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Latent TB Infection (LTBI) – *Mycobacterium tuberculosis* pathogenesis and the dynamics of the granuloma battleground

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ABSTRACT

Latent tuberculosis infection (LTBI) is established in over 90% of persons infected with *Mycobacterium tuberculosis* (*Mtb*), from whom new active TB cases will arise. Understanding the spatio-temporal dynamics of host immune responses in LTBI granulomas is essential to designing effective post-exposure therapies that inhibit progression to TB. Information arising from cancer studies and other modalities – where local chronic inflammation leads to immunopathology – can help provide insights into the biological pathways at play in LTBI granulomas. Translational studies using patient material as well as LTBI+ donor-derived tissue samples are instrumental in understanding the various components of granuloma dynamics, immunological landscapes therein and how this could help to identify therapeutic targets. Deep sequencing technologies may aid to decipher the genetic changes in lung granuloma and blood samples from LTBI+ individuals associated with progression to active TB disease. This may lead to advancement of development of targeted Host-Directed Therapies (HDTs) and their evaluation as adjunct TB therapies for improving treatment outcomes for LTBI and pulmonary TB.

Introduction

Tuberculosis (TB) has plagued humankind for centuries and has caused over one billion deaths. Today, an estimated 2 billion people worldwide have latent TB infection (LTBI). Humans are the main reservoir of *Mycobacterium tuberculosis* (*Mtb*) and human to human spread primarily occurs through inhalation of respiratory aerosols and secretions containing *Mtb* exhaled from a patient with active pulmonary TB. Despite intense study over the past six decades the sequence of human host-*Mtb* interactions and pathophysiological events following primary or secondary infection, remain a mystery (Zumla et al., 2011). The granuloma was thought to be a result of protective cell-mediated and has been central to the study of pathogenesis of TB and other intracellular pathogens (James and Zumla, 1999). A spectrum of granulomatous changes has been described from complete resolution, to continuing granuloma turnover with protective and deleterious inflammation, caseous necrosis, fibrosis and scarring of tissue leading to long-term functional disability (Drain et al., 2018). Technological advances over the years have enabled more detailed studies at molecular and cellular level, several recent publications have questioned whether the granuloma or its individual immune components make the granuloma a 'friend' or a 'foe' (Rubin, 2009; Galizia and Marti, 2018). Thus, gaining a deeper understanding of the host-pathogen interactions in persons harbouring LTBI may hold quintessential clues to achieving the WHO's goal of eliminating TB as a public health threat by 2050.

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Imaging studies of the TB granulomas have shed light on how individual lesions in the same patient evolve over time, just as how cancer lesions do. This evolutionary process is mediated and influenced by exposure to drugs, the hosts genetics as well as that of the infecting *Mtb* species (Ankrah et al., 2016). Studies show that position emission tomography (PET) with [18F]fluoro-2-deoxy-D-glucose (FDG) is able to monitor lesions in patients with subclinical TB – which could have arisen from a previously LTBI state – and the behavioural pattern of individual lesions which are likely to perpetrate the development of symptomatic clinical disease (Drain et al., 2018). Since most people infected with *Mtb* do not develop active clinical disease, LTBI presents a unique and challenging opportunity to understand the intricate relationship between host cells in the granuloma, the contribution of the stromal compartment to sustain infection and how the local immune responses is placed therein. Thorough dissection of these biological component which collectively govern host-pathogen interactions and pathogenesis will reveal essential information necessary for developing next-generation drugs and therapies.

Genetic aberrations in the *Mtb* genome usually make the bacilli refractory to antibiotics and resistant to immune attack. However, we have yet to uncover whether some of these mutations aid their long survival in the LTBI granuloma without attracting much attention from immune cells. Furthermore, an individual's human leukocyte antigen (HLA) background, naturally occurring variations in immune-related genes and selective pressure affecting gene expression patterns may influence local host-pathogen interactions in the LTBI granuloma and later, the development of clinical TB. Indeed, the genetic basis of susceptibility to TB is being roped back into the limelight due to vaccine efficacy studies and evaluation of new biomarkers for host-directed therapy (HDT) as well as to predict clinical responses to standard anti-TB treatment (Goletti et al., 2018).

