 Emerging trends in the formation and function of tuberculosis granulomas

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INTRODUCTION

"On the basis of my numerous observations I consider it established that, in all tuberculous affections of man and animals, there occur constantly those bacilli which I have designated tubercle bacilli and which are distinguishable from all other microorganisms by characteristic properties."

With those celebrated words in 1882, Koch announced the discovery of the etiological agent of one of the oldest recorded human afflictions (Koch, 1882). The term “tubercle” refers to an original description by Sylvius (in 1650) of the apparent lung nodules characteristic of the “consumption” disease, which became christened as “tuberculosis (TB)” by Schonlein (in 1839) in recognition of its intricate correlation with these structures (Sakula, 1982).

Today, these tubercles are known as granulomas, defined as organized immune cell aggregates that form in response to persistent TB infection (Ramakrishnan, 2012). The cellular composition of TB granulomas includes Mφs, neutrophils, monocytes, dendritic cells, B- and T-cells, fibroblasts, and epithelial cells (Russell, 2007; Ramakrishnan, 2012). Moreover, TB granulomas are characterized by a high-turnover rate of their Mφ population and by specialized differentiations taking place in mature Mφs such as tightly interdigitated cell membranes that make Mφs appear either epithelial (Adams, 1974), fusion into multinucleated giant cells (Helming and Gordon, 2007), or differentiation into foamy cells with a high lipid content (Russell et al., 2009). While granulomas have been studied for about 200 years, their role in TB etiology remains unclear. In 1819, Laënnec first proposed granulomas as the cause of TB (Sakula, 1982). Yet, about a century went by before Ghon correlated the presence of a single caseous granuloma in the mid-region of the lung with a corresponding nodal involvement (the Ghon complex) and the pathogen’s dissemination, thus serving as a marker for latent TB (Dorhoi et al., 2011). In spite of this, subsequent studies and clinical observations established the granuloma as a host-protective structure that “walls off” Mtb to prevent its dissemination, a notion that still predominates. Seminal studies by Ramakrishnan in zebrafish, however, have now evidenced mycobacteria actually exploit the granuloma into a tool for pathogenesis, suggesting its function can be modulated depending on the disease context (Ramakrishnan, 2012). Considering TB is still one of the leading causes of human death due to a single infectious agent, substantial insights into microbe physiology and host defenses rest in the attempt to better understand the mechanisms governing TB granulomas.

Here, we will focus exclusively in the role of Mφ polarization in the formation and function of TB granulomas. Likewise, we will provide a unique perspective on the significance of B-cells, whose immune-modulatory function has long been ignored in TB.

MACROPHAGE POLARIZATION IN TB GRANULOMAS

Mφ polarization is broadly classified into M1 and M2 programs (Goedt and Orfano, 1999; Gordon, 2003; Mantovani et al., 2004; Martinez et al., 2009). On one hand, the M1 program is a response to type-1 inflammatory conditions (e.g., IFN-γ), often associated with intracellular pathogen resistance (Quintana-Murci et al.,
2007; Benoit et al., 2008). IFN-γ is mainly responsible for the establishment of the M1 program, granting Mφs the capacity to kill mycobacteria (Flynn et al., 1993; Ehrt et al., 2001). The production of nitric oxide (NO) in Mφs (characteristic in murine models) is arguably one of the most important consequences mediated by IFN-γ, as mice deficient for NO production succumb to Mtb infection (Chan et al., 1992). In fact, the enzyme iNOS (inducible NO synthase) required for NO production is a bona fide marker of murine M1 Mφs (Xie and Nathan, 1993). Other marker genes, whose expression is induced in M1, include ido1, pigs2, il12b/il23a, socs3, marco, cd86, ifr5/irf5, and stat1/stats5, among others (Lawrence and Natoli, 2011; Murray and Wynn, 2011b). Collectively, the M1 program is part of the “common host response” against intracellular bacteria that endows Mφs with a non-permissible nature (Ehrt et al., 2001; Deretic et al., 2004; Martinez et al., 2009; Cairo et al., 2011; Murray and Wynn, 2011a). On the other hand, the M2 program is dictated by type-2 inflammatory signals (e.g., IL-4, IL-10), enabling Mφs to participate in the suppression of inflammation, phagocytosis, tissue remodeling, and repair, among others (Sica et al., 2008; Martinez et al., 2009; Murray and Wynn, 2011a). However, this program also renders Mφs poorly microbicidal against intracellular pathogens (Raju et al., 2008; Martinez et al., 2009). This is best illustrated by how the arginine metabolism is used in M2 Mφs, which shuts down NO production in favor of tissue reparation (Shearer et al., 1997). Indeed, M2 polarization is accompanied by ARG1 (type-1 arginase) expression that inhibits NO production by outcompeting iNOS to convert arginine into ornithine and urea (Munder et al., 1998; El Kasmi et al., 2008). Along arg1, other M2 marker genes include fizz1, chi311/chi312/chi313, mrc1, cd36, socs2, il-10, klf4, jmjd3/irf4, pparγ, and stat6, among others (Lawrence and Natoli, 2011; Murray and Wynn, 2011b). Altogether, Mtb might influence the granuloma function by controlling Mφ polarization, a premise that is presciently in line with the following findings, which for the purpose of conciseness, are mainly based on the use of the iNOS/ARG1 polarization axis.

