Resolving the immunological background of multiple sclerosis _ ResolveMS.

Short Project Description

Multiple sclerosis (MS) is a leading cause of neurological disability in the young adult population. Accumulating evidence point towards an autoimmune origin where a specific type of white blood cells called T cells reacts against self-proteins and antigens that are present in the central nervous system (CNS), such as the myelin sheath that covers the neuronal axons. Risk for developing the disease is driven both by differences/polymorphisms in immune genes and environmental factors. The two strongest established risk factors for MS are Epstein-Barr virus (EBV) seropositivity and the major histocompatibility complex (MHC) class II gene HLA-DRB1*1501. EBV is a member of the herpes virus family and infects another kind of white blood cells called B cells and establishes a chronic infection. 85-95% of all people are infected chronically with EBV but virtually all patients with MS are infected as confirmed also by a new study in Science, this year that received a lot of attention in the scientific world. EBV is thus a necessary but not sufficient factor to develop MS. Unique to Sweden, off-label rituximab (RTX; an anti-CD20 treatment approved for rheumatoid arthritis) has become the most prevalent MS treatment with about 5000 patients nationwide (approx. 50% of the total) of which >1000 in Stockholm.

Although progress has been made in revealing some of the underlying immunopathophysiological mechanisms of MS, actual mechanisms for how disease is triggered are still largely unknown. A crucial question is which antigens drive certain B and T cell populations to mount an attack on cells of the CNS. In the context of this project, we want to combine many different techniques and by decreasing individual variation to characterize immune cells in patients newly diagnosed with MS and compare them with those seen in patients who are successfully treated with rituximab (both during phase with total B cell depletion and when B cells have returned) as well as healthy controls. The goal is to be able to better characterize disease-causing cells and understand how they are regulated and also to find markers that allow them to be recognized in blood samples. Given the widespread use of RTX for MS in Sweden, this will be of high importance in order to optimize long term risk-benefit of this important type of treatment.

The **main hypothesis** for the project is that inflammatory disease activity in MS is driven by memory B cells, which in turn activate memory T cells, as supported by our recent publication (Jelcic, Al Nimer et al., Cell, 2018), and that this to a large degree is caused by EBV infection and carriage of certain HLA alleles. We also want to address the crucial question of what type of antigen specificity drives this pathological B- and T-cell dysregulation that cause MS disease activity, as well as a number of associated questions.

The study **objectives** are: 1) To determine how proteome, transcriptome, epigenome, and genome differ in immune cells, particularly B and T cells, in untreated MS versus controls. How are they affected by hereditary and environmental factors? 2) To determine how immune cell populations differ in cerebrospinal fluid from those found in blood. 3) To determine how serology, EBV T cell response and EBV DNA copies differ in B cells in untreated MS versus controls. 4) To determine what proteins are recognized by B and T cell clones established from untreated MS vs healthy donors. 5) To attempt to identify

disease-causing cells and activating antigens with tetramer technology. 6) To determine immune cell characteristics correlating with clinical disease progression. 7) To determine if MS specific immune cells can be affected in vitro by different compounds.

Methodology: We want to recruit newly diagnosed patients prior to start of first therapy and follow them over several years with routine diagnostic examinations with blood and spinal fluid tests, MRI, and clinical examination. As controls, we want to recruit a smaller number of patients with MS who have ongoing treatment with the drugs RTX or other treatments and healthy controls, where sampling is done on one occasion. This is summarized as follows: I) Newly diagnosed MS: blood and spinal fluid samples and leukapheresis in connection with diagnostic assessment and possible follow-up sampling with spinal fluid test and / or leukapheresis on a maximum of two further occasions with 6-24 months between sampling occasions. II. MS with ongoing treatment with RTX or natalizumab: Blood test and leukapheresis and / or spinal fluid test. One occasion. III. MS with previous treatment with RTX and where B cells have been repleted: Blood test and leukapheresis and / or spinal fluid test. One occasion. IV. Healthy donors: Leukapheresis and possible spinal fluid test.

The biological material will be characterized ex vivo / in vitro by the following immunological methods: a) Flow cytometry and sorting to identify disease-specific cells. Staining with specific tetramers as well as the fluorospot method will also be used. b) The cells will be characterized by -omics methods to study the disease-specific populations in terms of expression of different genes (transcriptomics) and proteins (proteomics). c) -Omics, along with other methods will be used to further study epigenetic mechanisms. d) In vitro functional immunological experiments. e) Analyses will be performed in combination with the above methods plus clinical data or data on hereditary and environmental factors from other studies (EIMS). f) Should additional material from multiple patients be needed for other immunological studies, we will combine and use biological material from patients participating in the STOPMSII study.

Relevance and expected results

The project addresses several important issues, not only scientifically but also regarding its clinical implications. As mentioned above, anti-CD20 therapy (rituximab; RTX), is increasingly used off-label for MS in Sweden, uniquely in Europe, with about 5000 patients on treatment. This project may provide the following: 1) By the use of leukapheresis we will be able to analyze samples with several different techniques and reduce the effect of individual genetic and environmental factors. Results will be then further confirmed in STOPMS larger patient material. In this way we aim to shed light on the role of immune cells in autoimmunity, which would make it possible to expand studies to further identify disease markers and mechanisms that will lead in the future to more tailored and specific treatment. 2) Identifying a specific mode of action for RTX in MS, thus providing a better rational for its clinical use, and perhaps also methods for selecting patients for this treatment, as well as identifying patients at increased risk for side effects. Given the heterogeneity of MS, finding biomarkers that predict response to this specific treatment, or risk for side effects, will allow us to maximize the benefit/risk ratio for patients treated with RTX and also the other anti-CD20 antibodies currently.

Budget

The laboratory costs of the project will be covered from other sources. We have already performed successfully two leukapheresis and would like to proceed. Here, we would like to apply for the cost of 7 leukapheresis in MS patients and 6 leukapheresis in healthy controls which will be performed at Aferesmottagningen, Karolinska, Huddinge; 13 x 20000 = 260.000 SEK

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