Summary

This thesis deals with three subjects: inflammatory neuropathies, the (human) immune system, and microbial pathogens. The work is mainly focussed on Guillain-Barré syndrome (GBS), an acute inflammatory neuropathy that is often induced by a bacterium called Campylobacter jejuni, and chronic inflammatory demyelinating polyneuropathy (CIDP). In the broadest sense, the goal is to reveal how activation of the immune system causes inflammatory neuropathy. Much less is known about the T cell response than about the antibody response in inflammatory neuropathy and therefore the thesis is entirely focussed on T cells. The T cell response to myelin and to the microbial pathogen C. jejuni, and the status of the T cells during inflammatory neuropathy are described. The newly obtained data will be put together into a hypothesis on the pathogenesis of GBS.

The presence of adhesion-, costimulatory-, and antigen presenting molecules on different cell types as conditions for local T cell activation in human sural nerve biopsies of CIDP and vasculitic neuropathy patients and healthy controls is described in Chapter 2. In biopsies from CIDP and vasculitic neuropathy patients, but not healthy controls, Schwann cells expressed the adhesion/T cell stimulatory molecule CD58 (LFA-3). Expression of the co-stimulatory molecule CD86 was detected on vascular tissue in patients with vasculitic neuropathy. Schwann cells of a single vasculitis patient strongly expressed CD1b, a molecule involved in the presentation of self glycolipids to T cells. There was no evidence for the presence of dendritic cells in sural nerve biopsies. These findings suggest that T cell activation can be perpetuated locally nerves of patients with CIDP and vasculitic neuropathy, but no support was found for the hypothesis that naive autoreactive T cells are locally activated to cause tissue damage.

In an attempt to gain insight into the reactivity of circulating T cells to peripheral nerve constituents, lipid extractions and protein fractions of normal peripheral nervous system myelin, and crude homogenate of CIDP-affected peripheral nerve were used to stimulate ex vivo T cells of GBS and CIDP patients and healthy controls. As described in Chapter 3, a strong memory T cell response against tetanus toxoid could be measured in all patients, but no response against any of the preparations of myelin could be demonstrated.

Because many patients with GBS have suffered from a preceding C. jejuni infection, the in vitro T cell response of healthy donors against this micro-organism was described in Chapter 4. We found a preferential expansion of peripheral blood γδ T cells after exposure to crude sonicates of C. jejuni. Expansion of γδ T cells was dependent on the presence of CD4+ T cells in the cultures or addition of exogenous IL-2 or IL-15. C. jejuni stimulation was mediated via the T cell
receptor and appeared to be induced by a non-proteinaceous bacterial antigen, most likely of phosphoantigenic origin.

In contrast, γδ T cells of acute GBS patients with antecedent *C. jejuni* infections completely failed to respond, as described in **Chapter 5**. GBS patients without evidence for antecedent *C. jejuni* infections and individuals with *C. jejuni* enteritis without GBS responded like healthy individuals. In some patients, the γδ T cell non-responsiveness could last for years after recovery from GBS. Supplementing cell cultures with the cytokines IL-2 or IL-15 resulted in restoration of the γδ T cell proliferative response, suggesting that γδ T cell non-responsiveness in GBS patients reflects a lack of production of cytokines required to activate γδ T cells. T cell non-responsiveness and ensuing defective autoimmune regulation may be a more general mechanism leading to autoimmune disease.

In **Chapter 6** the presence of *C. jejuni* DNA in blood mononuclear cells from GBS patients, *C. jejuni* enteritis patients, and healthy subjects was studied. Because persistent antigen can cause T cell non-responsiveness, the presence of *C. jejuni* in GBS patients may provide an explanation for the observed T cell non-responsiveness in these patients. Two target genes, the flagellin and the *ceuE* gene, were used for PCR identification of *Campylobacter* species. DNA extracted from blood mononuclear cells of approximately 30% of the healthy individuals and 50% of the patients contained *C. jejuni* DNA as verified by Southern blot analysis or sequencing of the PCR products. Cell sorting revealed that *Campylobacter* DNA was present in the CD14+ and CD33+ populations, indicating that cells from the myelomonocytic lineage are the *Campylobacter* DNA carrying cells. These data did not provide us with an explanation for the T cell non-responsiveness in GBS patients as observed in **Chapter 5**.

A hypothesis on the sequence of events leading to GBS, combining established ideas with the findings presented in this thesis may be formulated as follows. An infection with a ganglioside-mimicking pathogen is the first event in the development of GBS. Initially, a normal immune response will develop which consists of a bactericidal or cytotoxic innate lymphocyte response, followed by an adaptive T cell response, and antibody production. For as yet unknown reasons a subset of T cells becomes hyperactivated followed by anergy or a certain T cell population was already non-responsive to stimulation with antigen before the actual infection took place. If these nonresponsive T cells are the ones that should normally support regulatory T cells, or should perform a regulatory function themselves, a physiological immune response now turns into a pathogenic one, characterized by a long duration and heightened intensity. As a consequence, pathogenic autoantibodies and immune-stimulatory molecules are produced in high amounts and gain access to the peripheral nerve where complement and macrophage activation damage the
nerve tissues. In this scenario, molecular mimicry alone is not sufficient to cause autoimmunity, but it is a condition for a pathogenic response. Only the combination of defective downregulation and a peripheral nerve-mimicking epitope on a pathogen may lead to GBS.

The question that arises immediately is why T cells become non-responsive. Potential answers to this question are: 1) the infection is very severe due to an inherent property of the pathogen, or 2) due to an inadequate early defense of the host, both leading to hyperactivation and anergy, or 3) genetic host factors, or 4) other immunological host factors contribute to the observed T cell non-responsiveness. It may for example be crucial how the host immune system was primed before the GBS-inducing infection was established.