The animal models to study TB granulomas are discussed in detail elsewhere (Flynn, 2006). Here, we highlight recent findings in mice and zebrafish documenting the TB granuloma dynamics, supported by studies and clinical observations done in TB patients. It is widely postulated the onset of human pulmonary TB begins when inhaled Mtb is captured by Mφs and transported across the alveolar epithelium into the lung tissue. In zebrafish, the subsequent steps leading to a nascent granuloma have been captured in real-time imaging (Davis et al., 2002). While infected Mφs undergo apoptosis, they promote the recruitment of phagocytes, which upon arrival, display high motility conducive for scavenging apoptotic cells. The phagocytosis of dead Mφs leads to the formation of cell aggregates, fomenting bacterial growth. Subsequent rounds of this cycle promote the formation of a stable granuloma in 3 days post-infection (p.i.), a process that is dependent on the region of difference-1 (RDI) virulence locus of M. marinum and independent of T-cells (Davis et al., 2002; Volkman et al., 2004, 2010; Davis and Ramakrishnan, 2009). It is unclear whether zebrafish Mφs undergo polarization. Yet, since most transcription factors governing T-cell polarization are highly conserved in zebrafish (Mitra et al., 2010), along with physiological and pathological responses characteristic of type-1 and type-2 immunity (Aggad et al., 2010; Balla et al., 2010; Holt et al., 2011; Wittamer et al., 2011; Renshaw and Trede, 2012), it seems as a matter of time before Mφ polarization is identified and characterized in this teleost. By contrast, the early stage of Mtb infection in mice is marked by M1 Mφ polarization, reminiscent of clinical observations in TB patients (Benoit et al., 2008). In fact, transcriptomic analysis of infected murine Mφs revealed the gene modulation provoked by Mtb overlaps with that of IFN-γ to establish the M1 program (Ehrt et al., 2001). Type-1 inflammatory signals secreted by infected Mφs induce cell recruitment and formation of primary granulomas. Unlike zebrafish, however, granuloma formation in mice takes up to 3 weeks when Mycobacterium reaches a plateau and coincides with adaptive immunity involvement. For instance, nascent liver granulomas were visualized by intravital microscopy between 2 and 3 weeks after Mycobacterium bovis Calmette–Guerin (BCG) challenge (Egen et al., 2011). In another study, Mtb infection did not change the murine Mφ population (iNOS<sup>low</sup>ARG1<sup>low</sup>) in bronchoalveolar lavage (BAL) during the first week (Redente et al., 2010). At day 21 p.i., however, M1 Mφs (iNOS<sup>high</sup>ARG1<sup>low</sup>) dominated in BAL and granulomas, coinciding with a peak of IFN-γ in infected lungs (Redente et al., 2010). In humans, although NO production by monocyte-derived Mφs remains controversial, both iNOS and NO are detected in granulomas and alleles for NOS2 are associated to TB susceptibility (Nicholson et al., 1996; Facchetti et al., 1999; Choi et al., 2002; Schon et al., 2004; Moller et al., 2009). After 35–60 days p.i., while murine Mφs at the granuloma core remained iNOS<sup>high</sup>ARG1<sup>low</sup>, there was a dramatic shift toward the M2 program (iNOS<sup>low</sup>ARG1<sup>high</sup>) in Mφs surrounding the core, accompanied by elevated type-2 inflammatory signals (Redente et al., 2010). This is in line with ARG1 detection in human TB granulomas (Pessanha et al., 2012).

