoid follicles (ELF). Formation of such follicles was documented in TB patients [14,15], infected mice [14,16,17] and non-human primates (NHP) [18]. The presence of organized BCF close to active TB lesions raises the question about the role of these cells in local host-pathogen interactions. As multifunctional players in immune responses, B cells produce antibodies, different cytokines and chemokines, and serve as the professional antigen presenting cells (APC), thus modulating the activity of other immune cells in many different ways [19]. Although the role of B cells and humoral immunity in TB infection was reviewed a few years ago [20–22], new data accumulate rapidly making it worth of summarizing the state of our knowledge again. Our intention is to provide the reader with some comparative and intra-species aspects of the role of B lymphocytes in TB (see Fig. 1).

2. General role of B cells in TB immunity

The data concerning changes in B cell counts during clinical TB look controversial. In some studies, significantly lower B cell numbers in the peripheral blood of patients with active pulmonary TB [23–26] or latent infection (LTBI) [26] compared to healthy donors were reported. However, other studies demonstrated the lack of differences [26,27], or even increased B cell content, in the blood of TB patients [28]. Most possibly, these contradictory results are due to the differences between

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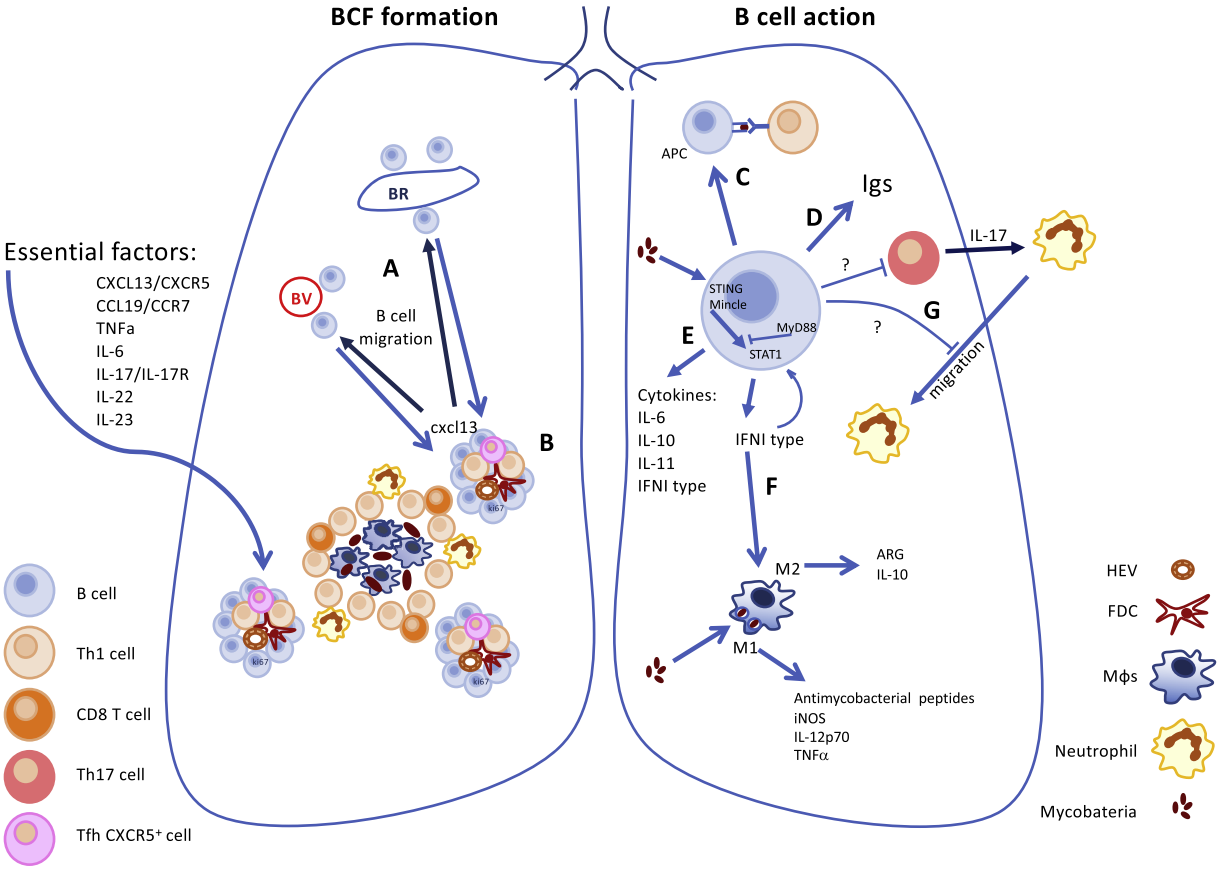

