Innate and adaptive host responses and their genetic control in tuberculosis : studies in Indonesia, a highly TB endemic setting

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TB can be cured with TB treatment for 6 months
Photo of billboard in front of PUSKESMAS DUREN SAWIT Jakarta

Summary and General Discussion

Tuberculosis (TB) is an infectious disease, caused by *Mycobacterium tuberculosis* (MTB) through inhalation of airborne MTB containing droplets. MTB infection does not necessarily progress to TB, as only 5-10% of exposed individuals in endemic areas develop clinical signs and symptoms of TB. Today, MTB has infected one-third of the global population, causing 8 million new cases and 2 million deaths per year, which makes TB the second leading cause of death from a single infectious disease worldwide after AIDS. The extraordinarily high numbers of morbidity and mortality are due to the ability of MTB to evade an effective immune response and to persist within the host for long periods of time. The pathogen MTB is not eradicated, but is contained by the immune system. Though TB can be cured by TB-chemotherapy, the complex and long-lasting treatment means compliance is often incomplete, resulting in a rising incidence of multidrug-resistant (MDR) strains. As the number of TB cases is growing -with HIV co-infection strongly increasing the risk of progression from latent to active MTB infection- and MDR strains are emerging, host responses and genetic markers for disease susceptibility or disease severity need to be defined better.

The host response to MTB depends on the immune status of the host, including concurrent co-infection, the stage of MTB infection, and perhaps also the MTB strain involved. A better understanding of the host response and how MTB modulates this might provide new tools and targets for developing improved strategies to control TB. Given the impact of mycobacterial exposure and the immunoregulatory consequences for host immunity, it is important to study the integrity and the regulation of immune responses and their downstream signaling pathways in TB endemic areas. We therefore performed a large study in Indonesia, since most individuals are likely to be exposed to tuberculous and environmental mycobacteria. With a case detection rate of 285/100,000 per year and >200 million inhabitants, Indonesia is a highly TB-burdened country and ranks third globally in TB cases after China and India. The cohort described in this thesis was carried out in Jakarta, using a case-control study design, including longitudinal cohort. Newly diagnosed pulmonary TB patients were included from an outpatient clinic "Perkumpulan Pemberantasan Tuberkulosis Indonesia" (PPTI), in a poor area in Central Jakarta; as control subjects, healthy individuals were recruited from the same area.

Variation in host immune response to TB: Innate and adaptive immune responses

Human host defense mechanisms are considered key elements in the pathogenesis of and protection from TB disease. An orchestrated series of innate immune pathways and T helper 1 (Th1) dominated adaptive immune pathways, which include activated macrophages, T cells and cytokines especially IFN-γ and TNF-α, are activated following MTB infection. MTB appears capable of interfering with multiple key steps in innate and adaptive IFN-γ dependent immunity. Successful control of MTB infection is dependent on a variety of immunological effector mechanisms that participate in the killing of MTB-infected host cells. In our study population, MTB-specific stimulation of IFN-γ production as well as IFN-γ receptor (IFN-γR) signaling was significantly down-regulated during active TB, correlated with TB disease severity and disease activity, but normalized during microbiological TB cure (**Chapter 2**). We hypothesize that the depression of both IFN-γ production and IFN-γR signaling synergises in contributing to defective host control of MTB infection, at the level of both innate and adaptive immunity. Decreased IFN-γ production had a MTB-specific component, which was reversible, as well as a non-specific component, which did not seem to normalise during treatment: PHA induced IFN-γ production remained lower in TB patients compared with controls, irrespective of treatment status or disease activity. Longer follow-up studies will be needed to substantiate the possible permanence of the decreased IFN-γ production in TB-susceptible individuals. IFN-γ is produced in response to IL-12/23. Although we found IFN-γ production to be low, there was no suppression of IL-12/23 p40 in active TB. The high IL-12/23p40 levels in active TB in the absence of high IFN-γ production suggest that there is active suppression of IFN-γ production, which is not dependent on IL-12/3p40 regulation. IL-12 likely has several different roles in the host response to infection. In contrast to IL-12 and IFN-γ production, there was a slight increase in TNF production during active TB. The shift towards a pro-inflammatory cytokine profile during successful treatment underlines the essential role of $CD4^+$ T-cells in TB protection. Furthermore, the IFN-γ/IL-10 ratio is increased during TB cure, providing a potential new biomarker profile of curative host responses.