The shift toward M2 Mφs during Mtb infection could have deleterious consequences for the granuloma as a host-protective structure (Figure 1). First, ARG1 expression in uninfected Mφs surrounding the granuloma core suggests the development of an immunosuppressive niche. Indeed, Mtb promotes its survival by inducing ARG1 expression through Myd88-dependent signaling pathways (El Kasmi et al., 2008; Qualls et al., 2010). At the transcriptional level, murine M2 Mφs displayed a diminished inflammatory response to Mtb as reflected by a reduced NO production and increased of iron availability, alluding ARG1 might also be implicated in nutrient deprivation mechanisms limiting microbial growth (Forbes and Gros, 2001; Kahnert et al., 2006; Cairo et al., 2011). Furthermore, M1 Mφs possess a “fail-safe” system sustaining optimum NO production based on citrulline recycling via argininosuccinate synthase (ASS1), which is absent in M2 Mφs (Qualls et al., 2012). Given the restrictive granuloma environment where arginine may be limited, the presence of this fail-safe system may become further accentuated. Second, M2 Mφs may represent a transitional state into the formation of “foamy” Mφs that are rich in cholesterol, a carbon source for microbial intracellular survival (Pandey and Sassetti, 2008; Peyron et al., 2008; Russell et al., 2009; Griffin et al., 2011). Recently, Mtb lipids were shown to trigger PPARγ, the master regulator of M2 polarization, to increase expression of CD36 and induce foam cell formation.
TB granuloma environment favoring bactericidal function

FIGURE 1 | A model illustrating the putative roles of Mφ polarization and B-cell involvement during the formation and function of TB granulomas. TB granuloma Mφs undergo various specialized transformations: they can look like epithelial characterized by tightly interdigitated cell membranes that link adjacent cells; they can fuse into multinucleated giant cells; or they can differentiate into foamy cells with a high content of intracellular lipids. While none of these specialized transformations in the granuloma Mφ population are well understood, we propose they might be reflection of the Mφ polarization status that may render the granuloma structure with a microbicidal capacity (top) or as a tool of pathogenesis (bottom). In the former scenario, the local Mφ population in lung undergoes a M1 polarization early on during Mtb infection and granuloma formation, distinguished by a cell-surface receptor repertoire responsive to pro-inflammatory signaling (e.g., IFN-γR<sup>high</sup>, MHC-II<sup>high</sup>, CD86<sup>high</sup>, IRF5<sup>positive</sup>), among others. These Mφs have been noted to be most frequently located in the necrotic center of a mature tuberculous granuloma where apoptotic and necrotic Mφs are abundant along with extracellular bacteria. Accompanying the M1 Mφ polarization is the recruitment of neutrophils and Th1 cells, whose migration and activation status might be influenced by a B-cell involvement likely characterized by a pro-inflammatory phenotype (e.g., IFN-γ<sup>high</sup>, IL-6<sup>high</sup>, IgG<sup>high</sup>). In the latter scenario, we propose a change in the TB granuloma environment during the late stages of Mtb infection, distinguished by the M2 Mφ polarization driven by the high expression of transcription factors (e.g., PPARγ<sup>high</sup>, STAT6<sup>positive</sup>) antagonistic for type-1 inflammation, and characterized by a cell-surface receptor repertoire promoting tissue repair activities (e.g., IL-4R<sup>high</sup>) and the formation of foamy cells (e.g., CD36<sup>high</sup>), while suppressing the microbial functions like NO production (e.g., ARG1<sup>high</sup>), among others. We envision M2 Mφ polarization might give rise to the formation of foam and multinucleated giant cells, whose presence is noted to be most frequently at the rim and center of mature TB granulomas, and which may favor the intracellular resilience of Mtb. Furthermore, classical M2 Mφs have been noted to be most frequently located surrounding the granuloma center and overwhelmingly in the local lung environment. Along with the M2 Mφ polarization is the inhibition of neutrophil recruitment while enhancing that of T<sub>REG</sub>s, activities that might be influenced by a B-cell involvement likely characterized by a anti-inflammatory phenotype (e.g., IL-10<sup>high</sup>, CD1d<sup>positive</sup>).

(Mahajan et al., 2012). Here, we postulate that factors governing M2 polarization establish additional anti-inflammatory signaling loops, like that of CD36, to increase microbial fitness within granulomas (Kuda et al., 2011). Third, the shift toward M2 Mφs may allow Mtb to control the antigen-presentation process to undermine adaptive immunity within granulomas (Benoit et al., 2008). Indeed, TB granulomas display a limited antigen-presentation to evoke significant T-cell responses (Egen et al., 2011). While Mφ polarization was not addressed in this study, M2 Mφs do inhibit the proliferation of CD4 T-cells while fomenting the activity of regulatory T-cells (Schebesch et al., 1997; Curiel et al., 2004; Biswas and Mantovani, 2010). Altogether, the shift toward M2 Mφs might also occur in human granulomas and contribute to Mtb pathogenesis given that TB susceptibility is often accompanied by elevated
type-2 inflammatory and immunosuppressant signals (Kahnert et al., 2006; Raju et al., 2008; Almeida et al., 2009; Schreiber et al., 2009).