Fig. 1. B cell follicle formation and action of B cells in the lungs during TB. During mycobacterial infection, B cells appear near the walls of blood vessels and bronchus (A) and then migrate to the interstitial area forming follicles in close vicinity of developing granuloma (B). Lung B cell follicles contain all prominent components of SLO, such as CXCR5$^+$ follicular Th cells, follicular dendritic cells, germinal centers, high endothelial venules and proliferating B cells (ki67$^+$). B cells from infected lungs present mycobacterial antigens to CD4$^+$ Th cells (C); produce almost all classes of Igs, except IgE, specific and nonspecific to mycobacterial antigens (D). It was also shown that type 1 IFN are the major cytokines produced by lung B cells during TB. They are capable to polarize lung macrophages toward anti-inflammatory M2 phenotype (E). Lung B cells influence neutrophil migration via regulation of the Th17/IL-17 response and, perhaps, some additional mechanisms independent of T cells early during TB infection (F).

groups of patients enrolled (form and stage of the disease, ethnicity, age, etc.).

The role of B cells has been studied in different experimental TB models, and the results again look contradictory. Thus, in the aerosol challenge model it was shown that B cell-deficient mice develop more severe disease compare to control animals, characterized by a pronounced lung neutrophil inflammation at the early stage of infection, irregular shape of granulomata and shorter life span [11]. However, in experiments with two congenic mouse strains, the wild type CBA and B cell-deficient CBA-xid, no to marginal differences in susceptibility to infection induced by virulent *M. tuberculosis* strains H37Rv [29] and Erdman [30] were found.

It is impossible to judge whether the shifts in B cell counts are the cause or the consequence of TB infection, and even observations made after direct B cell depletion are inconclusive. Thus, in TB-infected macaques, B cell depletion by administration of rituximab resulted in an increased mycobacterial burden and an altered cytokine production in some, but not other, individual granuloma [31]. B cell deficiency in rheumatoid arthritis patients treated by rituximab did not increase the risk of TB reactivation [32,33]. Most likely, B cell depletion at different phases of the infectious process leads to different physiological consequences, and B cells play different roles during the initial and advanced phases of infection.

It should be emphasized that both clinical observations and animal studies suffer from serious limitations. The vast majority of studies in TB patients utilized the circulating population of B cells isolated from peripheral blood. The numbers, phenotypes and secretory activity of these cells may differ profoundly from B cells from inflamed TB-affected tissues (lung, in the first instance). With this regard, studies utilizing BAL and pleural cavity B cells are clearly more relevant, but in these settings the issue of adequate control groups is a serious concern, since the corresponding extraction procedures are, certainly, not applicable to LTBI groups and rarely performed in non-TB clinics. Obviously, even less informative are the studies in which the results obtained *in vitro* were not validated in ex vivo/*in vivo* settings.

TB animal models possess their own limitations. Thus, gene knockout studies are based upon phenotypes resulted from complete elimination of a gene function. Mutations leading to complete dysfunctions of proteins playing important roles in host protection cause aberrations in protective mechanisms that are extreme. Such cases are very useful for the analyses of immune and biochemical mechanisms, but in general populations they are rapidly eliminated by natural selection and are not a common cause of susceptibility, except rare cases of Mendelian susceptibility to mycobacterial diseases (MSMD). In addition, the effect on TB may be mediated not by the targeted molecule, but indirectly, through other member(s) of the pathway. Adoptive transfers of cells, sera or antibodies are artifactual, regarding the concentrations of molecules and sizes of cell populations, thus, do not mimic physiological conditions. Finally, the vast majority of TB studies in mice were performed in the relatively resistant C57BL/6 strain, whose features of TB pathogenesis differ profoundly from clinical variants of the disease. As discussed previously, usage of genetically susceptible animals and

refined models of infection provides much more reliable and relevant results [34,35].

