Multi-epitope-engineered tolerogenic dendritic cells effectively dampen EAE

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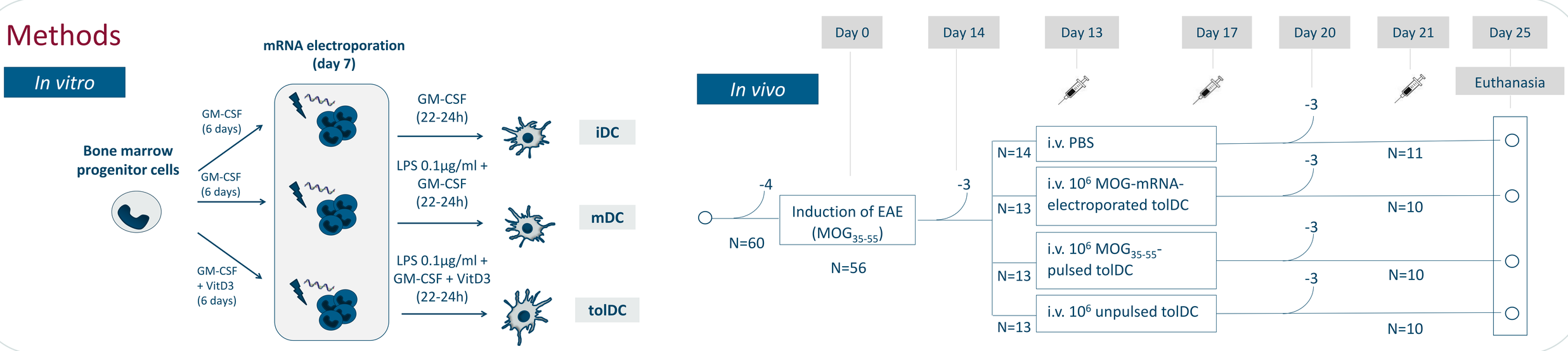
Background & Objectives

Although effective in reducing relapse rate, currently available therapies for multiple sclerosis (MS) do not halt disease progression. Whereas several mechanisms underlie this process, epitope spreading potentially is one of the driving forces. Hence, targeting a variety of disease-associated antigens, for instance by the use of myelin antigen-presenting tolerogenic dendritic cells (tolDC), is a promising strategy to re-establish tolerance in a myelin-specific manner. Electroporation with mRNA encoding full-length myelin proteins is an innovative technique to load tolDC with a variety of naturally-processed myelin epitopes. Therefore, we aimed to investigate the feasibility to generate tolDC presenting myelin oligodendrocyte glycoprotein (MOG) epitopes using mRNA electroporation. Next, we evaluated the efficacy of MOG mRNA-electroporated tolDC to dampen pathogenic T cell responses in experimental autoimmune encephalomyelitis (EAE).

Methods

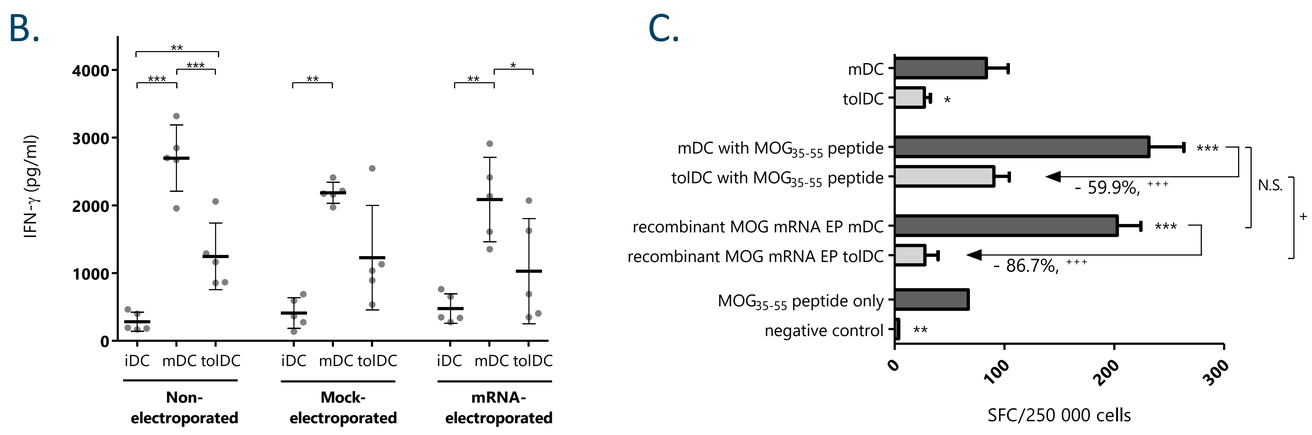


MOG mRNA-electroporated tolDC retain their maturation-resistant phenotype and effectively suppress MOG-specific splenocytes

A.

