Systems Approach to Understand the Immune Response in Tuberculosis: An Iterative Process between Mouse Models and Human Disease

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Tuberculosis remains a disease of considerable mortality and morbidity. The immune response determining whether individuals infected with the pathogen *Mycobacterium tuberculosis* control the infection, and remain latent, or go on to develop active tuberculosis disease is poorly understood. Our studies used microarray technology to derive blood transcriptional profiles of the host response during tuberculosis, which, combined with data from experimental systems, highlighted a potentially detrimental role for Type I interferons during infection, with important implications for vaccine and therapeutic development. Our studies have also provided candidate biomarkers, which may advance diagnosis and treatment monitoring. These studies thus exemplify the promise of a systems biology approach to understand the immune response to complex infectious disease such as tuberculosis, leading to improved experimental models and systems for improving our mechanistic understanding of why some individuals control the infection whereas others go on to develop active disease.

TUBERCULOSIS IS CAUSED BY AEROSOL INFECTION WITH *Mycobacterium tuberculosis*

Tuberculosis is predominantly a pulmonary disease, although the bacillus can infect and replicate in other organs. Tuberculosis remains a global health problem with reports of 9.4 million new cases and 1.4 million deaths in 2009 (WHO 2010, 2012). The majority of individuals infected with *Mycobacterium tuberculosis* remain asymptomatic, with a third of the world's population estimated to be latently infected with the bacteria (Barry et al. 2009; O'Garra et al. 2013). Of these individuals with latent tuberculosis, 5%–15% will develop active disease in their lifetime (Comstock et al. 1974; Storla et al. 2008). Despite increases in funding tuberculosis research globally, the factors which determine why some individuals control this pathogen, whereas others go on to develop disease, are still poorly understood. This is due to the difficulties in overcoming a number of hurdles presented by this disease. The current test for tuberculosis requires detection of the *M. tuberculosis* organism (currently by culture or by polymerase chain reaction [PCR]) and can only be of use for sputum detection, which is not possible in 30% of pulmonary tuberculosis cases or in extrapulmonary disease. In addition, although the tuberculin skin test, or *M. tuberculosis*-antigen-specific interferon (IFN) γ release assay of blood cells, can detect whether latent individuals have been exposed to *M. tuberculosis*, these assays cannot determine whether an individual with latent tuberculosis has subclinical disease or whether they will progress to active

tuberculosis (reviewed in O'Garra et al. 2013). This is a complex issue due to the heterogeneity of individuals with latent tuberculosis, who may have cleared the infection, may have controlled/persistent infection, or may have subclinical active disease (Barry et al. 2009; O'Garra et al. 2013). Thus these latent individuals can only be defined by immune reactivity to *M. tuberculosis* antigens. However, individuals with active tuberculosis will also react to these antigens and thus clinical symptoms together with culture or PCR detections of the *M. tuberculosis* bacilli, the latter which are not always possible, remain the current ways to determine whether an individual has active tuberculosis (reviewed in Young et al. 2008; Barry et al. 2009; O'Garra et al. 2013). Despite the recent global efforts in funding tuberculosis research, control of tuberculosis worldwide has been hindered by the lack of an effective vaccine (Kaufmann 2012; McShane et al. 2012; Pitt et al. 2013), the emergence of drug-resistant forms of *M. tuberculosis* and the lack of sensitive, specific, and rapid diagnostics (Young et al. 2008). Imperfect diagnostics also impact the monitoring of tuberculosis treatment and the appraisal of much-needed new drugs (Young et al. 2008; O'Garra et al. 2013). In addition, the immune response to *M. tuberculosis* is complex and incompletely characterized, which further impedes attempts to develop new tests, vaccines, and treatments.

Mechanisms determining whether protection or pathogenesis results from *M. tuberculosis* infection remain poorly understood. Tuberculosis results from the interaction between the environment, the host, and the pathogen, and risk factors include HIV coinfection, diabetes, over-

crowding, malnutrition, and general poverty. The immune response to *M. tuberculosis* is multifactorial and host factors such as the cytokines TNF, IFN- γ , and IL-12 have been shown to be required for protection in mouse and man (Flynn and Chan 2001; Casanova and Abel 2002; Cooper 2009; Alcais et al. 2010; O'Garra et al. 2013). Treatment of patients with biologics such as anti-TNF, for the treatment of rheumatoid arthritis or Crohn's disease, may result in reactivation of active tuberculosis in individuals who have previously been exposed to *M. tuberculosis* (termed latent) (Keane et al. 2001) (reviewed in Flynn and Chan 2001; Cooper 2009; O'Garra et al. 2013). This requirement for TNF for the control of *M. tuberculosis* infection had previously been reported in experimental mouse models of tuberculosis (reviewed in Flynn and Chan 2001; Cooper 2009). In addition, IL-12-, IFN- γ -, and the Th1-type responses have been shown to be required for protection against tuberculosis in both mouse models (Flynn and Chan 2001; Cooper 2009) and also in Mendelian susceptibility mycobacterial diseases and some Mendelian susceptibility tuberculosis in humans (reviewed in Casanova and Abel 2002; Alcais et al. 2010; O'Garra et al. 2013). However, collectively, these findings still do not explain why a large number of individuals still develop active tuberculosis, therefore requiring more in depth studies of the immune response to this pathogen (O'Garra et al. 2013). Based on the known host and bacterial factors that influence the outcome of exposure to *M. tuberculosis*, it is likely that combinations of host-genetics and *M. tuberculosis*-strain will be associated with an increased risk of active tuberculosis and/or disease severity (reviewed Casanova and Abel 2002; Alcais et al. 2010; Gagneux 2012; O'Garra et al. 2013).