3. Phenotypes of B cells involved in TB response

The general B cell population consists of different cell types, e.g., B1 and B2 lymphocytes, which play different roles in immunity [36]. In a few articles, the phenotype of B cells infiltrating lungs during TB was analyzed. Our study of TB infection in highly susceptible mice of the I/St strain [37] showed that about 85% of lung B cells express the $CD19^{+}B220^{hi}IgM^{lo}IgD^{hi}$ phenotype characteristic for conventional B2 population. However, lung B2 cells, unlike their counterparts in lymphoid organs, did not express the CD23 marker, a low-affinity Fc-receptor for IgE. Although explanation why lung B2 cells do not express CD23 receptor is lacking, it is tempting to speculate that in the lung tissue expression of the molecules potentially involved in allergy-linked pathways should be down-regulated. About 15% of lung B cells expressed the $CD19^{+}B220^{lo}IgM^{hi}IgD^{lo}$ phenotype typical for $CD5^{+}$ and $CD5^{-}$ B1 subsets, and the B1/B2 ratio in the lungs remained stable throughout the infection course. On the other hand, the ratio of B cell populations in pleural fluid (PF) changed along TB progression. The B1 subset was predominant in intact animals, but TB infection was accompanied by the growth of $CD23^{-/lo}$ B2-like population in pleural cavity [37].

Studies in TB patients describe diverse circulating B cell populations, subjected to alterations during the disease. In a big study describing blood B cell populations in patients with active and latent TB infection it was shown that, compared to healthy donors, populations of atypical $IgD^{-}CD21^{-}CD27^{-}$ and activated $IgD^{-}CD27^{+}$ B cells were increased, whereas the population of naïve $IgD^{+}CD27^{-}$ B cells was decreased in both groups of patients. In addition, atypical B cells were poorly proliferative, displayed defective total immunoglobulin production and produced low amounts of IL-6 and IL-10 [25]. These results align with those obtained by comparing blood B cell populations in patients with active TB and non-TB lung diseases. It was shown that the numbers of non-class switched marginal zone, mature B cells and memory $IgD^{+}CD27^{+}$ cells were decreased in TB patients [38], suggesting that mycobacterial infection somehow suppresses and/or exhausts B cell functions, similarly to what was observed in HIV-positive individuals [39].

Increased numbers of $CD19^{+}CD1d^{+}CD5^{+}$ B cells, capable to inhibit Th17, but not Th1, activity were reported in TB patients [40]. More recently, the population of activated B cells expressing the FAS ligand molecule was assessed in TB patients, and it was demonstrated that the size of this population increases after successful anti-TB treatment [41]. In addition significantly lower amounts of $FASL^{+}IL5RA^{+}$ B cells were present in pleural cavity of LTBI compare to active TB patients [42]. Comparison of MDR-TB patients with healthy donors showed that the MDR-TB patients display much lower frequencies of non-switched $IgD^{+}CD27^{+}$ memory B cells in the peripheral blood, reduction of the circulating plasma cell pool and an increased frequency of circulating type 1 transitional $IgD^{+}CD38^{++}$, $CD69^{+}$ and $TLR9^{+}$ B cells [43].

4. B lymphocytes interactions with other immune cells in TB

Macrophages play dual role in TB pathogenesis and protection. On the one hand, macrophages are key cells that directly kill mycobacteria, on the other hand, macrophage phagosomes is the major niche for mycobacterial multiplication, at least at the early stage of infection [44]. Given that the paramount condition of the effective immune response against mycobacteria is the balance between effector functions of immune cells and capacity to control overwhelming inflammation and lung tissue damage [45], it is reasonable to take a look at the lung macrophages from the side of inflammation development.

It is well established that macrophages undergo polarization toward pro- or anti-inflammatory functioning (M1 and M2 populations, respectively), depending on molecular signals which they receive from the environment [46], and that different types of macrophages influence TB pathology in different ways [47,48]. Conditions and signals influencing macrophage polarization are described in detail in many reviews [49–51], so here we will discuss only polarization under B cell control.

