EFIS Lecture

Immune response to tuberculosis: How to control the most successful pathogen on earth

Stefan H.E. Kaufmann

Max Planck Institute for Infection Biology, Department of Immunology, Berlin, Germany

Correspondence to: kaufmann@mpiib-berlin.mpg.de

Keywords: tuberculosis, vaccination, biosignature, macrophage, neutrophil

Abstract

Tuberculosis (TB) remains a major health threat and general agreement exists that better control measures are needed. These include better diagnostics, drugs and vaccines. In particular, vaccines will be critical for better TB control. Based on knowledge about protective immunity against TB, a vaccine was created, VPM1002, which shows high protective efficacy and safety in experimental animal models. The vaccine has proven safe and immunogenic in human adults and neonates and is currently assessed in clinical trials in the context of HIV exposure. As a next step, a phase III efficacy trial in adults and a phase IIb efficacy trial in neonates are being planned. Biosignatures that differentially diagnose TB disease versus latent infection with high sensitivity and specificity have been designed. Most recently, a prognostic biosignature which predicts progression from latent infection to active TB has been identified. Biosignatures are not only of great value for improved diagnosis and prognosis of TB, they can also provide guidelines for better understanding of molecular mechanisms underlying disease. Accordingly, distinct biomarkers of diagnostic and prognostic value but of unknown biological function are being characterized functionally. In this way, deeper insights have been obtained on the role of type I interferon and of neutrophils in TB in experimental animal models of TB. In conclusion, clinical and basic research further supported by computational biology can complement each other in the pursuit of knowledge-based development of improved intervention measures for TB control.

According to a report published in the highly prestigious science journal Nature in 2013 (1), over the last two centuries, more than one billion people have succumbed to tuberculosis (TB) – more than to smallpox, malaria, the plague, influenza and acquired immunodeficiency syndrome (AIDS) together, in this period of time. To place this in today's context: the most recent health crisis caused by the Ebola virus could be brought under control after less than 30,000 people had fallen ill and less than 12,000 had succumbed (2). Compare this with 180,000 new cases and 30,000 deaths due to TB in a single week, which adds up to 9.6 million new cases and 1.5 million deaths in 2014 (3). Clearly, TB is not a disease of the past, but a viable threat of today. Accordingly there is good reason to call *Mycobacterium tuberculosis* (Mtb) the most successful pathogen on earth.

The Ebola epidemic was a tragedy, not only from a humanitarian but also from an economic standpoint (2). Accordingly it has served as a wake-up call for improved global health security against communicable diseases in general which had been claimed conquered too often in the past. In economic terms, Ebola caused a reduction of 12% of the gross domestic product in the afflicted areas in Western Africa, amounting to a loss of at least 1 billion USD (2). Similarly, TB is costly ranging from 2 billion USD per year, if only drugs are considered in the equation, to nearly 20 billion USD if all medical costs are included, and to 100 billion USD if loss of human capital is added (4). Some 5 billion Euro are lost in the European Union alone due to TB (5). In view of this, the proposal of the StopTB Partnership on behalf of the World Health Organization to reduce morbidity and mortality caused by TB by the year 2035 to 90 or 95%, respectively, compared to 2015, appears highly ambitious (6). Yet for both humanitarian and economic reasons there is no way around the need to improve the situation markedly. So the question is not whether we should act, but rather what needs to be done to ameliorate this devastating situation.

General agreement exists that we first need to raise the standards of health delivery systems in resource-poor regions so they become as functional as they are in resource-rich regions (2). Second, we need better interventional tools, notably better drugs, diagnostics and vaccines (7). Here, biomedical research can make a difference. Accordingly, we have embarked on knowledge-based design of a novel vaccine and of diagnostic and prognostic biomarkers for TB (8-10). During our journey towards better intervention measures we soon realized that not only clinical application benefits from basic research but also that clinical studies can fuel hypothesis-driven basic investigations (11;12) (Figure 1). In the following, I will first describe how we brought forward a vaccine against TB from the immunologic drawing board into the clinical trial pipeline; second, how we identified biosignatures for TB diagnosis and for prognosis of future risk of progressing to active TB disease in individuals with latent TB infection (LTBI); and third, how we harness our knowledge about biomarkers for better understanding of basic mechanisms underlying pathogenesis of TB.

Immune response to tuberculosis

Immunity to TB is driven by T lymphocytes as mediators and mononuclear phagocytes as effectors (13-15) (Figure 2). After inhalation, Mtb enters the alveoli where it is taken up by macrophages and dendritic cells (DCs) which transport the bacteria to draining lymph nodes where antigen-specific T lymphocytes are stimulated. Some Mtb organisms enter the lung parenchyma where they initiate a focus of inflammation. Antigen-primed T cells enter the lung parenchyma 3 weeks after their initial encounter with Mtb, at the earliest. They sense inflammatory signals emitted by macrophages which have accumulated around Mtb in first attempt to contain it. Through the coordinated activation of macrophages at inflammatory sites by T lymphocytes, a granuloma develops, which comprises T cells and mononuclear

phagocytes as well as neutrophils, DCs and B cells (16-18). A solid granuloma is formed which contains Mtb in a dormant stage, but does not eradicate it. To improve containment, a fibrous wall is formed around the lesion. Solid granulomas can persist over years in healthy individuals with LTBI. Disturbances in the tight immune regulation can lead to cell death and necrosis. A necrotic granuloma has formed. Further cell damage leads to caseation of the granuloma, which now fails to control Mtb. The pathogen starts to become metabolically active and grows in the necrotic detritus in the center of the lesion (16). Moreover, rupture of the lesion into the alveolar space allows for dissemination into the environment and access to the blood stream promotes dissemination to other organ sites. Active TB disease has developed and the patient is contagious. Each granuloma acts as an independent entity, i.e., solid granulomas still capable of containing Mtb and causing minimal mutilation to the host and caseous granulomas where Mtb flourishes and tissue damage is high coexist in a single patient (16;19).

