Tuberculosis: an Infection-Initiated Autoimmune Disease?

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Abstract

Tuberculosis is caused by *Mycobacterium tuberculosis* and provided original proof that an infectious agent can cause human disease. However, key steps in tuberculosis pathogenesis remain poorly understood. We propose that autoimmunity is a critical and overlooked process driving pathology in tuberculosis, and present clinical and experimental observations supporting this hypothesis.

Tuberculosis (TB) is the leading cause of death by an infectious disease. Robert Koch identified the causative organism, *Mycobacterium tuberculosis* (Mtb), providing "Koch's postulates" to prove that a disease is caused by an infectious agent. Furthermore, many lines of evidence in humans and mice demonstrate that the immune system is essential in protecting the host against Mtb. Therefore, it seems counter-intuitive to suggest that TB has an autoimmune component. However, diverse evidence suggests that autoimmunity is a critical process exacerbating pathology in TB, leading to cavitation and transmission.

The Host Immune Response Is Essential to TB Transmission

Mtb is transmitted by patients with pulmonary TB. The host immune response is central to containing Mtb, as patients with HIV-induced immunocompromise have a greatly increased TB risk, but conversely also drives the underlying lung pathology. Patients with advanced HIV infection rarely develop cavitation, whereas during immune system reconstitution tissue damage often occurs. Pulmonary disease most frequently occurs in young adults with the strongest immunological response [1]. Similarly, in rabbit models, cavitation is accelerated by pre-sensitisation with purified protein derivative (PPD) to drive a strong delayed-type hypersensitivity reaction, but inhibited by immunosuppression. Immunity is thought to be directed against Mtb antigens, with progressive accumulation of total mycobacterial antigenic load over time precipitating inflammation and lung tissue destruction [2]. However, a striking feature of TB granulomas is the paucity of mycobacteria, while extensive pathology develops. It remains unclear how so few mycobacteria drive such a florid inflammatory immune response.

Diverse Autoimmune Phenomena Occur in Human TB

Physicians treating TB patients observe numerous clinical events associated with autoimmune diseases that are unexplained by current disease paradigms. For example, autoantibodies associated

with autoimmune diseases such as Wegener's granulomatosis and systemic lupus erythematosus are detected in 40% of TB patients [3]. Poncet's disease is an inflammatory polyarthritis occurring in TB patients in the absence of detectable mycobacteria in the joint spaces. In the eye, TB can cause uveitis without mycobacteria, which resolves with anti-mycobacterial treatment. Uveitis is typically associated with autoimmune diseases such as inflammatory bowel disease, Behcet's disease and ankylosing spondylitis. Similarly, erythema nodosum, an inflammatory cutaneous disorder affecting the shins, occurs in TB and autoimmune diseases such as Crohn's disease, ulcerative colitis and sarcoidosis.

Genomic analyses show single nucleotide polymorphisms that associate with TB severity also associate with autoimmune disease, and CTLA-4 autoimmunity associated genotype contributes to TB severity [4]. Additionally, autoreactive T cells are reported to be increased in TB patients. Systemic autoimmunity can be trigged by intra-vesical administration of *Mycobacterium bovis* BCG for bladder cancer. When considered together, these phenomena suggest that mycobacteria induce inappropriate host responses to self-antigens, causing autoimmune inflammation.

Furthermore, experimental studies link Mtb with development of autoimmunity. For example, Mtb antigens are used in Freund's adjuvant in animal models of autoimmune diseases [5], suggesting that these antigens seem to overcome tolerance to host antigens when co-administered. In experimental models, immunisation to Mtb can drive autoimmune arthritis by causing cross-reactivity with proteoglycan in cartilage [6]. Similarly, an acetone-precipitable fraction of Mtb can specifically drive release of the autoantigen proliferating cell nuclear antigen (PCNA) from human cells [7]. Whilst these attributes of Mtb inducing autoimmune phenomena have been extensively used experimentally for modelling of autoimmune diseases, the concept that the same process is occurring in TB patients is not considered.

