Summary

The role of B cells in immunological processes goes beyond antigen presentation and the provision of antibodies, namely B cells also shape immune responses through the production of cytokines. B cells limit the pathogenicity of autoimmune disease and the host defence towards bacterial infection via the secretion of the anti-inflammatory cytokine IL-10, on the other hand, B cells promote disease severity of multiple sclerosis (MS) via IL-6. Remarkably, autocrine production of pro-survival IL-6 is one hallmark of transformed B cells, aiding tumour progression (1, 2).

The signals that determine how B cells gain pro- or anti-inflammatory capacities are still poorly understood. Toll-like receptor (TLR) signaling is known to be essential for the regulatory role of B cells (3), yet the results presented here show that after *in vitro* activation with TLR agonists, B cells produce both, IL-10 and IL-6. Hence, this work was aiming towards a better understanding of the molecular mechanisms that regulate the balance between the production of IL-6 and IL-10 in TLR-activated B cells. Experiments that were done in the first part of this thesis intended to identify a molecular switch downstream of TLR signaling that might enable a targeted separation and manipulation of the factors inducing pro- and anti-inflammatory cytokines. Since the TLR accessory phosphatidylinositol 3-kinase (PI3K) pathway was shown to suppress pro- and favour the production of anti-inflammatory cytokines in innate immune cells (4-8), the impact of this pathway on B cell cytokine expression was investigated. Yet, I could show that inhibition of this pathway blocked the production of both cytokines in B cells. Analysis of B cells that express a constitutive active form of the PI3K downstream target Akt (Akt^{BOE} B cells) revealed that the effect of such genetic modification was not restricted to TLR signaling and subsequent cytokine production but had a major impact on B cell development, leading to an almost exclusive generation of B cells with a marginal zone (MZ) B cell-like phenotype according to surface marker expression. Of note, in wild-type (WT) mice, MZ B cells are the main IL-10 and IL-6 producers under stimulation and constitute 3-5% of total splenic B cells. Consistently, total B cells of Akt^{BOE} mice produced more cytokines than total B cells of control mice upon activation. However, further analysis could show that in direct comparison to WT MZ B cells, TLR-stimulated Akt^{BOE} MZ B cells had substantial lower frequencies of IL-10-expressing cells. They also secreted less IL-10 but more IL-6. Thus, in B cells, instead of having anti-inflammatory capacities, the PI3K pathway is required for IL-10 and IL-6 expression and could be linked to certain aspects of the development towards MZ B cells. Additionally, this work proposes that ribosomal protein S6 (rpS6), which is known as a downstream

target of S6 Kinase (S6K) and thus the mTOR pathway, can also be targeted by Akt directly and thereby participates in the development or maintenance of MZ B cells.

In the second part of this thesis, metabolic stress was identified as a novel inducer of IL-6 expression in B cells. The results presented here show that the endoplasmic reticulum (ER) stress pathway suppresses IL-10 production of TLR-activated B cells while in contrast, it acts in synergy with TLR signaling in the activation of the pro-inflammatory cytokine IL-6. The transcription factor Activating Transcription Factor 4 (ATF4) could be identified as a mediator of stress-related IL-6 expression, disclosing a mechanism that might play a role during chronic inflammation which is a prominent source of metabolic stress for immune cells. Importantly, apart from the pharmacological induction of ER stress, amino acid deprivation served as a trigger of IL-6 production in B-, and importantly, even plasma cells, which usually lose the ability to produce IL-6 during their development. This could provide a so far unrecognized link between autocrine IL-6 expression of myeloma cancer cells, aiding tumour progression and survival of transformed cells.

The last part of this work identified astrocytes as major *Il-6*-producers in the CNS of mice suffering from experimental autoimmune encephalomyelitis (EAE). The role of astrocytes during CNS inflammation or injury is still poorly understood since there is a lack of markers that would enable the definition of subpopulations of this functionally diverse cell type. Comparative mRNA expression analysis revealed that *Il-6* strongly correlated with *Gfap* expression which suggests that IL-6 might serve as a novel astrocyte marker with the potential to identify cells with a particular regenerative or pathogenic function. In addition, a similar mRNA expression profile of *Il-6* and the ER stress-related transcription factor spliced X-Box Binding Protein 1 (*Xbp1spl*) that was detected here could indicate that the ER stress pathway is involved in the induction of *Il-6*.