While the general mechanisms of protection and pathogenesis in TB are understood, our knowledge of the fine details is still incomplete. From the perspective of vaccine-induced long-term protection, T cells are of particular interest (Figure 3). In TB, both CD4 and CD8 effector T cells are generated implying that antigens from Mtb are processed through the major histocompatibility complex class II (MHC II) and MHC I pathways. Within host cells, Mtb primarily resides in a phagosome, allowing access to MHC II processing. Yet, evidence has been presented that Mtb can egress into the cytosol where its antigens can be introduced into MHC I processing (20). However, other mechanisms including crosspresentation and xenophagy are likely involved as well in efficient stimulation of both CD4 and CD8 T cells (21-23). The predominant T cell population required for protection are Th1 cells producing cytokines such as interferon gamma (IFN- γ), tumor necrosis factor (TNF)

and interleukin (IL)-2, and current knowledge holds that IFN- γ is the major inducer of tuberculostatic activities in macrophages. In addition to type 1 T helper (Th1) cells, Th17 cells are generated during the early inflammatory stage of TB, which seem to transiently pave the way for the buildup of protective immunity.

CD8 T cells also produce type 1 cytokines and in addition have been shown to kill Mtb-infected macrophages as cytolytic T cells (14). By means of perforin and granulysin they also kill Mtb directly (24). In addition to these conventional T cells, unconventional T cells are likely involved (25), including mucosal-associated invariant T (MAIT) cells specific for vitamin B2 derivatives presented by MR molecules, which are preferentially located in the airways (26); CD1-restricted T cells, which preferentially recognize glycolipids in the context of CD1 molecules (27), and gamma delta ($\gamma\delta$) T cells with specificity for small pyrophosphate-containing metabolites (28). These nonproteinaceous antigens are abundantly produced by mycobacteria supporting the notion that unconventional T cells also participate in immunity in TB.

CD4 and CD8 T cells develop into memory T cells which can be subdivided into central memory (T_{CM}) and effector memory T (T_{EM}) cells (29;30). The T_{CM} preferentially reside in lymphoid organs whereas the T_{EM} favor tissue sites. In various animal models the more recently defined tissue-resident memory (T_{RM}) cells have been identified as critical mediators of protective immunity in the lung (31). A vaccine that promotes long-term protection needs to target memory T cells, which guarantee the long-term containment of Mtb in solid granulomas.

On the side of effector cells, mononuclear phagocytes are of particular importance for Mtb containment (15). However, even after IFN- γ activation, some Mtb can persist in these

phagocytes, which therefore serve as both effector and as sanctuary. Neutrophil influx has been associated with exudative responses and hence they are considered harmful in TB (32). More recently, myeloid-derived suppressor cells (MDSCs), both of granulocytic and of monocytic origin have been identified in TB, which are thought to impair protective immunity (15;33;34). Further impairment is caused by regulatory T cells (Treg), which are frequently found in TB (35).

The vaccine pipeline

The first vaccine against TB was introduced into clinical practice in 1924 (36). Bacille Calmette–Guérin (BCG) is an attenuated strain of the causative agent of bovine TB. BCG is the most widely administered vaccine worldwide with more than 4 billion doses. It protects partially against severe extrapulmonary forms of TB in neonates and infants, but its protective efficacy against pulmonary TB, notably in adolescents and adults, is weak (37;38). Since pulmonary TB is the major source of spread of this disease, a vaccine that prevents pulmonary TB would not only benefit the individual vaccinee but also the population at large by reducing TB dissemination (7).

Although it is generally agreed that a better TB vaccine is urgently needed, not a single new vaccine that is better than BCG has emerged in the twentieth century. However, the last two decades have witnessed significant progress (8;9;14). Today, a dozen preventive vaccine candidates have entered or successfully completed phase I clinical trials, for safety and immunogenicity. Currently, half a dozen candidates have successfully progressed through phase IIa in their target population and one candidate is currently undergoing first efficiency assessment in phase IIb (8;9;14).

Preventive vaccines against TB come in two types (8). First, subunit vaccines are generally considered as boosters of prime with canonical BCG. These subunit vaccines are either viral-vectored vaccines expressing few Mtb antigens or adjuvanted fusion proteins of Mtb antigens. The second group of candidates comprises viable whole cell vaccines. They are either Mtb attenuated by genetic manipulation or recombinant (r)BCG with improved efficiency and/or safety. These vaccines are considered for BCG replacement. Early on, subunit vaccines were considered not only for pre-exposure vaccination of newborns, but also for post-exposure vaccination of adults. More recently, BCG replacement vaccines are also being considered for post-exposure vaccination of adults in addition to pre-exposure in infants.

Development of a recombinant BCG vaccine

We genetically engineered BCG by exchanging the urease C (ureC) gene with listeriolysin (hly) (39;40). The resulting strain rBCG Δ ureC::hly was licensed to Vakzine Projekt Management (VPM) in Hannover, Germany, who named it VPM1002, and then sublicensed to Serum Institute India (SII) in Pune, India (41). SII is the largest vaccine producer by doses and currently 2 of 3 children on this globe receive a vaccine produced at SII. The decision to create an improved BCG vaccine was based on our knowledge that BCG is capable of preventing TB over the first years of life. However, containment over prolonged time periods of Mtb is not achieved by BCG. Rather Mtb resists sterile eradication even in BCG-vaccinated individuals and persists within the host. As long as it is controlled efficiently, Mtb does not cause active TB disease. This stage is called LTBI. We assume that this transient and partial BCG-induced protection in infants is primarily due to CD4 Th1 cells (14). It is likely that unconventional T cell populations are targeted by BCG, as well (25). In contrast, CD8 T

lymphocytes are not stimulated by BCG (14). Hence, we considered the possibility that this is a major hole in the BCG-induced T-cell repertoire.