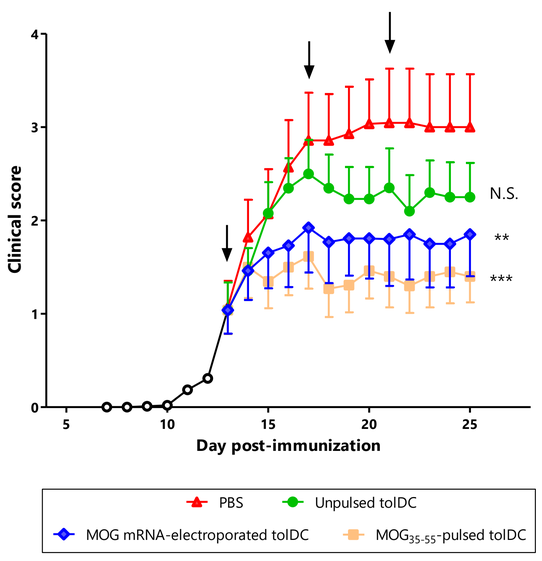
	iDC	mDC	tolDC	tolDC mock EP	tolDC mRNA EP
IL-12p70 (pg/ml)	3.22 ± 2.66 +++	317.4 ± 111.0 ***	87.02 ± 39.88 +++	71.51 ± 33.54 +++	88.48 ± 40.45 +++

TolDC demonstrate low secretion of IL-12p70 and low stimulatory capacity in an allogeneic mixed lymphocyte reaction following activation with a pro-inflammatory stimulus. This maturation-resistant phenotype was not affected by mock or mRNA electroporation (fig. **A.** and **B.**). Moreover, MOG mRNA-electroporated tolDC displayed a marked suppressive effect on MOG-reactive splenocytes, as evidenced by a 86,6±5,0% reduction on average of IFN-γ-producing cells as compared to splenocytes stimulated with electroporated mDC (fig. **C.**)



A. IL-12p70 secretion in the culture supernatant of iDC, mDC and tolDC (n=6). *statistically significant when compared to iDC, +statistically significant when compared to mDC. **B.** IFN-γ secretion by allogeneic splenocytes in coculture with different conditions of DC (n=5). **C**. Modulation of MOG-specific T cell responses by different conditions of tolDC (n=3). MOG_{35-55}-reactive splenocytes were cocultured with DC in a 1:10 ratio. Following overnight incubation, the number of IFN-γ-secreting T cells was quantified using ELISPOT. *statistically significant compared to mDC. All results are shown as mean ± SD. * p<0.05; ** p<0.01; *** p<0.001. N.S. not significant.

In vivo administration of MOG mRNA-electroporated tolDC abrogates further EAE development



Clinical follow-up of mice, showing mean clinical score. Arrows represent days of treatment. Error bars correspond to SEM. ** p<0.01 compared to PBS, *** p<0.001 compared to PBS, N.S. not significant compared to PBS.

Mice treated with MOG mRNA-electroporated or MOG_{35-55}-pulsed tolDC displayed a stabilization of clinical score from the first administration onwards, whereas clinical score worsened in mice treated with unpulsed tolDC and PBS. This resulted in a significantly decreased mean clinical score for the groups treated with MOG mRNA-electroporated and MOG_{35-55}-pulsed tolDC, but not with unpulsed tolDC, compared to PBS. No statistically significant difference between clinical course in the MOG mRNA-electroporated and the MOG_{35-55}-pulsed tolDC group could be observed.

