REVIEWS

Mycobacterium theory regarding pathogenesis of Takayasu's arteritis: Numerous unsolved dilemmas

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Abstract

Mycobacterium tuberculosis (Mtb) has been implicated in the pathogenesis of Takayasu's arteritis (TA), but there is no direct evidence substantiating the association. Several cases series and laboratory studies provide indirect evidence on the role of *Mtb* and other related species in the immunopathogenesis of TA. This association could be explained by the molecular mimicry between mycobacterium heat shock protein (mHSP 65) and the human homologue (hHSP60) driving immune response in TA. Two different histopathological studies that have evaluated the presence of mycobacteria in aortic tissue have reported contradictory results. This may be due to regional differences in the prevalence of *Mtb*, ethnicity, and study methodology. Recent progress in identifying susceptibility genes and study of *TNFα*-308 gene polymorphism has opened up new avenues for research on mycobacterium theory. Based on the currently available data, three different models have been proposed. Among these, two models favor the mycobacterium theory, while one does not. Transcriptomic and proteomic studies of mycobacteria could help in identifying specific or common traits of mycobacteria that are relevant to the development and reactivation of TA.

Keywords: Takayasu's arteritis, Tuberculosis, Histology, Molecular mimicry, TNF-alfa gene polymorphism

Introduction

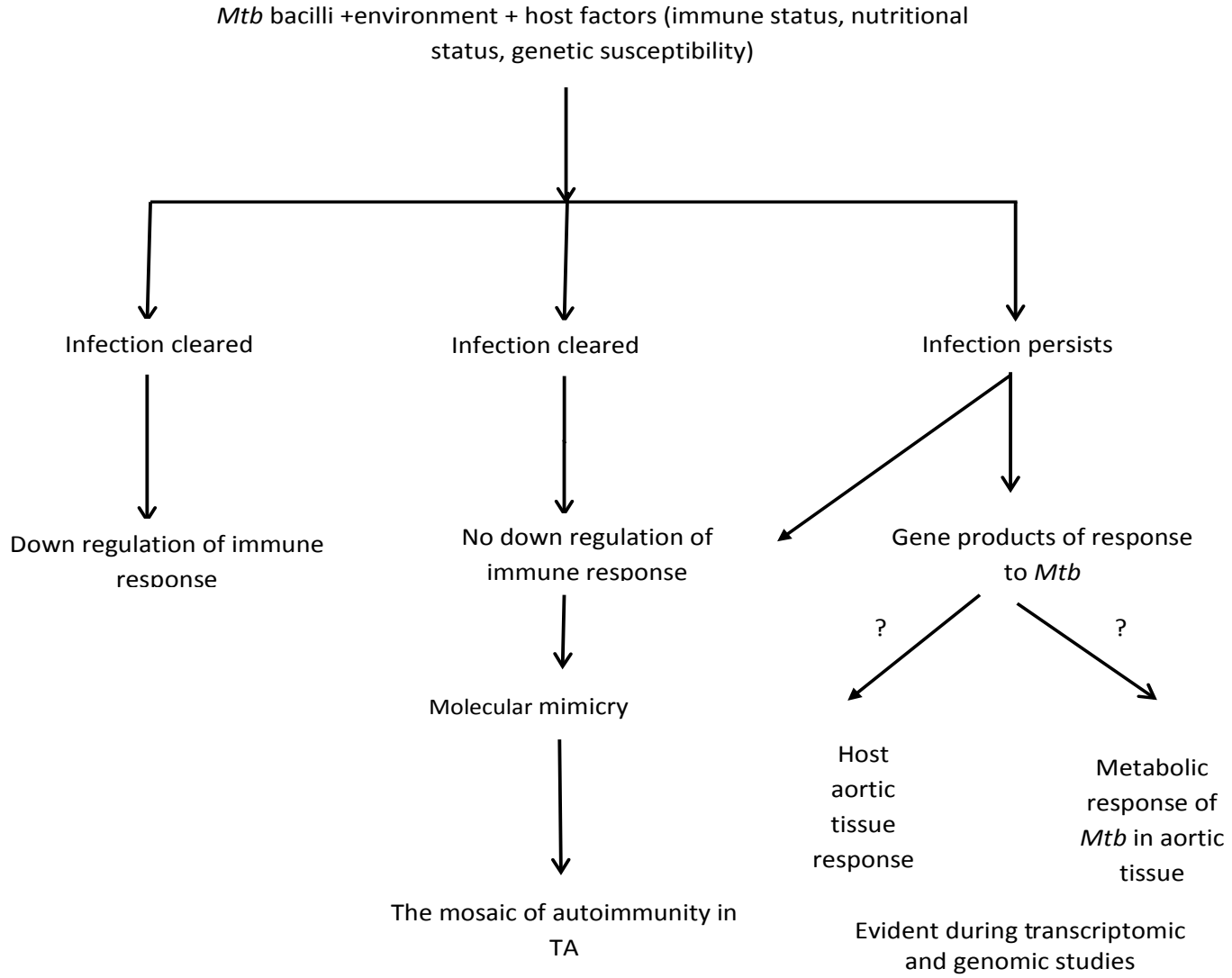
Takayasu's arteritis (TA) is a rare chronic pan arteritis of aorta and its major branches. Although genetic background has been implicated in the predisposition to TA and exogenous factors, particularly *Mycobacterium tuberculosis (MTb)* as possible etiological agents, there is no convincing data to substantiate the findings. This review evaluates the available literature evidence linking mycobacteria infections with the etiopathogenesis of TA.

