



Department of Immunology – Stefan H.E. Kaufmann

Research Program

A Introduction

Our research continues to focus on the crosstalk between *Mycobacterium tuberculosis* (Mtb) and its mammalian host. This includes:

- (i) basic investigations into both host and pathogen;
- (ii) targeted research towards better intervention measures;
- (iii) translation from preclinical into clinical studies;
- (iv) reverse-translation of clinical findings into basic research.

Tuberculosis (TB) remains a health threat of global dimension which affects Sub-Saharan Africa, China and India most severely (WHO Report 2018). At the same time, TB is a highly interesting target for basic research, since it reflects the outcome of a long-standing co-evolutionary process between pathogen and host. An estimated 1.7 billion individuals are infected with Mtb, of whom ca. 90-95% will carry the pathogen lifelong without developing active TB (figure 1.1). These individuals with so-called latent TB infection (LTBI) harbor dormant Mtb bacilli with low metabolic and replicative activity, which are controlled by an ongoing immune response. In 5-10% of LTBI cases, active TB disease develops within the first 2 years in half of the cases but also occasionally at later times of life. It is becoming increasingly clear that LTBI and TB are not distinct entities but form a continuum. Accordingly, protection and pathology in TB are the outcome of a complex and highly dynamic crosstalk between Mtb and the immune system.

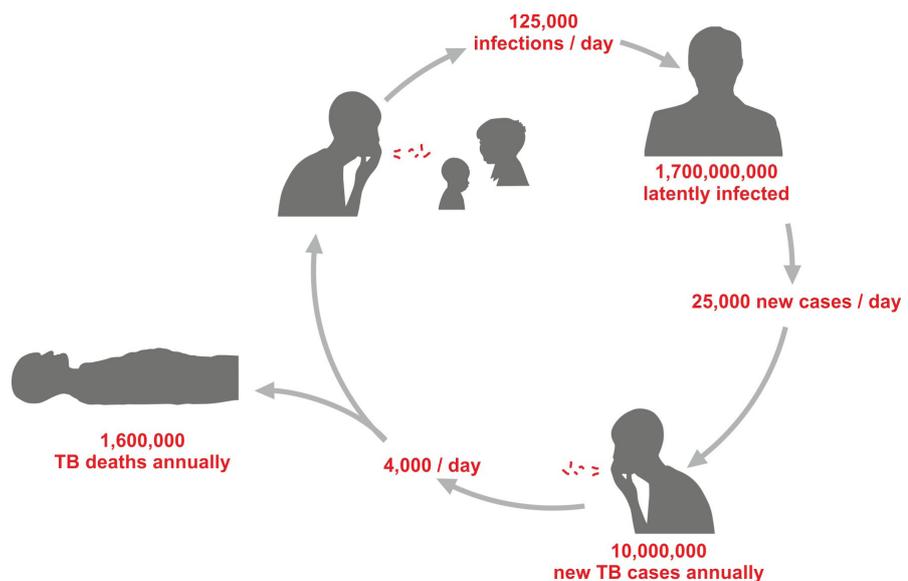


Figure 1.1: Epidemiology of TB (adapted from Kaufmann, Immunity 2010: Fig. 1, p. 568).