TB and Sarcoidosis Are Virtually Indistinguishable

Further evidence for autoimmunity TB comes from sarcoidosis, a human disease even considered part of the same disease spectrum as TB by some. Sarcoidosis is an autoimmune disease with no proof of an infectious aetiology, despite extensive investigation for mycobacteria within lesions, that resolves with systemic corticosteroid treatment. Sarcoidosis has several similarities with TB, suggesting a common fundamental process. Firstly, histological analysis shows well-organised granulomas formed from activated macrophages, multinucleate giant cells and peripheral T cells, and lesions are often histologically indistinguishable from TB granulomas (Figure 1). Secondly, both TB and sarcoidosis typically affect the lung upper lobes and the mediastinal lymph nodes. Thirdly, sarcoidosis and TB can affect other organs, including the central nervous system, and neurosarcoidosis and TB meningitis can be clinically indistinguishable. Finally, analysis of peripheral blood gene signatures show highly similar expression patterns [8]. Therefore, similarities between the autoimmune disease sarcoidosis and the infectious disease TB suggest common antigens drive the two diseases. Since sarcoidosis is an autoimmune disease that resolves with corticosteroids, the implication is that similar autoantigens contribute to pathology in TB. Adjunctive corticosteroids are routinely used in treatment of pericardial and meningeal TB and intriguingly, corticosteroids accelerate radiographic resolution in pulmonary TB and potentially reduce TB mortality.

Investigating an Autoimmune Process of Unknown Aetiology

The clinical phenomena presented suggest that the interaction of host with mycobacterial antigens elicits the subsequent development of an additional autoimmune inflammatory process, exacerbating pathology in TB. However, the host antigens involved are unknown, presenting a significant challenge. Nevertheless, the clinical and experimental associations are so strong that ignoring this possibility risks neglecting a central process in TB pathogenesis.

A bioinformatic approach may identify pathways up-regulated in granulomas, and indicate the antigen presenting molecules and T cell receptors most likely involved. One approach could be RNA-Seq analysis of TB granulomas, sarcoid granulomas and suitable control tissues. Analysis TB and sarcoid tissue, combined with protein interaction networks, may indicate pathways induced to inform *ex vivo* analyses. Alternatively, a hypothesis-driven approach may identify antigens. Mtb causes extensive extracellular matrix destruction and cleavage of collagen and elastin releases novel epitopes that may be autoreactive, which have been identified in other destructive pulmonary pathologies [9]. Therefore, investigating T cell responses to lung structural fibrils may be fruitful. Alternatively, bioinformatic approaches suggest cross-reactive T cell epitopes between the Mtb and human proteomes, leading these authors to suggest that autoimmunity contributes to pathology [10]. Additionally, direct evidence of cross-reactivity of antibodies to mycobacterial 65-kDa heat shock protein and human lactoferrin has been demonstrated.

Finally, and perhaps most likely, key antigens may be shared between Mtb and humans. For example, specific lipid antigens are common to both Mtb and the host, such as glycerophospholipids. These antigens include phosphatidylglycerol and phosphatidylinositol, which are presented by CD1d to NKT cells. Phosphatidylglycerol is also presented by CD1b molecules to CD1b self-reactive T cells. CD1-restricted presentation may explain why the HLA associations in TB, although described, are weak, and why mice do not develop typical human pathology, since they lack Group I CD1 molecules. These host lipids may include stress antigens such as cholesterol esters or squalene, presented by CD1c and CD1a respectively, to self-reactive T cells. We hypothesise that host lipids presented by Group I CD1 are the common antigens for TB and sarcoidosis.

Concluding Remarks

Diverse evidence from both clinical observation and animal studies suggests that development of autoimmunity is a fundamental process in human TB. This concept has wide implications and the

evidence is sufficiently strong to justify further investigation. Experimental data rarely challenge established dogma; instead, concepts must evolve and then experiments investigate these hypotheses [11]. Apoptosis of infected cells has recently been shown to induce self-reactive T cells to promote auto-inflammation in other infections [12], and extensive macrophage apoptosis occurs in TB, providing a potential central mechanism. Whilst it may seem implausible that Mtb induces autoimmunity to transmit, such ingenious immunological deception may explain why pathological processes in TB remain so enigmatic.