It was reported that peritoneal B1 cells shift macrophage polarization toward M2 variant *in vitro*, leading to an up-regulated expression of the genes for anti-inflammatory cytokines. Stimulation of macrophages obtained from B1-enriched VH12 transgenic mice resulted in a significantly higher expression of mRNA for TNFα, IL-1b and CCL3, but lower for IL-10, compare to the wild-type controls [52]. It was shown also that B1 cells drive the fusion of peritoneal macrophages *in vivo* [53]. This latter observation provides a suggestive explanation of macrophage behavior in the TB-infected lung. During TB infection, lung macrophages are able to fuse and form giant multinuclear cells, as well as to transit to the so-called foamy macrophages, containing high amounts of lipid bodies and presumably playing a central role in mycobacterial persistence and reactivation [54,55]. An indirect evidence for the existence of such relations between B1 cells and macrophages was provided by the observation that after infection with *M. tuberculosis* significantly higher amounts of foamy macrophages are recovered from lungs of the wild type CBA compared to the B1 cell-deficient CBA-xid mice [30]. However, much more experimentation is needed for reliable conclusions.

A recent work by Bénard and co-authors (2017) shed more light on the question of B cell participation in macrophage polarization. It was shown that upon mycobacterial stimulation both murine and human B cells produce type I interferons that modulate macrophage polarization. The fact that these molecules polarized macrophages along the M2 anti-inflammatory axis *in vitro* was confirmed by the *in vivo* experiments with mixed bone marrow chimeric mice bearing Myd88-deficient B cells overexpressing type I interferons and displaying increased numbers of anti-inflammatory macrophages in tuberculous lungs [56]. In line with this experimental evidence, production of type I interferons by B cells obtained from pleural cavities of TB patients was dramatically increased compared to B cells from the peripheral blood of TB patients or healthy donors. The authors concluded that type I interferons produced by B cells play an important local role at the sites of infection and inflammation (lung, pleural cavity) where they closely contact other immune cells [56].

A few investigations were published demonstrating an increased neutrophil influx to the sites of infection under conditions of B cell deficiency. As mentioned above, this was shown for TB-infected lungs of B-/- mice [11]. Another evidence of B cell influence onto neutrophil migration was obtained in our experiments demonstrating that B cells delay neutrophil migration towards the site of BCG injection which has serious consequences regarding the vaccination efficacy [57]. Later, Kozakiewicz and colleagues in a mouse model demonstrated that the influence of B cells on neutrophil migration is released via regulation of the Th17/IL-17 response and, perhaps, some additional mechanisms [58]. These results resonate with observations made by Zhang and colleagues in TB patients, describing an increase in the blood B cell population capable to suppress Th17 response *in vitro* in an IL-10-independent but contact-dependent manner [40].

General physiological and molecular aspects of interactions between antigen presenting B cells and $CD4^{+}$ T cells were characterized in considerable detail (reviewed in Ref. [59]). Surprisingly, our search for papers describing the B cell antigen presenting function in TB revealed only two works. Using B-cell deficient mice, it was shown that effective generation of memory cell precursors specific to *M. tuberculosis* antigens required presentation of these antigens to $CD4^{+}$ T-cells exactly by B cells [60]. In our study, it was directly shown that the MHC II expression on the surface of lung B-cells increases during TB infection and their capacity to present mycobacterial antigens to immune $CD4^{+}$ T-cells is similar to that of splenic B cells [37].

Due to obvious reasons, studies simultaneously analyzing B cells in blood, lymphoid organs, pleural fluid and lungs of the same TB patient are lacking, so we can only guess how B cell populations at different anatomic locations release their diverse roles in human TB.

5. B cells in tuberculous lung: follicles, cytokines, chemokines

Formation of B cell follicles is a very common feature of inflammation. Besides infectious diseases caused by viruses (influenza virus [61], murine gamma-herpes virus [62], modified vaccinia virus Ankara [63]), bacteria (*Pseudomonas aeruginosa* [64]), mycobacteria (see below) and fungi (*Pneumocystis* lung infection [65]), their appearance was described for nonspecific interstitial pneumonia [66], autoimmune conditions and tumors [67,68]. Lung B cell follicles contain all prominent components of SLO, such as $CXCR5^+$ follicular Th cells, follicular dendritic cells, germinal centers and high endothelial venules [10,11,15]. For tuberculous lung inflammation, formation of B cell follicles was reported in TB patients and experimental animals [12,13,15,16,69].