Emerging data from clinical studies provide staunch ground for accounting for immune-cell types as well as surface or effector molecule expression profiles to better gauge the pathophysiological status linking LTBI and clinical TB. Natural killer (NK) cells, previously underemphasised in protection to TB, are now acknowledged as important modulators of progression to active TB from LTBI (Roy Chowdhury et al., 2018). An increase of CD4+ and CD8+ mucosal-associated invariant T cells (MAIT) in peripheral blood of individuals harbouring LTBI compared to uninfected control subjects has also been reported (Paquin-Proulx et al., 2018). The existence of a clonotypic T-cell receptor (TCR) repertoire in the LTBI is associated with protection against clinical disease (Tully et al., 2005), which requires more detailed analysis in light of recent findings examining TCR diversity in patients with TB. More recent work has re-examined the structural diversity of the TCR repertoire in blood from patients with TB where – in comparison to age- and exposure-matched controls – a restricted TCR-repertoire exists and a broader TCR repertoire was associated with better clinical performance among patients (Luo et al., 2012). In addition, LTBI+ individuals exhibited a preferred usage of certain TCR clonotypes, which was further analysed in a different study where the TCR complementarity-determining region 3 (CDR3), which is responsible for antigen specificity, was shared among individuals in association with HLA restriction (Glanville et al., 2017). This helps elucidating the structural anatomy of the T-cell response in LTBI+ individuals, warrants a more detailed description of TB granulomas at different stages.

Differences in the TCR repertoire may be governed by the sub-anatomy of the TB granuloma as well as infection with different *Mtb* species or re-infection – which occurs more frequently than previously thought (Cohen et al., 2012). The notion that seeding of commonly shared TCR clonotypes in lung tissue occurs early in embryonal development suggests that a pre-wired TCR repertoire and dual recognition patterns with shared TCRs recognising evolutionarily preserved relevant target structures (Pogorelyy et al., 2017) exists from early life while demanding further investigation. The comparison of T-cell clones from twins, humans of different ages and cord blood showed that prenatally-formed T-cell clones persist in the mature organism for decades, providing a 'pre-programmed' immune-receptor repertoire (Pogorelyy et al., 2017). This suggests that the TCR repertoire recognising potential antigenic targets, such as tumour antigens, or pathogens, has been shaped earlier in life. The consequences of prenatal infections or exposure to other target antigens and productive anti-tumour immune responses is now a field of active investigation. Comparative studies of TCR repertoires from the TB granuloma with that from blood in conjunction with HLA allelic selection and expression profiles across patient cohorts in different geographical locations will yield valuable clinical data for mapping key host response patterns and characteristics.

The anatomy in granuloma lesions and lessons from tumour heterogeneity

A fresh look into the functional diversity and anatomy in TB granulomas can be inspired by cancer studies, comprising different subgroups based on immune-reactivity patterns that may also be linked to sub-anatomical areas within the lesion (Gerlinger et al., 2012; Thorsson et al., 2018). The local anti-tumour immune response landscape supports the notion that the spatial relationship of inflammatory cells is vital in conferring *in-situ* protective immune responses (Thorsson et al., 2018). Adaptive cellular immune responses herein may receive 'bystander' activation signals via alternate ligands, molecular mimicry or even 'in-built' cross-reactivity (Whiteside et al., 2018), as recently described for TCR gamma-delta (γδ) exhibiting exquisite antigen specificity as well as a broad recognition of stress signals via TCR germline-encoded motifs (Melandri et al., 2018). Endogenously-produced cytokines such as IL-15 and IL-7 expressed in TB granulomas (Maeurer et al., 1999) can stimulate memory CD8+ T cells and induce an effect comparable to specific TCR ligation, with subsequent production of IFN-γ, perforin and granzyme B (Liu et al., 2002). Thus, the interplay between various immune-cell subsets in the disease microenvironment i.e. interaction of CD8+ T cells and other immune cells, e.g. CD4+, NK cells, Tregs, macrophages and myeloid-derived suppressor cells (Liu et al., 2002) is critical for protective cellular immune responses. Furthermore, spatial proximity of T cells to cancer cells has been strongly associated with increased survival in patients with cancer (Carstens et al., 2017). Similar studies examining the 3D interaction of T cells in TB granulomas with other immune cell subsets, including *Mtb*-infected cells and epithelial cells – given the similarities between TB granulomas and cancerous lesions – warrant deeper insights.

Each TB granuloma appears to be 'unique' concerning the overall cytokine production pattern as well as the different areas within the same lesion, representing a challenge to applying immuno-modulatory HDTs (Ndlovu and Marakalala, 2016). Some TB granulomas may contain dysfunctional T cells associated with chronic antigen exposure and immune exhaustion akin to subpopulations of tumour-infiltrating T lymphocytes (TIL) in cancer (Anderson et al., 2017). The heterogeneity in TB granulomas may also be associated with a simple sample bias of the granuloma tissue area examined, such as described for tumour heterogeneity (Gerlinger et al., 2012; Thorsson et al., 2018). T-cell populations which are already resident in the lung are also of great importance in orchestrating host responses in the LTBI granuloma based on information from other modalities, as described in Table 1.

Table 1
Features of tissue-resident memory (TRM) T cells which may be pertinent to LTBI.

