Using multi-epitope-targeting tolerogenic dendritic cells to tackle myelin reactivity

Judith Derdelinckx[1,2] – Maxime De Laere[1] – Wai-Ping Lee[1,3] – Christel Bocken[5] – Jasmijn Daans[1] – Megha Meena[1] – Ibo Janssens[1] – Barbara Willekens[1,2] – Patrick Cras[3] – Peter Ponsaerts[1] – Niels Hellings[5] – Zwi N. Berneman[1,4] – Nathalie Cools[1,4]

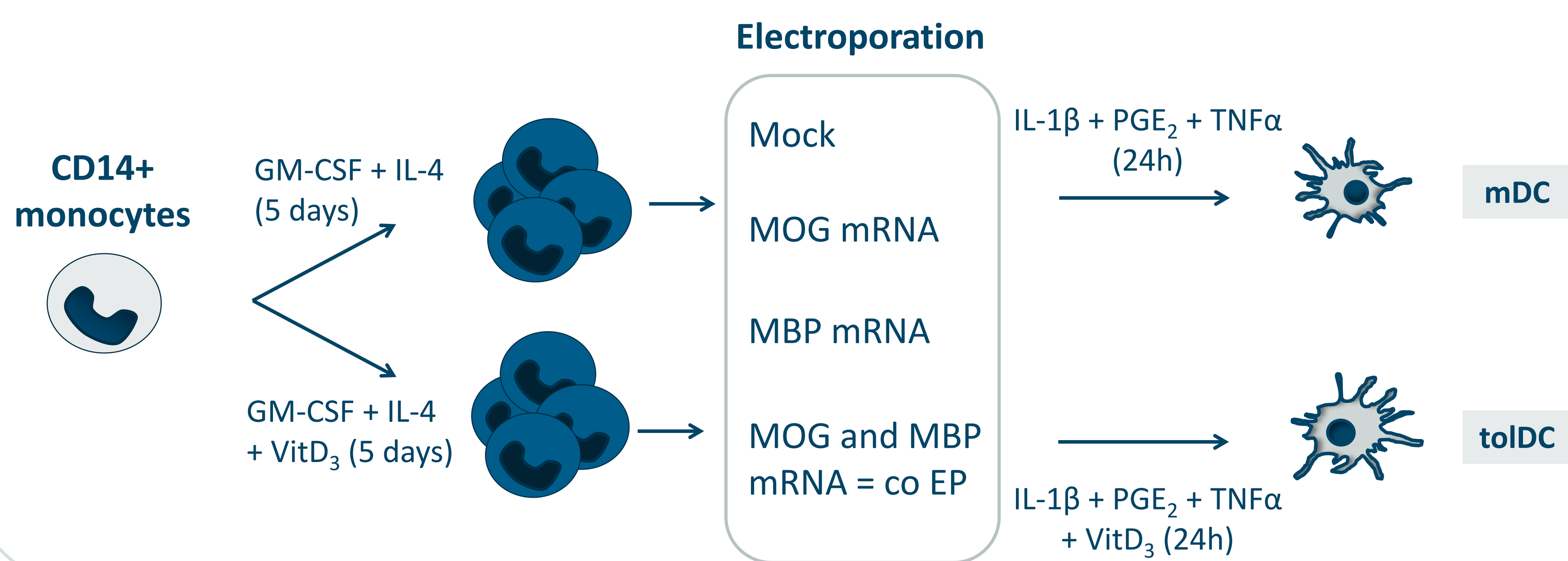
[1]Laboratory of Experimental Hematology, Vaccine and Infectious Disease Institute (Vaxinfectio), Faculty of Medicine and Health Sciences, University of Antwerp, Edegem, Belgium
[2]Division of Neurology, Antwerp University Hospital, Edegem, Belgium
[3]Department of Neurology, Antwerp University Hospital, Born Bunge Institute, Translational Neurosciences, Faculty of Medicine and Health Sciences, University of Antwerp Edegem, Belgium
[4]Center for Cell Therapy and Regenerative Medicine, Antwerp University Hospital, Edegem, Belgium
[5]Department of Immunology, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium

Contact address: Judith.Derdelinckx@uantwerpen.be

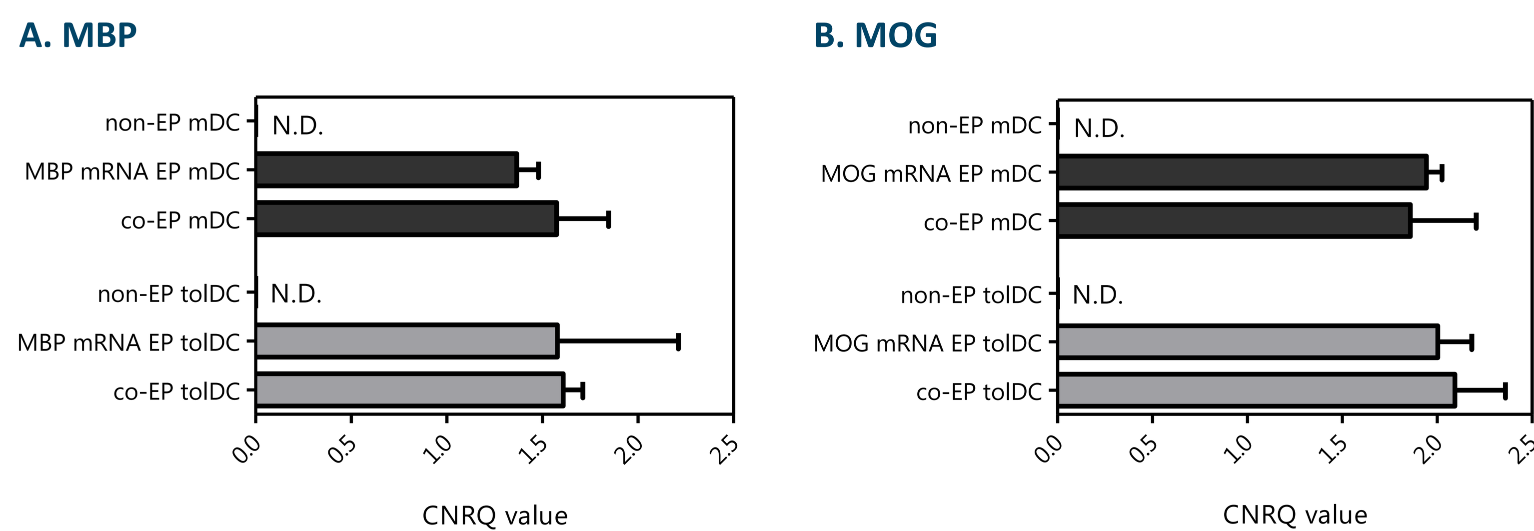
Introduction

The ultimate goal in the treatment of multiple sclerosis (MS) is to tackle the dysregulated immune response at its core by modulation of pathological myelin-specific immune responses. In this context, the use of myelin-specific tolerogenic dendritic cells (tolDC) is a promising strategy to re-establish tolerance in an antigen-specific manner. However, the antigen loading of these cells is complicated by several factors, including choice of target antigens. Even though myelin-derived epitopes are suggested to be the main target of the autoimmune reaction in MS, the patient-specific myelin reactivity profile and expansion of the myelin-reactive T cell repertoire in time, known as epitope spreading, complicate the choice for target antigens among the different myelin proteins. Transfection of DC with messenger RNA (mRNA) encoding myelin proteins offers the potential to overcome these issues by inducing presentation of a wide variety of naturally-processed myelin peptides.