Attempts to dissect molecular mechanisms that are involved in BCF formation during TB infection resulted in several important findings. Thus, it was demonstrated that mice with KO mutation in the *cxcr5* gene have increased susceptibility to TB due to abnormal T cell localization within lung parenchyma and that the presence of $CXCR5^+$ Tfh cells is mandatory for BCF formation [15]. Similar experimental approaches based upon knocking out genes for different cytokines and chemokines, combined with TB challenge, revealed the involvement in lung B-follicles formation of CXCL-13 and CCR7, mediating CCL19 and CCL21 signaling [10,70], IL-6 [10,15,67], IL-17 [71], IL-22 [68,69], IL-17R and IL-23 [72]. Using mice with conditionally knocked out *tnfa* gene in B cells, we demonstrated that the lack of TNF-α production by B lymphocytes themselves abrogates formation of BCFs in the lungs of infected mice but does not influence severity of TB infection [73].

Data concerning B cell cytokine activity during various intracellular infections are rapidly accumulating (reviewed in Refs. [74–76]), and it is likely that the B cell-derived cytokines are prominent factors of TB inflammation and pathology. Nevertheless, the role of B cell-secreted cytokines in TB immunity is incompletely defined. There is evidence that lung B cells participate in regulation of anti-inflammatory IL-10, since B cell-deficient infected CBA-xid mice display elevated IL-10 levels in the lung, but possible mechanisms have not been addressed [30]. B cell depletion in the NHP TB model did not change IL-17 and TNF-α production, but resulted in a decrease of IL-6 and IL-10 levels, suggesting their synthesis by B cells [31]. We demonstrated that lung B cells from infected TB-susceptible mice are prominent producers of pro-inflammatory IL-6 and IL-11, but do not secrete classical type I (TNF-a, IFN-γ), or anti-inflammatory type II (IL-10, TGF-b) cytokines [37]. However, atypical B cells with decreased levels of intracellular IL-6 were recovered from blood of TB patients [25]. An important observation was recently reported by Bénard A and colleagues (2017). They demonstrated that during TB infection type I interferons are key cytokines produced by lung B cells in mice and pleural B cells in patients. The authors emphasized that type I interferons are capable to polarize macrophages along the anti-inflammatory M2 axis, but their deleterious role in TB progression and inhibitory influence onto IFN-γ production [77] look like more important pathogenic factors.

Observations described above suggest that during mycobacterial infections formation of BCF may be considered rather as an additional factor of pathogenesis than a part of protective immunity. Similarly, addressing the question whether an impaired BCF formation influences anti-TB immune responses, Slight and colleagues argued that follicle formation is just a consequence of "correct" T cell localization within the lung but by itself may not be necessary for bacterial control [15]. Yet another argument supporting this concept is provided by our studies in mice infected with *Mycobacterium avium*. Since the key genetic regulator of this infection is *Nramp1* gene, mice of B6 (*Nramp1*^{s}) and I/St (*Nramp1*^{r}) strains display mirror-type picture of genetic susceptibility to *M. avium* and *M. tuberculosis*: B6 are susceptible to *M. avium* but resistant to TB, whereas the opposite is true for I/St mice [78]. We demonstrated that exactly susceptible mice are prone for BCF formation and that the numbers of lung BCFs in F2 hybrids co-segregate with the lung mycobacterial burden after infection with these two mycobacterial species [17,37,78]. Another example of deleterious rather than protective role of BCF formation is autoimmune rheumatoid arthritis, the disease associated with the local T-cell priming, autoantibody production, and correlation of B cell follicles formation with its severity [67].

On the other hand, it has been shown that during TB follicular $CXCR5^+$ Tfh cells accumulated within B cell follicles and produced pro-inflammatory cytokines that activated macrophages for optimal control of mycobacteria [15], whereas disorganized lymphoid aggregates with irregular $CXCR5^+$ T cell localization were associated with poor protection [14,15]. In the NHP TB model, formation of organized ectopic lymphoid tissue containing $CXCR5^+$ T cells within granulomata was associated with better protective outcomes, and in TB patients lung B cell aggregates were associated with containment of infection [71]. In a cancer mouse model, formation of B cell follicles in the lung positively correlated with survival time, suggesting protective role of these structures [67].