Main characteristics of TRM T cells	Strongly CD103+ CD69+; some exist in CD103- CD69- compartments Comprise 40% of lung T-cell pool; not found in blood (among PBMCs) Produce more perforin and less granzyme B May clear dormant *Mtb* reservoirs if activated/mobilised
TRM T-cell antigen specificity and priming	Local antigen exposure drives TCR diversity of TRM T cells Genetic make-up of individuals plays a bid role i.e. mutational load, pathogen invasion, HLA background Some HLA elements may protect against infection but induce autoimmunity i.e. HLA-B57 is a 'good antigen presenter' for influenza-specific epitope recognition yet it is also associated with drug-induced autoimmunity Certain HLA restriction elements may be associated with superior *Mtb* clearance, thus enabling sterilising immunity in some people – in-depth genetic analysis of clinical samples required
Effect of tissue microenvironment on TRM T-cell biology	Molecular studies have identified 6 distinct immune profiles in cancer lesions: wound healing, IFN-γ-dominant, inflammatory, lymphocyte depleted, immunologically 'silent' and TGF-β-dominant These phenotype-genotype combinations dictate the nature and biology of infiltrating as well as resident immune cells, response to immunotherapy as well as chemotherapy and survival Infecting pathogens also dictate the local immunological signature i.e. EBV is linked to lower B cells and HBV associates with high TCR γδ numbers and IL-17 expression Exaggerated pro-inflammatory cytokine production i.e. TNF-α, IL-6, IFN-γ, IL-18, type 1 interferons and vascular endothelial growth factor (VEGF) induces aberrant neovascularisation and affect immune-cell trafficking and movement within the tissue microenvironment – also shown in TB granulomas The number of PD-1+ tissue-infiltrating and TRM cells – particularly antigen-experienced CD8+ T cells – affects targeted immune responses to cancer and infected host cells alike IL-13, IL-4, IL-5 and TGF-β activate regulatory T-cell subsets, which dampen antigen-specific lymphocyte responses An intricate control of inflammatory responses by TRM subsets in LTBI lesions are likely to dictate whether active disease would ensure. TGF-β upregulation, for instance, is a known characteristic of patients with active TB
Memory phenotypes of TRM T cells	Effector memory cells (CD45- CCR7-) likely to be excellent at mediating disease regression in patients, based on adoptive cell therapy (ACT) studies in cancer Terminally-differentiated precursor-like T cells (CCR7+, CD45RA+) have reduced telomerase activity, shorter telomeres and lack tissue homing capacity i.e. tissue homing chemokine receptor CXCR3 expression CXCR3+ T cells in SCC tissue correlated positively with increased patient survival – also in the presence of pulmonary TB lesions
Other TRM T-cell subsets	Double-negative (DN) T cells may arise from CD4+ or CD8+ T cells May also exhibit a stem cell-like (CD103+, CD95+, CD117+) immunophenotype, consistent with favourable outcomes in cancer and TB TCR γδ T cells, MAIT cells, invariant NK T cells There is already some evidence of these unconventional T cells playing a role in TB immunopathology as well as protection i.e. Vγ9Vδ2 T cells and MR1-restricted MAIT as well as iNKT cells in LTBI. Their role early after infection and during LTBI establishment in humans warrants further elucidation

Changes in the gut and lung microbiomes and how this may influence establishment of LTBI, local granuloma-associated immune responses and or progression to active TB disease is worth investigating. The gut-lung axis is of particularly importance in augmenting immune responses originating in the gut and exerting effects in the thoracic region (Bacher et al., 2019). Integrating the gut and lung microbiome profile for predicting progressors from LTBI and patients with active TB who are likely to respond to therapy must be considered in future clinical studies.

Conclusion

Dissecting the heterogeneity and dynamics of LTBI granulomas will yield a plethora of information regarding important aspects of biology: immune responses, mutational burden induced by *Mtb* infection and an individual's microbiome profile. Cutting-edge technology such as deep sequencing and multiplatform immunological analysis will help develop a wholistic framework to better understand TB pathogenesis and the tenets of host protective mechanisms, which will collectively aid the development of effective HDTs.

Conflicts of interest

The authors declare no conflicts of interest.

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Ethical approval

Ethical approval was not required for this work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2019.02.035.

References

Anderson KG, Stromnes IM, Greenberg PD. Obstacles posed by the tumor microenvironment to T cell activity: a case for synergistic therapies. Cancer Cell 2017;31(3):311–25.

Ankrah AO, van der Werf TS, de Vries EFJ, Dierckx RAJO, Sathekge MM, Glaudemans AWJM. PET/CT imaging of *Mycobacterium tuberculosis* infection. Clin Transl Imaging 2016;4:131–44.