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Text box: Directions for Future Research

- What is the mechanism whereby *Mycobacterium tuberculosis* induces immune cells to respond to host antigens?
- Do Mtb adjuvants prime a response to bystander host antigens, and if so, what are these host antigens?
- Alternatively, are there antigens that are shared between host and pathogen?
- In addition to auto-immunity related phenomena, does immune dysregulation similar to human auto-inflammatory conditions play a role?
- What are the optimal experimental approaches and model systems to investigate this phenomenon?
    - Unbiased genomic and proteomic approaches
    - Hypothesis driven approaches, such as lung structural fibrils or CD1-presented lipids
    - Autoantibody profiling as a surrogate marker for T cell immunity
    - Human clinical investigation versus tractable model systems
- What novel experimental tools are required, such as for characterisation of CD1-restricted T cell responses in the peripheral circulation and in lung granulomas?
- If mycobacterial infection drives autoimmunity as an evolutionary strategy, what are the implications for novel vaccination approaches?
- Is autoimmune inflammation also the principal mechanism driving the TB-Immune Reconstitution Inflammatory Syndrome that occurs during HIV treatment?
- Will inhibiting autoimmune inflammation, whilst concurrently preserving anti-mycobacterial effector immune responses, reduce mortality of patients with advanced TB?

References

1. Comstock, G.W. et al. (1974) The prognosis of a positive tuberculin reaction in childhood and adolescence. Am J Epidemiol 99 (2), 131-8.

2. Hunter, R.L. (2016) Tuberculosis as a three-act play: A new paradigm for the pathogenesis of pulmonary tuberculosis. Tuberculosis (Edinb) 97, 8-17.

3. Kakumanu, P. et al. (2008) Patients with pulmonary tuberculosis are frequently positive for anti-cyclic citrullinated peptide antibodies, but their sera also react with unmodified arginine-containing peptide. Arthritis Rheum 58 (6), 1576-81.

4. Pagan, A.J. and Ramakrishnan, L. (2015) Immunity and Immunopathology in the Tuberculous Granuloma. Cold Spring Harb Perspect Med 5 (9).

5. Billiau, A. and Matthys, P. (2001) Modes of action of Freund's adjuvants in experimental models of autoimmune diseases. J Leukoc Biol 70 (6), 849-60.

6. van Eden, W. et al. (1985) Arthritis induced by a T-lymphocyte clone that responds to Mycobacterium tuberculosis and to cartilage proteoglycans. Proc Natl Acad Sci U S A 82 (15), 5117-20.

7. Haftel, H.M. et al. (1994) Induction of the autoantigen proliferating cell nuclear antigen in T lymphocytes by a mycobacterial antigen. J Clin Invest 94 (4), 1365-72.

8. Maertzdorf, J. et al. (2012) Common patterns and disease-related signatures in tuberculosis and sarcoidosis. Proc Natl Acad Sci U S A 109 (20), 7853-8.

9. Lee, S.H. et al. (2007) Antielastin autoimmunity in tobacco smoking-induced emphysema. Nat Med 13 (5), 567-9.

10. Chodisetti, S.B. et al. (2012) Potential T cell epitopes of Mycobacterium tuberculosis that can instigate molecular mimicry against host: implications in autoimmune pathogenesis. BMC Immunol 13, 13.

11. Behr, M.A. and Waters, W.R. (2014) Is tuberculosis a lymphatic disease with a pulmonary portal? Lancet Infect Dis 14 (3), 250-5.

12. Campisi, L. et al. (2016) Apoptosis in response to microbial infection induces autoreactive TH17 cells. Nat Immunol 17 (9), 1084-92.

