Permutations of time and place in tuberculosis

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Summary

Tuberculosis (TB) remains a global health pandemic. The current depiction of the *Mycobacterium tuberculosis* (Mtb) life cycle proposes that airborne bacilli are inhaled, phagocytosed by alveolar macrophages, resulting in formation of a granuloma which ruptures into the airways to re-initiate the infectious cycle. However, this widely proposed model overlooks the fact established 100 years ago that the initial site of Mtb implantation is in the lower zones of the lungs, while infectious cavitary pulmonary disease develops at the lung apices. The immunological events at these two pulmonary locations are different; cavitation does not occur in the bases, only the apices. However, the current conceptual model of TB considers the immunology of these two temporally and spatially separated events to be identical. One key consequence is that prevention of primary childhood TB at the lung bases is regarded adequate immunological protection, but extensive evidence demonstrates that greater immunity may predispose to immunopathology and transmission at the lung apex. Addressing time and place in human TB immunology suggests that much greater understanding of immunopathological mechanisms of TB is required before performing further pre-exposure vaccination trials.

The life cycle of *Mycobacterium tuberculosis* in man

Mycobacterium tuberculosis (Mtb) causes the disease tuberculosis (TB) and is an obligate human pathogen. TB may affect any organ [1], but the life cycle is only completed when it causes pulmonary immunopathology, which drives aerosolisation of bacilli and transmission to a new host [2]. Alberto Ghon identified in 1914 that the initial site of infection after exposure is in the well ventilated lower zones of the lungs [3] and this finding has been confirmed in many subsequent post mortem studies [4, 5]. Even before then, Morgagni and Laennec had identified that active pulmonary TB occurs predominantly in the apical segments of the upper lobes [6], although it may also cause cavities in the apices of the lower lobes. Therefore, the TB life cycle involves an initial seeding event in the well ventilated lung bases, followed by dissemination to a second location at the lung apices, where immunopathology may lead to lung destruction and transmission.

However, the widespread depiction in the scientific literature of the Mtb life cycle suggests that these events all occur at a single location [7-10] (Figure 1). While some simplification can be justified to summarise complex biological phenomena, we feel that this generalization is now hampering research and understanding of the disease. In the TB field, it appears that the recognized syndrome of theory-induced blindness, where data contradicting a long-held theory are dismissed [11], has taken hold. The current model omits key complexities in the host immune response to Mtb that may lead to the pursuit of ultimately flawed therapeutic and vaccination strategies.

The immunological events at the lung base and apex are different

The clinical features of human TB demonstrate that there are key differences in the host immune response to Mtb at the two pulmonary loci. When initially inhaled to the lower zones of the lung, Mtb will cause primary consolidative pneumonia or lymphadenitis in a small proportion of individuals

(less than 5%), most commonly in very young children [12], but cavitating pneumonia almost never develops (Figure 2A). Disseminated TB may occur either as miliary TB with small granulomas throughout both lung fields, or TB meningitis, but neither are associated with pulmonary cavitation. In contrast, from the age of puberty, secondary or reactivation of TB can develop, peaking in early adulthood [13]. This is a very different phenomenon. First, the location is either the lung apices or the apex of the lower lobes [14]. Secondly, progressive tissue destruction occurs, resulting in pulmonary cavity formation (Figure 2B). Histologically, the pulmonary lesions of primary TB differ from post-primary TB, with greater bacterial load, reduced lipid accumulation and an acute inflammatory response in primary disease compared to secondary disease [15]. The clinical spectrum of disease demonstrates that the immunological events at the two pulmonary loci must be significantly different.