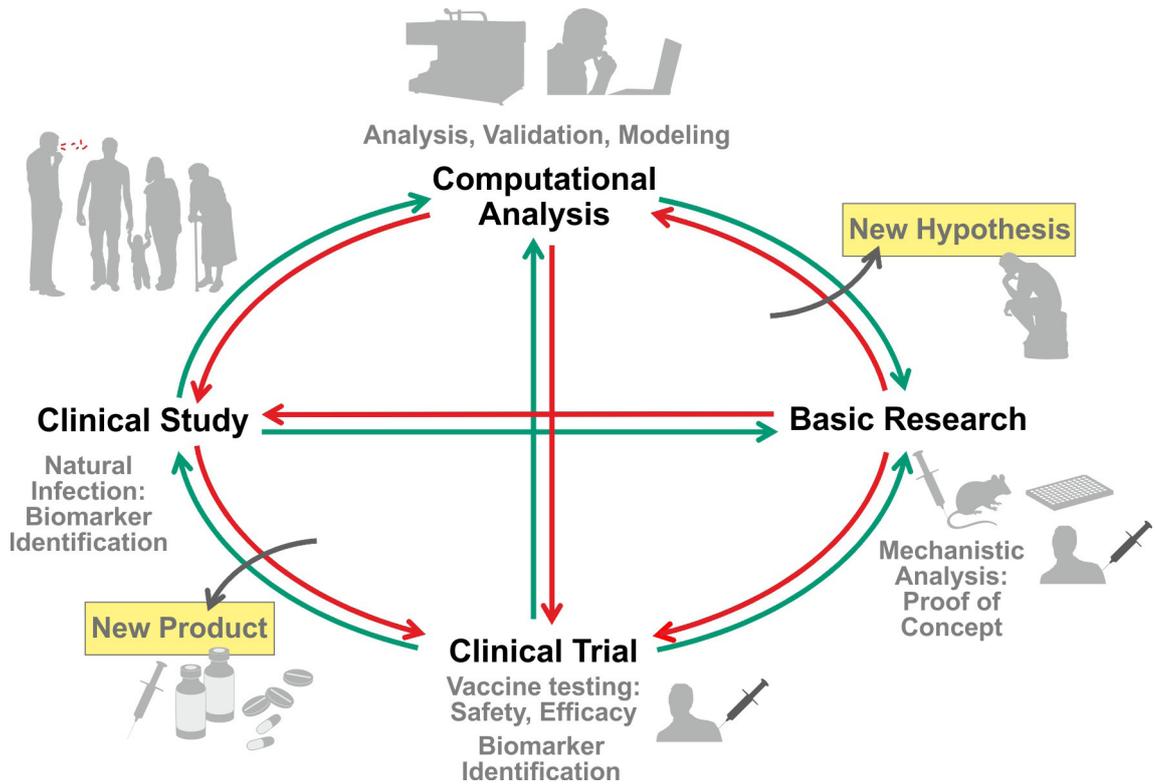


Figure 1.2: Research Strategy (adapted from Kaufmann et al, Curr Opin Immunol, 2014: Fig. 1, p 19).

At the cellular level, we focus on the crosstalk between Mtb and mononuclear phagocytes (MP), which serve as both effector cells and as habitat for Mtb. At the tissue level, our focus lies on granulomas, as solid granulomas contain Mtb, and necrotic and caseous granulomas cause tissue damage and promote growth and dissemination of Mtb.

Granuloma formation and sustenance crucially depend on migration of and coordinated interactions among different leukocyte populations through cytokines, chemokines and surface receptors. T lymphocytes are central regulators of protection and pathology. They depend on antigen-presenting cells, notably dendritic cells (DC) and MP, for appropriate activation. In addition, B cells and neutrophils are also found in granulomas. The role of B cells and antibodies in TB remains elusive. Even less is known about innate lymphoid cells (iLC) in TB.

Mtb influences the course of infection and disease by means of numerous factors which manipulate the host responses, as well as by its ability to alternate between dormancy and metabolic and replicative activity. We therefore remain interested in TB as a highly attractive target for a better understanding of basic mechanisms of protection and pathology, as well as for developing novel intervention measures in a rational way.

We pursue these aims through a highly intertwined and reciprocal strategy rather than independent avenues that includes translation of basic research into a medical product, as well as reverse translation of findings obtained in clinical studies back to basic research questions (figure 1.2). In the following the research output of our group over the last 3.5 years is described in short and accompanied by abstracts describing the different projects (authors in bold indicate members of the Department).

B Immunity in Tuberculosis and Other Bacterial Infections

Basic understanding of the innate and adaptive immune response to Mtb remained a core activity of our group. Our major focus has been on innate immunity, comprising both professional (MP, neutrophils, DC and transformed macrophages) and nonprofessional immune cells (epithelial and endothelial cells).

