Host-pathogen interactions during (myco)bacterial respiratory tract infections
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Host-pathogen Interactions During (Myco)bacterial Respiratory Tract Infections

General Introduction
Host immunity is classically divided into two functionally distinct immune systems, the innate and the acquired immune system.

The Innate Immune Response

When an organism infects the body, the defense systems already in place may be well sufficient to prevent replication and spread of the infectious agent, thereby preventing development of disease. These established mechanisms are referred to as the ‘innate’ immune system. The innate immune system is of crucial importance for the initiation of an efficient immune response against invading pathogens. In order to start an appropriate inflammatory response leading to the elimination of microorganisms, pathogens have to be distinguished from self. In addition to initiation of the inflammatory reaction, the innate immune response is thought to orchestrate the adaptive immune response. Although the innate immune response comprises several defense mechanisms, such as phagocytosis and intracellular killing of pathogens by macrophages and neutrophils, complement activation, and activation of NK cells and $\gamma$δT cells, we herein focus on the role of pathogen recognition receptors.

Toll-like receptors

The involvement of Toll in innate immunity was first described in Drosophila. Mutant flies that were defective in individual components of the Drosophila Toll signaling pathway (Toll, Spaetzle, Tube or Pelle) were highly susceptible to fungal infection due to the lack of production of the antifungal peptide Drosomycin (1). A year after the discovery of Drosophila Toll, the homology of the Toll/IL-1 receptor (TIR) domain in Drosophila and mammals, led to the discovery of mammalian Toll-like receptors (TLRs) (2).

It is now well established that TLRs represent a primary line of defense against invading pathogens in mammals, plants and insects (for review see (3, 4)). TLR are evolutionary conserved and recognize pathogen associated molecular patterns (PAMP) which are highly conserved motifs of micro-organisms. Cells expressing TLRs are diverse: TLR expression is found in typical cells of the innate immune system like monocytes/ macrophages and neutrophils but also on dendritic cells (DC) and epithelial cells. In general, activation of TLRs leads to the production of pro-inflammatory cytokines and chemokines, cyclooxygenase, nitric oxide and upregulation of adhesion molecules resulting in to initiation the inflammatory response. Different TLR and TLR combinations recognize distinct ligands (table 1). Stainings of TLRs reveal that TLR1, TLR2, TLR4, TLR5 and TLR6 are all localized to the plasma membranes whereas TLR3, TLR7, TLR8 and TLR9 are preferentially
<table>
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<th>RECEPTOR</th>
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<td>Triacyl lipopeptides, Soluble factors, Non-capped lipoarabinomannan</td>
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<td>Toxoplasma gondii</td>
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**TABLE 1: TLR and their ligands**

*These ligand preparations were possibly contaminated with lipopolysaccharide or other potent microbial components. Adapted with modifications from (4).

Present in intracellular compartments such as endosomes. The nature of the ligand recognized by individual TLRs seems to determine their expression pattern. For example, TLR3, 7, 8 and 9 all recognize nucleic acid structures whereas TLR1,
TLR2, TLR4, TLR5 and TLR6 generally recognize cell wall components (table 1). Of note, surface TLRs are not completely restricted in their expression pattern, for example, TLR2 can localize to phagosomes following exposure to certain ligands (5).

**Toll-like receptor signaling**

TLR4 was the first human TLR to be identified and was for long the prototypic TLR. The TLR4 receptor complex consists of TLR4, CD14 and MD-2 (fig. 1). LPS-binding protein is generally believed to associate more loosely with the complex. Moreover, CD14 has been reported to be important in TLR2 function (6, 7). Differences between TLRs are mainly emerging from the signaling pathways used. TLR-signaling pathways are dependent on intracellular adaptor proteins (Reviewed in (4)). Intracellular adaptor proteins directly interacting with TLRs contain the TIR domain, which is also part of the Interleukin (IL)-1 and IL-18 Receptors. After binding of a specific ligand to TLR2 (forming heterodimers with TLR1 or 6), TLR4, TLR5, TLR7, TLR8 and TLR9, the intracellular adaptor molecule MyD88 (myeloid differentiation primary response protein 88) mediates activation of IRAKs (IL-1-receptor-associated kinases) and TRAF6 (tumour-necrosis-factor-associated factor 6) resulting in the release of nuclear factor-κB (NF-κB). NF-κB translocates to the nucleus and induces expression of inflammatory cytokines. MyD88 is dependent on a second intracellular adaptor molecule, MAL (MyD88-adaptor like protein or TIRAP: TIR-domain-containing adaptor protein) (8, 9).