Once internalized by professional phagocytes, BCG neutralizes the phagosomal milieu at pH7 thereby interfering with phagosome maturation (15). By deleting the gene encoding ureC in rBCG Δ ureC::hly, which catalyzes ammonia (NH_4^+) production, we interfered with phagosome neutralization. Accordingly, the intracellular phagosomal milieu reaches an acidic pH of around 6.0, when rBCG Δ ureC::hly resides in macrophages (39). Integrating the gene encoding hly in the chromosomal ring of BCG allows perturbation of the phagosomal membrane (42). As a built-in safety mechanism, hly is rapidly degraded once in the cytosol (43). This in turn leads to the release of vaccine-derived mycobacterial antigens into the cytosol (39). Moreover, the acidic pH in the phagosome promotes phagosome maturation eventually leading to phagosome–lysosome fusion (41). The perturbed membrane likely allows release of lysosomal enzymes which cause apoptotic events (22;44). In addition, release of double-stranded (ds) DNA stimulates absent in melanoma 2 (AIM-2) and stimulator of IFN genes (STING) (45). Sensing of ds DNA by AIM-2 results in inflammasome activation ultimately leading to IL-1 β and IL-18 processing and secretion. Moreover, sensing of dsDNA by AIM-2 and STING causes LC3-II expression as indicator of autophagy/xenophagy (45). Both apoptosis and autophagy/xenophagy are known to improve antigen processing and presentation (46;47). This probably involves crosspriming through release of apoptotic vesicles containing mycobacterial antigens and pathogen-associated molecular patterns (PAMPs) (22;47). As a result, improved CD4 and CD8 T cell activation occurs {Farinacci, 2012 4011 /id}. Moreover, the rBCG Δ ureC::hly -stimulated CD4 T cells comprise both Th1 and Th17 subsets (48). We assume that Th17 cells contribute to protection during early stages

whereas Th1 cells act as major mediators of protection. Indeed more profound stimulation of Th17 cells after vaccination with rBCG Δ ureC::hly as compared to parental BCG vaccination has been found both in animal experiments and in human trials (48;49). Moreover, we observed superior induction of T_{CM} cells by the rBCG Δ ureC::hly (50). Current investigations are aimed at elucidating whether in addition to improved T_{CM} cells, also lung T_{RM} cells are being induced.

Improved protection of rBCG Δ ureC::hly over BCG was demonstrated in several mouse experiments (41). Typically, in these studies, mice were vaccinated with BCG or rBCG Δ ureC::hly and after 120 days, mice were aerosol-infected with the well-defined Mtb strain H37Rv or with a clinical isolate of the Beijing genotype family (39). During the infection period over 6 months, bacterial load was monitored by determining colony-forming units (CFUs). It was found that rBCG Δ ureC::hly reduces the pulmonary bacterial load more than 100 fold over nonvaccinated mice as compared to a 10-fold reduction stimulated by parental BCG when H37Rv serves as challenge organism. When mice were challenged with the Beijing strain, BCG failed to induce any significant protection whereas rBCG Δ ureC::hly caused a more than 100-fold reduction of bacterial load particularly at late time points. Hence, VPM1002 achieved 99.5% reduction of the Mtb burden. More recently we determined whether rBCG Δ ureC::hly was also effective when given post-exposure (51). In this experiment, mice were first infected with Mtb. Ca. 40 days later, mice were drug-treated to reduce bacterial load to less than 10 CFUs per lung. These subclinically infected mice were then vaccinated and the cause of infection determined over the following months. The rBCG Δ ureC::hly causes significantly better protection than parental BCG. Hence, VPM1002 is capable of inducing profoundly improved protection over parental BCG when given either pre- or post-exposure.

Similarly impressive are safety data achieved with VPM1002 (41). For example, in SCID mice, systemic high-dose infection with rBCG Δ ureC::hly does not affect survival of mice over 100 days whereas mice receiving a similarly high dose of BCG succumb by day 30 (39). Because of these promising preclinical data, VPM1002, entered the clinical trial pipeline.

A phase I trial in Germany (Clinical Trial registration no. NCT 00749034) was successfully completed in 2009 (49). In these experiments, tuberculin skin test-positive (TST⁺) and -negative (TST⁻) individuals were given three escalating doses of 10⁴, 10⁵, 10⁶ CFUs of VPM1002 and compared with immunization with 10⁶ CFUs of BCG. TST⁺ individuals were negative in IFN- γ release assay (IGRA⁻) indicating they were BCG-vaccinated, and not Mtb-infected (52). Hence VPM1002 is safe and immunogenic both as prime and as boost. A similar phase I trial but with TST⁺ young adults only was then performed in a TB-endemic area in South Africa (NCT 01113281). This trial proved its immunogenicity and safety in a TB-endemic setting. Next, a phase IIa trial was performed in South Africa with three escalating doses administered to newborns (NCT 01479972). Doses were 10⁴, 10⁵, 10⁶ VPM1002 and 10⁶ BCG as comparator. VPM1002 proved safe and immunogenic in neonates. Currently the vaccine is undergoing a phase II trial in South Africa (NCT 02391415) in HIV-unexposed and HIV-exposed neonates. This trial has the objective of clarifying whether VPM1002 is safe and immunogenic in HIV-exposed infants.

Encouraged by our findings that VPM1002 also induces protection when given post-exposure in an animal model (51), application for a phase III vaccine trial is currently prepared in India to determine whether VPM1002 can prevent TB recurrence in former TB patients after successful drug treatment. It is unclear whether TB recurrence is due to relapse (i.e. reactivation of dormant Mtb despite successful drug treatment) or due to reinfection with a new Mtb organism. The recurrence rate in drug-cured individuals is as

high as 10%. Hence, “cured” TB patients represent a high risk group making feasible a phase III trial with numbers of study participants in the 1000s, rather than the 10,000s. We consider this trial as a first step towards targeting the population of adolescents and adults with VPM1002 independent from them being naïve or previously exposed to Mtb. Even in high TB-endemic areas, clinical trials with individuals with LTBI can easily reach participant numbers exceeding 10,000 individuals per group. Hence stratification for individuals with LTBI with elevated risk of developing TB during the trial would reduce group size, shorten trial length and consequently reduce cost of the study (12;53). This issue could be solved by the availability of a prognostic biosignature which predicts risk of developing TB.