DC and iLC are critically involved in the early stages of Mtb infection. DC primarily orchestrate the acquired T cell response, whilst iLC participate in the early control of infection by providing the cytokine milieu that guides DC, MP and T cells. Regarding DC, we identified two novel markers, BTLA and CD163, which allow characterization of different subsets of conventional DC (cDC), which are known to display anti-mycobacterial activity and to promote T cell activation in TB. Our studies suggest that BTLA is a marker of cDC2 differentiation associated with progressive TB. Moreover, we could distinguish two subsets of cDC2 on the basis of expression levels of BTLA and CD163. One population was found to express poor phagocytic function but high antigen presentation capacity, whereas the other one had high phagocytic activity and released proinflammatory cytokines. Regarding iLC we focused on type 2 iLC in TB and performed a comprehensive gene expression analysis using single cell sequencing. In this way, iLC2 were identified as critical cells in the preservation and reconstitution of lung integrity in TB.

Since the granuloma is the hallmark of TB, other studies focused on the role of different cell types in control of TB infection and in lung pathology. We further characterized the role of myeloid-derived suppressor cells (MDSC) in TB and showed that they affect granuloma persistence. Moreover, we identified a unique role of Mtb antigen-independent memory CD8⁺ T cells in early protection against TB. These T cells with specificity for other antigens are stimulated by IL-18 from Mtb infected DC. Such cells could bridge the gap between innate and acquired immunity. The role of antibodies in the control of TB remains controversial. We therefore embarked on studies aimed at gaining a better understanding of the role of antibodies in TB together with the group of Hedda Wardemann. These studies revealed isotype dependency of the antibody response in human TB. Experiments in mice with focus on the neonatal Fc receptor deciphered the role of this receptor for specific IgG isotypes in mucosa-associated responses against Mtb.

Chemokines and cytokines are the main soluble mediators of the immune system. IL-36 γ , a recently described IL-1 family cognate, has been identified as a potent stimulator of antimicrobial peptides in Mtb-infected MP. Consequently, IL-36 γ plays a critical role in defense against TB and more precisely by modulating cholesterol metabolism in TB. The cytokine transforming growth factor-beta (TGF β) has been implicated in granuloma formation and immune suppression in TB for some time. Although it is highly upregulated during active TB, its precise role on the molecular level remains incompletely understood. Utilizing genetically modified mice, the role of TGF β in TB has been deciphered. Thus, we identified a critical involvement of TGF- β 2 signaling in myeloid cells in the killing of Mtb.

At the molecular level, the crosstalk between Mtb and host molecules remains of highest interest to us. Thus, we are highly interested in receptors that sense infection and drive inflammation. A major highlight of our primarily hypothesis-driven investigations was the identification of the Aryl hydrocarbon receptor (AhR) as a pattern recognition receptor (PRR) for bacterial pigmented virulence factors, namely phen-

zines from *Pseudomonas aeruginosa* and naphthoquinones from Mtb, as pathogen-associated molecular patterns (Alves et al., Nature, 2014). More recent studies aimed at further understanding the role of the AhR in the context of: (i) bacterial quorum sensing; (ii) recognition of other naphthoquinones, such as lawsone, a major component of henna that has been implicated in skin disease and (iii) drug therapy. First, we demonstrated that quorum sensing molecules released from *P. aeruginosa* and other gram-negative bacteria are recognized by the AhR. “Spying” of bacterial quorum sensing by the AhR allows the host to determine infection dynamics and thereby control of host defense. At low concentrations of *P. aeruginosa*, which are harmless to the host, AhR signaling is suppressed. At high levels, when bacteria produce quorum sensing molecules, the AhR is enabled to mobilize host defense. Whilst quorum sensing allows bacteria to only invest in energy at high bacterial density when they are ready to overwhelm the host, the AhR allows host cells to only invest in energy when needed for defense against high bacterial load as indicated by quorum sensing molecules. Second, we identified a number of plant pigments, such as the naphthoquinone Lawson, as AhR ligands. Lawson is the major pigment in henna, which has been used for therapeutic and cosmetic purposes. We revealed that Lawson has both beneficial and detrimental effects after application to the skin (as is the case for henna) depending on the physio-pathological context. Third, we determined that the TB drugs rifampicin and rifabutin are sensed by the AhR and that this sensing impacts on TB drug metabolism. Whilst rifampicin inhibited AhR-mediated signaling, rifabutin activated the signaling cascade. Accordingly, rifabutin was degraded more rapidly than rifampicin. This effect, however, could be reverted by a small molecule inhibitor of the AhR, which improved outcome of TB treatment with rifabutin. We, therefore, propose that the AhR is a candidate target for host-directed therapy in adjunct to canonical drug treatment in TB. Finally, in collaboration with the group of Michael Kolbe, we continue to pursue the structural characterization of the AhR.

