

Liposomal drug delivery: targeting therapies for neuroinflammation



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Background

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) for which there is no known cure (1). Existing therapies target the immune system responsible for causing damage in the disease, but are not fully effective and have a range of side effects (2).

Liposomes are drug delivery nanoparticles which may be suitable for improving treatment targeting in MS (3). Liposomes accrue at sites of disrupted vasculature, such as the inflamed central nervous system, so may offer improved spatial targeting for treatment. Further, liposomes are internalised by phagocytic immune cells, which may offer direct targeting to key pathogenic phagocytic immune populations (4).

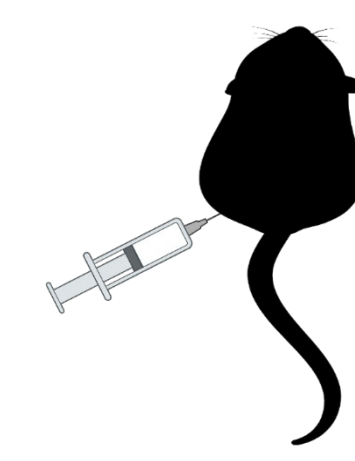
As such, **we propose that a novel liposomal formulation of MS therapy mitoxantrone will be more effective in treating neuroinflammation than its freely delivered counterpart, potentially due to enhanced delivery characteristics.**

Aims

- To determine the impact of liposomal encapsulation on drug efficacy in the context of neuroinflammation, using liposomal mitoxantrone (LMTX) as proof-of-concept.
- Delineate liposome uptake by different immune cell populations and assess the mechanistic importance of these interactions.

