Immune regulation by mimethyl fumarate (DMF) in relapsing-remitting multiple sclerosis patients

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Valorisation addendum

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In the Western world, multiple sclerosis (MS) is the most common neuroinflammatory disorder of the central nervous system (CNS) in young adults aged 20 – 40 years. MS is the main cause of neurological impairment and disability amongst young adults, affecting more than 2 million people worldwide (1). The prevalence and incidence rates of MS have been gradually increasing worldwide over the past two decades (2). The prevalence of MS in Belgium is approximately 118 individuals per 100,000 inhabitants and in the Netherlands it is estimated to be 88 per 100,000 inhabitants (392, 393). MS patients have a wide range of symptoms, from numbness in limbs and paralysis to fatigue and cognitive symptoms (1, 24). Furthermore, these symptoms cause MS patients to be vulnerable to depression since the quality of life is decreased through physical, social and psychological losses or problems. In terms of employment in Belgium, a high rate of 56% of MS patients below the retirement age are unemployed (394). For the MS patients who have the possibility of being employed, productivity at work is affected in 85% of the patients. This is mostly due to fatigue and cognitive difficulties, reflecting a high economic burden on society as a whole, and a financial burden on the patients themselves (394). Mean annual costs for an MS patient with an Expanded Disability Status Scale (EDSS) between 0 and 3 (mild MS) is €26,400 and is more than double for patients with an EDSS between 7 and 9 (severe MS, progression). In addition, the mean cost of a relapse was estimated to be €3,000 (394). Since MS adversely affects the quality of life and is accompanied by a high socio-economic burden, it is important to study the disease mechanisms of MS in more detail. In addition, treating MS patients as early as possible with an effective treatment is crucial in order to delay disease progression.

Until now, there still is no cure available for MS. However, over the past two decades, the treatment landscape has changed tremendously and several novel disease modifying therapies (DMTs) have been added to the armamentarium of MS treatment. At this moment, 12 treatments have been approved for MS, which present 10 different substance classes with different mechanisms of action (181). Although some of these treatments are target specific, multiple MS treatments have no clearly defined mechanism of action or a multifactorial and not fully

unraveled mechanism at the moment of commercial release (181). In order to maximize patient benefit, appropriate selection of MS therapies is crucial. For this reason it is important to have insight into the working mechanism of these treatments. Although much has already been learnt about the pathogenesis of MS by the administration of treatment, unravelling the unknown mechanisms of action additionally leads to improved insight into and more specific knowledge about the role of the different immune cell subtypes in MS pathology.

The research that I performed for this thesis revealed different modes of action of dimethyl fumarate (DMF) therapy that are responsible for the immunomodulatory and positive clinical effects that have been seen in MS patients.

The importance of investigating the working mechanism of DMF therapy in MS patients

At the start of this study, the therapeutic mechanism of action of DMF in MS patients was largely unclear. Our research led to a better understanding of the different immune cell subtypes targeted by DMF in MS patients. For instance, our research indicated that DMF redirects the disrupted immune balance of MS patients away from a pro-inflammatory immune response by specifically decreasing memory and effector memory T and B cells. The same immune cell subtypes were shown to be targeted by DMF in different study populations originating from different countries (Spain, Germany, USA and Belgium) (199, 304, 308, 309, 316). This indicates that the effects of DMF on the immune system are reproducible, even though it has multiple working mechanisms. Additionally, the fact that similar results are observed in different geographical regions suggests that the therapy is reliable and that its effects are vast. The effect of DMF on specific immune cell subtypes can be used to build upon with additional research, to enable to make predictions in the future which specific MS patient/sub group would benefit from this therapy. By enabling physicians to make these informed decisions based on the specific patients' immune cell profile, these patients would be treated sooner with a more suitable treatment. When patients would benefit from an effective treatment earlier in their disease, the disease would remain stable for a longer period of time, which results in reduced chances to develop severe disabilities. Additionally, a patient with a more stable disease course would be able to fully participate in society for a longer period of time,

which has positive effects on the ability to remain employed, the quality of life, financial state, physical and emotional wellbeing, the healthcare system, stress on the family, friends and caregivers of the patient, and society as a whole.

In this study, side effects that were observed due to the use of DMF were mainly flushing and gastro-intestinal symptoms. This caused two patients to drop out of the study. Therefore, careful clinical evaluation of severe flushing and gastro-intestinal symptoms should be considered. Furthermore, one MS patient dropped out due to lymphopenia. DMF causes pronounced lymphopenia in 4-6% of MS patients (361). Thus, several studies, including our study, have demonstrated that when administering DMF to MS patients it is important to monitor the absolute lymphocyte number, as this will reduce the risk of developing prolonged lymphopenia and will secure the safety of the patient. The relatively low incidence and severity of adverse events, as well as the high efficacy of the drug make DMF therapy a popular treatment option, which can be used to treat the majority of relapsing remitting MS (RRMS) patients in the first-line setting. Additionally, in most patients, some side effects that do occur will decrease in severity or will even completely disappear after a period of time. Because of this, a long adherence to DMF treatment is possible, with positive effects on the disease course. The longer a patient can benefit from first-line treatment, the longer a treatment switch to second- or third line can be postponed, potentially protecting the patient from more severe adverse events down the line. In addition, since DMF is an oral treatment, the drug can be prescribed by a specialist, but also by a general practitioner (for repeated doses). The drug can be taken orally by the patients at home, limiting the hospital visits and increasing the comfort for the MS patients.