In the majority of individuals, the initial primary infection is self-limiting. It is estimated that 90% of those exposed to Mtb will control infection life-long [16], while in approximately 5% of exposed individuals, pulmonary pathology will develop at a later time point at the lung apices resulting in cavitation and transmission. The precise mechanistic events are poorly understood but some inferences can be made from clinical observations. CD4+ T cells play a complex role in the host-pathogen interaction; CD4 T cells are necessary for protection from clinical disease, as manifested by the high incidence of TB in the context of HIV infection, but at the same time are necessary for immune-mediated tissue destruction, since patients with advanced HIV infection very rarely develop pulmonary cavitation [17]. Furthermore, as the immune system reconstitutes with anti-retroviral therapy, immunopathology with lung infiltrates and cavitation often develops [17]. Whilst the current paradigm suggests that granulomas rupture into the airway [8], post-mortem studies suggest that cavities in fact develop in areas of necrotizing lipoid pneumonia, where infected foamy alveolar macrophages predominate, in association with infarction due to vasculitis and bronchial obstruction [15, 18]. The process of cavitation in the lung apices is driven by a very low bacterial load and in association with strong host immunity [15], which contrasts necrotic mouse models where the bacterial load is extremely high [19].

Cavitation typically occurs at the apex of the upper lobes, though can also occur at the apices of the lower lobes. Historically, this regional distribution was thought to be due to differential oxygen distribution [6]. However, alternative hypotheses have been suggested, such as differential lymphatic drainage [20] or reduced blood flow [15]. Enzymes such as the collagenase matrix metalloproteinase-1 must be the final effectors of extracellular matrix destruction that leads to cavity formation [21, 22], and collagen breakdown favours Mtb in the host-pathogen interaction [23]. Therefore, alternative hypotheses for the apical localization would include increased susceptibility to extracellular matrix breakdown, due to reduced availability of plasma antiproteases and the increased susceptibility of collagen under tension to enzymatic cleavage [24], or the modulation of cellular biology by tissue stiffness [25] . Excessive neutrophil influx may exacerbate pathology [26]. Investigation of these competing hypotheses is hindered by the need to study relatively large animal models to investigate cavity formation, such as the rabbit [27]. Therefore, the precise dissection of the underlying mechanisms, and the specific role of T cells in cavitation, has been hindered by the lack of suitable models [28]. Consequently, we do not understand the key mechanisms which lead to disease transmission, nor why over 90% of exposed individuals do not develop such pathology [29].

Implications of distinguishing primary and secondary disease

A current central focus of TB research is vaccination, including both pre-exposure and post-exposure strategies[30-32]. BCG vaccination protects from childhood but not adult TB [33], demonstrating that protection from primary disease does not equate to protection from secondary reactivation of TB. The recent phase 2b trial of the novel vaccine candidate, MVA85A, did not show any efficacy preventing TB infection in infants, but the 37 month follow-up period is insufficient to determine whether the modulation of the cell-mediated immune response will increase the incidence of cavitary pulmonary

TB after puberty. Since 90% of individuals will control infection lifelong, any perturbation of the immunological equipoise that leads to containment of infection and leads to cavitary pathology may inadvertently increase disease incidence. Such an increase in infectious patients has the potential to greatly increase transmission in the longer term [2]. However, this potential harm is currently rarely considered and follow-up periods in clinical trials are too brief to exclude this adverse outcome.

Furthering this concern, experimental data demonstrate that vaccination can worsen immune-mediated tissue damage in the mouse [34], which lacks the key collagenases driving tissue destruction [35]. In the rabbit model, pre-sensitisation with PPD to drive a strong cellular immune response increases the frequency of pulmonary cavitation [36] and perhaps unexpectedly according to current paradigms, immunosupression with azathioprine prevents cavity formation [37]. In humans, Virchow recognised that tuberculin treatment could cause breakdown of pulmonary granulomas [38] and epidemiological studies demonstrate that a stronger Mantoux test in childhood is associated with the subsequent development of pulmonary disease in adulthood [39]. In advanced HIV infection where the inflammatory innate response is decreased [40], cavitation does not occur but may develop during immune reconstitution after antiretroviral treatment [17]. Taken together, these observations demonstrate that an enhanced immune response can drive lung destruction and pathology at the lung apices, but this will only become apparent in early adulthood.

Is prevention of transmission the solution?

