To achieve great things,

Two things are needed:

A plan, and not quite enough time.

(Bernstein)

Summary

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SUMMARY

Human beings are in constant interaction with microbial pathogens and evolution has mostly selected for peaceful coexistence. Successful survival is determined by host-pathogen interactions at a cellular level: coexistence with mutual advantages on the one hand and protective immune responses on the other hand. Human immune responses to infectious diseases are aimed at removal of the causative pathogen but are also frequently responsible for damage to the host cells and tissues. Variation of genes encoding for immune receptors and signaling proteins affect the susceptibility to and severity of infectious diseases such as bacterial meningitis (BM).

The studies described in this thesis aim to unravel the details of the pathogenesis and host immune response to BM and how genetic variation affects these processes and thereby the outcome of disease.

PART I focuses on meningitis caused by *Mycobacterium tuberculosis* (*M. tub*). *Chapter 2* describes a retrospective cohort study of 554 children with tuberculous meningitis (TBM) in South Africa, a country with incidence figures of this disease ranging among the highest worldwide. In this study the relationship between presenting clinical characteristics and outcome of pediatric TBM was described. Variables independently associated with poor clinical outcome were ethnicity, stage of disease, headache, convulsions, motor function, brainstem dysfunction, and cerebral infarctions. TBM starts with nonspecific symptoms and is mostly diagnosed when brain damage has already occurred, so the focus should be on early diagnosis.

In order to better understand the details of TBM pathogenesis and subsequent inflammatory responses, animal studies are of eminent importance. *Chapter 3* summarizes animal models that have studied TBM pathogenesis, especially granuloma formation, and local immune responses inside the central nervous system (CNS). Animal models are crucial to our understanding of both host and bacterial factors in TBM. The non-human primates are irreplaceable for drug and vaccine testing. The rabbit model seems to most closely mimic human TBM. The murine model however, is superior in terms of studying the immunological response and is ideal to study the genetics of host defense.

Chapter 4 describes the development of a new murine model to study the pathogenesis of TBM focusing on inflammatory mediators in the CNS. Direct intracerebral inoculation with *M. tub* induced development of granuloma in the mouse brain and mimics human TBM pathogenesis with secondary subependymal *Rich foci* after primary pulmonary infection. Although the cytokine and chemokine patterns differ from human disease, this model may well be used to study detailed inflammatory

responses inside the brain, identifying the receptors and proteins involved and the genetic basis it depends on.

PART II focuses on meningitis caused by *Neisseria meningitidis* (NM) and *Streptococcus pneumoniae* (SP) and how genetic variation influences the innate immune responses upon BM, thereby affecting susceptibility, course of disease and prognosis. In *Chapter 5*, studies that describe associations with single nucleotide polymorphisms (SNPs) in large cohorts of patients with invasive pneumococcal and meningococcal disease, including meningitis, were summarized and applied to the pathogenesis of BM. Studies on genes involved in adhesion to epithelial surfaces, pathogen recognition, complement and cytokine responses, important steps in meningitis pathogenesis, were the focus of this review. Although several SNPs with a significant effect on susceptibility to pneumococcal and meningococcal disease have been described, no study focused exclusively on BM.

In *Chapter 6* the discovery of a significant association with variation in the Toll-like receptor 9 (TLR9) gene and the susceptibility to develop meningococcal meningitis is described. The *TLR9* +2848-A allele was significantly more frequent in 392 controls than in 389 survivors of meningococcal meningitis indicating a protective effect of this SNP. The biological consequence of this SNP seemed that it alters the ability of TLR9 to respond to molecular motifs of meningococci, resulting in upregulated immune responses and thus protecting against severe disease upon meningococcal infection.

Chapter 7 describes a study that compares *TLR9* genotypes amongst BM survivors clustered according to variables determining severity of disease. Both *TLR9* -1237 and *TLR9* +2848 SNPs are associated with a decreased incidence of bacteremia with NM and also with a more pronounced inflammatory response inside the CNS in terms of significantly higher levels of pleocytosis and significantly more decreased glucose levels in cerebrospinal fluid. Thus, people carrying either of these SNPs will be relatively protected to develop meningococcemia upon infection with *N. meningitidis*. However, in case of unanticipated bacteremia and possible subsequent meningitis, they are able to develop more pronounced TLR9 induced inflammatory responses inside their brain immune cells, resulting in more efficient removal of meningococci from the CNS but also more neuronal damage, reflected by an increased chance to develop post meningitis hearing loss.

In *Chapter 8* a study on BM susceptibility using a multigene approach is described. Genotype frequencies of SNPs in *TLR2*, *TLR4*, *NOD1*, *NOD2*, and *CASP1* in 473 survivors of childhood BM were compared to healthy ethnically matched controls. *TLR4* +896 and *NOD2* SNP8 were significantly associated with susceptibility to develop MM. Besides, we identified two genetic traits consisting of the combinations

of *TLR2* and *TLR4* SNPs as well as *TLR4* and *NOD2* SNPs, both strongly associated with susceptibility to meningococcal meningitis.

Chapter 9 describes a study that compares genotypes of 11 SNPs in seven immune response genes (*TLR2*, *TLR4*, *TLR9*, *NOD1*, *NOD2*, *CASP1* and *TRAIL*) amongst 393 BM survivors clustered according to 13 clinical validated severity variables. *TLR4* +896 mutant alleles are highly associated with post-meningitis hearing loss. In a multigene analysis, combined carriage of the *TLR2* +2477 wild type with *TLR4* +896 mutant alleles increases the risk of hearing loss. Carriage of one or both mutant alleles in *TLR4* +896 and *TLR9* -1237 also increases the risk of post-meningitis hearing loss.

SNPs in immune response genes contribute to differences in susceptibility and clinical severities as well as outcome of BM. Innate immune responses play an important role in host defense to BM and subsequent neuronal and cochlear damage. Genetic markers may be used for identification of high-risk patients by creating prediction rules for post-meningitis hearing loss and other sequelae, and provide more insight in the complex immune response in the CNS, possibly resulting in new therapeutic interventions.