In the near future, we envision the role of Mφ polarization in the granuloma context will be tested directly in different ways. First, we expect further advances in real-time imaging in both zebrafish and mouse models. Highly conserved Mφ polarization markers are ideal candidates for the development of novel animal reporter lines expressing different fluorochromes to target the different Mφ subsets. Second, specific gene inactivation of Mφ polarization markers with the use of morpholinos (in zebrafish), siRNA-based technology, or gene-knockout strategy (including conditional strategies), may be used at different stages of granuloma formation in animal models. The strategies above could be used in combination with global array-based transcriptomics and proteomics approaches in order to assess the granuloma and local lung environment in the presence or absence of Mφ subsets. Collectively, we expect there would be more future efforts to bridge results obtained in animals into the human context as discussed in the conclusion section.

A ROLE FOR B-CELLS IN GRANULOMATOUS DISEASES

Alterations in the lung environment by Mtb and/or subsequent immune responses likely affect the infection outcome. None of these is more apparent than the type-1 inflammatory storm that is unleashed in murine lungs at 3 week p.i., when a peak of IFN-γ/TNF coincides with CD4+ T-cell involvement, an event that impacts the organization of nascent granuloma structures. Yet, mice in which CD4+ T-cells are unable to produce IFN-γ/TNF are still resistant to TB, suggesting a complex scenario for protection (Torrado and Cooper, 2011). In this perspective article, we propose that, beside T-cells, B-cells modulate the TB granuloma formation and function through interaction with their cellular components.

Despite extensive evidence for anti-Mtb antibody production in TB patients (Kunnath-Velayudhan et al., 2010, 2012), and a higher susceptibility of pIgR (IgA receptor)-deficient mice (Tjarndlund et al., 2006), initial studies examining the role of antibodies in TB indicated a modest impact in protective immunity, with benefits limited to passive administration of anti-Mtb antibodies (Glatman-Freedman and Casadevall, 1998; Roy et al., 2005; Abebe and Bjune, 2009). This contributed to the notion B-cells played a minor role in TB immunity, if any. Yet, recent studies now provide compelling reasons to revisit the role of B-cells in TB (Cooper, 2009; Maglione and Chan, 2009; Flynn et al., 2011; Philips and Ernst, 2012). First, B-cells infiltrate the lungs of Mtb-infected mice and humans (Tsiai et al., 2006), where they organize in ectopic B-cell follicles at the periphery of granulomas (Gonzalez-Juarrero et al., 2001; Ulrichs et al., 2004; Kahnert et al., 2007; Maglione et al., 2007). These foci are the predominant sites of cellular proliferation in the infected lungs attesting to the importance of B-cells in shaping the local environment during infection (Ulrichs et al., 2004). Moreover, B-cells also infiltrate the granuloma structure, as shown in non-human primates where activated B-cell clusters are found in close contact with T-cells (Phuah et al., 2012), and in the lungs from cattle with natural tuberculosis (Beytut, 2011). Mtb-specific B-cells also exist at local sites of infection in pleural fluids, a strategic place to influence the immunity against Mtb (Feng et al., 2011). Beyond TB, B-cells are well-known cellular components in

Table 1 | Characteristics of B-cells identified in non-TB granulomatous diseases.

| Disease or model | Type of B-cells | Reported role in disease | Specie | Reference |
|-----------------|-----------------|--------------------------|--------|-----------|
| Wegener’s granuloma | Undefined | Detrimental | Humans | Voswinkel et al. (2008), Holle et al. (2012) |
| Sarcoidosis | Undefined | Unknown | Humans | Fukuda et al. (1997) |
| Churg–Strauss syndrome | Undefined | Detrimental | Humans | Donvik and Omdal (2011) |
| Crohn’s disease | B1a | Unknown | Humans | Geboes et al. (1988) |
| Schistosomiasis | Undefined | Favor protective Th2 immunity; inhibit T-cell-mediated immunopathology; granuloma formation | Mouse | Hernandez et al. (1997), Ferrui, Jankovic et al. (1998), Jacobs et al. (1999), Ji et al. (2008) |
| Leishmaniasis | Include B2b as well as CD5+CD1d+IL-10 producing regulatory Breg cells | Limits immunopathology; favor protective Th2 immunity; favor granuloma formation | Mouse | Smelt et al. (2000), Ronet et al. (2010), Moore et al. (2012) |
| Coccidioidomycosis | IL-10 producing Breg | Unknown | Humans | Li et al. (2005) |
| Paracoccidioides | B1, IL-10 producing Breg | Detrimental | Mouse | Popi et al. (2008) |
| Cat-scratch disease | IL-10 producing Breg | Unknown | Humans | Vermi et al. (2006) |
| Pristane induced oil granuloma response | Undefined | Granuloma formation | Mouse | Chen et al. (2010) |

*B1 cells: developmentally defined; innate-like B-cells in the mouse; CD5+ or CD5− subpopulation poorly defined in humans.