Experimental set-up

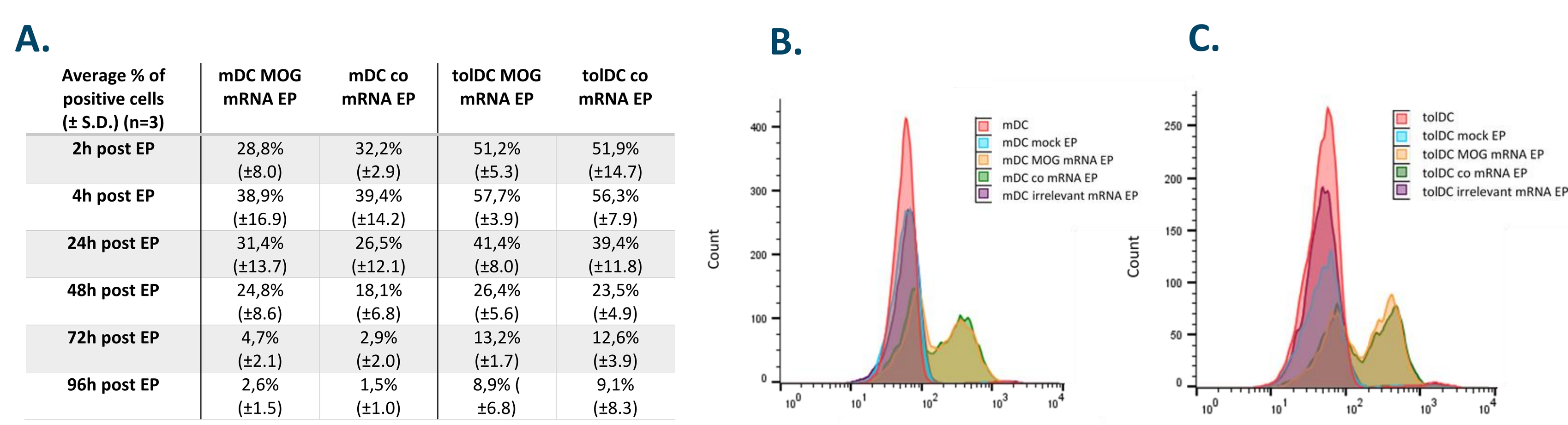


MOG and MBP mRNA can be detected intracelullarly following elecroporation



Intracellular levels of mRNA were analyzed 2 hours following electroporation using RT-qPCR analysis. Successful transfection of mDC and tolDC after MOG and MBP mRNA electroporation was demonstrated, with no statistically significant differences in the intracellular presence of MOG or MBP mRNA following single mRNA electroporation versus mRNA co-electroporation (n=3). Expression kinetics are shown as mean log CNRQ value ± SD, relative to the expression of the reference gene GAPDH.