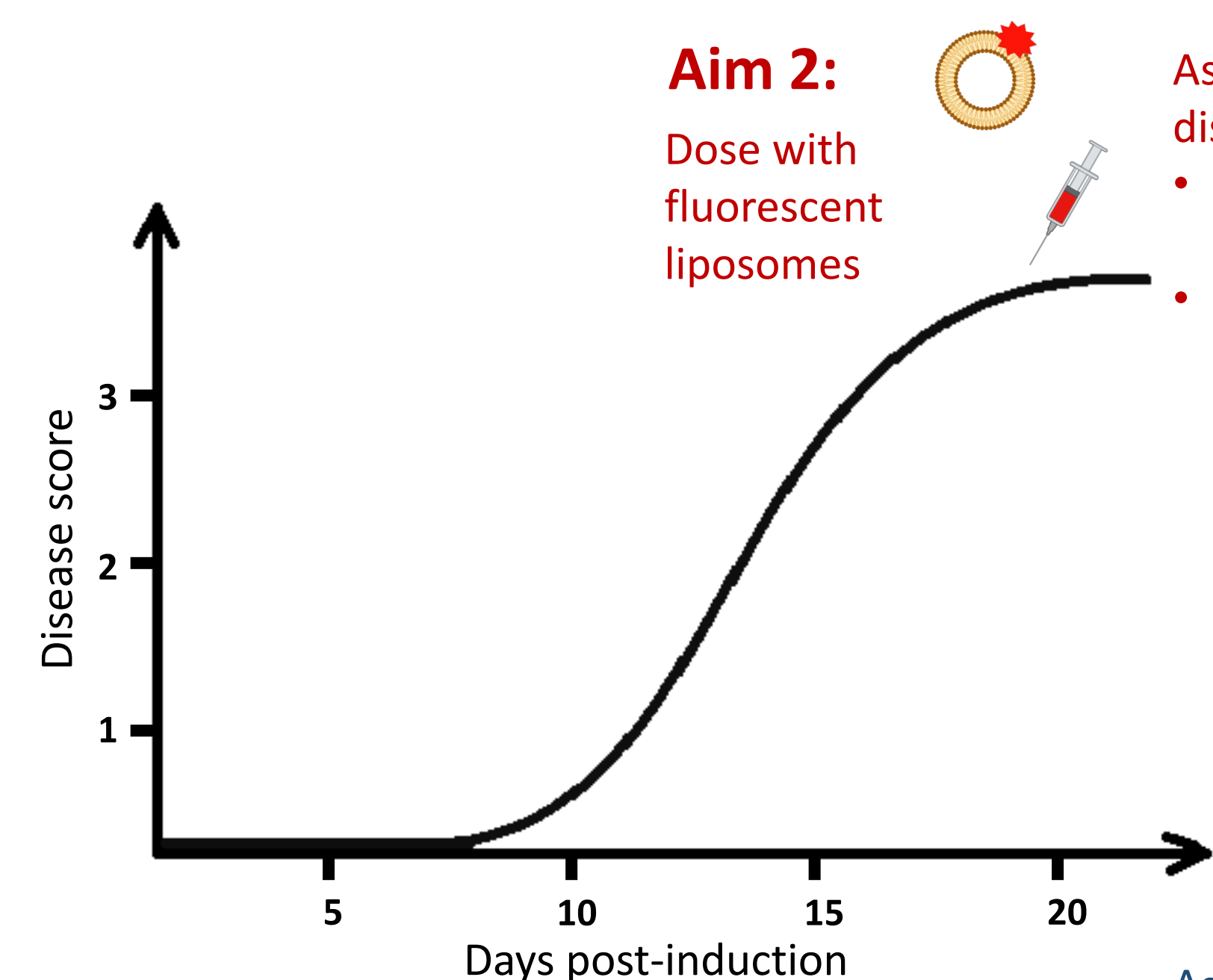
Methods

Project aims will be investigated using **experimental autoimmune encephalomyelitis (EAE)**, an inducible mouse model of neuroinflammation as occurs in MS.



EAE scoring

- Loss of tail tone
- Impaired rolling reflex
- Hind limb paralysis
- Hind and forelimb paralysis



Aim 2:
Dose with fluorescent liposomes

Assess liposome distribution:
• CNS delivery (IVIS imaging)
• Liposome-interacting immune cell populations (flow cytometry)

Aim 1:
Dose with LMTX or free MTX

Assess treatment outcomes:
• EAE severity
• Pathogenic immune cell abundance (flow cytometry)