Helper $CD4^+$- and cytotoxic $CD8^+$ T -cells secrete not only IFN-γ but can also express granulysin, as an effector molecule in host defense mechanisms. Granulysin, co-localized with perforin and granzyme-B in cytolytic T-cell granules, has a broad antimicrobial spectrum, being able to kill bacteria, fungi and parasites as well as tumor cells in vitro. Granulysin is able to kill extracellular MTB directly, and also intracellular MTB when in the presence of perforin. Concentrations of granulysin measured in the plasma of active TB patients were found to be low during acute disease, to increase to normal levels in the stage of convalescence, and to rise to exceed concentrations in control subjects after completion of TB therapy. The increased concentration of circulating granulysin in plasma likely is functionally relevant, presumably reflects the activity of cytotoxic T-/NK-cells and represents a new soluble biomarker of (innate and/or adaptive) cellular immune activity to MTB (**Chapter 3**). It is not clear whether or when the increased levels of circulating granulysin would decrease back to the value of control individuals following TB cure. Additional follow-up studies are necessary to obtain better insight into the dynamics of plasma granulysin levels. Interestingly, circulating IFN-γ levels in the plasma were detected in active TB, which were significantly higher than those after therapy or in controls. The circulating levels of IFN-γ in the plasma may result from the local production and spill-over of IFN-γ levels in the plasma from activated lymphocytes sequestered at the site of MTB infection or alternatively be produced by NK-cells (**Chapter 3**). Taken together, chapter 2 and 3 show that type-1 cytokines, IFN-γ / IL-10 ratio's and granulysin levels may provide new biomarker signatures of TB disease and curative host response in TB. Our results for the first time also show that there is a systemic depression of IFN-γR signaling during active TB.

Variation in genetic background

TB is the manifestation of the outcome of a complex crosstalk between the human host, the microbe and its environment. Unraveling of such complex interactions by studying host responses and genetic determinants of susceptibility to disease and manifestations of disease has proved challenging. Numerous studies, however, have produced evidence for host genetic factor in the control of TB, but contradictory results have been reported from one population to another, since particular alleles/haplotypes were not

consistently associated with protection or disease predisposition in different ethnic background. We have explored several genetic markers that have been identified to affect susceptibility to TB, including the genes that encode type-1 cytokines and type-1 cytokine receptors such as *IL12B*, *IL12RB1*, *IFNG* and *IFNGR1*. Mutations in genes of the IL-12/23 / IFN-γ axis are known to cause extreme susceptibility to infection with environmental mycobacteria, and subtle variations in these genes may influence susceptibility to more virulent mycobacteria such as MTB. In our study population, we have detected nine known single nucleotide polymorphisms (SNPs) and two new silent variations e.g. 135G>A (S45S in exon 3) and 1056C>T (N532N in exon 10) in the *IL12RB1*. The *IFNGR1* allele CA_{12} and genotype CA_{12}/CA_{12} were associated with protection from pulmonary TB (**Chapter 4**). Interestingly, *IL12B* (ins/del) promoter heterozygosity was associated with protection from TB in BCG vaccinated individuals. This could indirectly support the role for IL-23 in generating memory T cells and for IL-12 in optimal immunity against TB, since these heterozygous individuals produce indeed higher levels of IL-12p40, an essential component of both IL-12 and IL-23, in response to LPS. This observation is in line with the notion that developing adequate memory in response to BCG vaccination is IL-12p40-dependent.