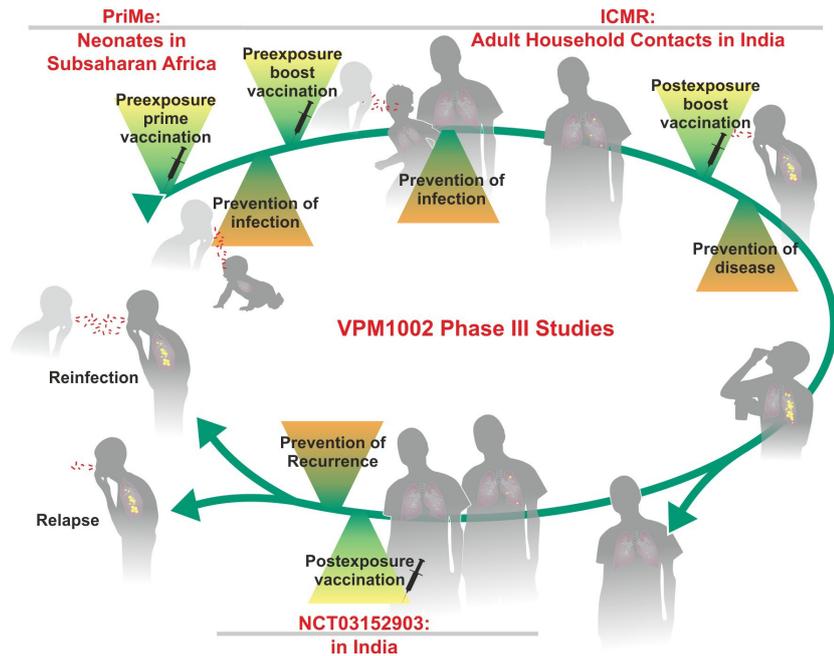
Other studies revealed a role for syndecans (Sdc) in the invasion of lung epithelial cells. It appears that the mycobacterial heparin-binding hemagglutinin adhesin (HBHA) participates in binding of Mtb to Sdc. Epithelial cells can serve as a protective niche for Mtb, and knockdown of distinct Sdc involved in Mtb binding rendered mice more resistant to Mtb colonization. Autophagy/xenophagy is emerging as an important cell autonomous defense mechanism against intracellular pathogens. Hence, molecules involved in modulating autophagy/xenophagy are of great interest. These molecules include NEDD4 which was identified as essential part of the autophagy/xenophagy inducing signaling cascade. NEDD-4 is an E3 ubiquitin ligase which is activated by membrane-perturbing-intracellular bacteria such as Mtb, *Listeria monocytogenes* and also the vaccine candidate VPM1002. NEDD-4 is involved in killing of these bacteria through activating autophagy/xenophagy by increasing the protein level of the essential autophagic component Beclin-1. Another intracellular sensor that caught our interest is cGAS which is known to participate in sensing of cytosolic DNA. A recent study in our laboratory elucidated the additional role of cGAS as sensors of extracellular cyclic dinucleotides (eCDNs). We observed that clathrin-dependent endocytosis promotes internalization of eCDNs and subsequent sensing of endocytosed eCDNs by cGAS. This leads to stimulation of type I IFN. In continuation of this work, a novel role of the nuclear cGAS in malignancies was identified: It suppresses DNA damage and promotes tumorigenesis. NOD1/2 are well known intracellular pattern recognition receptors (PRR) which recognize bacterial peptidoglycans. Our most recent studies revealed that NOD1/2 also sense host-derived molecules. This allows NOD1/2 to monitor cellular homeostasis and intracellular stress. Hence, NOD1/2 do not only recognize PAMP (Pa-

thogen Associated Molecular Patterns), but also stress-associated molecular patterns (SAMP). More precisely, we identified cytosolic sphingosine-1-phosphate (S1P) as SAMP for NOD1/2. Thus, NOD1/2 serves as a molecular hub that can integrate signals from both bacteria and host cells. Finally, our work identified galactin-9 as sensor of mycobacterial arabinogalactan. We therefore consider galactin-9 as novel PRR involved in TB and arabinogalactan from Mtb as PAMP.