![Figure 1: Scheme of TLR signaling pathways.](image-url)
In addition to this MyD88 dependent signaling, there is more: TRIF (TIR-domain-containing adaptor protein inducing interferon (IFN); also known as TIR-domain-containing molecule 1; TICAM1), is a MyD88 independent adaptor molecule which is important in signaling of TLR3 and TLR4 (10-12). The adaptor molecule TRAM (TRIF-related adaptor molecule; also known as TIR-domain-containing molecule, TICAM2) is important for TRIF function (13-15) and activation of TRIF/TRAM mediates activation of Interferon Regulatory Factor 3 (IRF-3) and "late" NF-κB activation. IRF-3 activation results in gene transcription of IFNα and IFNβ in turn activates STAT1 and induces several IFN-inducible genes (16, 17). In case of TLR4, "late" activation of NF-κB is referred to as the MyD88 independent signaling pathway (18, 19).

Excessive production of mediators released upon TLR activation contributes to the pathogenesis of inflammation, as observed in sepsis. Several molecules such as SIGIRR (single immunoglobulin IL-1R-related molecule), sMyD88 (a spliced variant of MyD88), ST2, IRAK-M, A20 or SOCS 1 (Suppressor of Cytokine Signaling) have been described to regulate TLR signaling by suppressing the TLR signaling cascade (Reviewed in (20)).

Cytokines and chemokines
Among the various mechanisms of innate immunity, cytokines and chemokines play a major role in orchestrating the antimicrobial host defense. Cytokines/chemokines are broadly defined as molecules that are made by one cell and act on another. Roughly, cytokines can be divided in two classes: Pro-inflammatory (e.g. IL-1 and Tumor Necrosis Factor α (TNF)) and anti-inflammatory cytokines/soluble cytokine receptors or cytokine receptor blockers (IL-10, transforming growth factor (TGF)-β, soluble TNF receptors, IL-1 receptor antagonist). Cytokine/chemokine activities are dependant on many factors like the amount cytokine produced, the nature of the target cell, the nature of produced cytokines, the timing and the sequence of cytokine action (21). Cellular recruitment on chemokine gradients and trafficking and recirculation of leukocytes towards the site of inflammation, is a multi-step process including rolling, adhesion, and migration across endothelial barriers.

C-type lectins
DCs are professional antigen presenting cells that are seeded throughout peripheral tissues to act as sentinels that process and presents thereby enabling adequate immune responses (22). Recently, many novel cell-surface molecules have been identified that are involved in antigen capture by DCs. Herein, a large diversity of C-type lectin receptors (CLR) has been identified on DCs that are involved in the recognition of carbohydrate structures (23, 24). Recently it is becoming clearer that some CLR, like Dectin-1, DC-Specific intercellular adhesion molecule
grabbing nonintegrin (DC-SIGN) and the mannose receptor, are more than scavenger receptors. Moreover, it was demonstrated that bacteria and viruses van use CLR in order to suppress immune function or to disseminate from mucosal surfaces to the draining lymph nodes (25, 26). *Mycobacterium tuberculosis* modulates the immune function by interaction of its cell wall component mannose-capped lipoarabinomannan. It blocks TLR induced DC maturation and induces IL-10 while inhibiting IL-12 production (26, 27). Five murine homologues of DC-SIGN exist but cellular expression has been only described for two of these: murine DC-SIGN (mDC-SIGN) and SIGN Related (SIGNR)-1 (28-31). mDC-SIGN is expressed by DC (32, 33). In contrast, SIGNR1 is abundantly expressed in lymph nodes by medullary and subcapsular macrophages, in spleen by marginal zone macrophages and in the liver by the sinusoidal endothelial cells (31). In addition, Taylor et al. demonstrated that mSIGNR1 is also expressed on resident peritoneal macrophages (30). The function and binding capacities of SIGNR1 have been studied in detail and are similar to human DC-SIGN (31, 34, 35). The function of these murine homologues of DC-SIGN during infection is largely unknown.

The Adaptive Immune Response

The main feature distinguishing the adaptive immune response from the innate is that specific memory of infection is imprinted in the adaptive immune system, so that should a subsequent infection with the same organism occurs, a specific and effective response is evoked fairly quickly. Classically, the adaptive immune response can be divided in two parts: The cellular immune response and the humoral immune response.