Developing a novel pre-exposure vaccine is hindered by the limitations of current animal models, none of which fully reflect the complexity of human disease in man, and the decades of follow-up needed for human pre-exposure vaccination trials. Since we cannot be certain that pre-exposure vaccination will not inadvertently worsen the TB pandemic without prohibitively long studies, alternative approaches should be explored [41]. For example, post-exposure vaccination that prevents

infectious pulmonary TB would break the cycle of transmission. Vaccination of 16 to 20-year-olds with immunological evidence of Mtb exposure would require approximately three years follow-up to determine if such an approach would improve the host immune response at the lung apex, thereby reducing infectious pulmonary disease. This has the potential to reduce the transmission of Mtb and thereby the incidence of childhood TB.

An alternative approach to vaccination would be to combine improved case finding with novel treatment regimes. The TB control strategy of active case finding is well established [42], and in combination with chemoprophylaxis of latent TB is a mainstay of controlling TB in low incidence countries [43]. For the developing world, active case finding could be strengthened by incorporation of novel biomarkers of active pulmonary TB [44]. The development of near-patient assays would revolutionize population screening. Assays could combine both pathogen and host-derived factors such as liporabinomannan and matrix degradation products to identify the aerosol super-shedders who drive the pandemic [45, 46]. A panel of biomarkers may permit assays that can be implemented for screening the entire adult population. The diagnostic test does not require high specificity, only high sensitivity, as the next investigation for an individual with a "possible pulmonary TB" result would be a chest x-ray and sputum smear, which is very specific for pulmonary TB [47]. Novel treatment approaches may incorporate host-directed therapies to reduce pathology and accelerate bacterial clearance [48]. By identifying and treating infectious individuals, this approach would break the infectious cycle and prevent childhood TB, thereby achieving exactly the same goal as pre-exposure vaccination without the concern of delayed adverse events.

Conclusion

Reconsidering long-established features of human TB identifies a fundamental inconsistency between clinical disease and widely-proposed paradigms of TB immunology in the research arena. Primary

TB is a distinct clinical and immunological entity from secondary disease, the form that transmits infection. There is a need to reconsider the conceptual framework of the optimal host TB immune response to incorporate both time and place. One immediate consequence is that complex issues regarding pre-exposure vaccination approaches are apparent and a better understanding of immunological events in primary and secondary TB is urgently required. Approaches such as post-exposure vaccination of young adults and innovative methods of TB case finding and treatment should be a central strategy of future TB research.

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Contributors

PE wrote the first draft of the manuscript and JSF and PE serially edited it.

Conflict of interest statement

We declare that we have no conflicts of interest.