Biomarkers

With a good dozen vaccine candidates undergoing clinical assessment, overall financial investment in the development of TB vaccines is increasing incrementally, calling for appropriate gating strategies to push forward the most promising candidates (12). First-in-human phase I trials assessing safety and providing first evidence for immunogenicity employ relatively small study groups. Accordingly, financial investment can be less than 1 million euros. A phase IIa trial to define optimum dose and route in the target population requires already a two- to three-fold higher investment of some 2.5 million euros. Phase IIb trials aiming at first efficacy assessment in the target population, frequently increases financial investment by a factor of 2–4 to some 5-10 million euros and a phase III trial for final determination of vaccine efficacy by another factor of 5 to a total of some 25-50 million euros. Thus, the further advanced a vaccine, the more important becomes the question of cost. One way to overcome such an obstacle is the involvement of high-risk groups in phase IIb and phase III trials. Testing whether a vaccine can prevent TB recurrence as described

above for VPM1002 is such a way to reduce numbers of study participants and related cost. As nonmutually exclusive alternative, biosignatures that (i) rapidly diagnose active TB at its onset, (ii) prognose risk of progression to active TB and (iii) predict protective efficacy of vaccine candidates are to be considered (12). Aside from this, such biosignatures can be developed for point-of-care diagnosis of TB for identifying individuals at risk of progressing to active TB and for prognosis of success of TB drug treatment. It is for these reasons that we have embarked on the design of biosignatures for TB (12;53). Our studies primarily focus on transcriptomics and are complemented by metabolome analysis (10;54-58).

Research into TB biosignatures by several groups including ourselves has revealed a number of robust biomarkers which allow differential diagnosis of TB patients versus healthy individuals or patients suffering from unrelated diseases (10;54-62). These signatures include downstream signaling cascades of IFN type I and type II, downstream signaling cascades of Fc gamma receptor (FcγR), and signaling cascades involved in IL-2 receptor-induced T cell regulation. These biological signatures can provide deeper insights into the mechanisms underlying pathogenesis of, and host resistance against, TB (see below). However, since members of a given signaling cascade are often coregulated, they possess lower discriminatory power than unrelated markers. Hence, we embarked on the design of a signature composed of a minimum number of distinct markers for robust point-of-care diagnosis. These biosignatures include as core markers: the FcγRI, which binds antibodies through the FcγR and is expressed on professional phagocytes; lactoferrin, which is involved in iron uptake; members of the large GTPase protein family, guanylate-binding proteins (GBPs) which are IFN-induced and involved in inflammasome assembly and cell-autonomous immunity as core markers. These markers can be complemented by the cytotoxic molecule granzyme A and the antimicrobial polypeptides defensin α1 and α4. A signature composed

of these markers allows TB diagnosis with >85% specificity and >85% sensitivity in different regions including Southern, Eastern and Western Africa as well as Bangalore, in India and Minsk, in Eastern Europe. Similar markers were also identified by other researchers as part of TB diagnostic signatures (59-62). In agreement with this, a computational analysis of all discriminatory markers of TB found in the public domain revealed that discrimination of TB patients versus healthy individuals or versus patients with other diseases can be performed at >90% specificity and >90% sensitivity by selected sets of biomarkers (Weiner et al., unpublished).

In addition to transcriptome analyses we also performed metabolome analyses (58). This led to a TB-specific biosignature composed of 15–20 metabolites capable of discriminating TB patients from healthy individuals. The major discriminatory metabolite markers comprise small molecules involved in hypoxia-induced inosine and methionine metabolism with anti-inflammatory activity; fibrinopeptides indicative of fibrotic lesions; lysophosphatidyl cholines indicative of massive cell death, either by apoptosis or necrosis and members of tryptophan metabolism with immunosuppressive activity.

Our ongoing studies on prognostic biomarkers benefit from contributions of a large consortium [the Grand Challenge 6, (GC6) consortium supported by the Bill and Melinda Gates Foundation] comprising several sites in Africa, research laboratories in Europe and the US. Blood samples of more than 4,500 HIV⁻ household contacts of newly diagnosed TB patients (who served as index cases) were included in this longitudinal observational study (GC6 <http://www.biomarkers-for-tb.net/>). Following diagnosis of TB of an index case, household contacts were recruited and blood samples collected immediately after inclusion into the study, as well as 6, 18 and 24 months later. Three percent of study participants developed TB whereas 97% remained healthy over the 2 year-study period. Prognostic

signatures are being created on the basis of transcriptomics and metabolomics which allow prognosis of TB before onset of clinical TB disease. Samples from the GC6 study were also employed for validating data generated in a so-called adolescent cohort study (63). The signature defined in this study could prognose risk of progression to active TB in the GC6 study participants, thereby generating proof-of-principle for feasibility of a robust biosignature of TB risk biosignature. This signature comprises 16 markers, most of which had already been identified in different studies aimed at determining biosignatures for TB diagnosis. It is most likely that this biosignature diagnoses subclinical incipient TB thereby prognosing risk of clinical TB disease.

It is hoped that biosignatures of this type will ultimately allow identification of individuals at risk of becoming diseased and contagious, who can then be recommended for preventive drug therapy. This would not only benefit the affected individual but also reduce spread amongst contacts in the community. Moreover it is hoped that by allowing stratification for TB risk such prognostic signatures will reduce trial size, shorten trial length, and consequently lower cost. Only once clinical trials on vaccine or drug efficacy have been completed it will become possible to define prognostic biosignatures of vaccine or drug efficacy, which can then further guide design of future clinical trials (12).