*B2 cells: developmentally defined; include “conventional” follicular B-cells as well as “innate-like” marginal zone B-cells.

* Breg: functionally defined; present among various B-cell populations including CD5+CD1d+ B-cells; can produce IL-10.
several other granulomatous diseases (Table 1). Not only B-cells are present in granuloma but also they could be important for their maturation. This is suggested in pristane induced oil granuloma formation (Chen et al., 2010) and during Schistosoma japonicum infection (Ji et al., 2008) where the absence of B-cells results in a marked delay in granuloma formation. In the context of the TB, although granulomas form in the absence of B-cells, their numbers and size remain lower and they hardly become inflammatory (Bosio et al., 2000; Maglione et al., 2007). This could be the result of the well-known ability of B-cells to contribute to the organization of secondary and tertiary lymphoid organs (Moseman et al., 2012).

Second, although this is a rare event, occurrence of mycobacterial infections was reported upon rituximab-mediated depletion of B-cells, suggesting a protective role for these lymphocytes (Winthrop et al., 2008; Gea-Banacloche, 2010). However, other granulomatous diseases were successfully treated with rituximab (Donvik and Omdal, 2011; Holle et al., 2012), cautioning B-cells may be detrimental depending on the disease context. Finally, beyond antibody production, B-cells display diverse roles in the immunity against multiple pathogens that could operate during TB. In this regard, Salmonella infection, though not occasioning granuloma formation, represents a paradigm for antibody-independent roles of B-cells against an intracellular bacterium with the evidence that B-cells producing IL-10 (Breg) impairs the control of natural and vaccine-induced immunity to Salmonella (Neves et al., 2010). Since this role cannot simply be recapitulated in animal models lacking B-cells (Mastroeni et al., 2000; Mittrucker et al., 2000), this exemplifies how deletion of the B-cell compartment eclipses specific functions of these cells.

B-cells express adaptive and innate receptors to recognize pathogens (Blumenthal et al., 2009; Rawlings et al., 2012). Beyond antibody production, B-cells secrete various signals including cytokines, and serve as antigen-presenting cells (Rawlings et al., 2012). In addition IL-23-deficient mice also have poor levels of IL-17 and IL-22. These deficiencies resulted in a marked alteration of CXCL13 production, the chemokine responsible for B-cell recruitment and follicle formation (Khader et al., 2011; Zhang et al., 2011). In the mouse model, IL-17A (Okamoto Yoshida et al., 2010) or IL-23-deficient (Khader et al., 2011; Zhang et al., 2011) animals have marked defects in the formation of granulomas and/or B-cell follicles. In addition IL-23-deficient mice also have poor levels of IL-17 and IL-22. These deficiencies resulted in a marked alteration of CXCL13 production, the chemokine responsible for B-cell recruitment and follicle formation (Khader et al., 2011; Zhang et al., 2011). It is not known if IL-10 production by B-cells is at the initiation or a secondary consequence of the alterations in IL-17 levels. These observations might provide an explanation for the links reported in TB patients between Th17 and formation of B-cell foci and IL-10 (Zhang et al., 2011, 2012).

Evidence obtained in non-TB diseases argue B-cells favor Th1 polarization (involved in TB protective immunity) through IL-6 and IFN-γ production during Salmonella infection, or promote Th2 differentiation (thought to be detrimental during TB) through either IL-2 (Wojciechowski et al., 2009) or IL-10 (Ferru et al., 1998; Popi et al., 2008; Ronet et al., 2010) in the control of different parasites. Conversely, B-cells also suppress T-cell activity as best illustrated in mice with a targeted deletion of Myd88 in B-cells during Salmonella infection (Neves et al., 2010). Finally, evidencing the role of B-cells as antigen-presenting cells, mice with a targeted deletion of MHC-II in B-cells displayed a reduction of IL-2 and IFN-γ by CD4+ memory T-cells during
**CONCLUSION**

Among trends emerging in TB etiology, the notion that the local lung environment shifts from a host-protective nature toward one favorable to microbial resilience is discussed here at the granuloma level and in the context of Mφ polarization and B-cell function (see also an illustration in Figure 1). Exploring these issues will likely bring us closer to uncover the enigma concealed by TB granulomas. One can envisage that studies investigating the role of genes involved in host tolerance (Medzhitov et al., 2012) might be a good way to explore these aspects of the disease. Although in humans this could be limited to immunogenetic studies, more mechanistic studies could be conducted in animal models where selective inactivation of those genes could provide new insights on the consequences on the pathology. These studies could go along with more sophisticated approaches based on single cell analysis such as those involving laser microdissection or more global phenotypic signatures obtained from mass cytometry, in order to further identify cell subsets involved at different stages of granuloma formation and TB.