There is evidence that B cell follicles in tuberculous lung are tightly associated with well-organized granulomata and T-cell distribution [15], and our recent findings provide some additional supportive information. Not only we observed disappearance of B cell follicles in susceptible mice along progression of lung pathology [37], recently we found that confluence of granulomata and development of destructive pneumonia is accompanied by a decrease in lung B cell content [5]. General impression is that interaction of B cells with other immune cells in tuberculous lung occurs predominantly within highly organized follicles, where B cells proliferate themselves, serve as APC for proliferating $CD4^+$ T-cells and produce inflammatory cytokines and immunoglobulins [18,35]. Ineffective host control of TB progression results in disintegration of B cell follicles [25], and B cell deficiency leads to alterations in TB granuloma structure [11]. There is little doubt that structural organization of lung B cells is very important for the TB pathogenesis and immune response; however, exact mechanisms of their actions still remain to be defined.

An important cytokine-producing B cell subtype is often referred to as "regulatory B cells" (Breg), whose definition, however, continues to be somewhat vague. Initial description of B cells with immunosuppressive functions operating during autoimmune conditions emphasized IL-10 production by these cells as their key characteristic [79–81]. Further studies demonstrated their capacity to support persistence of Treg cells and introduced the term "Breg cells" [80,81]. Since neither regulatory cytokine profiling [73,82-85], nor linage-specific phenotype description [86] and search for Breg specific transcriptional factor(s) [87] were successful yet [73,86], for today IL-10 production remains the only reliable characteristic of Breg cells [88]. Consequently, readers of the literature are compelled to deal with a peculiar mixture of works in which regulatory functions are either ascribed to a certain population of B cells, but the term "Breg" is omitted, or to the "Breg population" which is not phenotypically defined. Nevertheless, a few examples of "Breg" participation in TB control can be provided. Thus, Zhang and colleges have shown that in humans Breg cells suppress *M. tuberculosis* antigen-specific IL-22 production by peripheral blood mononuclear cells, which returns to normal after successful treatment [89]. A minor subset of B cells that express cell death-inducing ligand FasL was described as the FasL-positive regulatory (or killer) B cell population, which putatively is involved in TB control since these cells were induced during anti-TB treatment [90]. Subsequent study [42] demonstrated that both FASL/IL5RA-positive and CD24/CD38-positive B cells with regulatory functions are present during latent and active TB, and that the size of the FASL/$IL5RA^+$ population is larger in LTBI patients and further increases after BCG re-

stimulation. However, direct evidence for involvement of these cells in protection are lacking.

6. Antibodies and tuberculosis infection

Antibodies (Ab) are prominent players in host interactions with various intracellular pathogens (reviewed in Ref. [92]); however, their role in TB continues to be controversial for many decades [91–94]. For the purposes of this review, it is convenient to split the studies in two general categories: (i) Ab produced by mycobacteria-infected organism itself, and (ii) passive Ab immunization against TB.

6.1. Internal Ab production in TB

During the last decade, when attention to B cell follicles in tuberculous lung have re-emerged the interest to the role of B cells in TB, a few experimental works were published concerning Ab production in infected animals. We have shown that lung B cells of TB-infected I/St mice produced Ab of all isotypes, except IgE. Interestingly, these lung B cell-originated Ab, unlike serum Ab, showed very weak reactivity with mycobacterial antigens, which underlines principal difference between tissue and circulating B cells. From a panel of monoclonal Ab obtained from lung B cells, only few clones demonstrated specificity to mycobacteria or lung tissue extract, while specificity of the vast majority of clones remained unidentified [37]. In another recent study in mice, it was demonstrated that the $CD23^{-}CD43^{+}$ B1 cells from peritoneal cavity produced high amounts of IgM after *in vitro* stimulation with total lipids from *M. tuberculosis* H37Rv or the whole live *M. bovis* BCG [95]. In a macaque TB model, it was shown that infected animals produce cultural filtrate protein-specific IgG in the lung tissue [18]. In another NHP study, it was shown that low levels of Ab response during early stage of infection were associated with TB reactivation in the latently infected animals, suggesting that stress-induced reactivation occurred exactly in the animals that failed to rapidly develop Ab against mycobacteria [96].