MOG mRNA electroporation induces long-term MOG protein expression

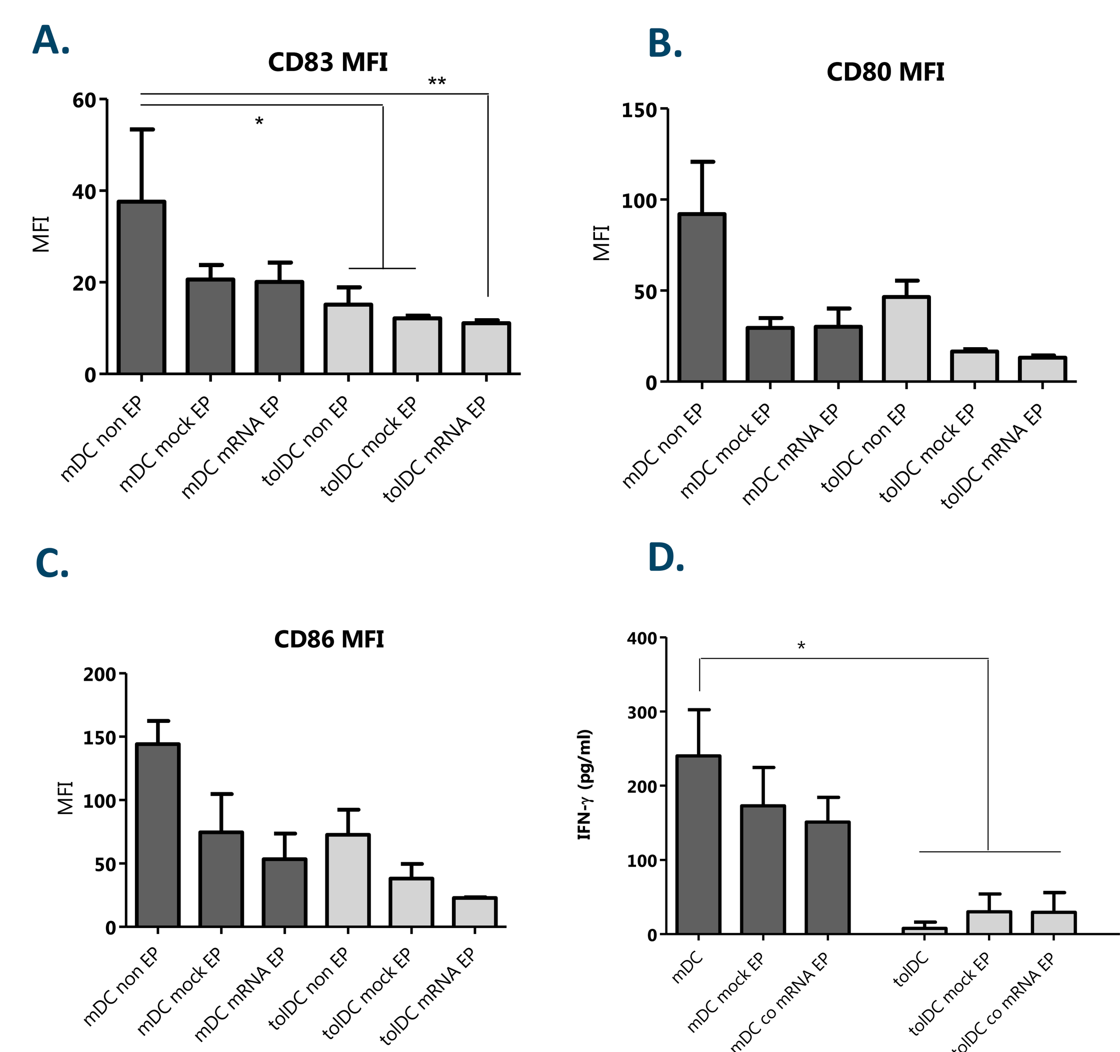


A.

Average % of positive cells (± S.D.) (n=3)	mDC MOG mRNA EP	mDC co mRNA EP	tolDC MOG mRNA EP	tolDC co mRNA EP
2h post EP	28,8% (±8.0)	32,2% (±2.9)	51,2% (±5.3)	51,9% (±14.7)
4h post EP	38,9% (±16.9)	39,4% (±14.2)	57,7% (±3.9)	56,3% (±7.9)
24h post EP	31,4% (±13.7)	26,5% (±12.1)	41,4% (±8.0)	39,4% (±11.8)
48h post EP	24,8% (±8.6)	18,1% (±6.8)	26,4% (±5.6)	23,5% (±4.9)
72h post EP	4,7% (±2.1)	2,9% (±2.0)	13,2% (±1.7)	12,6% (±3.9)
96h post EP	2,6% (±1.5)	1,5% (±1.0)	8,9% (±6.8)	9,1% (±8.3)