Reverse translation from biomarkers to hypothesis-driven research

Aside from their value for better TB control, biomarkers can also guide hypothesis-driven research towards a better understanding of mechanisms underlying TB (10;11). While some biomarkers represent transcripts for gene products of well-known functions in infection with, and protection against, Mtb, the functional roles in TB of other markers remain

incompletely understood. We have harnessed our knowledge about TB biomarkers for better understanding their biological functions in the disease process. Although type I IFN had already been associated with TB, its precise role was incompletely understood until recently (64-67). Biomarker studies had provided evidence for a correlation between type I IFN and neutrophils and TB disease (55;60;62). We employed a highly susceptible mouse strain, 129S2 and a highly resistant strain C57BL/6 to decipher in more depth the role of type I IFN in TB (65;68). These studies revealed elevated production of the pro-inflammatory cytokines IL-1 β , IL-1 α , TNF α , IL-6; the granulocyte-colony-stimulating factor (G-CSF); and the chemokines CXCL1, CXCL3, and CCL2 in the susceptible 129S2 mice compared to resistant C57BL/6 mice. Elevated abundance of proinflammatory cytokines and of chemokines is paralleled by increased influx of neutrophils and inflammatory macrophages into the lung. Moreover, susceptible 129S2 mice develop aggravated lesions and succumb to low-dose aerosol infection with Mtb before day 30, whereas C57BL/6 mice survive the whole duration of the experiment (100 days). Intriguingly, genetic deletion of the receptor for type I IFN in 129S2 mice reduces abundances of analyzed cytokines and chemokines to those seen in the resistant C57BL/6 mice, and ameliorates lesions. Accordingly, the IFNR1 knockout (KO) mice survive the duration of the experiment (100 days) with significantly lower bacterial load. Phenotypic characterization of the leukocytes in the lung of infected KO mice and wildtype 129S2 littermates revealed a significantly reduced influx of neutrophils and inflammatory macrophages into the lung of IFNR1 KO mice. Reciprocally, these KO mice harbour higher numbers of alveolar macrophages. Finally, depletion of neutrophils in 129S2 mice by treatment with monoclonal antibodies (Mab) against Ly6G, rescues recipients from early death. Thus, our study provides new insights into the pathogenic role of neutrophils and type I IFN in TB.

Recent studies, mostly in the cancer field, have revealed an inhibitory role of certain neutrophilic and monocytic phagocytes which have been termed myeloid-derived suppressor cells (MDSCs) (69;70). Although these cells do not represent a distinct subset, but rather a developmental stage, we considered it worthwhile to determine whether MDSCs participate in pathogenesis of TB (34). MDSCs are characterized by the coexpression of the type I macrophage (M1) marker enzyme inducible nitric oxide synthase (iNOS) and the M2 marker enzyme, arginase 1. Indeed immunohistology of Mtb-infected lung granulomas of susceptible 129S2 mice reveals profound abundances of these cells in lesions, which are significantly reduced after treatment with anti-granulocyte receptor (Gr)-1 Mab. Further phenotypic characterization of these cells demonstrates influx of both monocytic and neutrophilic MDSCs into the lungs of Mtb-infected susceptible 129S2 mice. We also observed influx of these cells during Mtb infection in the resistant C57BL/6 mice, albeit at lower abundance. Intriguingly, MDSCs of either neutrophilic or monocytic origin harbor high numbers of Mtb. In susceptible 129S2 mice, ca. 40% of the neutrophilic and ca. 20% of the monocytic MDSCs isolated from the lung are infected and on average four to six Mtb organisms are found per cell. MDSCs express the IL-4 receptor and in vitro culture with IL-4 promotes Mtb infection of MDSCs. Finally, in vitro studies demonstrate that these MDSCs inhibit secretion of the protective IFN- γ by T cells. Studies in the cancer field have identified all-trans retinoic acid (ATRA) as a small molecule that affects MDSC functions (71). Accordingly, we interrogated whether ATRA treatment would ameliorate TB (34). To this end, 129S2 mice were aerosol-infected with a low dose of Mtb and subsequently treated with ATRA. Twenty days later, impact of ATRA on the course of TB was determined. In ATRA-treated mice, proportions of myeloid and neutrophilic MDSCs in the lung are decreased, paralleled by significantly reduced bacterial load, and ameliorated lesions. We take these

experiments as evidence for the generation of MDSCs in TB, and for their disease-exacerbating function. Our experiments are in agreement with a study demonstrating high prevalence of MDSCs in lung lavage cells of TB patients (33). Together, they suggest that ATRA treatment should be exploited for host-directed therapy of multidrug-resistant TB in adjunct to conventional drug therapy (72;73).

In sum, our findings provide compelling evidence for a pathogenic role of neutrophils through two mechanisms. First, they act as highly aggressive effectors, which evade tight control and primarily harm the host by destroying the tissue surrounding sites of Mtb residence. Second, they act as MDSCs impairing the protective immune response, thereby further exacerbating detriment to the host.

Concluding remarks

In this treatise I describe different but intertwined research avenues we have pursued over the last two decades. Starting from a basic research approach we rationally designed a vaccine candidate against TB, which has now reached an advanced state of clinical trial assessment. In parallel, clinical observational studies have allowed us to define biosignatures that can diagnose and prognose clinical TB disease. As an added value, these clinical studies also stimulate hypothesis-driven research. Thus the combination of field studies and basic research, further supported by computational analyses, serves as a highly successful strategy for in-depth understanding of the complex tactics developed by the most successful pathogen on earth, Mtb (see Figure 1). We are convinced that this approach can facilitate rational design of improved intervention measures to ultimately gain an upper hand against the highly devastating communicable disease, TB.

Acknowledgements

Work of the author is currently supported by The European Union's Seventh Framework Programme (EU FP7) ADITEC (HEALTH-F4-2011-280873); by the EU Horizon 2020 project TBVAC 2020 (grant no. 643381); The Bill & Melinda Gates Foundation (BMGF) GC6-2013, #OPP 1055806 and #OPP 1065330); the Bundesministerium für Bildung und Forschung (BMBF) Infect ERA project “Anti-Bacterial Immune Regulation” (ABIR) (grant no. 031A404) and BMBF project “Infect Control 2020.” The author is coinventor of the TB vaccine VPM1002.

Figure Legends

Figure 1. Reciprocity between basic and clinical research. Basic research has led to the rational design of a vaccine candidate against tuberculosis (TB) which is currently progressing through the clinical trial pipeline. Observational clinical studies have revealed biomarkers for TB diagnosis and for risk of progressing to TB. They also provide guidance for basic research to better understand the biological role of these biomarkers in pathogenesis of and protection against TB. Basic and clinical research need support from computational biology to integrate information into the context of latent infection and active disease. Adapted from Kaufmann et al. Curr Opin Immunol 2014 Feb 18;28C:18-26.