Case studies suggesting an association between TA and tuberculosis

Possible relationship between TA and latent and active tuberculosis (TB) was first suggested by Shimizu and Sano in 1948.[1] This hypothesis was based on the detection of Langhans giant cells granulomas in the arterial specimens of patients with TA. These granulomas morphologically resembled to those found in the tuberculous lesions. Subsequent research has shown that pseudoaneurysms and vascular damage secondary to abdominal TB were similar to those found in TA.[2] Arteritis of vessels near tuberculosis cavities in the lung parenchyma, termed as Rasmussen's aneurysm, has been demonstrated in patients with pulmonary TB.[3] The placement of mycobacterial theory in the conceptual frame of autoimmunity mosaic in TA is shown in figure 1. Studies done during the last fifty years in Japan, Korea, India, and South Africa have reported either tubercular hypersensitivity or evidence of active TB in patients with TA series (Table 1).[4]

A case series of twenty TA patients from India has found that the chances of having TA in active TB patients were 44.8 times more when compared to general population, in whom the prevalence rate of TB was 1.5 times in the year 1970.[5] A systematic search of the Medline database has identified 9 cases of TA with active TB, mainly in the lymph nodes and lungs and occasionally in the internal organs such as kidneys.[6, 7] One case had a rare form of skin TB (papulonecrotic tuberculide), while another subjects had cold agglutins and cryoglobulins, which disappeared

Fig. 1: The placement of mycobacterial theory in the conceptual frame of mosaic of autoimmunity in TA



MTB = *Mycobacterium tuberculosis bacilli*

after treatment for TB lymphadenitis.[8, 9] One of the most recent cases suggesting the TA-TB association is of a young female with granulomatous colitis. The patient was initially diagnosed to have Crohn's disease, and repeated endoscopic, radiological and histopathological studies suggested a diagnosis of intestinal tuberculosis with associated TA with absent pulses and non-recordable blood pressure in the right upper limb. The diagnosis of intestinal tuberculosis was confirmed by PCR-based, Gen-Probe Amplified *Mycobacterium Tuberculosis* Direct Test (AMTD) system and a positive mycobacterium tuberculosis culture on Lowenstein-Jensen media.[10]

Achievement of symptomatic remission including return of pulses after anti-TB treatment in two of the aforementioned anecdotal cases raises the possibility of a causal (not coincidental) association of TB and TA.[4, 10]

Assessment of latent tuberculosis in TA with tuberculin skin test and interferon-gamma release assays

Several case series on TA from different countries have demonstrated higher frequencies of tuberculin skin test (TST) positivity in TA patients as compared to the general population (Table 1). Amongst the TSTs, Mantoux test is well suited for use in resource poor settings. However, the major drawback is its low specificity in high TB prevalence population.