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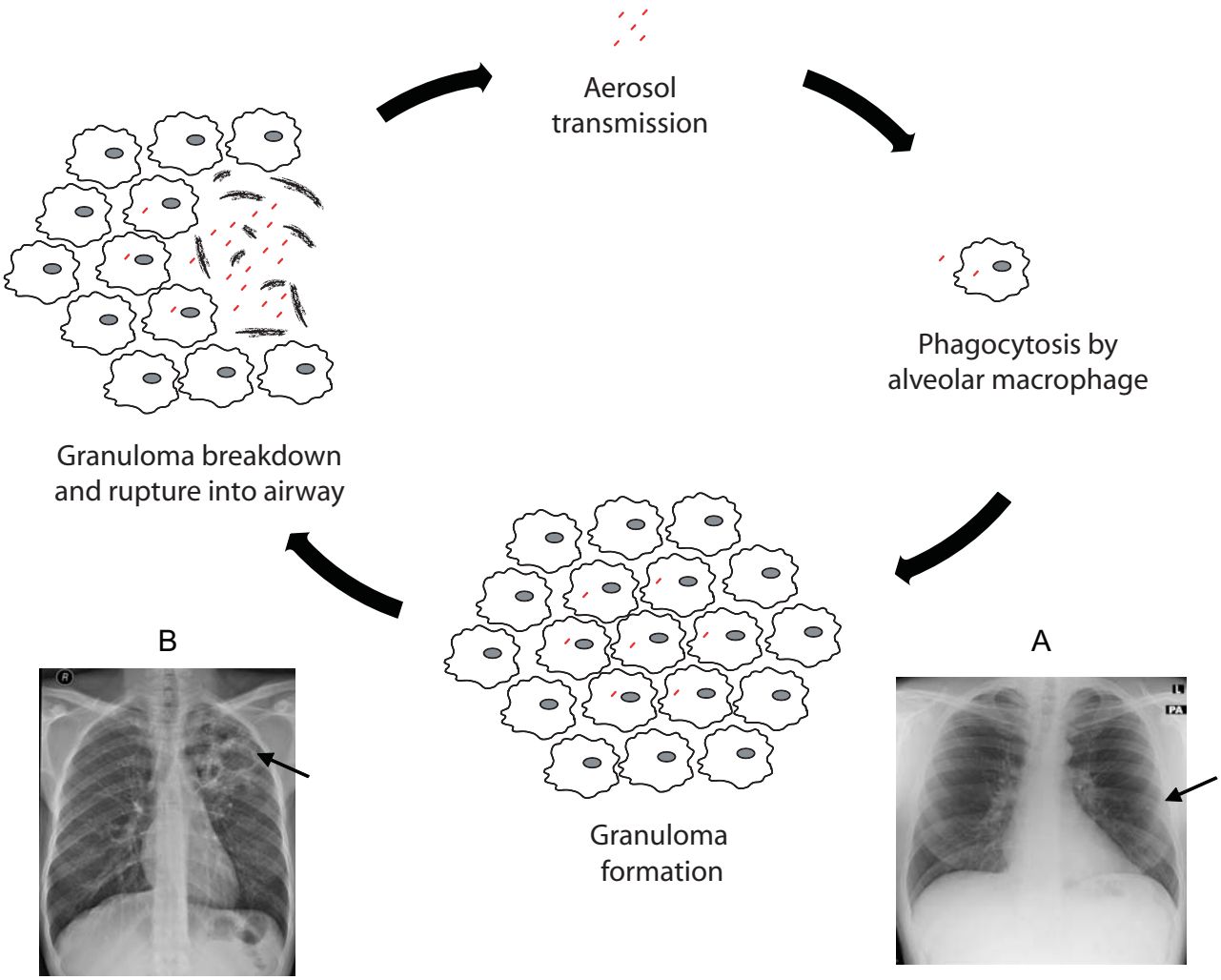
Figure legends

Figure 1: The TB life cycle involves two distinct immunological loci. The widely depicted life cycle of *Mycobacterium tuberculosis* implies all events happen at a single locus. Aerosolised Mtb is inhaled, phagocytosed by alveolar macrophages, a granuloma forms which ultimately breaks down and ruptures into an airway, completing the cycle. However, this neglects the fact that the initial implantation of aerosolised Mtb is the lung bases (A, arrow indicates calcified Ghon focus), while infectious cavitary pulmonary TB occurs at the lung apices (B, arrow indicates cavity). Cavitation does not occur at the site of initial implantation, demonstrating that the immunology of these loci is different, but the current paradigm implies a single immunological process.

Figure 2: Immunological events differ between the lung base and apex. Childhood pulmonary TB causes a primary pneumonia in the lower zones of the lungs, which does not cavitate (A). In contrast, adult pulmonary TB occurs at the lung apices, and causes extensive cavitation (B).



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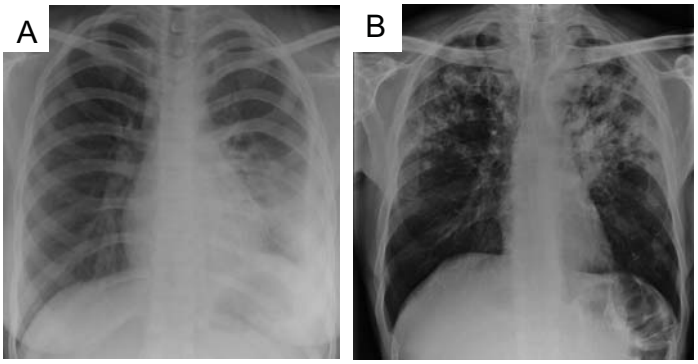

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