Next to the IL-12/IFN-γ axis, we were interested to explore genes involved in the innate immune recognition of MTB. Host cells sense the presence of MTB by using multiple recognition systems in which different classes of receptors interact with each other. Recognition of MTB by pattern recognition receptors (PRR) is crucial for activation of both innate and adaptive immune response. PRR are key components in determining the outcome of infection. In our large scale genetic studies, Toll like receptor (TLR) 8, DC-SIGN, complement component and scavenger receptor were found to be associated with TB susceptibility (**Chapter 5**). Natural resistance associated macrophage protein (NRAMP1), a metal transporter protein, is believed to play a role in iron transport in macrophages and increases the susceptibility to infection in other populations as described in e.g. a study in The Gambians. *NRAMP1* polymorphisms from our study were, however, not associated with susceptibility to TB (Chapter 6). *NRAMP1* polymorphisms show a large difference between populations. Moreover, the distribution of *NRAMP1* alleles in our study is different compared to other study in Asia. Distribution of alleles may differ between continents and also between

populations on the same continent, which may reflect differences in selective pressure in the past. A large-scale association studies from various populations are essential to more precisely elucidate the true association between polymorphisms and susceptibility to TB.

Variation in disease presentation of tuberculosis

Much of the considerable variation in outcome to exposure and infection with MTB and also in TB disease severity can probably be attributed to a complex interplay between the host, the pathogen and environmental factors. TB can present in the clinic with various degrees of severity, for example pulmonary TB and extrapulmonary TB. In our study we have included only pulmonary TB. The disease severity in pulmonary TB can be graded from mild, moderate to advance TB, based on chest X-ray radiography (CXR). CXR reading is however difficult to assess as the quality of radiographs as well as the interpretations of the radiologists who read the CXR may differ.

Disease severity can also be predicted by analyzing clinical or hematological data. For example, TB is associated with malnutrition as evidenced by lower body mass index; anaemia is found in >60% of TB cases. Interestingly, erythrocyte sedimentation rate (ESR) -which is commonly used in the clinic in Indonesia because it is easy, rapid and cheap- and C-reactive protein (CRP) seem to be sensitive inflammation predictors and also associated with disease severity (**Chapter 2**). In TB endemic areas where many other infections are common, one should bear in mind that anaemia can result not only from malnutrition or iron deficiency but also from infections. Additional (and cheap) measurements such as ESR should be sufficient to exclude infection cases before prescribing iron supplementation. Clearly, the anaemia described in chapter 6 is mainly the result of anaemia of chronic disease, in this case TB, more than of iron deficiency. Iron supplementation for TB patients is not necessary, because iron parameters improved towards control values and haemoglobin levels normalized after successful TB therapy even without iron supplementation. Therefore, iron supplementation, which is often prescribed by medical doctors in primary health care in Indonesia for anaemia cases, should be restricted for those who have the highest prevalence of iron deficiency, e.g. children and women in the reproductive age.