1. LMTX treatment offers enhanced protection against EAE relative to free drug

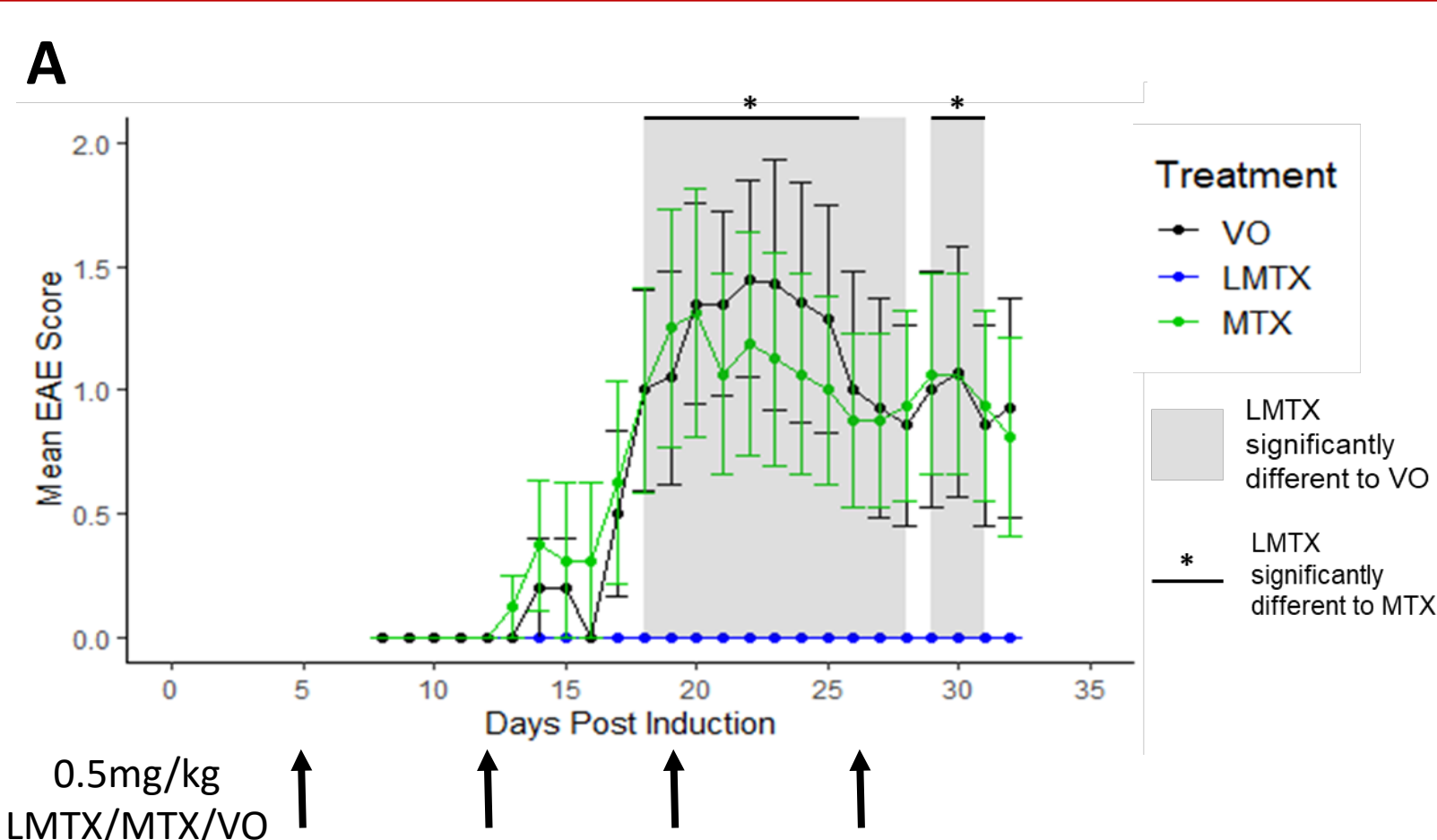
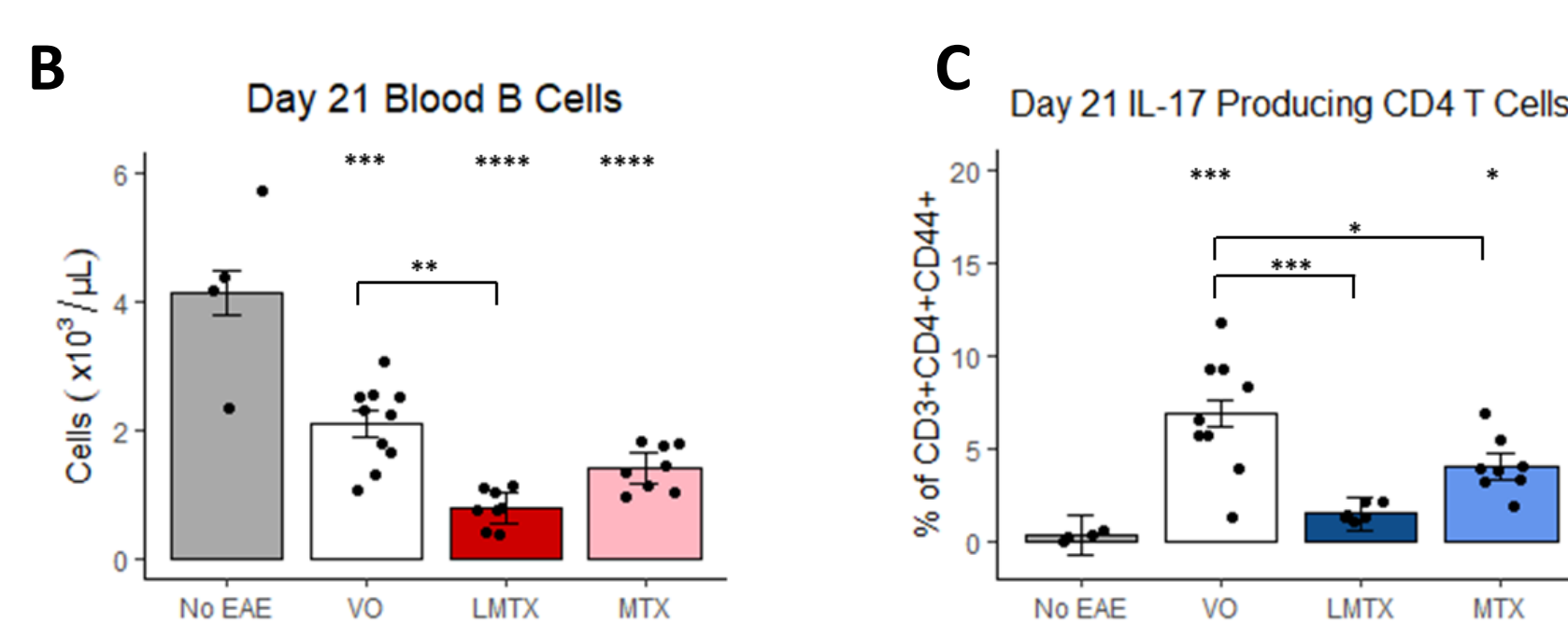


