Influence of adjuvants in murine experimental autoimmune encephalomyelitis - towards modelling regulatory B cell function

Background and current knowledge:

Multiple sclerosis (MS) is one of the most common neurological disease among young adults with a suggested autoimmune origin. Over the last years, it has been assumed that not only T cells but also B cells play a key role in its immunopathogenesis. Here, B cells are presumed to contribute to disease initiation and progression by their three major functions, namely antigen presentation, antibody production as well as the secretion of cytokines. Over the last year however, evidence condensed that B cell-secreted cytokines are not only driver of inflammation, but furthermore important for the regulation of autoimmunity.

Experimental autoimmune encephalomyelitis (EAE) is the most commonly used animal model for MS, which has been established by many researchers over the last decades. In C57BL/6 mice MS-like spinal cord lesions can be induced via immunisation with myelin oligodendrocyte glycoprotein (MOG) in Complete Freund’s adjuvants (CFA), followed by repetitive injection of pertussis toxin. Weber et al. demonstrated that mice immunised with MOG protein, a model in which B cells are activated via the B cell receptor (BCR) and involved in a pathogenic manner, benefited from anti-CD20-mediated B cell depletion. In contrast, mice immunised with MOG peptide, a setting in which B cells do not recognize the antigen via their BCRs and therefore remain mainly naïve, showed a more severe course of EAE upon B cell depletion. This led to the assumption that pan B cell depletion may not exclusively be beneficial in all settings and strengthened the idea that it is necessary to further investigate the regulatory functions of B cells in EAE.

To be able to make relevant conclusions it is important to understand what impact the individual components of immunisation have on the peripheral immune cells and especially on B cells. B lymphocytes cannot only be activated by binding antigen to the B cell receptor, but also by other routes such as stimulation of the toll-like receptors (TLR). As CFA contains heat-killed mycobacteria tuberculosis and has ligands for TLR 2, 4 and 9, we aim to investigate whether the used adjuvant CFA influences regulatory B cell properties and if so to what extent.

Thus, the goal of my doctoral thesis is to understand how regulatory B cells can be fostered in EAE with the aim to establish an animal model where the role of regulatory B cells can be studied particularly.

Research plan and methods:

Two central questions outline the subject of the proposal on hand. The first step is to find how the frequencies of splenocytes differ under various immunisation factors. For this task, we will examine what influence CFA has on the peripheral immune system in contrast to incomplete Freund’s adjuvant (IFA). In comparison to CFA, IFA does not contain mycobacteria components. Furthermore, we will analyse how the peripheral immune system changes after injection of CFA/MOG peptide in comparison to IFA/ MOG peptide. For each group three mice will be used, and the experiments will be repeated at least three times. To examine the splenocytes FACS analysis will be made. Hence, it is necessary to find a suitable extracellular staining first.

As mentioned above, the second task is to establish an animal model for EAE where the role of regulatory B cells can be studied particularly. Hence, it is important to investigate to what extend immunisation factors change the regulatory functions of B cells. To study this aspect, FACS analysis with intracellular staining for IL-10, a representative of the anti-inflammatory cytokine group and IL-6, a representative of pro-inflammatory cytokines will be established and performed. Further research via ELISA of IL-10 and IL-6 producing B cells is required to consolidate a quantitative result and to match the frequencies analysis. To make sure the given results are analysed in the correct way, IL6- und IL10 knock-out mice will be used as control groups.

All animal experiments are carried out in accordance with the guidelines of the Central Department for Animal Experiments, University Medical Center, Göttingen and approved by the Office for Consumer Protection and Food Safety of the State of Lower Saxony.