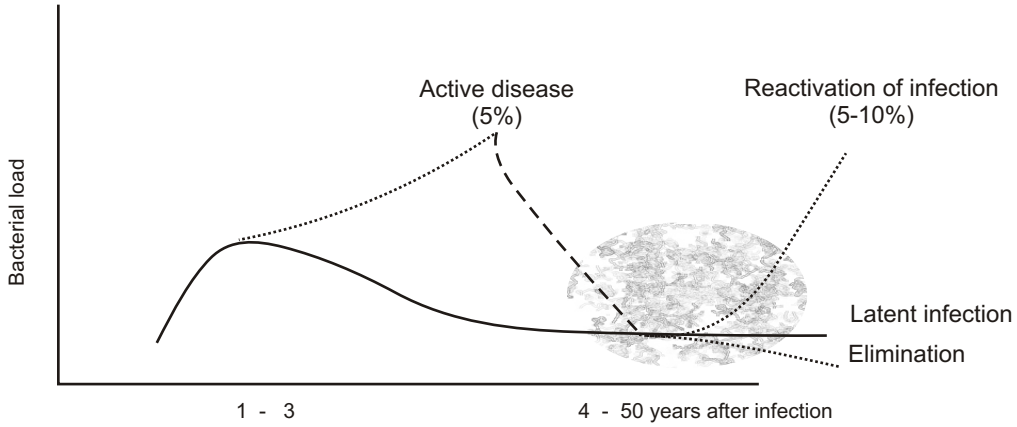

Figure 1. The development of MTB infection. In the vast majority of individuals who are infected by MTB latent TB will develop. Individuals in this latent phase are a potential reservoir for MTB transmission as 5-10% of these may reactivate to active TB later in life. The grey area is an interesting study area and subject for follow-up study.

The global epidemic increase of type 2 diabetes (DM) has reached also developing countries, where TB is endemic. Type 2 DM has become one of the risk factors for TB. In our study, TB with concomitant type 2 DM presented with a lower Karnofsky-index and more symptoms, especially with more frequent weight loss. However, there was no evidence for more severe disease based on blood testing, bacteriological or radiological examinations (**Chapter 7**). Interestingly, DM has a negative effect on the outcome of TB treatment with 22.2% of cultured sputum specimens were still positive for MTB, underlining the need to improve the care for TB patients with concomitant DM. As type 2 DM is present in 14.8% of our TB patients in Indonesia screening fasting blood glucose in TB patients, in particular for those above 35 years, is clinically important.

FUTURE OUTLOOK

The initial phase of MTB infection is characterized by rapid bacterial growth. About 5% of infected individuals develop primary disease in the first years after infection (Figure 1). In the vast majority, the formed granulomas can contain MTB infection, probably in a state of dormancy, in which MTB is deprived of oxygen and nutrients. This results in a latent stage of infection with minimal bacterial replication phase. As 5-10 % of latently infected individuals will reactivate to active TB, individuals in the latent phase are a potential reservoir for transmitting MTB later in life. As latent TB infection constitutes a chronic infection, the immune system is chronically exposed over a period of many years, a situation that has important implications for the generation and maintenance of T-cell memory. How this chronic infection shapes the immune response and its dominance repertoire is an important question that remains unsolved. Why and when susceptible individuals will develop TB disease due to reactivation of latent infection should be studied in longer follow-up studies. Knowledge of the host response in individuals with latent phase infection may help to identify protective antigens and protective immunity. This will help to develop post-exposure vaccines to prevent reactivation of TB, and to facilitate diagnosis of LTBI. Our studies have raised several questions that need to be addressed in follow-up studies investigating the dynamics of host responses towards TB. For example, the dynamics of PHA-induced IFN-γ production did not seem to normalize during treatment (**Chapter 2**), and granulysin levels following TB therapy remained unexpectedly high above the level of controls following treatment (**Chapter 3**). With additional longer follow-up studies we might substantiate the possible permanence of the decreased IFN-γ production in TB-susceptible individuals, and asses whether the low granulysin levels observed in active TB precede or result from active infection. In addition, it will be of interest to determine whether control individuals with low granulysin and IFN-γ levels have a higher risk of acquiring TB. Moreover, follow-up studies of household controls who have lived with TB patients during active disease manifestation might add to the knowledge of the latent phase of TB infection. Analysis of host responses may provide new information that can be further used for better TB interventions. Currently, we are planning follow-up studies to answer these new research questions.

To conclude, immunological studies of well-defined patient populations representing the natural history of TB will likely answer several of these questions and help to better define protective immunity. The long-standing collaborative studies between the Netherlands and Indonesia have addressed various aspects of tuberculosis in a TB-endemic setting, e.g. clinical epidemiology, immunology, immunogenetics, microbiology and pharmacology. Further fruitful research collaboration is needed to strengthen the quality of this research, and to address these important problems related to TB in Indonesia. These studies will have implications for both vaccine development and diagnosis of LTBI and TB disease.