TolDC treatment in EAE mice reduces MOG-specific splenocyte reactivity and prevents epitope spreading

		MOG_{35-55}	MOG_{92-106}	MBP_{4-14}	MBP_{84-97}	$PLP_{139-151}$	$PLP_{178-191}$	PLP_{56-70}
	Healthy mice	0/2 (1,25 ± 0,12)	1/2 (1,11 ± 0,63)	0/2 (0,72 ± 0,55)	0/2 (1,06 ± 0,39)	0/2 (0,81 ± 0,90)	1/2 (1,11 ± 0,36)	N/A
	Immunized mice	3/3 (11,42 ± 11,03)	0/3 (1,25 ± 0,11)	0/3 (1,17 ± 0,25)	0/3 (1,08 ± 0,04)	1/3 (1,26 ± 0,50)	0/3 (0,99 ± 0,24)	N/A
IDay 20 pi	PBS	3/3 (8,21 ± 1,30)	3/3 (1,93 ± 0,49)	0/3 (0,97 ± 0,33)	1/3 (1,44 ± 0,39)	1/3 (1,30 ± 0,49)	2/3 (1,57 ± 0,62)	N/A
	Unpulsed tolDC	0/3 (1,16 ± 0,16)	0/3 (1,15 ±0,04)	0/3 (1,02 ± 0,11)	0/3 (0,94 ± 0,13)	0/3 (1,05 ± 0,13)	0/3 (0,95 ± 0,08)	N/A
	MOG_{35-55}-pulsed tolDC	1/3 (1,23 ± 0,34)	0/3 (1,07 ± 0,05)	0/3 (1,05 ± 0,06)	0/3 (1,04 ± 0,55)	0/3 (1,07± 0,03)	0/3 (1,07 ± 0,26)	N/A
	mRNA-electroporated tolDC	0/3 (1,23 ± 0,13)	0/3 (1,11 ± 0,14)	0/3 (1,05 ± 0,14)	0/3 (0,97 ± 0,20)	0/3 (1,10 ± 0,06)	0/3 (0,97 ± 0,13)	N/A
Day 25 pi	PBS	9/9 (8,18 ± 4,81)	2/9 (1,24 ± 0,43)	1/9 (1,09 ± 0,33)	1/9 (1,07 ± 0,31)	0/9 (0,93 ± 0,24)	0/9 (1,05 ± 0,19)	0/6 (0,81 ± 0,18)
	Unpulsed tolDC	1/10 (1,20 ± 0,27)	0/10 (1,08 ± 0,10)	0/10 (1,07 ± 0,19)	0/10 (1,05 ± 0,12)	0/10 (1,08 ± 0,17)	0/10 (1,03 ± 0,14)	0/7 (1,05 ± 0,21)
	MOG_{35-55}-pulsed tolDC	1/10 (1,30 ± 0,27)	0/10 (1,03 ± 0,08)	0/10 (1,09 ± 0,09)	0/10 (1,04 ± 0,06)	0/10 (1,02 ± 0,13)	0/10 (0,99 ± 0,13)	0/7 (0,98 ± 0,06)
	mRNA-electroporated tolDC	0/9 (1,02 ± 0,14)	0/9 (0,96 ± 0,09)	0/9 (1,06 ± 0,11)	0/9 (1,00 ± 0,08)	0/9 (0,99 ± 0,13)	0/9 (0,98 ± 0,12)	0/6 (1,01 ± 0,07)

Whereas splenocytes from untreated and PBS-treated mice exhibited strong reactivity towards MOG_{35-55}, this was effectively suppressed in tolDC-treated mice. Interestingly, although EAE was induced using MOG_{35-55} immunization, epitope spreading could be detected in untreated and PBS-treated EAE mice, but not in tolDC-treated mice.

Myelin-specific IFN-γ ELISPOT responses at different time points in the EAE disease course. Immunized mice: untreated EAE mice sacrificed at day 14 pi. Number of responder mice (ratio of antigen-specific spot count ≥ 1.5) are shown per myelin peptide, with between brackets the mean ratio of antigen-specific spot count over background spot count ± SD.

Discussion

MOG mRNA-electroporated tolDC effectively suppress EAE splenocytes *in vitro* and *in vivo*, which is reflected by a beneficial effect on the clinical course of EAE following intravenous administration. Additionally, we demonstrated the need for MOG presentation for optimal clinical efficacy of tolDC treatment, underlining the importance of a disease antigen-specific therapeutic approach.

Literature

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