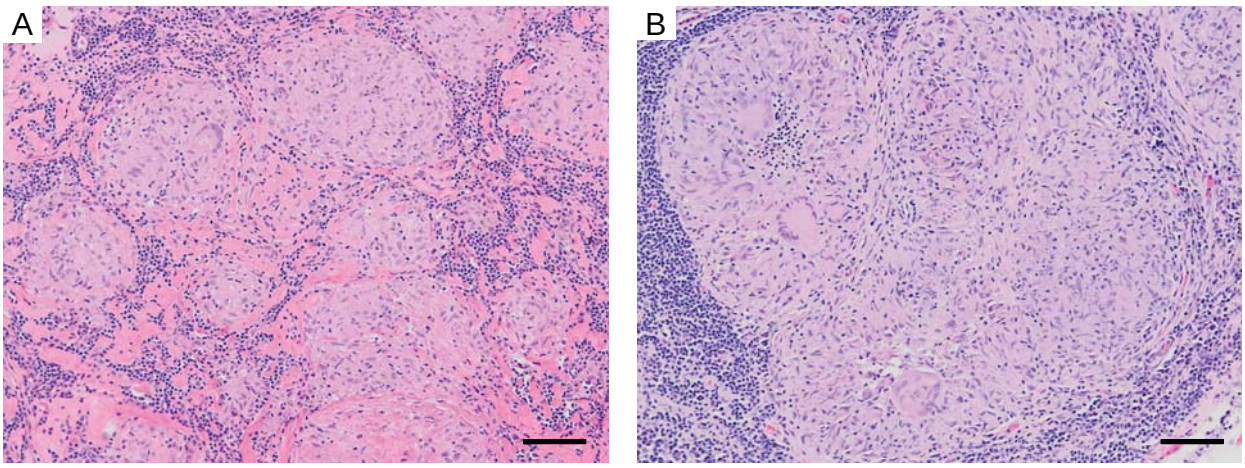

Figure 1: The histological appearances of sarcoidosis and tuberculosis are often indistinguishable. **A)** Lymph node biopsy of sarcoidosis; the patient improved with systemic immunosuppression with corticosteroids. **B)** Lymph node biopsy of tuberculosis; *Mycobacterium tuberculosis* culture was positive and the patient recovered with antibiotic therapy. Scale bar 100µm.

Supplemental table 1: Additional references supporting the hypothesis that autoimmunity occurs in TB

Clinical observations	
Cavitation does not occur in advanced HIV infection, but may during immune reconstitution	Kwan, C.K. and Ernst, J.D. (2011) HIV and tuberculosis: a deadly human syndemic. Clin Microbiol Rev 24 (2), 351-76.
A strong immune response associates with subsequent TB	Comstock, G.W. et al. (1974) The prognosis of a positive tuberculin reaction in childhood and adolescence. Am J Epidemiol 99 (2), 131-8.
Circulating autoantibodies are common in patients with TB	Kakumanu, P. et al. (2008) Patients with pulmonary tuberculosis are frequently positive for anti-cyclic citrullinated peptide antibodies, but their sera also react with unmodified arginine-containing peptide. Arthritis Rheum 58 (6), 1576-81.
	Shen, C.Y. et al. (2013) Autoantibody prevalence in active tuberculosis: reactive or pathognomonic? BMJ Open 3 (7).
Uveitis associates with TB and diverse autoimmune diseases	Cordero-Coma, M. et al. (2010) The value of an immune response to Mycobacterium tuberculosis in patients with chronic posterior uveitis revisited: utility of the new IGRAs. Eye (Lond) 24 (1), 36-43.
Erythema nodosum occurs in TB and several autoimmune diseases	Garcia-Porrua, C. et al. (2000) Erythema nodosum: etiologic and predictive factors in a defined population. Arthritis Rheum 43 (3), 584-92.
Erythema induratum is a vasculitis associated with TB	Cho, K.H. et al. (1996) Erythema induratum of Bazin. Int J Dermatol 35 (11), 802-8.
Poncet's disease is a TB-related culture-negative polyarthritis	Rueda, J.C. et al. (2013) Clinical features of Poncet's disease. From the description of 198 cases found in the literature. Clin Rheumatol 32 (7), 929-35.
Autoimmune-associated genotype is linked to disease severity in TB	Thye, T. et al. (2009) CTLA4 autoimmunity-associated genotype contributes to severe pulmonary tuberculosis in an African population. PLoS One 4 (7), e6307.
Autoreactive T cells are increased in patients with TB	Del Gallo, F. et al. (1990) Increased autoreactive T cell frequency in tuberculous patients. Int Arch Allergy Appl Immunol 91 (1), 36-42.

