Essay #6 06/20/19 Stephanie Fischinger

The search for biomarkers in tuberculosis for progression/ reversion

Infection with *Mycobacterium tuberculosis* (Mtb) results broadly in two different sates: active and latent tuberculosis (Tb). A person with active Tb shows symptoms, bacteria are multiplying, and the individual can infect other people whereas latent Tb is a dormant infection without symptoms. Although both states of tuberculosis are treatable with antibiotics, there are still many difficulties in diagnosing and distinguishing between active and latent Tb, which is due to the complexity of the disease as well as the absence of clear diagnostic markers. Infection with Mycobacterium tuberculosis (Mtb) can lead to different disease trajectories in humans, therefore Tb can present itself as a spectrum of disease[1]. The bacterium is highly infective, and just a few bacteria inhaled into the lung can lead to a manifestation of Tb disease[2]. Although the trajectory of Tb is broadly categorized into two disease-states, it is actually much more nuanced[3]. The research field has acknowledged the differences in the disease spectrum, for example, patients with active disease but no symptoms have been described as having subclinical or incipient Tb. This makes it hard to distinguish between active disease, latently infected individuals, infected individuals who have cleared their bacterial burden, and asymptomatic disease states[3].

Nevertheless, most Tb-infected individuals do not experience symptoms but rather remain in a latent, quiescent state of infection, so called latent tuberculosis infection (LTBI)[4]. The transition from being uninfected to becoming latently infected is called conversion. Here, converters change status in their interferon-Gamma Release Assay (IGRA), such as Quantiferon test (QFT) or tuberculin skin test (TST) test to positive because lymphocytes react to Tb-specific antigens and release detectable IFN-γ[5]. Spontaneous reversion of latent Tb state defined by IGRA or TST testing does also occur, but it's clinical implication remain unclear[6]. Around 10% of latently infected people progress to the active Tb state with manifestation of active disease. In latent and active tuberculosis, the bacteria are thought to be a mix of replicating and non-replicating organisms. In latent disease state, the non-replicating bacteria seem to be predominant and are kept in check by the immune system[7]. Newly infected individuals have a higher chance of progression to an active disease state, possibly because newly infected patients with latent disease have not yet reached a balance between the organism and the immune system[4,8]. After reaching equilibrium, the bacteria can only progress to active Tb if the immune system fails and the replicating population grows to be the majority. Individuals who transition from latent to active disease are classified as progressors with a positive IGRA/TST test as well as a clinical diagnosis.

Until now, there have been no clear markers to predict progression or understand what is needed for the immune system to regain control over active Tb disease. To further dissect the humoral immune profile that is associated with progression or reversion, the Adolescent Cohort Study was employed[9]. Briefly, 6363 healthy adolescents aged 12-18 were enrolled, whereas half the participants were evaluated every 6 months during a 2-year follow up, and the other half was evaluated at enrollment and at 2 years. Individuals who developed active tuberculosis during the study were classified as progressors. Adolescents who got infected and transitioned to a latent stage were included as converters (QFT positive). Reverters were identified as individuals who revert from QFT positive status (latent) to QFT negative and non-progressors as people who don't

progress from latent to active disease. Previous analysis of the progressor cohort has shown that blood transcriptome changes are associated with progression, and a correlation of risk (COR) signature including 16 interferon-stimulated genes (ISG) has been identified to be enriched in progressors up to 18 months before progression[9,10]. Apart from changes in Type I/II interferon signaling and complement cascade, monocyte and neutrophil gene modulations coincided with active Tb diagnosis[10]. Recently, a 3-protein signature was developed on the ACS progressor subcohort consisting of Complement component 9 (C9), creatine kinase M- and B-type (CKMB) as well as C1q tumor necrosis factor-related protein 3 (C1qTNF3)[11].

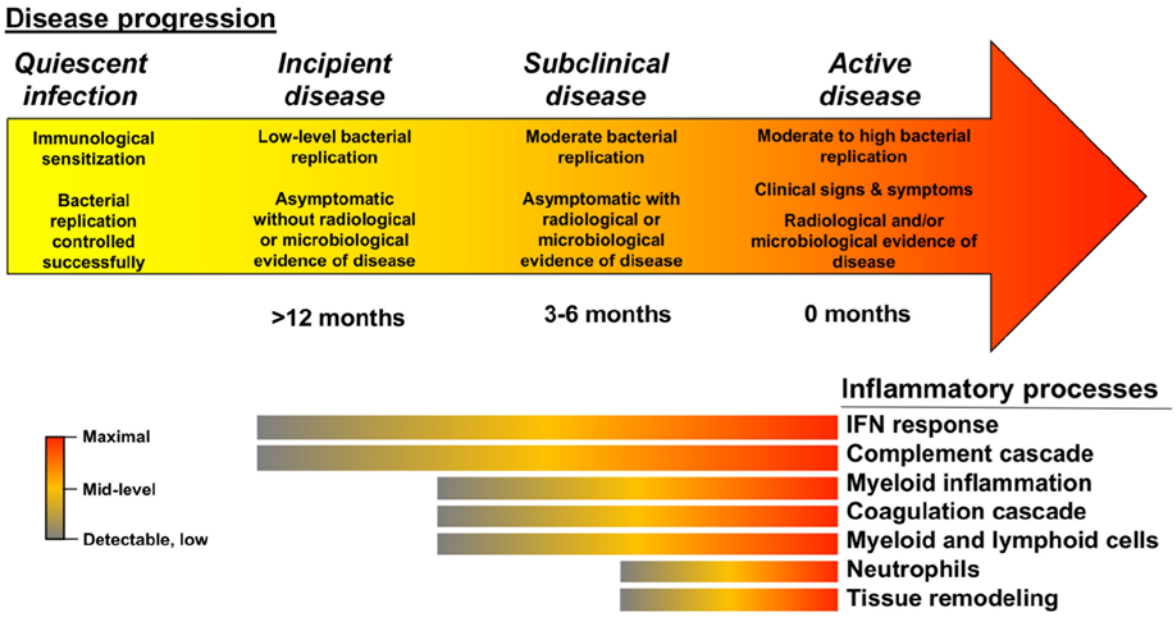

Figure 1: Disease progression of tuberculosis, starting from a silent infection without positive IGRA/TST testing towards active clinically diagnosed Tb disease. Inflammatory processed involving the complement cascade as well as IFN-depended responses by lymphocytes has been associated with disease progression. Adapted from Scriba TJ and Penn-Nicholson A in PLoS Pathogen 2017.

These analyses have been defined based on markers involved in cellular immunity, but very little is known about the humoral profile associated with the TB disease trajectory. Therefore, we want to apply the systems serology approach to decipher the unique antibody profile associated with disease progression and well as QFT reversion. Previous studies have been able to distinguish latently infected individuals and patients with active disease based on antigen-specific non-neutralizing antibody-dependent effector functions[12]. PPD specific antibody-dependent cellular cytoxicity (ADCC) as well as NK cell activation were enriched in latently infected individuals whereas patients with active Tb showed elevated levels of antibody-dependent cellular phagocytosis (ADCP)[12]. Moreover, IgG levels against Tb-specific antigens have been shown to be higher in patients with pulmonary Tb compared to healthy household contacts[13] and in latently infected individuals compared to uninfected persons[14]. These data together with the known changes in cellular immunity give rise to the hypothesis that antibody functional profiles change with Tb disease state. Our longitudinal sample set provides the opportunity to detect potential changes in healthy individuals over time and to generate longitudinal profiles of disease progression and associated antibody profiles.

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