SYSTEMS APPROACHES USING BLOOD TRANSCRIPTOMICS CAN PROVIDE KEY INFORMATION ON INFECTIOUS DISEASES OF THE LUNG, SUCH AS TUBERCULOSIS

We took an unbiased systems biology approach to determine why, upon infection with *M. tuberculosis*, some individuals remain latent and others go on to develop active tuberculosis. We used an unbiased systems approach to analyze the transcriptome in blood of active tuberculosis patients, diagnosed as culture positive, against individuals with latent tuberculosis, as defined by the tuberculin skin test (TST) and IFN- γ release assays (IGRAs), and against healthy controls. Our approach has provided knowledge in the form of an "immune signature" of the immune response in active tuberculosis and identified potential factors leading to pathogenesis of tuberculosis disease. Our success was based on using strict clinical criteria, sufficient sample size for the initial description of an immune signature, and validating this signature in two additional sets of tuberculosis patients and healthy controls, from intermediate and high burden countries. Using complementary analytical approaches of modular-, pathway-, and gene-level analysis, we identified a striking IFN-inducible

neutrophil-driven signature of active tuberculosis, and a previously unappreciated association with Type I IFN-inducible genes and disease susceptibility (Berry et al. 2010). This IFN-inducible signature significantly correlated with the extent of lung radiographic disease and disappeared during successful treatment (Berry et al. 2010), as early as 2 wk post successful *M. tuberculosis* treatment corresponding to eradication of the tubercular disease in the lung (Bloom et al. 2012). Thus the immune response in blood as measured using a transcriptomic approach can reflect disease and likely the immune response occurring at the site of disease in the lung. These findings present strategies for measuring the immune response during other organ-specific human diseases (inflammatory or infectious), where human tissue is not often available, to provide an understanding of the factors underlying host pathogenesis. Subsequent studies from independent groups, independently confirmed these findings in different geographical regions, using varied blood collection methods and microarray platforms, showing also a robust blood transcriptional signature of active tuberculosis, dominated by overrepresentation of IFN-inducible transcripts (Maertzdorf et al. 2011, 2012; Ottenhoff and Kaufmann 2012), which strengthens the approach considering significant technical differences, diverse ethnicity, differing environments, and presumably infection with varying strains of *M. tuberculosis*.

Increased Levels of Type I IFN Induction during *M. tuberculosis* Infection Contribute to Enhanced Disease

Using pathway analysis we revealed the novel findings that the blood IFN-inducible gene signature of active tuberculosis is represented by Type I IFN, as well as IFN- γ -inducible genes (Berry et al. 2010). The transcriptional signature reflected changes in cellular composition and altered cytokine gene expression in discrete cells. A dominant IFN-inducible gene profile in neutrophils purified from the blood of active tuberculosis patients accounted for this profile in whole blood, suggesting that overactivation of neutrophils by IFNs during infection may contribute to disease pathogenesis in tuberculosis (Berry et al. 2010).

Our findings that the overabundant IFN-inducible gene signature in active tuberculosis, constituting genes downstream from both IFN- γ and Type I IFN- α/β receptor (IFN- $\alpha\beta$ R) signaling, have major implications for the immunopathogenesis of tuberculosis. Although IFN- γ has been shown to be protective during immune responses to intracellular pathogens, including mycobacteria, the role of Type I IFN is less clear. Signaling through the IFN- $\alpha\beta$ R is crucial for defense against viral infections; however, IFN- $\alpha\beta$ signaling has been shown to be detrimental during intracellular bacterial infections (Trinchieri 2010), the mechanism of which is only starting to be understood. However, the role of IFN- $\alpha\beta$ in tuberculosis infection is unclear; many papers suggest a harmful role, although others do not (reviewed in O'Garra et al.

2013). Although IFN- $\alpha\beta$ R-deficient mice show increased survival upon infection with highly virulent *M. tuberculosis* strains, which induce higher levels of Type I IFNs and are more reflective of clinical isolates (Manca et al. 2001, 2005), small or no effect was seen with less-virulent strains of *M. tuberculosis* (reviewed in O'Garra et al. 2013). The pronounced IFN-inducible signature that we have reported (Berry et al. 2010) in the blood of active TB patients, comprising a large number of Type I IFN-inducible transcripts, and correlating with disease severity, provides the first data to support a role for Type I IFNs in the pathogenesis of human tuberculosis disease. This may also explain why the most pronounced effects of Type I IFNs have been observed during infections with virulent isolates of *M. tuberculosis* infection in mice where higher levels of IFN- $\alpha\beta$ are induced (Manca et al. 2001, 2005) or as more recently reported by coinjection of Poly(I)C during *M. tuberculosis* infection which results in Type I IFN-

dependent exacerbation of disease (Mayer-Barber et al. 2011).