TLRs serve an important role in bridging innate and adaptive immunity. TLRs sense microbial products, trigger the production of cytokines and chemokines and induce DC maturation. DC express a broad range of TLRs through which they can recognize a variety of pathogens. After challenge with microbial or inflammatory stimuli, immature DC undergo a complex process of maturation, resulting in their migration from tissues to secondary lymphoid organs. During this migration, DC upregulate major histocompatibility complex (MHC) and costimulatory molecules and present antigen-specific peptides to the T cell receptor (TCR). All T cells have one specific TCR that is specialized for binding to the complex of MHC molecule and peptide derived from the micro-organism. Recognition of the peptide in combination with MHCII and the presence of co-stimulatory molecules primes the T cell. There are many lymphocyte co-stimulatory molecules among which interaction of CD28 and B7 is fairly prominent. Amonst others, an important co-stimulatory pair belonging to the TNF receptor family is CD27 and its ligand CD70 (36, 37). Naïve T cells are generally long-lived in their resting state. Upon activation, T lymphocytes reenter the cell cycle and divide rapidly to produce progeny that will differentiate into
General Introduction

One of the most important events in the induction of cellular immunity is the differentiation of CD4⁺ T cells into the two major classes of CD4⁺ effector T cells. The milieu of cytokines and co-stimulatory molecules in which T cell priming takes place determines the T helper type (Th) polarization of the adaptive immune response (38).

**Cellular Mediated Immunity (Th1)**

T cells that are initially stimulated with IFN₇ and IL-12 generally develop along a Th1 pathway, in part because IFN₇ inhibits Th2-cell development. The induction of IFN₇ is regulated by IL-12, IL-1, TNF and IL-18, produced by accessory or antigen presenting cells such as macrophages and DCs. Th1 CD4⁺ T cells are potent producers of the typical Th1 cytokine IFN₇. CD8⁺ T cells are also important cells in the cellular immune response. CD8⁺ T cells recognize peptide fragments in the context of MHC class I which bind to the TCR or in the context of CD1 present on professional antigen presenting cells (39). CD8⁺ T cells, also called cytotoxic T lymphocytes, exert their effects through lysis of the target cell presenting a foreign peptide or through production of the protective cytokine IFN₇ and TNF following antigen presentation.

IFN₇ is of crucial importance during several infectious diseases in particular during intracellular infections such as caused by *M. tuberculosis*. IFN₇ exhibits its main effect on macrophages that are considered to be important effector cells of the cellular immune response. Macrophages can be activated by IFN₇, by other cytokines like TNF or by direct contact with activated T cells. IFN₇ stimulates phagocytosis, the oxidative burst and intracellular killing of microbes. Moreover IFN₇ can activate macrophages to restrict growth of intracellular mycobacteria like *M. tuberculosis*. For a long time nitric oxide synthase 2 (NOS2) was considered to be the key player in IFN₇ induced anti-microbial activity. By NOS2 action, nitric oxide and its metabolites are generated from L-arginine and represent one of the major antimycobacterial defense mechanisms of macrophages (40, 41). A second antimicrobial defense mechanism is based on reactive oxygen, which is generated by transfer of an electron from NADPH to molecular oxygen by NADPH-oxidase. The latter mechanism seems to be of less importance since mice deleted of the gene for NOS2 were highly susceptible to *M. tuberculosis* infection, whereas mice incapable of making NADPH-oxidase were only slightly or not susceptible (42-45). Recently, another important IFN₇ inducible anti-microbial molecule was found; LRG-47, a GTPase that works independently of NOS2 (46). Newly published work has revealed autophagy as a novel facet of IFN₇’s antibacterial activities (47). In addition, IFN₇ upregulates MHC class I and class II on a variety of cells, thereby stimulating antigen presentation to both CD4⁺ and CD8⁺ T cells.
Humoral Immunity (Th2)

Antibodies are synthesized by host B lymphocytes when exposed to foreign antigens in combination with MHC class II. Each antibody has a variable recognition site that is complementary to the surface of the foreign antigen and enables it to bind with varying degrees of strength to the antigen. The invariable part of the antibody (Fc tail) is specialized in the effector function of the antibody such as activating the complement system and binding to Fc receptors inducing phagocytosis by macrophages or neutrophils. Hence, when a microbial antigen is coated with specific antibodies, they induce complement fixation and phagocytosis ultimately leading to activation of innate defense mechanism of the acute inflammatory response. Moreover, complement assembly can result in formation of the membrane attack complex, which may help killing the microorganism by disturbing the membrane stability of the organism. Another function of specific antibodies is inhibition of microbial reactions such as blocking of bacterial toxins or attachment of the microorganism to its host receptor.