Another important finding is that DMF is fully effective on immune cells after 6 months of treatment. The absolute lymphocyte number plateaued after 12 months of DMF treatment (361). These results indicate that after 6 months of treatment, physicians can cautiously assess the effectiveness of the treatment on immune cells, treatment adherence and side-effect profile. A next step would be to further investigate the most critical immune cell subtypes affected by DMF in this study. Continued monitoring of these subtypes in a large cohort may then improve our ability to identify poor responders and improve treatment outcomes.

In this thesis I showed that pro-inflammatory cytokine production by T cells and B cells is reduced in DMF-treated MS patients, shifting the Th1/Th17 and pro-inflammatory B cell response to an anti-inflammatory response. Functionally, DMF treatment induces apoptosis of B cells and reduces the expression of survival, antigen presentation and costimulatory markers on B cells. It may be possible to extrapolate these uncovered working mechanisms of DMF to other auto-immune diseases where DMF could possibly be used as a treatment. Furthermore, the uncovered mechanism of action of DMF and its ability to shift the immune profile can facilitate the development of next generation therapies for MS. In addition, the discovery of specific immune cell subtypes that are targeted by DMF could lead to tailored therapy. Finally, if in the future it is desired to combine several MS therapies with DMF for a better treatment efficacy, the acquisition of sufficient knowledge about the mechanism of action of DMF as performed in this study would ensure patient safety. Finally, understanding the mechanism of action of DMF on the immune system of MS patients gave us more insight in the role of these immune cells in the pathogenesis of MS.

The importance of DN B cells in the pathology of MS

Monoclonal antibodies that target CD20 expressing B cells have been shown to be a highly effective treatment option for MS patients (395). However, depleting the total B cell population can result in lowered resistance against pathogens and higher rates of cancer and infection (395). Furthermore, therapies such as rituximab that target all CD20-positive B cells have been shown to also kill non-pathogenic B cells. For these reasons it is important to identify disease-relevant B cell subtypes among the vastly diverse peripheral B cell compartment to find alternative targeted therapies. DN B cells may be one of the B cell subtypes with potential as therapeutic target. Our research group previously showed that DN B cells are abnormally elevated in the peripheral blood and cerebrospinal fluid (CSF) of MS patients. Moreover, DN B cells showed pro-inflammatory functional characteristics indicating that they might play a role in the pathogenesis of MS (68). In addition, we observed that DMF treatment decreased the frequency of DN B cells in MS patients. In this thesis, the developmental phenotype and migratory capacity of DN B cells from MS patients were investigated in more detail. We showed that DN B cells have a mature and memory phenotype, though with earlier maturation features compared to class-switched memory (CSM) B cells.

Furthermore, we have shown that DN B cells can become activated and that they have a high migratory capacity towards pro-inflammatory chemokines involved in B cell trafficking to the CNS in MS. Unraveling the phenotype of DN B cells is important in order to understand their functional relevance and possible involvement in MS pathogenesis. The finding of a high migratory capacity of DN B cells towards pro-inflammatory cytokines provided more evidence that DN B cells could possibly contribute to the pathogenesis of MS, making them even more desirable to be targeted by biologics. The knowledge obtained from our studies could contribute to future targeting or modulating DN B cells for MS treatment. Furthermore, by next-generation sequencing of the B cell receptor immunoglobulin genes (383), our research group showed that DN B cells of MS patients and healthy individuals share a common developmental pathway. Thus, information gathered on DN B cells of MS patients could also be implemented in the development of therapies for immune aging which could also contribute to the improvement of general healthcare.

When specifically targeting pathologically relevant DN B cells, the risk of bacterial and viral infections and neoplasms would be diminished compared to targeting the entire B cell population. This means that less side effects are expected when tailoring such a more selective B cell therapy. Furthermore, various other B cell subtypes produce anti-inflammatory cytokines, induce Treg and/or inhibit effector T helper cells, which counteracts the inflammatory processes and thus is beneficial for the pathogenesis of MS. However, previous research performed by our research group and results in this thesis show that the effect of targeting DN B cells would not only be contained to DN B cells only, as this B cell subtype could also have an indirect effect on T cells. DN B cell depleting therapy could eventually be used as a personalized treatment for patients with an increased frequency of DN B cells. However, more research is needed in order to determine whether anti-DN B cell therapy has a significant effect on the pathogenesis of MS by itself. When anti-DN B cell therapy alone is not sufficient, the therapy could also be used as an add-on treatment.

However, in order to tailor a therapy for depleting DN B cells in MS patients further research is needed to find out which markers or targets are specifically expressed by DN B cells and are absent in other B cell subtypes. This could be performed by

RNA sequencing. Furthermore, it would be interesting to investigate DN B cell-associated signaling pathways that may assist us to better understand the association between the elevations of DN B cells in aging and in autoimmune disease. A possible scenario in the search for a marker or target would be to consider the pathways related to DN B cell cytokine production, migratory pathway, activation pathway, IL-21/granzyme B pathway, T-bet or Toll-like Receptor pathways. If one of aforementioned pathways is relevant and can be targeted for treatment, the first step would be to review drugs that are already on the market or in development, for instance for the treatment of other diseases, that have an effect on proteins that are involved in previously discussed pathways. Another approach would be to target the involved proteins with monoclonal antibodies that may or may not already be developed. At this moment several drugs that block the above mentioned pathways are in development. For instance Human r-IL-21 is available for clinical use to target tumors (396, 397). Several TLR7 or 9 antagonists are tested in clinical phase II trials in order to treat diseases like psoriasis, optic neuritis, diffuse large B cell lymphoma and Sjögren's syndrome (398). In conclusion, although in our research some characteristics of DN B cells were unraveled, it is clear that more research is needed to eventually target DN B cells for therapeutic purposes.