Figure 2. Immunopathology of tuberculosis. (A) *Mycobacterium tuberculosis* (Mtb) in the alveolar space is engulfed by alveolar macrophages (blue), neutrophils (orange) and interstitial dendritic cells (DCs). DC transport Mtb to draining lymph nodes where they activate T cell responses. (B) Under the guidance of T cells, a solid granuloma is formed which contains Mtb thereby preventing progression to active disease. However, Mtb is not eliminated and hence persists in the solid granuloma. (C). A dysfunctional acquired immune response results in necrosis and subsequent caseation of the granuloma. The caseous granuloma causes severe tissue damage. Its rupture into alveoli, allows dissemination to the environment affecting other individuals and its rupture into the blood stream results in systemic infection and extrapulmonary disease. Adapted from Andersen P, Kaufmann SH. Novel vaccination strategies against tuberculosis. Cold Spring Harb Perspect Med 2014 Jun;4(6). pii: a018523.

Figure 3. A more refined view of infection and immunity in TB. (A) Antigen presentation by dendritic cells (DCs) results in stimulation of CD4 and CD8 T-cells. These T cells develop into memory T cells performing multiple functions. During the infection state, Mtb is metabolically active. (B) As long as memory T cells can sustain the solid granuloma, Mtb is controlled and individuals remain healthy but latently infected. During latency, Mtb enters into, and remains in, a dormant stage. (C) Immunoregulatory mechanisms impair T cell responses, thereby allowing caseation of granulomas and progression to active disease. During disease, Mtb regains metabolic and replicative activity. **Abbreviations:** B, B cell; CD4, CD4 T cell; CD8, CD8 T cell; CTL, cytotoxic T lymphocyte; CTLA-4, CTL-associated protein 4; IFN- γ , interferon gamma; IL, interleukin; M Φ , macrophage; MHC, major histocompatibility complex; Mtb, *Mycobacterium tuberculosis*; PMN, polymorphonuclear neutrophil; T, T cell; TB, tuberculosis; T_{eff}, effector T cell; TGF- β , transforming growth factor beta; Th, T helper cell; T_M, memory T cell; TNF, tumor necrosis factor; T_{reg}, regulatory T cell. Adapted from Ottenhoff THM, Kaufmann SHE (2012) PLoS Pathog 8(5): e1002607.

Reference List

- (1) Scully T, Editor. Tuberculosis. Nature 2013;502(7470):S2-S16.
- (2) Commission on a Global Health Risk Framework for the Future, National Academy of Medicine S. The Neglected Dimension of Global Security: A Framework to Counter Infectious Disease Crises. National Academies Press; 2016. Report No.: DOI: 10.17226/2191.
- (3) WHO. Global Tuberculosis Report 2015. Geneva: WHO Press; 2015.
- (4) Review on Antimicrobial Resistance. Antimicrobial resistance: Tackling a crisis for the health and wealth of nations. HM Government & Wellcome Trust; 2014.
- (5) Diel R, Vandeputte J, de VG, Stillo J, Wanlin M, Nienhaus A. Costs of tuberculosis disease in the European Union: a systematic analysis and cost calculation. Eur Respir J 2014 Feb;43(2):554-65.
- (6) StopTB Partnership, WHO. The Global Plan to Stop TB 2011-2015. http://www.stoptb.org/assets/documents/global/plan/TB_GlobalPlanToStopTB2011-2015.pdf 2011
- (7) Abu-Raddad LJ, Sabatelli L, Achterberg JT, Sugimoto JD, Longini IM, Jr., Dye C, et al. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. Proc Natl Acad Sci U S A 2009 Aug 18;106(33):13980-5.
- (8) Andersen P, Kaufmann SH. Novel vaccination strategies against tuberculosis. Cold Spring Harb Perspect Med 2014 Jun;4(6):a018523.
- (9) Kaufmann SHE. Tuberculosis vaccines: Time to think about the next generation. Sem Immunol 2013;25:172-81.
- (10) Weiner J3, Kaufmann SH. Recent advances towards tuberculosis control: vaccines and biomarkers. J Intern Med 2014 May;275(5):467-80.
- (11) Kaufmann SH, McElrath J.M., Lewis DJ, Del GG. Challenges and responses in human vaccine development. Curr Opin Immunol 2014 Feb 18;28C:18-26.
- (12) Kaufmann SH, Evans TG, Hanekom WA. Tuberculosis vaccines: time for a global strategy. Sci Transl Med 2015 Feb 25;7(276):276fs8.
- (13) O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP. The immune response in tuberculosis. Annu Rev Immunol 2013;31:475-527.
- (14) Ottenhoff TH, Kaufmann SH. Vaccines against tuberculosis: where are we and where do we need to go? PLoS Pathog 2012;8(5):e1002607.
- (15) Kaufmann SHE, Dorhoi A. Molecular determinants in phagocyte-bacteria interactions. Immunity 2016;44(3):476-91.
- (16) Gengenbacher M, Kaufmann SHE. *Mycobacterium tuberculosis*: success through dormancy. FEMS Microbiol Rev 2012;36:514-32.