There are two types of antigens that stimulate B cells: The ones that require T cell help and the ones that do not (T cell independent antigens or TI antigens). The majority of antigens will stimulate B cells only if they have the assistance of antigen specific and primed Th cells. The B cell receptor captures complementary antigens, after which the antigens are internalized and peptides in association with endogenous MHCII are presented. A primed T cell recognizes this complex and causes stimulation of the B cell with subsequent activation, proliferation and maturation leading to the production of specific antibodies and to antibody memory. TI antigens consist of two types: TI-1 antigens contain several molecular features that enable them to stimulate a variety of receptors on the B cell independently of the specific B cell receptor. TI-1 antigens are also referred to as polyclonal activators. TI-2 antigens are characterized by high molecular weight, repeating epitopes and slow degradability in vivo. Phosphorylcholine (PC) from the pneumococcal polysaccharide capsule is an example of a TI-2 antigen important in host defense against infectious diseases (48-51). TI-2 induced antibodies against antigens present in the digestive tract and low-affinity natural antibodies against pneumococcal polysaccharides are produced by B-1a B cells in the mouse and IgM memory cells in man (49, 51). These natural antibodies are considered part of the natural defense against infection. During the first days of infection with *S. pneumoniae*, more protective IgM against pneumococcal TI-2 antigens is formed by B-1b cells (51).
Pulmonary Infectious Diseases

As a result of the daily inhalation of 10,000 litres of air, the human lung is exposed to a large number of airborne pathogens which can result in a variety of respiratory tract infections.

**Tuberculosis**

Tuberculosis is a disease of the lungs caused by the acid fast rod *M. tuberculosis*. *M. tuberculosis* is a well-adapted and very successful human pathogen. This has infected at least one-third of the world's population and is responsible for more than 2 million deaths each year (52). Only a subset of infected people progress to primary tuberculosis. Most infections are controlled by the immune response and are asymptomatic; nonetheless, the bacilli persist in the host (termed latent tuberculosis). It is estimated that an infected person has a 10% lifetime change of reactivating the latent *M. tuberculosis* infection and progressing to active disease. The chance of developing tuberculosis increases to 10% per year if latent tuberculosis patients are co-infected with the human immune-deficiency virus (HIV). During active tuberculosis, infected individuals are contagious and capable of transmitting the infection to others. *M. tuberculosis* is famous for its strategies to circumvent immune recognition which starts early after ingestion by macrophages. Immune evasive strategies associated with *M. tuberculosis* are inhibition of phagosome-lysosome fusion, inhibition of proton pumps to stop lowering the pH in phagosomes and the reduction of MHC class II expression by host macrophages (reviewed in (53)).

**Innate immune response to M. tuberculosis**

After inhaling the bacillus, macrophages are the phagocytic cells that initially ingest *M. tuberculosis*, and provide an important cellular niche during infection. After entrance of *M. tuberculosis*, the bacterium is recognized by receptors of the innate immune system thereby triggering the inflammatory response. Up till now, several studies investigated the role of TLRs and TLR associated molecules in mycobacterial infection (54-66). It appears as striking that these studies are divergent in their results; they differ from none, moderate or even strong effects due to the lack of a TLR or TLR associated molecule. Several studies in which protective roles for TLR2, TLR4 or MyD88 were found, deficiencies of these molecules resulted in exaggerated inflammatory responses most likely due to the increased mycobacterial load, providing a more potent proinflammatory stimulus (57, 59-62, 64). Interestingly, mice deficient for the proinflammatory cytokines TNF and IL-1 are susceptible to tuberculosis (67-72).
Adaptive immune response to M. tuberculosis