Indeed we now show that mice that have elevated the levels of Type I IFN resulting from deletion of the MAP kinase *tpl-2*, which we have shown previously negatively regulates IFN- β production by macrophages (Kaiser et al. 2009), when infected with *M. tuberculosis* (particularly strains resulting in enhanced Type I IFN production) or the intracellular bacterium *Listeria monocytogenes*, show increased bacterial loads (McNab et al. 2013). This was accompanied by increased levels of the suppressive cytokine IL-10. The TPL-2-ERK1/2 signaling pathway regulated production by macrophages of several cytokines important in the immune response to *M. tuberculosis* as well as regulating induction of a large number of additional genes, many in a Type I IFN-dependent manner. The exacerbation of disease and promotion of IL-10 production resulting from bacterial infection in the ab-

Strategy for Elucidating Determinants of Protection or Disease in Tuberculosis:
An Iterative Process between Human Disease and Experimental Models

Figure 1. Integrating systems biology analysis of human disease and experimental models with mechanistic studies to advance our understanding of the immune factors involved in pathogenesis of tuberculosis. Current individual animal models do not always recapitulate all features of human disease; in turn difficulties in sampling from the anatomical location of the infection in humans limit the level of research permissible. Comprehensive profiling tools, such as microarrays, sequencing, metabolomics, and proteomics, allow an unbiased survey of the human response to *M. tuberculosis* in vivo. Comparisons of the blood transcriptome in mouse models of tuberculosis with lung pathology will allow their improvement to more accurately reflect the human disease. Furthermore, studying identical compartments (such as whole blood) in animals and humans will enhance our ability to make direct comparisons between model systems and human disease. Taking advantage of the differences between animal models (e.g., using the zebrafish for studying early innate responses and the nonhuman primate for studying latency) and integrating data from these different systems will also advance our understanding of the immune factors involved in control of *M. tuberculosis* infection and those resulting in chronic disease. Global analysis of the composite immune response in experimental models and human disease can then direct mechanistic studies toward novel areas for the development of therapeutic agents, diagnostic biomarkers, and vaccines. (Modified from O'Garra et al. 2013 and Berry et al. 2013.)

sence of TPL-2 signaling in vivo, resulted from excess Type I IFN production (McNab et al. 2013). This supports previous studies from ours and other laboratories that induction of the suppressive cytokine IL-10 by *M. tuberculosis* can promote chronic infection (Turner et al. 2002; Beamer et al. 2008; Cooper 2009; Redford et al. 2010, 2011; O'Garra et al. 2013). Type I IFN not only enhances the levels of IL-10 during *M. tuberculosis* infection, but also led to decreased levels of the protective cytokine IL-12 (McNab et al. 2013) in part by blocking the activation of macrophages by the protective cytokine IFN- γ , for production of cytokines and control of bacterial load in response to *M. tuberculosis* infection (McNab et al. 2013) and (FW McNab, J Ewbank, A O'Garra, et al., unpubl.). In addition, we also have evidence that coinfection with influenza virus and *M. tuberculosis* results in exacerbated tuberculosis disease and mycobacterial loads in the lung, which could be ameliorated by perturbation of signaling through the Type I IFN- $\alpha\beta$ receptor using knockout mice (Redford et al. 2013). Thus, various mechanisms resulting in enhanced levels of Type I IFN production during *M. tuberculosis* infection may contribute to exacerbation of tuberculosis disease in humans and may also potentially interfere with successful vaccination.

CONCLUSIONS AND IMPLICATIONS

Transcriptional profiling studies of human blood from patients with active tuberculosis, integrated with clinical data and immunological profiling has showed a previously unrecognized role for Type I IFN in the exacerbation of disease. Work in experimental models by manipulation of the strain of *M. tuberculosis*, coinfection with viruses which induce Type I IFN, or perturbation of pathways regulating the levels of Type I IFN all contributed to exacerbation of tuberculosis in mouse models, supporting its role in pathogenesis in human tuberculosis disease. These studies have highlighted that information obtained by using a systems approach to understand the immune response underlying human disease can inform on studies in experimental systems where mechanisms can more readily be dissected. All approaches, complemented by comparisons of publicly available data, highlighted the potentially detrimental role of Type I IFNs in tuberculosis, with important implications for vaccine development and immunotherapies. Incorporation of additional model systems, and further development of analyses of complex data, coupled with mechanistic studies exemplify the promise of systems biology approaches to further our understanding of complex infectious disease (Fig. 1).

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