- (17) Reece ST, Kaufmann SH. Floating between the poles of pathology and protection: can we pin down the granuloma in tuberculosis? *Curr Opin Microbiol* 2012 Feb;15(1):63-70.
- (18) Ulrichs T, Kaufmann SHE. New insights into the function of granulomas in human tuberculosis. *J Pathol* 2006 Jan;208(2):261-9.
- (19) Lin PL, Ford CB, Coleman MT, Myers AJ, Gawande R, Ioerger T, et al. Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. *Nat Med* 2014 Jan;20(1):75-9.
- (20) van der Wel N, Hava D, Houben D, Fluitsma D, van ZM, Pierson J, et al. *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. *Cell* 2007 Jun 29;129(7):1287-98.
- (21) Schaible UE, Winau F, Sieling PA, Fischer K, Collins HL, Hagens K, et al. Apoptosis facilitates antigen presentation to T lymphocytes through MHC-I and CD1 in tuberculosis. *Nat Med* 2003;9(8):1039-46.
- (22) Winau F, Weber S, Sad S, de Diego J, Hoops SL, Breiden B, et al. Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. *Immunity* 2006 Jan;24(1):105-17.
- (23) Deretic V. Autophagy, an immunologic magic bullet: Mycobacterium tuberculosis phagosome maturation block and how to bypass it. *Future Microbiol* 2008 Oct;3(5):517-24.
- (24) Stenger S, Hanson DA, Teitelbaum R, Dewan P, Niazi KR, Froelich CJ, et al. An antimicrobial activity of cytolytic T cells mediated by granulysin. *Science* 1998 Oct 2;282(5386):121-5.
- (25) Godfrey DI, Uldrich AP, McCluskey J, Rossjohn J, Moody DB. The burgeoning family of unconventional T cells. *Nat Immunol* 2015 Nov;16(11):1114-23.
- (26) Napier RJ, Adams EJ, Gold MC, Lewinsohn DM. The Role of Mucosal Associated Invariant T Cells in Antimicrobial Immunity. *Front Immunol* 2015;6:344.
- (27) Brigl M, Brenner MB. CD1: antigen presentation and T cell function. *Annu Rev Immunol* 2004;22:817-90.
- (28) Scotet E, Nedellec S, Devilder MC, Allain S, Bonneville M. Bridging innate and adaptive immunity through gammadelta T-dendritic cell crosstalk. *Front Biosci* 2008;13:6872-85.
- (29) Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol* 2004;22:745-63.
- (30) Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. *Nat Rev Immunol* 2008 Apr;8(4):247-58.
- (31) Park CO, Kupper TS. The emerging role of resident memory T cells in protective immunity and inflammatory disease. *Nat Med* 2015 Jul;21(7):688-97.
- (32) Dallenga T, Schaible UE. Neutrophils in tuberculosis-first line of defence or booster of disease and targets for host-directed therapy? *Pathog Dis* 2016 Apr;74(3).
- (33) du Plessis N., Loebenberg L, Kriel M, von Groote-Bidlingmaier F, Ribechini E, Loxton AG, et al. Increased frequency of myeloid-derived suppressor cells during active tuberculosis and after

recent *Mycobacterium tuberculosis* infection suppresses T-cell function. Am J Respir Crit Care Med 2013 Sep 15;188(6):724-32.

- (34) Knäul JK, Jorg S, Oberbeck-Mueller D, Heinemann E, Scheuermann L, Brinkmann V, et al. Lung-residing myeloid-derived suppressors display dual functionality in murine pulmonary tuberculosis. Am J Respir Crit Care Med 2014 Nov 1;190(9):1053-66.
- (35) Larson RP, Shafiani S, Urdahl KB. Foxp3(+) regulatory T cells in tuberculosis. Adv Exp Med Biol 2013;783:165-80.
- (36) Calmette A, Guérin C, Boquet A, Nègre L. La vaccination préventive contre la tuberculose par le "BCG". Paris: Masson; 1927.
- (37) Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. JAMA 1994 Mar 2;271(9):698-702.
- (38) Colditz GA, Berkey CS, Mosteller F, Brewer TF, Wilson ME, Burdick E, et al. The efficacy of bacillus Calmette-Guerin vaccination of newborns and infants in the prevention of tuberculosis: meta-analyses of the published literature. Pediatrics 1995 Jul;96(1 Pt 1):29-35.
- (39) Grode L, Seiler P, Baumann S, Hess J, Brinkmann V, Nasser EA, et al. Increased vaccine efficacy against tuberculosis of recombinant *Mycobacterium bovis* bacille Calmette-Guerin mutants that secrete listeriolysin. J Clin Invest 2005 Sep;115(9):2472-9.
- (40) Hess J, Miko D, Catic A, Lehmensiek V, Russell DG, Kaufmann SH. Mycobacterium bovis Bacille Calmette-Guerin strains secreting listeriolysin of Listeria monocytogenes. Proc Natl Acad Sci U S A 1998 Apr 28;95(9):5299-304.
- (41) Kaufmann SH, Cotton MF, Eisele B, Gengenbacher M, Grode L, Hesselting AC, et al. The BCG replacement vaccine VPM1002: from drawing board to clinical trial. Expert Rev Vaccines 2014 May;13(5):619-30.
- (42) Palmer M. The family of thiol-activated, cholesterol-binding cytolysins. Toxicon 2001 Nov;39(11):1681-9.
- (43) Decatur AL, Portnoy DA. A PEST-like sequence in listeriolysin O essential for *Listeria monocytogenes* pathogenicity. Science 2000 Nov 3;290(5493):992-5.
- (44) Farinacci M, Weber S, Kaufmann SH. The recombinant tuberculosis vaccine rBCG DeltaureC::hly(+) induces apoptotic vesicles for improved priming of CD4(+) and CD8(+) T cells. Vaccine 2012 Dec 14;30(52):7608-14.
- (45) Saiga H, Nieuwenhuizen N, Gengenbacher M, Koehler AB, Schuerer S, Moura-Alves P, et al. The recombinant BCG deltaureC::hly vaccine targets the AIM2 inflammasome to induce autophagy and inflammation. J Infect Dis 2015 Jun 1;211(11):1831-41.
- (46) Jagannath C, Lindsey DR, Dhandayuthapani S, Xu Y, Hunter RL, Jr., Eissa NT. Autophagy enhances the efficacy of BCG vaccine by increasing peptide presentation in mouse dendritic cells. Nat Med 2009 Mar;15(3):267-76.
- (47) Winau F, Hegasy G, Kaufmann SH, Schaible UE. No life without death--apoptosis as prerequisite for T cell activation. Apoptosis 2005 Aug;10(4):707-15.