Adaptive immunity to M. tuberculosis is principally a function of the cellular immune response. Consequently, initiation and maintenance of adaptive immunity to tuberculosis require several specific cellular interactions. The requirements for immune control of tuberculosis are reasonably well defined. Both CD4+ and CD8+ T cells are essential for protective immunity against M. tuberculosis (reviewed in (73)). Th1 polarized CD4+ effector T cells are important for the host defense against tuberculosis, as indicated by the high susceptibility of mice lacking IFNγ, CD4+ T cells or IL-12p40 (74-79). Granulomas are the characteristic histopathological lesions of tuberculosis that can form in any tissue infected with M. tuberculosis. Granulomas in humans are characterized by a necrotic core, surrounded by macrophages (some of which adopt an epitheloid morphology or become multinucleated “giant cells”) and lymphocytes. A mature granuloma is surrounded by fibroblasts. In murine tuberculosis the architecture of granuloma is different; no necrotic core can be found and the presence of multinucleated macrophages is very rare (fig. 2B). Evidence suggests that TNF plays a crucial role in the formation and the maintenance of the tuberculous granuloma in both mice and humans. Paradoxically, TNF also contributes significantly to the development of immunopathology. As long as the granuloma remain intact, M. tuberculosis is held at bay and this state is termed “latent” tuberculosis. However, when the immune status of the host changes, the disease can reactivate or re-infection can occur inducing open tuberculosis.

Figure 2: Granuloma. A: Simplified scheme of human granuloma without fibrotic capsule; 1B: Representative lung histology of a granuloma in wild-type mice, 6 weeks after intranasal infection with 1000 CFU of M. tuberculosis Erdmann. H&E staining, original magnification x 10

Host factors defining susceptibility to tuberculosis

Compromised hosts are more prone to develop infectious diseases and especially M. tuberculosis is known to affect immunocompromised patients. The host can be compromised in many ways. This can include defects of both the innate and the
adaptive immune response and can be roughly divided in two categories: primary immunodeficiencies, which are inherited (gene based) or occur through exposure in utero, and secondary or acquired deficiencies, which are due to an underlying disease or occur as a result of treatment. Regarding tuberculosis and mycobacterial infections other than tuberculosis (such as caused by *M. avium* or *M. kansasii*), the primary and secondary factors affecting the adaptive immune response are of great importance. There are several genetic causes of tuberculosis hyper-susceptibility, which affect the Th1 response necessary to combat *M. tuberculosis*. Examples in humans are the recessive mutations in IFNγ-receptor ligand binding chain, the IL-12p40 subunit and the IL-12 receptor β chain (80). Moreover, Pan et al. have recently described the sst1 (super susceptibility to tuberculosis) locus in mice and identified a candidate tuberculosis susceptibility gene, intracellular pathogen resistance (lpr)1 within this locus. Ipr1 gene controls *M. tuberculosis* replication in vitro and in vivo and regulates an apoptotic cell death pathway in macrophages (81).

Immunodeficiencies due to genetic defects are rare. In contrast, secondary immune defects are a big threat. Worldwide, two forms of secondary adaptive immunodeficiencies are primarily responsible for the high numbers of tuberculosis cases and spread of the disease: malnutrition and Human immunodeficiency Virus (HIV) infection. The major form of malnutrition, protein-energy malnutrition, presents with a wide range of disorders and results in reduced circulating numbers of T cells leading to inadequate T cell responses, structural changes in the lymphoid organs and reduction of production of complement components and antibodies. During HIV infection, CD4+ T cells are depleted. Consequently, in both malnutrition and HIV infection, the Th1 response necessary for keeping *M. tuberculosis* in the granuloma is disturbed giving rise to open tuberculosis. The bacillus replicates, affects more parts of the lung and spreads via the lymphatic system throughout the body.

Immunodeficiencies associated with western medicine and life style are also associated with the occurrence of tuberculosis. The use of anti-TNF therapy in Crohn’s disease and rheumatoid arthritis renders the patients more vulnerable to developing tuberculosis (82). Moreover, latent tuberculosis is to be considered before organ transplantations that require immunosuppressive therapy.

**Atypical mycobacterial infections**

*Mycobacterium kansasii*

*M. kansasii* is one of the most frequent nontuberculous mycobacterial pathogens isolated from clinical specimens. Infection with *M. kansasii* can cause pulmonary disease similar to tuberculosis in patients with various immune deficiencies, in particular HIV infection or in patients with pre-existing pulmonary disease like
asthma and chronic obstructive pulmonary disease (83-86). Since the start of the AIDS epidemic, a vast increase in M. kansasii infection incidence has been observed (87). However, little is known about pathogenicity, mode of transmission and natural reservoir of M. kansasii. The organism has been recovered occasionally from rivers and lakes but also from tap water, showerheads, and drinking water distribution systems and is thought to be acquired from the environment rather than from contact with infected patients. Unlike M. tuberculosis, culturing of M. kansasii from human sources is not exclusive proof of disease: as many as one-third of isolates has been reported to represent colonization or indolent infection of the respiratory tract rather than disease (88).