A retrospective analysis of 88 consecutive patients with TA demonstrated 16 patients (18.2%) with strongly positive

Table 1: Case studies suggesting an association between TB and TA

Cases reported	Year of publication	Country	Percentage of positive tuberculin test	Percentage of cases and site of active TB in TA patients
Case series (n=474)[4]	1961-1977	Japan, Korea, India, Mexico, South Africa	81-100%	21-70%
Anecdotal cases (n=9)[6]	1981-2013	USA, Turkey, India, South Africa, United Kingdom, Yemen		Lymph node, Lungs Kidney, Skin
Anecdotal case (n=1)[10]	2015	India		Pre-existing Crohn's disease with intestinal TB and TA

skin test for TB. However, a thorough investigation revealed active pulmonary TB in only three patients. The diagnosis of TB in the remaining 13 patients was presumptive, based on combination of strong positive skin test and elevated ESR.[11]

The case-control study by Karadaq *et al*. has assessed the possibility of latent TB infection (LTBI) in TA using TST and Quantiferon TB Gold (QFT-G). TST values >5mm for TA patients and >15mm for controls were considered as TST positivity. Although TST positivity was higher in TA group (55 patients (62.5%) vs 24 controls (41.4%), P = 0.008), QFT positivity was similar in the two groups (21 patients (22.3%) vs 24 controls (22.4%), P = 0.005). QFT was negative in two of the six TA patients with previous TB history. The study has concluded that QFT-G may be a useful and favorable test compared to TST in detecting LTBI in cases where anti-TNF treatment is considered for refractory TA.[12]

The concept of molecular mimicry

Diverse mechanisms have been proposed by which infections initiate and/or exacerbate autoimmune diseases. Molecular mimicry is one of these mechanisms, where sequence or structural similarities exist between foreign- and self-antigens.

Heat shock proteins (HSP) serve as early warning and protective mechanism for cells subjected to stress and they are also implicated in folding and managing proteins in the 'Stressed cell'. Studies indicate the role of 60kDa HSP in humans and its homologue mycobacterial 65kDa HSP in the commencement of innate immune response in TA. Increased expression of 65kDa HSP in aortic tissue as well as T-cell and IgG response to both human 60kDa HSP (hHSP60) and mycobacterial 65kDa HSP (mHSP65) has been observed in patients with TA when compared to healthy controls. This finding suggests that the cross-reactivity of immune response between mHSP65 and hHSP or related antigens may be an important cause for developing autoimmunity in TA.[13]

Understanding the pathophysiology of TA

The stimulus triggering the 65kDa HSP expression in aortic tissue is unknown. The stimulus in turn induces the major histocompatibility complex class I chain related A (MIC-A) on vascular cells. Recognition of MIC-A on vascular smooth muscles by the γδ cells and NK cells expressing NKG2D receptors, and subsequent release of perforin causes vascular inflammation.[14] Pro-inflammatory cytokines increase the recruitment of mononuclear cells within the vascular wall. T cells infiltrate and specifically recognize one or few antigens presented by a shared epitope in association with specific major histocompatibility complex alleles on the dendritic cells, the latter being activated through toll-like receptors. Occasionally, Th1 lymphocytes drive the formation of giant cells through the production of interferon-γ. Activated macrophages release vascular endothelial growth factor (VEGF), resulting in increased neovascularization, and platelet-derived growth factor (PDGF) promote smooth muscle migration and initial proliferation. Th17 cells induced by the IL-23 microenvironment also contribute to vascular lesions through activation of infiltrating neutrophils. Corticosteroids suppress the Th17, but not the Th1 response, in both the blood and within the vascular lesions through suppression