Protective role of antibodies against mycobacterial antigens was demonstrated both in experimental systems and in TB patients. Thus, the AID(−/−)μS(−/−) mice, in which secreted Ab are totally lacking, controlled mycobacterial growth less effectively and died earlier compared to control B6 animals [77]. Immune serum from *M. tuberculosis*-infected animals improved anti-TB response in the lungs of B cell-deficient mice, also suggesting a protective effect of Ab, although the authors avoided definite conclusions because of peculiarities in the composition of B cell population in AID(−/−)μS(−/−) mice [53]. The role of secreted IgA, the main isotype present in mucosal secrete, was demonstrated using vaccination approach [96]. Intra-nasal immunization with the mycobacterial antigen PstS-1 induced strong local IgA response and protection in the wild-type mice, whereas polymeric IgR knockout mice (pIgR−/−), which do not secrete IgA, were more permissive for mycobacterial growth in organs and demonstrated decreased production of pro-inflammatory IFN-γ and TNF-α in the lungs at the early phase of the disease [97].

Regarding the importance of general Ab response in human TB, the first body of indirect evidence was provided by studies in infants. In early studies, it was observed that in the cohort of children aged from 6 to 36 months those were at the highest risk to develop disseminated TB who did not produce Ab against LAM [98]. Similar observation has been reported for Ab against mycobacterial 38-kDa antigen [99]. Later, it was shown that the most vulnerable age of infants is 2–12 months when the level of maternal Ab begins to descend [100,101]. Recently, association of high titers of IgG against Ag85A with a reduced risk of developing active disease was demonstrated in an infant case-control study [102]. The latest study in infants (sub-study of MVA85A vaccine trial) showed that the BCG-vaccinated children with negative QuantiFERON-TB Gold In-tube test results (QFT-) displayed a significant increase in total IgG titers when re-tested, compared to the IgG titers at the baseline, which was not observed in QFT^{+} infants [103].

Protective Ab effect in adults was recently studied by comparing healthcare workers with patients suffering active disease in a TB hospital in Beijing. Irrespective to mycobacteria-reactive Ab titers, only Ab from healthy or latently infected donors provided protection, as measured by the CFU counts *in vitro* in the whole blood assay and in the mouse TB model *in vivo*, in which Ab were injected a few hours prior to aerosol challenge [104]. The operating mechanism, however, remains obscure since in the human system mycobacteria-non-specific, IFN-γ-negative $CD4^{+}$ T cells were the crucial component of effective antibacterial response *in vitro*. Profiling of plasma-derived IgG from latently and actively infected patients revealed that Ab isolated from LTBI individuals display a specific profile of Fc-fragment's glycosylation and bind FcRγIIIa with significantly higher affinity. It was shown that Ab from LTBI patents activated inflammasomes, promoted phagolysosome maturation and reduced bacterial survival within macrophages *in vitro* [105].

An interesting aspect of antibody response to mycobacteria is the spectrum of antigens recognized. One study describes the analysis of sera from patients with diagnosis of active TB and LTBI using mycobacteria proteome-vide high-throughput microarray assay [106]. It has been demonstrated that in the active TB antibody response was focused on an approximately 0.5% of the proteome enriched for a small number (13) secreted extracellular proteins, and that the level of response directly correlated with bacillary burden. In LTBI, Ab response was focused on membrane-associated mycobacterial antigens [106]. Another microarray study from the same research team compared reactivity of sera from patients with different smear bacillary counts and from infected macaques displaying different disease stages (active disease, latent infection, reactivation disease). The level of Ab response correlated with the activity of the disease: active and reactivated diseases were accompanied with increased Ab levels, whereas in latently infected animals Ab levels peaked rapidly after infection and then gradually declined. In addition, the level of anti-mycobacterial Ab in humans suggestively differentiated between smear-positive (high), smear-negative (intermediate), and non-tuberculosis (low) patients [96].

Taken together, these observations convincingly demonstrate that Abs participate in immune protection against TB, but there is a huge gap in our knowledge concerning molecular mechanisms of their activity.

6.2. Passive immunization

Protective effect of passively injected mAb specific to mycobacterial antigens was demonstrated in a few studies. Thus, administration of mAb against mycobacterial arabinomannan or lipoarabinomannan, major structural carbohydrate antigens of mycobacterial surface, localized the pathogen within lung granuloma centers of infected mice, suggesting that mAb conferred protection by enhancing cellular immune response [107]. Analogously, some protective effect in murine studies was demonstrated for anti-α-crystalline mAb [108,109]. In mice challenged with mycobacteria coated with mAb to heparin-binding haemagglutinin adhesin (HBHA) via intranasal route, dissemination to lymphoid organs was decreased compare to the control group [110]. Injection of mAb directed against lipoarabinomannan prior to or together with intravenous challenge with *M. tuberculosis* strain Harlingen diminished bacterial loads in lungs and spleens, reduced body weight loss and increased survival [111].