**Mycobacterium smegmatis**

*M. smegmatis* was identified in 1884, following the identification of *M. tuberculosis* in 1882. This fast-growing mycobacterial species that can form visible colonies in three days (in contrast to three weeks in case of *M. tuberculosis*) is a saprophyte, an opportunistic bacterial pathogen that rarely leads to infectious disease. In humans, *M. smegmatis* primarily is associated with soft tissue and wound infections. *M. smegmatis* rarely causes lung infection in the immunocompetent host (89). However, in severely immuno-compromised patients like patients with inherited IFNγ receptor 1 deficiency *M. smegmatis* infection can disseminate and can be lethal (90). In addition, *M. smegmatis* can cause pulmonary infections in patients with an underlying condition such as lipoid pneumonia (89, 91-93). Little is known about the pathogenesis of infection by either virulent or avirulent fast-growing mycobacteria like *M. smegmatis*.

**Gram-positive pneumonia**

*Streptococcus pneumoniae*

Although many pathogens have been associated with community acquired pneumonia (CAP) a small range of pathogens is responsible for most cases. The predominant pathogen in CAP is *Streptococcus* (S.) pneumoniae (pneumococcus), which is the causative pathogen for about two-thirds of all cases of bacteremic pneumonia and responsible for an estimated 10 million deaths annually (94, 95). *S. pneumoniae* is a Gram-positive diplococcus and a facultative anaerobic bacterium. It exists in encapsulated and non-encapsulated forms. The capsule defines the virulence of the bacterium and as a rule, only the encapsulated serotypes can cause disease. Ninety different serotypes of *S. pneumoniae* have been described. Groups at risk for diseases caused by pneumococci include children, elderly people and patients with immunodeficiencies.

Protection against pneumococcal infections is mediated by opsonin-dependent phagocytosis. Antibody-initiated complement-dependent opsonisation, which
activates the classical pathway of complement activation, is thought to be a major immune mechanism against *S. pneumoniae* infections (96-98). Important antigens present on the pneumococcal capsule are polysaccharides and phosphorylcholine (PC) which are both TI-2 antigens. B-1 cells, predominantly located in peritoneal and pleural cavities, are the major source of natural antibodies against PC (low affinity IgM) that occur in uninfected, “antigen-free” mice, and these natural antibodies are important in controlling *S. pneumoniae* infection (48, 49, 51). Moreover, early IgM or IgG responses against pneumococcal polysaccharides are necessary to protect from infection (49, 51). The mechanism of clearance of *S. pneumoniae* depends on the interaction of type-specific antibodies, complement, macrophages, neutrophils and epithelial cells in lung, liver and spleen. Herein, activation of phagocytes through TLRs triggered by *S. pneumoniae* specific PAMPs is feasible and a crucial role for TLR2 was described in a model of pneumococcal induced meningitis (99, 100).

**Gram-negative pneumonia**

*Non-typeable Haemophilus influenzae*

In addition to *S. pneumoniae* several other bacteria that can cause CAP. Another typical bacterium that causes CAP is *H. influenzae*, a pleomorphic, non-motile and Gram-negative rod (101-104). The organism is a facultative anaerobe and characterized by an absolute requirement for supplemental NAD (factor V) and a source of heme (factor X) for growth under aerobic conditions (102). Isolates from *H. influenzae* can be subdivided into encapsulated (type a-f) and nonencapsulated. Only a minority of strains have a polysaccharide capsule. The ‘non-typable’ unencapsulated form (NTHi) is a commensal organism in the human upper respiratory tract and in addition to CAP, it can cause localized infections such as middle ear infection, sinusitis and conjunctivitis. Moreover NTHi is frequently implicated in exacerbations of underlying chronic obstructive pulmonary disease or cystic fibrosis (101-104). Patients suffering from NTHi infections are mostly adults and the infection rarely leads to bacteremia. The pathogenesis of NTHi involves contiguous spread within the respiratory tract. This is usually a consequence of abnormalities in either innate or adaptive immune responses (102). For example, viral upper respiratory infection disrupts mucociliary clearance, mucosal integrity, and neutrophil function and predisposes to NTHi infection.