C Rational Vaccine Development

The development of TB vaccines has entered a new stage, with several vaccine candidates having advanced through the clinical trial pipeline. Currently, several different clinical endpoints are being used for efficacy testing of TB vaccine candidates. These include **prevention of infection (PoI)** for vaccines which are given early in life and therefore prior to infection with Mtb, and **prevention of disease (PoD)** in individuals who have not been exposed to Mtb previously and for individuals who already have LTBI. Thus, PoD is being assessed for both pre-exposure and post-exposure vaccination. Finally, **prevention of recurrence (PoR)** is being pursued more actively. This strategy assesses the efficacy of a vaccine to prevent recurrence of TB in previously drug-treated and apparently cured patients. Recurrence occurs in 10% of patients within 12 months after completion of treatment in areas with high TB prevalence (see figure 1.3).

Figure 1.3: Clinical efficacy trials with VPM1002 depicting the different clinical endpoints and study groups.



The recombinant vaccine BCG *AureC::hly*, which has been developed by our group over the last 2 decades, has now entered an exciting stage. The vaccine has been licensed to Vakzine Projekt Management (VPM) GmbH in Hannover, who termed it VPM1002 and sublicensed to the largest vaccine producer in the world, Serum Institute India Pvt. Ltd. in Pune, India. The vaccine has already successfully completed two phase I clinical trials in adults: the first in Germany and the second in South Africa. Moreover, a phase IIa clinical study in infants in South Africa has been successfully completed. These trials revealed that VPM1002 is safe and immunogenic in adults and infants. Most recently a clinical phase II trial with 416 HIV-exposed and unexposed neonates has been completed in South Africa, although unblinded data have so far not been relea-

sed. Based on this trial, an adoptive phase III trial (priMe) with near to 7000 infants (HIV-exposed and unexposed) is being initiated in 2019. This multicentric trial will encompass several sites in Sub-Saharan Africa including South Africa, Uganda, Kenya, Tanzania and Gabon. This trial will determine safety and PoI of VPM1002 with BCG as comparator. Moreover, an efficacy trial assessing both PoI and PoD in household contacts as been launched by the Indian Council of Medical Research (ICMR) in 2019. This multicentric phase III trial will include VPM1002, a second vaccine and controls, totaling to 12,600 participants. In the meantime, a phase III multicentric efficacy trial on PoR comprising 2000 participants already started in early 2018. The concept for this clinical trial on PoR was based on an experimental vaccination model in mice, in which post-exposure vaccination with VPM1002 was shown to induce protection against subclinical TB. In conclusion, VPM1002 has become one of the frontrunners of novel TB vaccines.

In addition, VPM1002 is being tested as a new therapeutic agent against bladder cancer. Current treatment of choice for bladder cancer patients is BCG. However, a relatively large proportion of bladder cancer patients do not tolerate BCG treatment. This group of patients has been selected for a clinical trial with VPM1002 as alternative treatment choice. The vaccine has successfully passed phase I and is now under phase II assessment. Recruitment has been completed and study results are expected by the end of 2019.

The frequent relapse of drug treated TB patients raises the question where Mtb hides and thereby evades drug treatment. One particular cell type, which could serve as niche for Mtb was identified both in human individuals with LTBI and in an experimental mouse model. The cell was defined as a long-term repopulating pluripotent hematopoietic stem cell which rests in the bone marrow and eventually recirculates in the body. The demonstration of viable Mtb residing in these stem cells indicates that they serve as a refuge for Mtb against drug treatment and, therefore, could provide an explanation for relapse after apparently successful drug treatment of TB. These experiments were performed together with the group of Fritz Melchers. An alternative cellular niche for Mtb was further investigated, namely adipocytes. Our investigation revealed a profound modulatory capacity of Mtb on adipose tissue biology.