Anti-PD1 antibody treatment may associate with acute TB	Fujita, K. et al. (2016) Anti-PD1 Antibody Treatment and the Development of Acute Pulmonary Tuberculosis. J Thorac Oncol.
BCG treatment for bladder cancer can elicit autoimmunity	Shoenfeld, Y. et al. (2001) BCG and autoimmunity: another two-edged sword. J Autoimmun 16 (3), 235-40.
TB and sarcoidosis blood transcriptomes are highly similar	Maertzdorf, J. et al. (2012) Common patterns and disease-related signatures in tuberculosis and sarcoidosis. Proc Natl Acad Sci U S A 109 (20), 7853-8.
	Koth, L.L. et al. (2011) Sarcoidosis blood transcriptome reflects lung inflammation and overlaps with tuberculosis. Am J Respir Crit Care Med 184 (10), 1153-63.
TB blood transcriptome is Type I interferon driven	Cliff, J.M. et al. (2015) The human immune response to tuberculosis and its treatment: a view from the blood. Immunol Rev 264 (1), 88-102.
Type I interferons are associated with autoimmune disease	Pascual, V. et al. (2006) Systemic lupus erythematosus: all roads lead to type I interferons. Curr Opin Immunol 18 (6), 676-82.
Corticosteroids accelerate improvement of radiographic changes in pulmonary TB	Smego, R.A. and Ahmed, N. (2003) A systematic review of the adjunctive use of systemic corticosteroids for pulmonary tuberculosis. Int J Tuberc Lung Dis 7 (3), 208-13.
Corticosteroids tend to reduce overall mortality in TB	Critchley, J.A. et al. (2013) Corticosteroids for prevention of mortality in people with tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis 13 (3), 223-37.
Autoimmunity to lung structural fibrils develops in other destructive pulmonary pathology	Lee, S.H. et al. (2007) Antielastin autoimmunity in tobacco smoking-induced emphysema. Nat Med 13 (5), 567-9.
Experimental evidence	
Immunosuppression can prevent cavitation in the rabbit model of TB	Yamamura, Y. et al. (1974) Prevention of tuberculous cavity formation by desensitization with tuberculin-active peptide. Am Rev Respir Dis 109 (6), 594-601.
Mtb antigens are used to drive autoimmunity in other disease models	Billiau, A. and Matthys, P. (2001) Modes of action of Freund's adjuvants in experimental models of autoimmune diseases. J Leukoc Biol 70 (6), 849-60.