Figure 1: disease parameters in EAE-induced mice treated weekly with 0.5mg/kg LMTX/MTX/drug vehicle only (VO).

A. disease severity as determined by daily clinical scoring. Blood analysis was performed on day 21 of the model to determine the impact of treatment on immune cell populations including **B.** B cells and **C.** IL-17-producing CD4 T cells. $n = 6$ per group, except untreated group where $n=5$. Linear mixed effects model with Tukey pairwise comparisons. Asterisks indicate significant difference to No EAE group except where otherwise indicated.



2. Liposomes accumulate in the CNS of EAE mice but not healthy mice up to 5 days following dosage

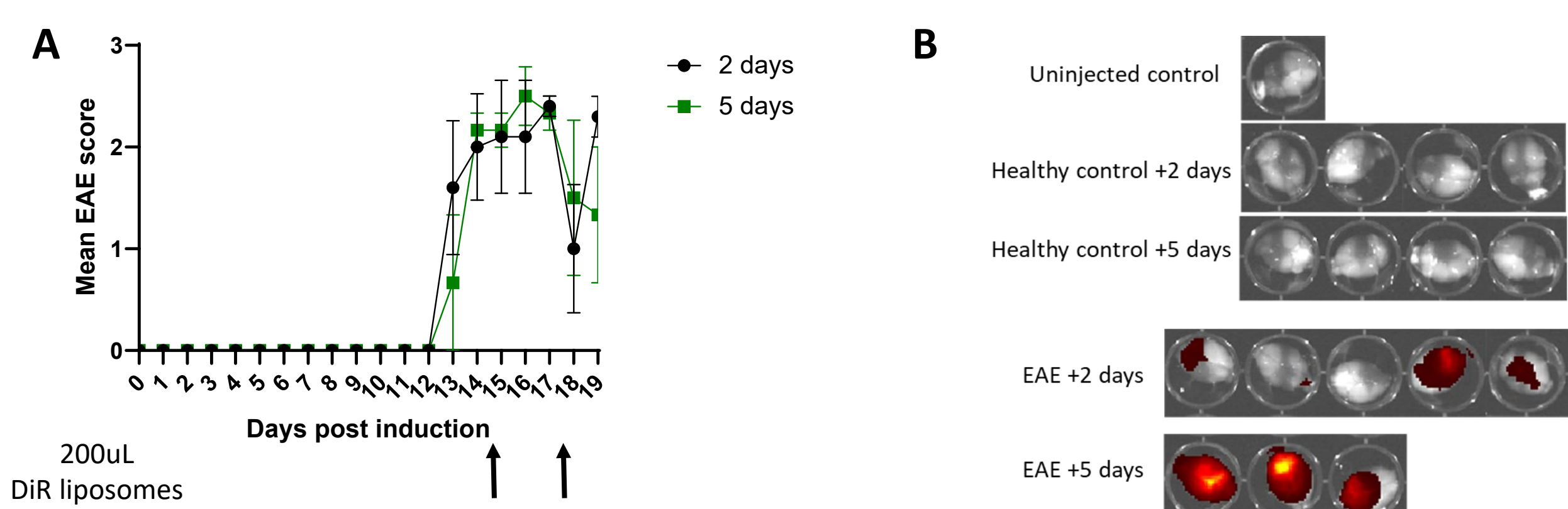


Figure 2: assessment of liposome biodistribution in CNS of EAE-induced mice at multiple timepoints following dosage.