In parallel, we aim at a better understanding of TB vaccination in animal models. Using an unbiased mass spectrometry-based approach, we could identify novel antigens of VPM1002 and Mtb which could be harnessed for rational TB vaccine design. In an attempt to improve the already profound protection afforded by VPM1002, we deleted the anti-apoptotic *nuoG* gene in VPM1002. Our work not only identified a role for *nuoG* in autophagic/xenophagic pathways but also demonstrated further improvement of vaccine efficacy while sustaining the excellent safety profile. In addition to creating new vaccines, we were also interested in the impact of the route of administration on the efficacy of BCG. To this end, we showed improved vaccine efficacy of BCG administered by the aerogenic mucosal route over the parenteral route. Intriguingly, mucosal BCG vaccination induced airway resident memory T cells of both CD4 and CD8 subpopulations. These findings indicate that a shift from intradermal to mucosal vaccination could further improve efficacy of BCG-based vaccines. In a further attempt to test novel routes of vaccine administration, we performed tattoo immunization with a cDNA encoding Mtb antigens as a boost to BCG. However, the cDNA boost provided only a minor improvement in lung pathology compared to BCG alone. During our vaccination studies of mice with VPM1002 and BCG we obtained evidence for gender differences in vaccine efficacy. Accordingly, we further analyzed the role of gender in

vaccine efficacy and found that for both BCG and VPM1002 female mice were better protected than male ones. We propose that future vaccine studies should analyze both genders separately.

D *Mycobacterium tuberculosis*: Biology, Biochemistry, Omics and Drug Susceptibility

Mycobacterium tuberculosis (Mtb) can persist in humans throughout the lifetime of its host. We seeked to understand the mechanisms that enable Mtb to resist and endure stresses presented by the intracellular milieu, host immunity and anti-TB drug therapy with the following approaches.

Using a holistic systems biology approach, an atlas of the comprehensive proteome of Mtb, in absolute terms of both composition and dynamics during dormancy and reactivation, was successfully established. The data obtained from different omics approaches led to the construction of a regulatory network of Mtb infection of the host cell *ex vivo*. This work was done in collaboration with the group of Uwe Sauer.

We are studying the cellular respiration and energy metabolism of Mtb during its transition into the hypoxic/anoxic state. We predicted that mycofactocin, a unique ribosomally derived electron carrier might fulfill the role of alternate reducing equivalents to energize respiration and oxidative phosphorylation. Consistently, targeted mutagenesis of these gene clusters and phenotypic analyses of the mutant/s thereof confirmed the role of mycofactocin in redox homeostasis and hypoxia/anoxia survival. In addition, mycofactocin is required to maintain NADH/NAD⁺ and intracellular ATP levels during Mtb growth at lower oxygen concentration. Therefore, understanding the mycofactocin-dependent regulatory mechanism may pinpoint vulnerabilities, which could help in developing interventions to treat LTBI and significantly reduce the risk of reactivation of TB. Given the critical role of copper in the control of bacterial pathogens including Mtb, we demonstrated that a small compound utilizes copper within phagosomes to augment killing of Mtb via reactive oxygen intermediates. These findings pave the way for a next generation of multifunctional antimicrobials. This work was done in collaboration with A.M. Angeles-Boza. We also identified a novel host-directed treatment approach in adjunct to canonical TB drug treatment: Inhibition of host lactate dehydrogenase A by a small molecule potentiated the bactericidal activity of the widely used TB drug isoniazid. Importantly, this molecule limited Mtb replication and onset of necrotic lesions in NOS2-deficient mice and augmented isoniazid effects in Mtb killing.

We have repurposed our established murine models recapitulating the immunopathophysiology of human pulmonary TB for testing the efficacy of anti-TB drugs. In this regard, we were actively engaged with academic and industry laboratories under the umbrella of the Innovative Medicines Initiative (IMI) to discover, develop, and evaluate new TB drugs in humanized mice and NOS2-deficient mouse models presenting hypoxic granulomas similar to human granulomas (solid versus caseous types). Notably, our experiments point to NOS2-deficient mice with hypoxic necrotizing lung lesions as experimental predictor of outcome of treatment with novel drugs in humans. Moreover, our humanized mouse model could be successfully harnessed for preclinical testing of novel TB drug regimens. In conclusion, the NOS2-deficient and the humanized mouse models developed by our group mimic pathology in human TB more precisely and provide more reliable preclinical models for prediction of outcome of TB drug treatment. These studies are accompanied and strengthened by *in vitro* cultures of human lung tissue.