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**REFERENCES**

Abebe, F., and Bjune, G. (2009). The role of antibody response during Mycobacterium tuberculosis infection. *Clin. Exp. Immunol.* 157, 235–243.

Adams, D. O. (1974). The structure of mononuclear phagocytes differentiating in vivo. *Sequential fine and histologic studies of the effect of Bacillus Calmette-Guérin (BCG). Am. J. Pathol.* 76, 17–48.

Aggad, D., Stein, C., Sieger, D., Mazel, M., Boudinot, P., Herbelin, P., et al. (2010). In vivo analysis of Ifn-gamma1 and Ifn-gamma2 signaling in zebrafish. *J. Immunol.* 185, 6774–6782.

Almeida, A. S., Lugo, P. M., Boechat, N., Huard, R. C., Lazzarini, L. C., Santos, A. R., et al. (2009). Tuberculosis is associated with a down-modulated, tubular immune response that impairs Th1-type immunity. *J. Immunol.* 183, 718–731.

Andreu, P., Johannson, M., Affara, N. I., Pucci, F., Tan, T., Junankar, S., et al. (2010). FeRgamma activation regulates inflammation-associated squamous carcinogenesis. *Cancer Cell 17*, 121–134.

Balla, K. M., Lugo-Villarino, G., Spitsbergen, J. M., Stachura, D. L., Hu, Y., Banuelos, K., et al. (2010). Eosinophils in the zebrafish: prospective isolation, characterization, and eosinophilia induction by helminth determinants. *Blood* 116, 3944–3954.

Barr, T. A., Brown, S., Mastroeni, P., and Gray, D. (2010). TLR and B cell receptor signals to B cells differentially program primary and memory Th1 responses to Salmonella enterica. *J. Immunol.* 185, 2783–2788.

Benoit, M., Desnues, B., and Mege, J. L. (2008). Macrophage polarization in bacterial infections. *J. Immunol.* 181, 3573–3579.

Berry, M. P., Graham, C. M., McNab, F. W., Xu, Z., Bloch, S. A., Oni, T., et al. (2010). An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* 466, 973–977.

Bertut, E. (2011). Immunohistochemical analysis of surfactant proteins and lymphocyte phenotypes in the lungs of cattle with natural tuberculosis. *Res. Vet. Sci.* 91, 119–124.

Biswas, S. K., and Mantovani, A. (2010). Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat. Immunol.* 11, 889–896.

Blumenfeld, A., Kobayashi, T., Pierini, L. M., Banaei, N., Ernst, J. D., Miyake, K., et al. (2009). RPI015 facilitates macrophage activation by Mycobacterium tuberculosis lipopolysaccharides. *Cell Host Microbe* 5, 35–46.

Bosio, C. M., Gardner, D., and Ellkins, 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. *J. Immunol.* 164, 6417–6425.

Cai, G., Recalcati, S., Mantovani, A., and Locati, M. (2011). Iron trafficking and metabolism in macrophages: relationship to the polarized phenotype. *Trends Immunol.* 32, 241–247.

Chakravarty, S. D., Zhu, G., Tsai, M. C., Mohan, V. P., Marino, S., Kirschner, D. E., et al. (2008). Tumor necrosis factor blockade in chronic murine tuberculosis enhances granuloma- tious inflammation and disorganizes granulomas in the lungs. *Infect. Immun.* 76, 916–926.

Chan, J., Xing, Y., Magliozzo, R. S., and Bloom, B. R. (1992). Killing of virulent Mycobacterium tuberculosis by reactive nitrogen intermediates produced by activated murine macrophages. *J. Exp. Med.* 175, 1111–1122.

Chen, H., Liao, D., Henn, T. M., Snowden, P., Ueda, Y., and Kelsoe, G. (2010). Genetic regulation of pristane-induced oil granuloma responses. *J. Exp. Pathol.* 91, 472–483.

Choi, H. S., Rai, P. R., Chu, H. W., Cool, C., and Chan, E. D. (2002). Analysis of nitric oxide synthase and nitrotyrosine expression in human pulmonary tuberculosis. *Am. J. Respir. Crit. Care Med.* 166, 178–186.

Cooper, A. M. (2009). Cell-mediated immune responses in tuberculosis. *Annu. Rev. Immunol.* 27, 393–422.

Curzi, T. J., Coukos, G., Zoa, L., Alvarez, X., Cheng, P., Motttram, P., et al. (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat. Med.* 10, 942–949.

Davis, J. M., Clay, H., Lewis, J. L., Ghori, N., Herbold, F., and Ramakrishnan, L. (2002). Real-time visualization of mycobacterium-macrophage interactions leading to initiation of granuloma formation in zebrafish embryos. *Immunity* 17, 693–702.

