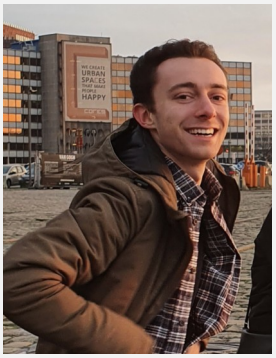
ImmunoTools *special* Award 2021



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Elucidating the Mode of Action of Tolerogenic Dendritic Cells: Extracellular Vesicles in Multiple Sclerosis

Multiple sclerosis (MS) is a chronic auto-immune disorder of the central nervous system (CNS) and the leading cause of non-traumatic disabling disease in young adults [1, 2]. MS is a complex disease characterized by a heterogeneous clinical course and symptoms. Although the exact cause of MS remains to be elucidated, it is accepted that both genetic and environmental factors affect a complex immunological response [3]. Based on studies showing the involvement of interferon-γ and interleukin (IL)-17 producing T cells in active MS, as well as their presence in brain lesions, T helper (Th) 1 and Th17 cells are considered key players in the disease pathology [4]. To date, there is no cure for MS, but several immune-modifying and/or -suppressive treatments have been developed over time. However, these have varying efficacy, limited long term effectiveness, and sometimes life-threatening side effects [5, 6]. Hence, there is an unmet need for new and more effective treatment strategies. In this perspective, cell therapy is a promising form of immunotherapy [7, 8]. Tolerogenic dendritic cells (tolDC) are characterized by a low expression of co-stimulatory molecules, decreased expression of maturation markers and an altered cytokine secretion [9, 10]. Our lab completed a phase I clinical trial in which vitamin D_3 ($vitD_3$)-tolDC are evaluated for the treatment of MS (NCT02618902) [11]. Various mechanisms by which tolDC can establish tolerance have been proposed, including T cell anergy, T cell depletion, cytokine deviation, induction of regulatory T cells (Tregs) and recently also induction of Bregs [12]. However, no arguments that $vitD_3$-treated tolDC induce T cell anergy or Tregs were found in our hand [9, 13]. **Much remains to be understood about the molecular and cellular functions of tolDC**.

We hypothesize that tolDC modulate the auto-reactive response via extracellular vesicles (EV). EV are nanosized membrane vesicles that are released by almost every cell type [14]. Interestingly, EVs are capable of transporting cargo including mRNA, miRNA, proteins, lipids, and metabolites [14]. It is known that DC-derived EV can provide activating signals that participate in the induction and maintenance of T-cell tolerance [15]. EVs can surpass several drawbacks associated with DC therapy such as limited migratory capacity, low yield and decreased viability post-production [16]. Moreover, with only 5% of DC reaching the lymph nodes [17], we expect a role for EV in the therapeutic effect of DC vaccination. EV can be modified by several loading mechanisms to carry a specific target [18]. For instance, DC-EV

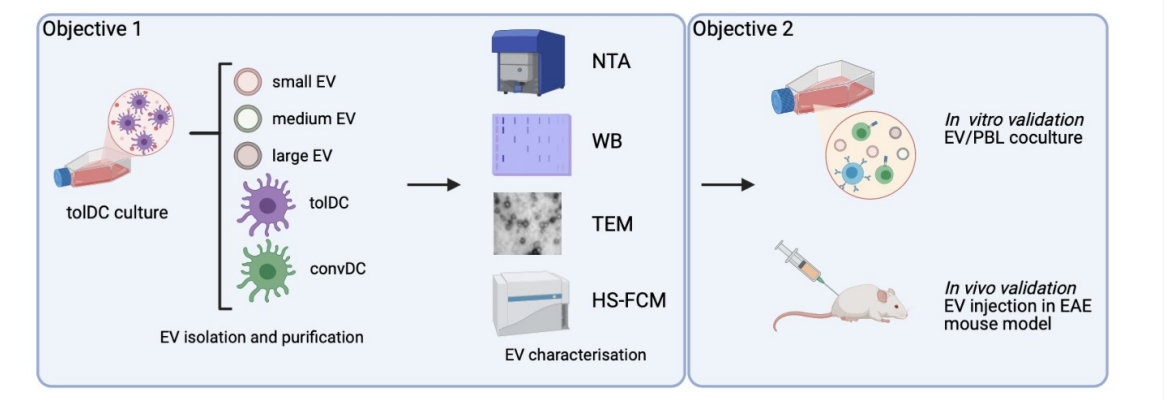
engineered to carry transcription growth factor (TGF)-β1 and IL-10 by sonication, were able to attenuate DC maturation and increase the induction of Tregs [19]. Hence, elucidating the role of EV in the mode-of-action of tolDC will broaden our knowledge on the regulators of immunity and could result in the development of new therapeutic strategies.

Objectives

A better understanding of tolDC immunobiology will open many possibilities for enhancing or redirecting their therapeutic activities. Here, we aim to assess the role of EV in the mode-of-action of tolDC and hypothesize that tolDC-EV have the potential to regulate tolerance-inducing molecular pathways. For this, the following objectives have been set forth:

1. To purify and characterize tolDC-EV from MS patients and healthy controls
2. To evaluate the immunoregulatory properties of tolDC-EV *in vitro* and *in vivo*

Methods using ImmunoTools reagents



In this project, we aim to isolate tolDC-derived EV from tolDC culture supernatants. EV will be characterized using nanoparticle tracking analysis (NTA), western blot (WB), transmission electron microscopy (TEM) and high-sensitivity flow cytometry (HS-FCM). To characterize, EV will be stained using fluorochrome-conjugated antibodies CD63-PE and CD9-FITC from ImmunoTools. The interplay between EV and T cells will be investigated by coculture with peripheral blood lymphocytes as part of the *in vitro* evaluation of the tolerogenic capacity of tolDC-EV. Assessing cytokine secretion by EV-challenged T cells could add to a better understanding of the tolerance-inducing mechanisms. For this, IL-10 and IL-4 secretion will be evaluated using ImmunoTools ELISA assays.

Together, the ImmunoTools award would allow for a strong basis of the tolerogenic capacity of tolDC-EV and will contribute to a better understanding of the immunoregulatory properties. This project will provide unique information that will impact the development of tolerance-inducing therapies by elucidating the mechanisms of tolerance induction.

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