A. Following clinical onset of EAE model, EAE-induced or healthy mice were dosed intravenously with 200uL DiR-tagged liposomes 2 or 5 days prior to imaging. **B.** IVIS fluorescence images of brain identify liposome infiltration into the CNS of EAE-induced, but not healthy, mice.

3. Liposomes interact preferentially with a distinct dendritic cell population

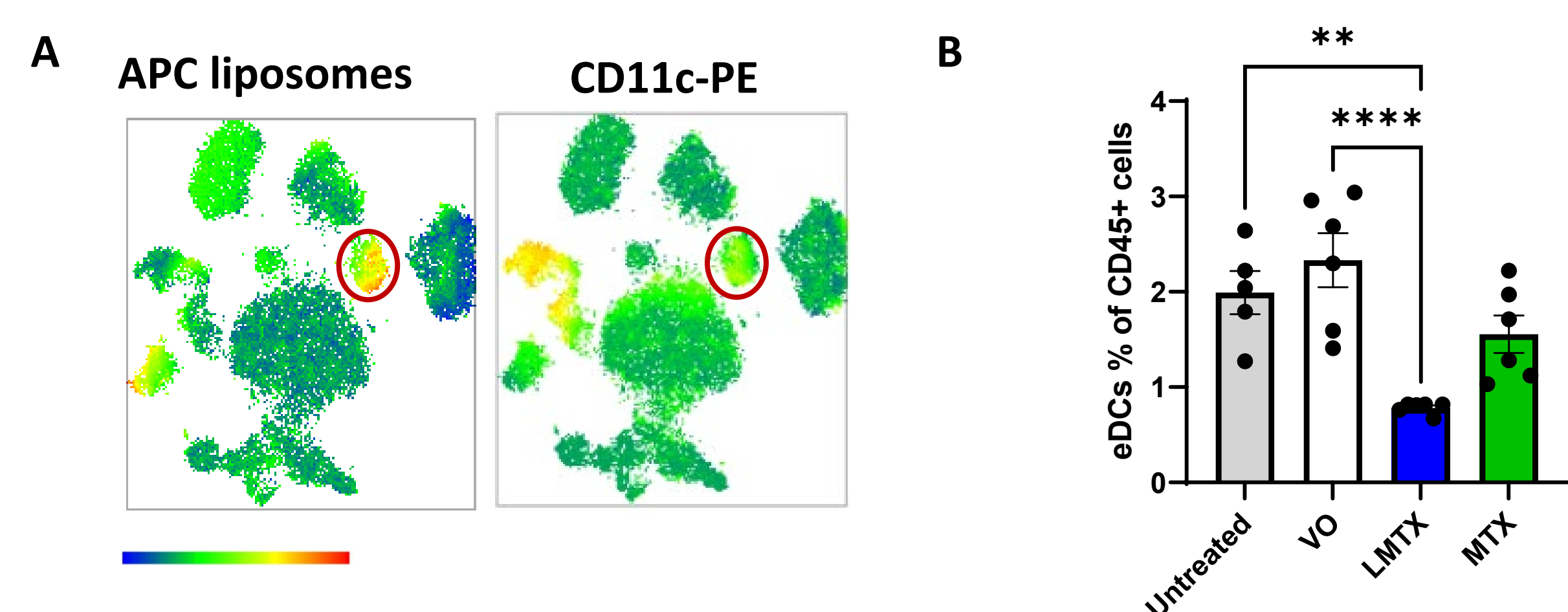


Figure 3: liposomes interact with a dendritic cell population which is depleted in LMTX-treated mice. **A.** tSNE analysis of mouse spleen 2 days following dosage with DiIc18(5)-DS-labelled liposomes identifies a specific CD11c-expressing cell population (referred to here as eDCs, indicated with red circle) which interacts with liposomes. **B.** Blood immunophenotyping on day 20 of EAE model shows eDC cell population to be depleted by LMTX treatment.