Davis, J. M., and Ramakrishnan, L. (2002). The role of the granuloma in expansion and dissemination of early tuberculous infection. *Cell* 136, 57–69.

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Investigation of chromosome 17 candidate genes in susceptibility to TB in a South African population. Tuberculosis 89, 189–194.

Moore, J. W., Beattie, L., Dalton, J. E., Owens, B. M., Maroof, A., Coles, M. C., et al. (2012). B cell T cell interactions occur within hepatic granu- lomas during experimental visceral leishmaniasis. PLoS ONE 7:e34143. doi:10.1371/journal.pone.0034143

Moseman, E. A., Iannace, M., Bosurgi, L., Tonti, E., Chevrier, N., Tumanov, A., et al. (2012). B cell maintenance of subcapsular sinus macrophages protects against a fatal viral infection independent of adaptive immunity. Immunity 36, 415–426.

Munder, M., Eichmann, K., and Mod- ocells, M. (1998). Alternative meta- bolic states in murine macrophages reflected by the nitric oxide syn- thase/arginase balance: competitive regulation by CD4+ T cells corre- lates with Th1/Th2 phenotype. J. Immunol. 160, 5347–5354.

Murray, P. J., and Wynn, T. A. (2011a). Obstacles and opportunities for understanding macrophage polar- ization. J. Leukoc. Biol. 89, 557–563.

Murray, P. J., and Wynn, T. A. (2011b). Protective and pathogenic functions of macrophage subsets. Nat. Rev. Immunol. 11, 723–737.

Neves, P., Lampropoulos, V., Calderon- Gomez, E., Roch, T., Stervbo, U., Shen, P., et al. (2010). Signaling via the MyD88 adaptor protein in B cells suppresses protective immunity dur- ing Salmonella typhimurium infection. Immunity 33, 777–790.

Nicholson, S., Bonecini-Almeida Mda, G., Lapu e Silva, J. R., Nathan, C. X., Qiu, W. M., Murnford, R., et al. (1996). Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. J. Exp. Med. 183, 2293–2302.

O’Garra, A., Stapleton, G., Dhar, V., Pearce, M., Schumacher, J., Rugo, H., et al. (1990). Production of cytokines by mouse B cells: B lymph- phones and normal B cells pro- duce interleukin 10. Int. Immunol. 2, 821–832.

Okamoto Yoshida, Y., Umemura, M., Yahagi, A., O’Brien, R. L., Ikuta, K., Kishihara, K., et al. (2010). Essential role of IL-17A in the forma- tion of a mycobacterial infection- induced granuloma in the lung. J. Immunol. 184, 4414–4422.

Pandey, A. K., and Sassetti, C. M. (2008). Mycobacterial persistence requires the utilization of host cholesterol. Proc. Natl. Acad. Sci. USA 105, 4376–4380.

Pessanha, A. P., Martins, R. A., Mattos- Guardali, A. L., Vinna, A., and Mor- eira, L. O. (2012). Arginase-1 expres- sion in granulomas of tuberculo- sis patients. FEMS Immunol. Med. Microbiol. 66, 265–268.

Peyron, P., Vaubourgeix, J., Poquet, Y., Levillain, F., Botanch, C., Bardou, F., et al. (2008). Foamy macrophages from tuberculosis patients’ gran- ulomas constitute a nutrient-rich reservoir for M. tuberculosis per- sistence. PLoS Pathog. 4:e1000204. doi:10.1371/journal.ppat.1000204

Philips, J. A., and Ernst, J. D. (2012). Tuberculosis pathogenesis and immunity. Annu. Rev. Pathol. 7, 353–384.

Phua, J. Y., Mattila, J. T., Lin, P. L., and Flynn, J. L. (2012). Activated B cells in the granulomas of nonhuman primates infected with Mycobac- terium tuberculosis. Am. J. Pathol. 181, 508–514.

Popi, A. E., Godoy, L. C., Xander, P., Lopes, J. D., and Mariano, M. (2008). B-1 cells facilitate Paracoccidioides brasiliensis infection in mice via IL-10 secretion. Microbes Infect. 10, 817–824.

Qualls, J. E., Neale, G., Smith, A. M., Koo, S. M., Defreitas, A. A., Zhang, H., et al. (2010). Argi- nine usage in mycobacteria-infected macrophages depends on autocrine paraacrine cytokine signaling. Sci. Signal. 3, ra62.

Qualls, J. E., Subramanian, C., Rafi, W., Smith, A. M., Balouzan, L., Defreitas, A. A., et al. (2012). Sustained generation of nitric oxide and control of mycobacterial infection requires argininosuccinate synthase 1. Cell Host Microbe 12, 313–323.