*Klebsiella pneumoniae*

*Klebsiella* (*K.*) species are opportunistic pathogens that can give rise to severe diseases such as septicemia, pneumonia, urinary tract infections and soft tissue infection (105). Typically, *Klebsiella* infections are nosocomial and mainly caused by *K. pneumoniae*, the medically most important species of the genus. The
hospitalized, immunocompromised patient with underlying diseases is the main target of these bacteria. *K. pneumoniae* is a Gram-negative, non-motile, usually encapsulated rod-shaped bacterium of the family of Enterobacteriaceae.

*Acinetobacter baumanii*

Members of the genus *Acinetobacter* recently gained interest as bacterial pathogens that can cause severe bacterial infections in critically ill patients in intensive care units (106). *Acinetobacter (A.) baumanii* is a Gram-negative, nonmotile saprophyte, an opportunistic bacterial pathogen that is a leading cause of opportunistic nosocomial pneumonia worldwide. The rising numbers of infections, the high incidence of mortality, the high antibiotic resistance of *A. baumanii* and the widespread colonization of skin, mucosal membranes and medical equipment makes *A. baumanii* a pathogen of high importance and concern (107-115). Knowledge of host defense mechanisms against this opportunistic Gram-negative bacterium is limited.

**Aim and Outline of this Thesis**

The general aim of this thesis was to obtain more insight into host-pathogen interactions that contribute to an adequate response to microorganisms that invade the respiratory tract. The specific objectives of each individual investigation are delineated in the respective chapters. In this thesis, we focused on pneumonia as a model of acute pulmonary infection and tuberculosis as a model of chronic pulmonary infection.

The first part of this thesis focuses on the role of TLRs and TLR signaling during pneumonia induced by several human pathogens: In Chapter 2, we investigated the roles of TLR4, CD14 and TLR2 during pneumonia induced by NTHi. In addition, we focused on the relative function of the MyD88 dependent and MyD88 independent signaling pathways of the TLR4 receptor complex. In Chapter 3 we studied the roles of TLR4, CD14 and TLR2 in pneumonia caused by *A. baumanii*, whereas in Chapter 4 the contribution of TLR2 to the immune response during pneumonia caused by *S. pneumoniae* was evaluated.

In Chapter 5, we investigated the role of CD14 during tuberculosis in mice. In Chapter 6 we sought to determine which TLR is important for the induction of inflammation by non-mannose capped lipoarabinomannan, a prominent cell wall component of fast growing, non-pathogenic mycobacteria, *in vivo*, by making use of TLR2, TLR4 and CD14 deficient mice.

In next part of this thesis, we studied the role of the murine C-type lectin SIGNR1 in two different models: First during *S. pneumoniae* induced pneumonia
(Chapter 7) and second during chronic lung infection resulting from infection with *M. tuberculosis* (Chapter 8).

*M. kansasii* is, after *M. avium*, the most common pathogen inducing mycobacterial infection other that *M. tuberculosis*. In Chapter 9 we investigated the role of the Th1 effectors CD4\(^+\) T cells, IFN\(_\gamma\) and IL-12 during pulmonary *M. kansasii* infection in mice. In addition, we studied the role of the pro-inflammatory cytokine IL-1 in this murine model of atypical mycobacterial infection (Chapter 10).

The Th1 response is crucial for successful host defense against mycobacterial infections and mice lacking CD27 have an altered host defense against viral infection of the respiratory tract (116). Therefore, another focus of interest of this thesis was the role of the co-stimulatory molecule CD27 during chronic and acute infection (Chapter 11 and Chapter 12). A prominent host factor that weakens Th1 responses and thus increases host susceptibility towards infection is malnutrition. Although several links between malnutrition and the immune system exist (e.g. deficiencies of proteins, zinc, iron or vitamins), leptin, the product of the *obese* gene could be another. By using mice mutant in the *obese* gene, we studied the role of leptin during *M. tuberculosis* infection (Chapter 13). Since leptin was demonstrated to also exert effects during the acute phase of inflammation, we also investigated the role of leptin during Gram-positive and Gram-negative pneumonia (Chapter 14).

**References**

CHAPTER 1


General Introduction


Generall Introductio n