4. CNS infiltration of liposome-interacting cell population is associated with severe disease in EAE

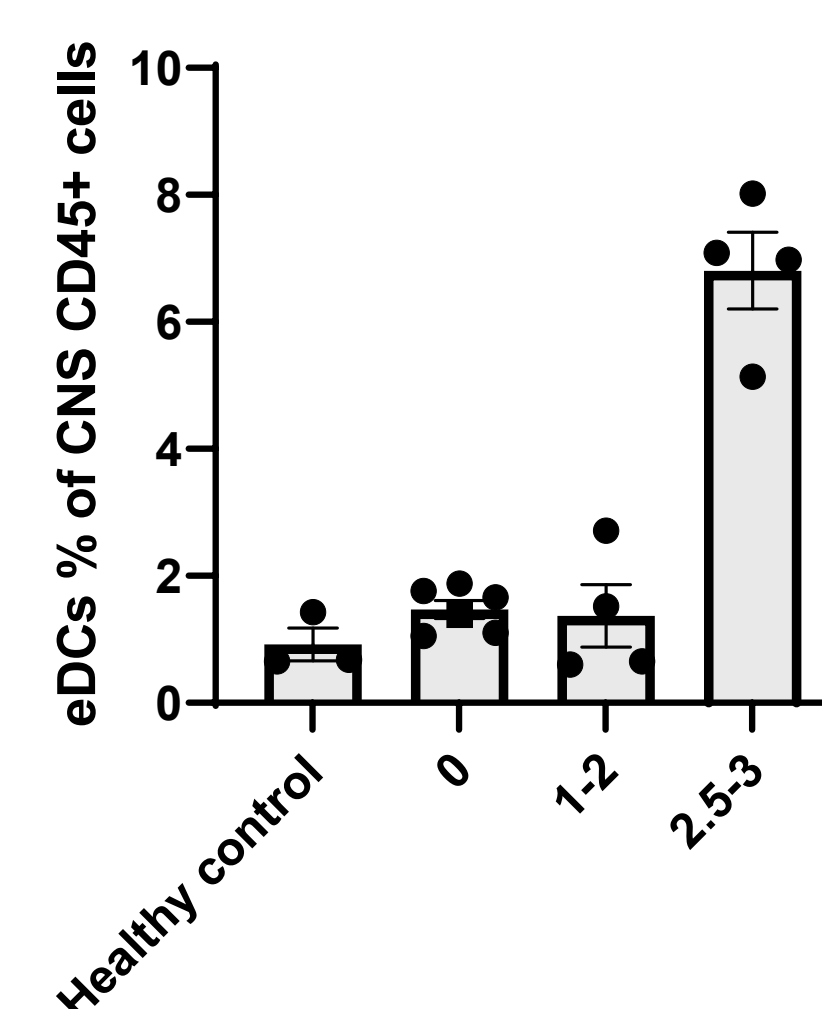


Figure 4: analysis of CNS immune infiltrates in EAE (day 20) identifies that the liposome-interacting eDC population is enhanced in the CNS of mice with severe EAE symptoms (2.5-3) compared to healthy controls or EAE-induced mice displaying no symptoms (0) or mild symptoms (1-2).

Conclusions and future directions

- LMTX has an enhanced impact on EAE neuroinflammation relative to MTX
- Liposomes are able to enter the CNS of EAE-induced mice
- A specific liposome-interacting dendritic cell population (eDCs) is enhanced in severe EAE and can be targeted by LMTX treatment

Future directions:

- Additional treatment trials and dose titration of LMTX in EAE
- Further characterisation of liposome-interacting eDCs, and mechanistic assessment of any contribution to neuroinflammation

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