Quintana-Murci, L., Alcais, A., Abel, L., and Casanova, J. L. (2007). Immunology in natural: clinical, epidemiological and evolutionary genetics of infectious diseases. Nat. Immunol. 8, 1165–1171.

Raju, B., Hoshib, Y., Belitskaya-Leya, L., Dawson, R., Ress, S., Gold, J. A., et al. (2008). Gene expression pro- files of bronchoalveolar cells in pul- monary TB. Tuberculosis (Edinb.) 88, 39–51.

Ramakrishnan, L. (2012). Revisiting the role of the granuloma in tuberculo- sis. Nat. Rev. Immunol. 12, 352–366.

Rawlings, D. J., Schwartz, M. A., Jack- son, S. W., and Meyer-Bahlburg, A. (2012). Integration of B cell responses through Toll-like recep- tors and antigen receptors. Nat. Rev. Immunol. 12, 282–294.

Redente, E. F., Higgins, D. M., Dwyer- Nield, L. D., Orme, I. M., Gonzalez- Juarrero, M., and Malkinson, A. M. (2010). Differential polariza- tion of alveolar macrophages and bone marrow-derived monocytes following chemically and pathogen- induced chronic lung inflammation. J. Leukoc. Biol. 88, 159–168.

Renshaw, S. A., and Trede, N. S. (2012). A model 450 million years in the making: zebrafish and verte- brate immunity. Dis. Model Mech. 5, 38–47.

Ronet, C., Hayon-La Torre, Y., Revaz- Breton, M., Mastelic, B., Tachin- Cottier, E., Louis, J., et al. (2010). Regulatory B cells shape the develop- ment of Th2 immune responses in BALB/c mice infected with Leish- mania major through IL-10 produc- tion. J. Immunol. 184, 886–894.

Ray, E., Stavropoulos, E., Brennan, J., Coode, S., Grgoriuva, E., Walker, B., et al. (2005). Therapeutic efficacies of high-dose intravenous immunoglobulin in Mycobacterium tuberculosis infection in mice. Infect. Immun. 73, 6101–6109.

Russell, D. G. (2007). Who puts the tubercle in tuberculosis? Nat. Rev. Microbiol. 5, 39–47.

Russell, D. G., Cardona, P. J., Kim, M. J., Allain, S., and Altare, F. (2009). Foamy macrophages and the pro- gression of the human tuberculo- sis granuloma. Nat. Immunol. 10, 943–948.

Russo, R. T., and Mariano, M. (2010). B-1 cell protective role in murine primary Mycobacterium bovis bacillus Calmette-Guerin infection. Immunobiologen 215, 1005–1014.

Sakula, A. (1982). Robert Koch: cente- nary of the discovery of the tubercle bacillus, 1882. Thorax 37, 246–251.

Schebesch, C., Koolja, V., Muller, C., Hakki, N., Bisson, S., Orfano, C. E., et al. (1997). Alternatively activated macrophages actively inhibit prolif- eration of peripheral blood lympho- cytes and CD4+ T cells in vitro. Immunology 92, 478–486.

Schon, T., Elmerger, G., Nessega, Y., Pando, R. H., Sundqvist, T., and Brit- ton, S. (2004). Local production of nitric oxide in patients with tuberculosis. Int. J. Tuberc. Lung Dis. 8, 1134–1137.

Schreiber, T., Ehlers, S., Heitmann, L., Rausch, A., Mages, J., Murray, P. J., et al. (2009). Autocrine IL-10 induces hallmarks of alterna- tive activation in macrophages and suppresses antituberculous effector mechanisms against M. tuberculosis T cell immunity. J. Immunol. 183, 1301–1312.

Shearer, J. D., Richards, R. J., Mills, C. D., and Caldwell, M. D. (1997). Dif- ferential regulation of macrophage...
arginine metabolism: a proposed role in wound healing. Am. J. Physiol. 272, E181–E190.

Sica, A., Larghi, P., Mancino, A., Rubino, L., Porta, C., Totaro, M. G., et al. (2008). Macrophage polarization in tumour progression. Semin. Cancer Biol. 18, 349–355.

Smith, S. C., Cotterell, S. E., Engwerda, C. R., and Kaye, P. M. (2000). B cell-deficient mice are highly resistant to Leishmania donovani infection, but develop neutrophil-mediated tissue pathology. J. Immunol. 164, 3681–3688.

Tjarnlund, A., Rodriguez, A., Cardona, S., Jorg, S., Pradl, L., Titukhina, M., et al. (2012). CD19(+)CD1d(+)CD5(+) B cell frequencies are increased in patients with tuberculosis and suppress Th17 responses. Cell. Immunol. 274, 89–97.

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