

Department of Immunology

Director: Stefan H. E. Kaufmann

2. Immunity in Tuberculosis and Other Bacterial Infections

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Basic understanding of the innate and adaptive immune response to Mtb remains a core activity of our group. Our major focus has been on innate immunity, comprising both professional phagocytes (MPs, neutrophils [1;2], DCs [10] and transformed macrophages [4]) and nonprofessional immune cells (epithelial and endothelial cells [5]). In part, this is due to the fact that most biomarkers characteristic for TB patients are related to innate immunity (see Section 5). In a reverse translational approach, we have concentrated our efforts on the characterization of biological functions of biomarkers of unknown or incompletely understood functions in TB, because we assume that they are important players in immunity to TB. These studies have addressed the role of microRNA 223 on the post-transcriptional level [6], the role of type I interferon (IFN) [7] in signaling events via IFNR/IFNAR and of neutrophils on the cellular level [1;2;8]. According to our findings, neutrophils primarily play a harmful role in TB [1;6;7]; first because they cause damage to lung tissue in TB and second because granulocyte-like myeloid-derived suppressor cells (MDSCs) impair protective immunity [2]. IFN-induced molecules of interest include the guanylate-binding proteins (GBPs), which are consistently upregulated at the transcriptional level in biomarker studies in TB patients and in individuals with high risk of TB [9].

We also remain 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 phenazines from *Pseudomonas aeruginosa* and naphthoquinones from Mtb, as pathogen-associated molecular patterns [10]. Future studies aim at further understanding the role of the AhR in the context of TB drug therapy, *P. aeruginosa* infection and recognition of other naphthoquinones, such as lawsone, a major component of henna, and its role in skin disease. Finally, in collaboration with the group of Michael Kolbe (MPIIB, Structural Systems Biology), we continue to pursue the structural characterization of AhR.

At the molecular level, the crosstalk between Mtb and host molecules remains of high interest to us. Our studies indicate a role for syndecans (Sdc) in the invasion of lung epithelial cells [15, added in proof]. 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 knockout (KO) of distinct Sdc involved in Mtb binding rendered mice more resistant to Mtb colonization. Autophagy is emerging as an important cell autonomous defense mechanism against intracellular pathogens. Hence, molecules involved in modulating autophagy are of great interest [11]. The molecules of interest include galectin-8, which serves as a versatile sensor for vesicle damage induced by pathogens, Rab24, which coordinates mTOR signaling, and NEDD4, which is involved in autophagy. In fact, apoptosis and autophagy remain of great interest both in the context of infection and of vaccination (see also Section 3, "Rational Vaccine Development"). A recent study in our laboratory elucidated the role of cGAS and STING 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 antibacterial activities in macrophages independent of STING/type I IFN.

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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 MPs. Consequently, IL-36 γ has been postulated to play a critical role in defense against TB [12]. 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 a molecular level remains incompletely understood. Utilizing genetically modified mice, the role of TGF- β in TB lung granulomas is being deciphered. The chemokine family is well known for its high redundancy as best illustrated by the large number of chemokines which utilize a single receptor. It was therefore surprising to us to identify a unique role of CXCL5 in TB since it shares the CXCR2 receptor with CXCL1, CXCL2, CXCL3, CXCL6 and CXCL15 in TB [5]. Absence of CXCL5 markedly reduced exacerbated pathology in experimental TB of mice infected with a high dose inoculum of Mtb.

Other studies have focused on the role of different cell types in control of TB infection and in lung pathology. The granuloma is the hallmark of TB. Careful dissection of different macrophage populations in the lung identified both arginase 1- and nitric oxide synthase-2 (NOS2)-expressing macrophage subsets, reflecting the presence of both M2 and M1 macrophages in granulomas [13]. Intriguingly, not only NOS2-expressing macrophages of M1 type, but also arginase-expressing macrophages of M2 type, participate in control of Mtb. M2 macrophages gain increasing importance when NOS2 is rendered ineffective by hypoxia. Platelets have been largely ignored in TB. Our recent findings suggest that platelets promote monocyte differentiation into multinucleated giant cells, thereby exacerbating lung granuloma pathology [4]. Aside from MPs, numerous nonprofessional phagocytes have been claimed to serve as quiescent or protective niche for Mtb, notably epithelial cells. We have also started to characterize the role of adipocytes as well as of hematopoietic stem cells (together with Senior Group Leader Fritz Melchers) as safe sanctuary for Mtb in LTBI. Characterization of DCs and presentation of mycobacterial antigens to T cells revealed crosstalk between myeloid and plasmacytoid DCs in induction of T cell responses [3]. Moreover, we identified a unique role of Mtb antigen-independent memory CD8⁺ T cells and natural killer (NK) cells, in early protection against TB [14; 16, added in proof]. 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 Independent Research Group Leader Hedda Wardemann.

In a more holistic view, a human lung tissue culture system has been established to analyze global tissue responses to Mtb invasion. Aside from whole-tissue omics, selected cell types (including MPs, epithelial and endothelial cells, and different lymphocyte subsets) will be analyzed with a focus on their role as effectors or host cells for Mtb. We have also succeeded in establishing a humanized mouse model for TB, which will be harnessed for better understanding of human immunity in an *in vivo* setting, including studies focused on vaccination against, and drug treatment of, TB.

In conclusion, research on the immunology of TB remains of high priority and is fueled by (i) direct hypotheses and (ii) questions raised by clinical studies.

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