of Th17 lymphocytes, but not interfering Th1-promoting cytokines.[15] Cytokines such as IL-6 and IL-18 have been shown to be elevated in the serum of TA patients and there is also evidence of upregulation of mRNA gene expression of TNF-α and IFN-γ in the peripheral blood mononuclear cells of such patients.[16, 17] These results substantiate the use anti-TNF and anti-IL-6 biologics in the treatment of TA.

TNF α-308 promoter gene polymorphism: A possible link between TA and TB

Tumor necrosis factor α (*TNFα*), a proinflammatory cytokine, has been shown to play a pivotal role in host defense against infections and autoimmune diseases.[18] Among the five polymorphisms (at position +691,-308,-851, and -857) of *TNFα* gene, *TNFα*-308 polymorphism is associated with several autoimmune diseases including RA.[19] The genetic variation on position -308 constitutes two allelic forms, i.e. the presence of guanine (G) represents the common variant and the adenine (A) defines the less common one. *TNFα*-308 A-allele displays increased gene transcription as compared to the common allele G. It has been shown to produce 6-7 fold higher levels of TNF-α transcription.[20]

There are only two published studies on *TNFα* promoter gene polymorphism.[18, 21] A study of TA in Han-Chinese population has reported absence of *TNFα* promoter polymorphism in TA patients.[21] On contrary, a preliminary Indian study has showed that G allele at *TNFα*-308 was more common in TA patients and controls, similar to that in other Indian as well as Japanese populations. Compared to the Western population, A allele was relatively less common in study subjects.

Based on these findings, the following hypothesis could be postulated: Patients with *TNFα*-308 with GG allele might be less competent than those with the GA or AA allele in eliciting an immune response against tuberculosis. The persistence of tuberculous antigen in individuals with GG allele may induce the development of autoimmunity. The presence of genetic and environmental risk factors along with autoimmunity could ultimately lead to TA.[18]

Histopathological features

Analysis of morbid anatomic features in TA is mainly based on the specimens excised during autopsy or bypass surgery. Two recently published histopathological studies have reported contradictory results on TB-TA association.

The histopathological study by Arnaud *et al*. has evaluated the presence of *Mtb* in fresh arterial samples, obtained from TA patients, using three different methods: acid fast and auramine-fluorochrome staining, mycobacterial cultures, and nucleic acid amplification. The study did not detect the presence of *Mtb* complex RNA in arterial lesions. Although no direct evidence has been reported by the researchers on the role of *Mtb* in the pathogenesis of arterial lesions, they did not rule out the likelihood of a cross reaction between mycobacterial and arterial antigens.[22]

The second study by Soto *et al*. has evaluated the presence of IS*6110* and *Hup B* genes in aorta of patients, as the former gene sequence helps in identifying the *Mtb* complex and the latter in establishing the differences between *Mtb* and *M. bovis*. The study considered Mexican patients with TA, TB (as a positive control), and atherosclerosis (as disease control). IS*6110* and *Hup B* gene sequences of *Mtb* were found in the aortic tissues of 23 to 33 (70%) patients with TA, in contrast to 17 of 53 (32%, P = 0.004) patients with atherosclerosis, and 27 to 33 (82%) patients with TB.[21] The study supports the hypothesis that TA may occur due to a (latent) TB infection.

The introduction of nucleic acid amplification by molecular techniques is one of the major developments in the diagnosis of mycobacterial infection. The substitution of conventional PCR by the RT-PCR technology aids in simultaneous detection and quantification of a targeted DNA molecule. Two recent studies have highlighted the importance of RT-PCR in detecting *Mtb* in formalin fixed paraffin embedded specimens (FFPE) such as the aortic tissue obtained at surgery or at post mortem.