Bacher P, Hohnstein T, Beerbaum E, Röcker M, Blango MG, Kaufmann S, Röhmel J, Eschenhagen P, Grehn C, Seidel K, et al. Human anti-fungal Th17 immunity and pathology rely on cross-reactivity against *Candida albicans*. Cell 2019;176:1–16, doi:http://dx.doi.org/10.1016/j.cell.2019.01.041.

Carstens JL, Correa de Sampaio P, Yang D, Barua S, Wang H, Rao A, et al. Spatial computation of intratumoral T cells correlates with survival of patients with pancreatic cancer. Nat Commun 2017;8:15095.

Cohen T, van Helden PD, Wilson D, Colijn C, McLaughlin MM, Abubakar I, et al. Mixed-strain *Mycobacterium tuberculosis* infections and the implications for tuberculosis treatment and control. Clin Microbiol Rev 2012;25(4):708–19.

Drain PK, Bajema KL, Dowdy D, Dheda K, Naidoo K, Schumacher SG, et al. Incipient and subclinical tuberculosis: a clinical review of early stages and progression of infection. Clin Microbiol Rev 2018;31(4) e00021-18.

Galizia J, Marti MA. Reactive nitrogen and oxygen species: friend or foe in the tuberculosis fight. Tuberculosis 2018;113:175–6.

Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366(10):883–92.

Glanville J, Huang H, Nau A, Hatton O, Wagar LE, Rubelt F, et al. Identifying specificity groups in the T cell receptor repertoire. Nature 2017;547:94.

Goletti D, Lee MR, Wang JY, Walter N, Ottenhoff THM. Update on tuberculosis biomarkers: from correlates of risk, to correlates of active disease and of cure from disease. Respirology 2018;23(5):455–66.

James D, Zumla A. The granulomatous disorders. United Kingdom: Cambridge University Press; 1999.

Liu K, Catalfamo M, Li Y, Henkart PA, Weng NP. IL-15 mimics T cell receptor crosslinking in the induction of cellular proliferation, gene expression, and cytotoxicity in CD8+ memory T cells. Proc Natl Acad Sci U S A 2002;99(9):6192–7.

Luo W, Su J, Zhang XB, Yang Z, Zhou MQ, Jiang ZM, et al. Limited T cell receptor repertoire diversity in tuberculosis patients correlates with clinical severity. PLoS One 2012;7(10)e48117.

Maeurer M, Seliger B, Trinder P, Gerdes J, Seitzer U. Interleukin-15 in mycobacterial infection of antigen-presenting cells. Scand J Immunol 1999;50(3):280–8.

Melandri D, Zlatareva I, Chaleil RAG, Dart RJ, Chancellor A, Nussbaumer O, et al. The γδTCR combines innate immunity with adaptive immunity by utilizing spatially distinct regions for agonist selection and antigen responsiveness. Nat Immunol 2018;19(12):1352–65.

Ndlovu H, Marakalala MJ. Granulomas and inflammation host-directed therapies for tuberculosis. Front Immunol 2016;7:434.

Paquin-Proulx D, Costa PR, Terrassani Silveira CG, Marmorato MP, Cerqueira NB, Sutton MS, et al. Latent *Mycobacterium tuberculosis* infection is associated with a higher frequency of mucosal-associated invariant T and invariant natural killer T cells. Front Immunol 2018;9:1394.

Pogorelyy MV, Elhanati Y, Marcou Q, Sycheva AL, Komech EA, Nazarov VI, et al. Persisting fetal clonotypes influence the structure and overlap of adult human T cell receptor repertoires. PLoS Comput Biol 2017;13(7)e1005572.

Roy Chowdhury R, Vallania F, Yang Q, Lopez Angel CJ, Darboe F, Penn-Nicholson A, et al. A multi-cohort study of the immune factors associated with *M. tuberculosis* infection outcomes. Nature 2018;560(7720):644–8.

Rubin EJ. The granuloma in tuberculosis—friend or foe?. N Engl J Med 2009;360 (23):2471–3.

Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, et al. The immune landscape of cancer. Immunity 2018;48(4) 812–30.e14.

Tully G, Kortsik C, Hohn H, Zehbe I, Hitzler WE, Neukirch C, et al. Highly focused T cell responses in latent human pulmonary *Mycobacterium tuberculosis* infection. J Immunol 2005;174(4):2174–84.

Whiteside SK, Snook JP, Williams MA, Weis JJ. Bystander T cells: a balancing act of friends and foes. Trends Immunol 2018;39(12):1021–35.

Zumla A, Atun R, Maeurer M, Mwaba P, Ma Z, O'Grady J, et al. Viewpoint: scientific dogmas, paradoxes and mysteries of latent *Mycobacterium tuberculosis* infection. Trop Med Int Health 2011;16(1):79–83.