- (48) Desel C, Dorhoi A, Banderhann S, Grode L, Eisele B, Kaufmann SH. Recombinant BCG {Delta}ureC hly+ induces superior protection over parental BCG by stimulating a balanced combination of type 1 and type 17 cytokine responses. *J Infect Dis* 2011 Sep 20;204(10):1573-84.
- (49) Grode L, Ganoza CA, Brohm C, Weiner J, III, Eisele B, Kaufmann SH. Safety and immunogenicity of the recombinant BCG vaccine VPM1002 in a phase 1 open-label randomized clinical trial. *Vaccine* 2013 Jan 2;31:1340-8.
- (50) Vogelzang A, Perdomo C, Zedler U, Kuhlmann S, Hurwitz R, Gengenbacher M, et al. Central memory CD4+ T cells are responsible for the recombinant bacillus Calmette-Guerin deltaureC::hly vaccine's superior protection against tuberculosis. *J Infect Dis* 2014 Jun 18;210(12):1928-37.
- (51) Gengenbacher M, Kaiser P, Schuerer S, Lazar D, Kaufmann SHE. Post-exposure vaccination with the vaccine candidate Bacillus Camette-Guerin deltaureC::hly induces superior protection in a mouse model of subclinical tuberculosis. *Microbes Infect* 2016;doi.org/10.1016/j.micinf.2016.03.005.
- (52) Diel R, Goletti D, Ferrara G, Bothamley G, Cirillo D, Kampmann B, et al. Interferon-gamma release assays for the diagnosis of latent Mycobacterium tuberculosis infection: a systematic review and meta-analysis. *Eur Respir J* 2011 Jan;37(1):88-99.
- (53) Ottenhoff TH, Ellner JJ, Kaufmann SH. Ten challenges for TB biomarkers. *Tuberculosis (Edinb)* 2012 Mar;92 Suppl 1:S17-S20.
- (54) Jacobsen M, Repsilber D, Gutschmidt A, Neher A, Feldmann K, Mollenkopf HJ, et al. Candidate biomarkers for discrimination between infection and disease caused by Mycobacterium tuberculosis. *J Mol Med (Berl)* 2007 Jun;85(6):613-21.
- (55) Maertzdorf J, Repsilber D, Parida SK, Stanley K, Roberts T, Black G, et al. Human gene expression profiles of susceptibility and resistance in tuberculosis. *Genes Immun* 2011 Jan;12(1):15-22.
- (56) Maertzdorf J, Weiner J, III, Mollenkopf HJ, Bauer T, Prasse A, Muller-Quernheim J, et al. Common patterns and disease-related signatures in tuberculosis and sarcoidosis. *Proc Natl Acad Sci U S A* 2012 May 15;109(20):7853-8.
- (57) Maertzdorf J, McEwen G, Weiner J, III, Tian S, Lader E, Schriek U, et al. Concise gene signature for point-of-care classification of tuberculosis. *EMBO Mol Med* 2016;8(2):86-95.
- (58) Weiner J, III, Parida SK, Maertzdorf J, Black GF, Repsilber D, Telaar A, et al. Biomarkers of inflammation, immunosuppression and stress with active disease are revealed by metabolomic profiling of tuberculosis patients. *PLoS ONE* 2012;7(7):e40221.
- (59) Anderson ST, Kaforou M, Brent AJ, Wright VJ, Banwell CM, Chagaluka G, et al. Diagnosis of childhood tuberculosis and host RNA expression in Africa. *N Engl J Med* 2014 May 1;370(18):1712-23.
- (60) Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* 2010 Aug 19;466(7309):973-7.

- (61) Kaforou M, Wright VJ, Oni T, French N, Anderson ST, Bangani N, et al. Detection of tuberculosis in HIV-infected and -uninfected African adults using whole blood RNA expression signatures: a case-control study. *PLoS Med* 2013 Oct;10(10):e1001538.
- (62) Ottenhoff TH, Dass RH, Yang N, Zhang MM, Wong HE, Sahiratmadja E, et al. Genome-wide expression profiling identifies type 1 interferon response pathways in active tuberculosis. *PLoS ONE* 2012;7(9):e45839.
- (63) Zak DE, Penn-Nicholson A, Scriba TJ, Thompson E, Suliman S, Amon LM, et al. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. *The Lancet* 2016;doi.org/10.1016/S0140-6736(15)01316-1.
- (64) Mayer-Barber KD, Andrade BB, Oland SD, Amaral EP, Barber DL, Gonzales J, et al. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. *Nature* 2014 Jul 3;511(7507):99-103.
- (65) Medina E, North RJ. Resistance ranking of some common inbred mouse strains to *Mycobacterium tuberculosis* and relationship to major histocompatibility complex haplotype and *Nramp1* genotype. *Immunology* 1998 Feb;93(2):270-4.
- (66) Antonelli LR, Gigliotti RA, Goncalves R, Roffe E, Cheever AW, Bafica A, et al. Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. *J Clin Invest* 2010 May;120(5):1674-82.
- (67) Manca C, Tsenova L, Bergtold A, Freeman S, Tovey M, Musser JM, et al. Virulence of a *Mycobacterium tuberculosis* clinical isolate in mice is determined by failure to induce Th1 type immunity and is associated with induction of IFN- α / β . *Proc Natl Acad Sci U S A* 2001 May 8;98(10):5752-7.
- (68) Dorhoi A, Yermeev V, Nouailles G, Weiner J, III, Jorg S, Heinemann E, et al. Type I IFN signaling triggers immunopathology in tuberculosis-susceptible mice by modulating lung phagocyte dynamics. *Eur J Immunol* 2014 Aug;44(8):2380-93.
- (69) Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* 2012 Apr;12(4):253-68.
- (70) Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. *Nat Rev Cancer* 2013 Oct;13(10):739-52.
- (71) Kusmartsev S, Cheng F, Yu B, Nefedova Y, Sotomayor E, Lush R, et al. All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination. *Cancer Res* 2003 Aug 1;63(15):4441-9.
- (72) Zumla A, Rao M, Wallis RS, Kaufmann SH, Rustomjee R, Mwaba P, et al. Host-directed therapies for infectious diseases: current status, recent progress, and future prospects. *Lancet Infect Dis* 2016 Apr;16(4):e47-e63.
- (73) Kaufmann SH, Lange C, Rao M, Balaji KN, Lotze M, Schito M, et al. Progress in tuberculosis vaccine development and host-directed therapies--a state of the art review. *Lancet Respir Med* 2014 Apr;2(4):301-20.

Figure 1

Figure 2

Figure 3