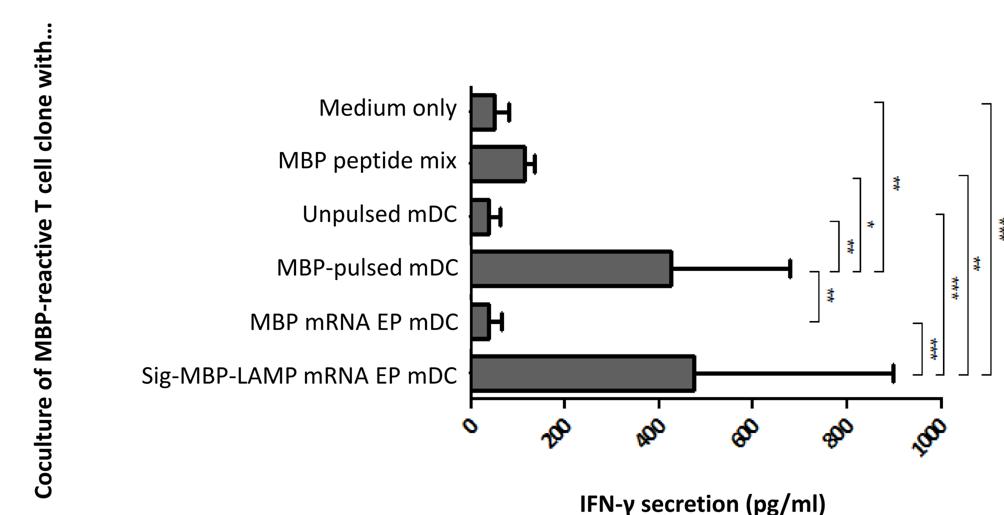
The kinetics of MOG protein expression following electroporation of mDC and tolDC with MOG and MBP mRNA (co-electroporation) were evaluated using flow cytometric analysis (**A**, n=3). A representative histogram for MOG expression for one donor is shown for mDC (**B**) and tolDC (**C**), 4 hours following electroporation.

Based on immunophenotyping and T cell-stimulatory capacity, tolDC retain their tolerogenic phenotype following mRNA electroporation



TolDC display no difference in expression of costimulatory markers CD83 (**A**), CD80 (**B**) and CD86 (**C**) following MOG and MBP mRNA co-electroporation (n=3) as assessed by flow cytometric analysis. Following 5 days of coculture of allogeneic peripheral blood lymphocytes with different conditions of mDC and tolDC (ratio 10:1), the concentration of IFN-γ in the supernatant was determined as a measure for T cell stimulation (**D**). Interestingly, the capacity to induce T cell hyporesponsiveness by tolDC was not affected by mock or MOG and MBP mRNA co-electroporation. * $p<0.05$, ** $p<0.01$ using Kruskal-Wallis test.

MBP mRNA-electroporated mDC are capable of presenting MBP peptides to T cells



Peptide-pulsed or mRNA-electroporated mDC from HLA-DR2+ donors were co-cultured for 24h with a full-length MBP-specific T cell clone to determine antigen-loading efficiency, as reflected by IFN-γ production of the T cell clone (n=3). The flanking Sig-LAMP mRNA sequence targets the translated protein to the lysosomal compartment, hereby enhancing antigen presentation through MHC class II molecules to CD4+ T cells. Results are shown as mean value ± SD. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ using one-way ANOVA.

Discussion

In the present work, we aimed to induce antigen-expressing human tolDC following mRNA electroporation. Co-electroporation with myelin mRNA constructs resulted in **long-term myelin expression** by tolDC without affecting their maturation-resistant phenotype. Moreover, myelin mRNA-electroporated control DC were able to stimulate myelin-specific T cell clones, demonstrating **effective myelin processing and presentation**. The *in vitro* down-modulation of autologous myelin-specific T cell responses by co-electroporated tolDC is currently being evaluated in healthy controls and MS patients. Our findings suggest that therapeutic vaccination with tolDC electroporated with mRNA encoding full-length myelin-derived proteins may lead to a more effective therapy for MS by induction of tolerance to a wide range of myelin-derived antigens and this without hampering the normal surveillance and effector function of the immune system.

Literature

Derdelinckx J, Berneman ZN, Cools N. GMP-Grade mRNA Electroporation of Dendritic Cells for Clinical Use. Methods Mol Biol. 2016;1428:139-50.

Universiteit Antwerpen
fwo Opening new horizons