Additional evidence of Ab impact on TB immunity was obtained in studies using polyclonal antisera. For example, passive administration of hyper-immune sera from immune competent animals, either infected or immunized with mycobacterial extracts, into SCID mice partly protected them from TB reactivation after withdrawal of INH + RIF chemotherapy, decreasing bacterial burden in the lungs and reducing granuloma size [112]. Administration of immune sera from infected animals to μMT mice lacking B cells prevented high neutrophil influx

into lungs after aerosol *M. tuberculosis* challenge [58]. It was also demonstrated that intravenous treatment of *M. tuberculosis*-infected mice at the early or late stages of infection with high doses of immunoglobulins from healthy donors resulted in substantially reduced bacterial loads in lungs and spleens. The effect was lacking in the *nude* recipients, suggesting the involvement of T cells [113].

6.3. Fc-receptors in TB immune response

The role of Ab receptors in anti-mycobacterial response was firstly demonstrated in a few *in vitro* studies. In the early work, it was shown that co-cultivation of bacteria with rabbit antisera promotes Fc-associated phagocytosis leading to successful phagosome-lysosome fusion, which, in turn, reduced bacterial viability [114]. Later, it was shown that mycobacterial phagocytosis by human macrophages via complement receptor binding leads to decreased levels of cytoplasmic Ca^{2+}. This seriously influences several antimicrobial responses, including generation of reactive oxygen and nitrogen intermediates, secretion of microbicidal proteins and peptides, and synthesis of inflammatory cytokines, e.g., TNF-α [115,116]. Phagocytosis of Ab-opsonized mycobacteria mediated by FcγR reversed the impairment of macrophage Ca^{2+} signaling induced by mycobacteria and reduced their intracellular survival [117].

More recently, activation and inhibition roles played by FcR-γ in TB immunity was characterized in greater detail [21]. B6-(RIIB$-/-$) mice lacking inhibitory receptor FcγRIIB controlled mycobacterial growth better than the wild-type animals and developed milder lung pathology. These mice displayed stronger Th1 response, higher amounts of lung $CD4^+$ IFN-γ-producing T cells and higher levels of APC activation and production of the p40 component of the Th1-promoting cytokine IL-12. In contrast, mice lacking the γ-chain mutual for activation-involved FcγR displayed an exacerbated TB immunopathology, elevated mycobacterial burdens in organs and produced more immunosuppressive IL-10. Similarly, transcription profiling of blood cells from HIV-TB co-infected patients revealed significantly higher expression of activating FcRγIa in patients with active TB compared to those with LTBI irrespective to their HIV status [118]. It was suggested that activating and inhibiting FcR-γ have reciprocal functions during TB infection and a precise balance between two signals is required for optimum immune response [119].

7. Conclusion remarks

The summary of recently obtained results concerning B cell functioning during TB infection in the lungs of patients and infected laboratory animals clearly shows that there are still serious gaps in our understanding of the role of B cell response in TB. Many questions await to be addressed in experimental and clinical settings, and here we formulate a few. Given B cell-neutrophil interactions described above, does the location of B cell follicles around active TB granulomata regulate neutrophil influx/efflux at the sites of granuloma formation? Do $CD4^+$ T cells proliferating within B cell follicles belong to the Th17 subset, and what is the role of follicular B cells in balancing IL-10/IL-17/IL-6 responses in tuberculous lungs? What is the nature of a signal inhibiting expression of the CD23 receptor on lung B2 cells, and is the $CD23^-$ phenotype TB-specific or more general? Is the type 1 interferon production by lung B cells STING activation-dependent, and what is its contribution to mycobacteria-advantageous elevation of cAMP levels in the vicinity of sites where mycobacterial multiplication occurs? How different B cell subsets migrate to and redistribute in TB-affected lung? These, as well as numerous other problems, should be the subject of further research within the frames of an exciting problem “B cells in TB”.

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