The study by Lee *et al*. has examined 129 FFPE specimens and performed AFB staining, nested *Mtb* PCR, and real time-*Mtb* PCR. The sensitivity and specificity values noted for the corresponding techniques were AFB staining: 37% and 98.2%, nested *Mtb* PCR: 68.3% and 98.5%, and real time-*Mtb* TB PCR 74.6% and 98.5.[23] An Indian study has reported that the sensitivity and specificity of detecting *Mtb* by RT-PCR were 93.87% and 98.69% respectively, taking positive culture results as reference standards.[24]

Models favoring and refuting the mycobacterium theory regarding pathogenesis of TA

A) Models favoring the theory

An autopsy study on 5760 specimens has found increased

expressions of IS*6110* and *Hup B* genes in aortic tissues of TA patients, attributing that arterial damage may occur due to previous *Mtb* infection. The probable hypothesis put forth by the researchers indicates that *Mtb* might be able to evade the immune response and the aorta represents a highly oxygenated tissue, enabling the bacteria to adapt to the stress generated on the host tissue.[21]

A preliminary Indian study has reported that G allele at *TNFα*-308 was more common in both TA patients and controls. The study proposes that TA patients with GG allele are less competent in eliciting an immune response against TB and eliminating them when compared to those with the GA or AA allele. Moreover, the persistence of TB antigen may predispose individuals with the GG allele to develop autoimmunity.[18]

B) Models refuting the theory

Some of the studies propose that the disease is triggered by loss of tolerance against self-proteins and the innate immune system might be responsible for the initiation and amplification responses.[25]

The extensive sequence homology between mHSP65 and hHSP60 leads to the epiphenomenal cross-reactions mediated by the adaptive immune system. If this postulation is proven, it would support the safe use of anti-TNF therapy in patients with TA.[26]

Future research directions on mycobacterium theory

- Further studies evaluating the role of mycobacterial products causing inflammation in TA should also explore the aortic tissue affinity of the microbe and the local factors that control the immune response.[27]
- Newer methods for rapid identification of clinical mycobacterial isolates such as protein profiling using matrix associated laser desorption ionization time of flight mass spectrometer (MALD-TOF MS) may assist in answering resolving some of the queries related to mycobacterial theory. [28]
- There is little evidence on the *in vivo* physiological and metabolic state of TB bacillus particularly in dormant/ latent/ persistent infections. A novel method called differential probability (DPA) of transcriptome data has shown that the response of bacteria to the macrophage environment is through shutting down of central metabolic pathways and increasing activity in lipid biosynthetic pathways. This could be speculated as an effort to remodel the surface to tackle the host defense attacks. The analysis also indicates that it is possible to deconstruct the response of *Mtb* to the intracellular environment into components that can be studied *in vitro*.[29] Such studies on patients of TA, in whom mycobacterial genes have been demonstrated in aortic tissue, can clarify whether TA results from latent TB infection.

Expert opinion

Compared to the Northern European countries and USA, TA is more prevalent among Asian, African, and Mediterranean populations. Future research should explore the possibility of important differences existing in the aorta and other large vessels and whether they enhance disease susceptibility in patients from Asia, Africa, and Mediterranean regions. Microarray techniques and proteomics are useful in studying the pattern variation within spared and involved aortic specimens in patients with TA. This will help in understanding the microbe/ host interaction in TA, particularly in the context of *Mtb* infection.

Conflict of Interest Statement

All the authors declare that there is no conflict of interest

Funding Statement

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Citation

Chogle AR, Jain S, Kushwaha H. Mycobacterium theory regarding pathogenesis of Takayasu's arteritis: Numerous unsolved dilemmas IJRCI. 2015;3(1):R2.

Submitted: 5 March 2015**, Accepted:** 6 April 2015, **Published:** 14 July 2015

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