T cell responses to Mtb are cross-reactive with host proteoglycans	van Eden, W. et al. (1985) Arthritis induced by a T-lymphocyte clone that responds to Mycobacterium tuberculosis and to cartilage proteoglycans. Proc Natl Acad Sci U S A 82 (15), 5117-20.
Mtb antigens can increase autoantigen expression	Haftel, H.M. et al. (1994) Induction of the autoantigen proliferating cell nuclear antigen in T lymphocytes by a mycobacterial antigen. J Clin Invest 94 (4), 1365-72.
Bioinformatics predict molecular mimicry between Mtb and humans	Chodisetti, S.B. et al. (2012) Potential T cell epitopes of Mycobacterium tuberculosis that can instigate molecular mimicry against host: implications in autoimmune pathogenesis. BMC Immunol 13, 13.
Cross reactivity occurs between human and Mtb proteins	Esaguy, N. et al. (1991) Mycobacteria and human autoimmune disease: direct evidence of cross-reactivity between human lactoferrin and the 65-kilodalton protein of tubercle and leprosy bacilli. Infect Immun 59 (3), 1117-25.
Mtb heat shock protein can induce autoimmune disease	Steinhoff, U. et al. (1999) Autoimmune intestinal pathology induced by hsp60-specific CD8 T cells. Immunity 11 (3), 349-58.
Glycerophospholipids are common to both humans and Mtb	Van Rhijn, I. and Moody, D.B. (2015) CD1 and mycobacterial lipids activate human T cells. Immunol Rev 264 (1), 138-53.
Glycerophospholipids are presented by CD1d	Brigl, M. et al. (2006) Conserved and heterogeneous lipid antigen specificities of CD1d-restricted NKT cell receptors. J Immunol 176 (6), 3625-34.
Glycerophospholipids is presented by CD1b	Van Rhijn, I. et al. (2016) Human autoreactive T cells recognize CD1b and phospholipids. Proc Natl Acad Sci U S A 113 (2), 380-5.
Mice lack group 1 CD1 molecules	De Libero, G. and Mori, L. (2014) The T-Cell Response to Lipid Antigens of Mycobacterium tuberculosis. Front Immunol 5, 219.
Mtb infection of macrophages disrupts host cell lipid metabolism	Lovewell, R.R. et al. (2016) Chewing the fat: lipid metabolism and homeostasis during M. tuberculosis infection. Curr Opin Microbiol 29, 30-6.
CD1c presents cholesterol esters to self-reactive T cells	Mansour, S. et al. (2016) Cholesteryl esters stabilize human CD1c conformations for recognition by self-reactive T cells. Proc Natl Acad Sci U S A 113 (9), E1266-75.
Human CD1 transgenic mice T cell lines become autoreactive after Mtb stimulation	Felio, K. et al. (2009) CD1-restricted adaptive immune responses to Mycobacteria in human group 1 CD1 transgenic mice. J Exp Med 206 (11), 2497-509.
Other infections can upregulate autoreactive T cells via autophagy	Campisi, L. et al. (2016) Apoptosis in response to microbial infection induces autoreactive TH17 cells. Nat Immunol 17 (9), 1084-92.

Mtb causes autophagy	Keane, J. et al. (1997) Infection by Mycobacterium tuberculosis promotes human alveolar macrophage apoptosis. Infect Immun 65 (1), 298-304.
General references	
TB overview	Wallis, R.S. et al. (2016) Tuberculosis-advances in development of new drugs, treatment regimens, host-directed therapies, and biomarkers. Lancet Infect Dis 16 (4), e34-46.
TB proven to be caused by *Mycobacterium tuberculosis*	Kaufmann, S.H. and Schaible, U.E. (2005) 100th anniversary of Robert Koch's Nobel Prize for the discovery of the tubercle bacillus. Trends Microbiol 13 (10), 469-75.
Pulmonary pathology is critical to the Mtb life cycle	Elkington, P.T. and Friedland, J.S. (2015) Permutations of time and place in tuberculosis. Lancet Infect Dis 15 (11), 1357-60.
Cavitation drives transmission	Yoder, M.A. et al. (2004) Cavitary pulmonary tuberculosis: The Holey Grail of disease transmission. Current Sci 86 (10), 74-81.
Mycobacteria are very scare within human TB lesions	Park, D.Y. et al. (2003) Comparison of polymerase chain reaction with histopathologic features for diagnosis of tuberculosis in formalin-fixed, paraffin-embedded histologic specimens. Arch Pathol Lab Med 127 (3), 326-30.
TB and sarcoid are highly similar	Agrawal, R. et al. (2016) Tuberculosis or sarcoidosis: Opposite ends of the same disease spectrum? Tuberculosis (Edinb) 98, 21-6.
Antigen microarrays may be a surrogate for T cell immunity	Yeste, A. and Quintana, F.J. (2013) Antigen microarrays for the study of autoimmune diseases. Clin Chem 59 (7), 1036-44.
TB is characterised by lung destruction	Elkington, P.T. et al. (2011) Tuberculosis immunopathology: the neglected role of extracellular matrix destruction. Sci Transl Med 3 (71), 71ps6.
Protease activity generates novel matrix-derived epitopes	Van den Steen, P.E. et al. (2004) Generation of glycosylated remnant epitopes from human collagen type II by gelatinase B. Biochemistry 43 (33), 10809-16.