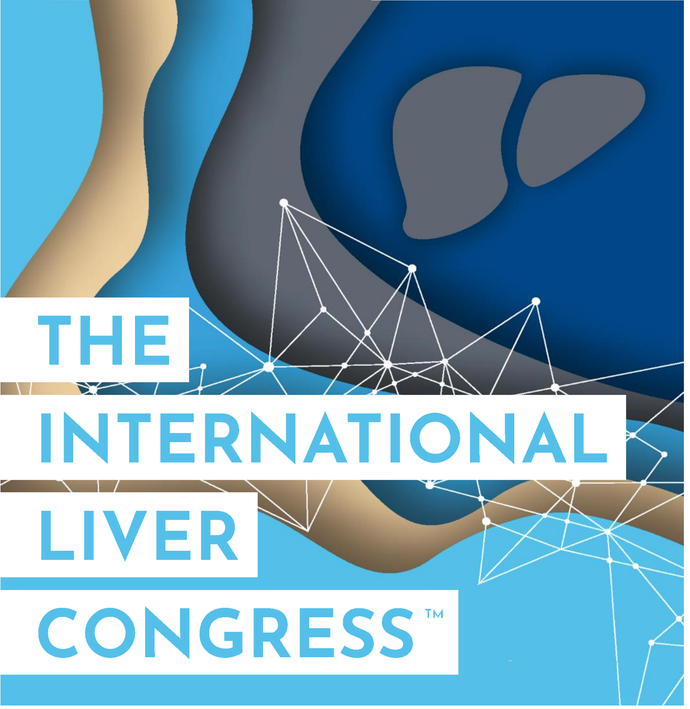

CD4 T cell tolerance induced by nanoparticle-mediated autoantigen delivery to liver sinusoidal endothelial cells depends on interferon-gamma

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1 Introduction

Targeted gene expression of myelin basic protein (MBP) in hepatocytes can induce MBP-specific CD4 T cell tolerance and prevent MBP-driven neuroinflammation in experimental autoimmune encephalomyelitis (EAE)[1]. The induction of tolerance depended on IFNG-CXCL9-CXCR3-mediated accumulation of autoreactive T cells in the liver and upregulation of CTLA-4[2]. Alternatively, MBP-specific CD4 T cell tolerance and suppression of EAE can be induced by delivery of MBP peptides to liver sinusoidal endothelial cells (LSECs) via nanoparticles (NP)[3].

2 Aim

It is unclear whether autoantigen delivery to LSECs with NP uses the same or different hepatic tolerance mechanisms as those activated by gene targeting to hepatocytes.

3 Method

B10.PL mice were immunised to MBP and injected with LSEC-targeting unloaded (NP) or MBP-loaded NP (MBP-NP). One day later, CD45.1- MBP-specific tg4 T cells were adoptively transferred into the CD45.1+ recipients. Additionally, mice received blocking IFNG antibody or isotype control. The transferred tg4 T cells were re-analysed *ex vivo* by flow cytometry on day seven after immunisation. Moreover, the clinical EAE course was monitored.

5 Conclusions

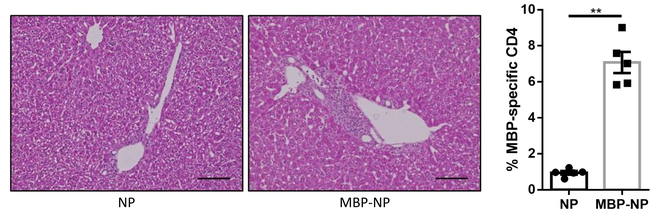
These results demonstrate that protection from autoimmune disease by autoantigen-delivery to LSECs is dependent on the induction of the IFNG-CXCL9-CXCR3 axis and upregulation of CTLA-4 on autoreactive T cells. Thus, these mechanisms are similar to those induced by hepatic autoantigen gene transfer, suggesting that LSEC-controlled transmigration of autoreactive lymphocytes into the liver parenchyma is essential for NP-mediated tolerance.

6 References

[1] Lüth, S. et al. Ectopic expression of neural autoantigen in mouse liver suppresses experimental autoimmune neuroinflammation by inducing antigen-specific Tregs. J Clin Invest, 2008. 118(10): p. 3403-3410.
[2] Krzikalla, D. et al. Interferon-gamma and CTLA-4 drive liver-induced systemic immune tolerance. EASL abstract in: Journal of Hepatology 2020; Vol. 73, S82
[3] Carambia, A. et al. Nanoparticle-based autoantigen delivery to Treg-inducing liver sinusoidal endothelial cells enables control of autoimmunity in mice. J Hepatol, 2015. 62(6): p1349-56.

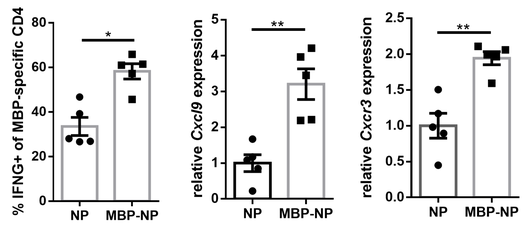
4 Results

MBP-specific T cells accumulate in livers of MBP-NP-treated mice



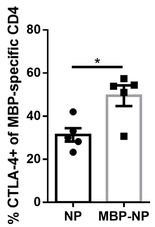
Infiltrating immune cells in the liver were visualised via HE staining (left) on day seven after immunisation. NPCs were isolated and the frequency of transferred, MBP-specific T cells was analysed via flow cytometry (right).

MBP-specific T cell accumulation is dependent on the IFNG-CXCL9-CXCR3 axis



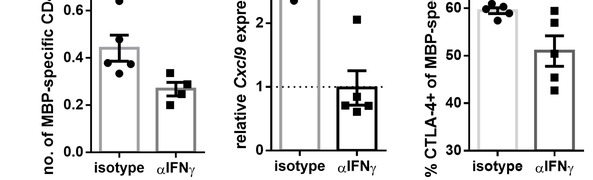
On day seven after immunisation, production of IFNG was analyzed in MBP-specific T cells after 4h stimulation with PMA/ionomycin (left). Expression of *Cxcl9* (middle) and *Cxcr3* (right) was determined in whole liver tissue.

CTLA-4 is upregulated on MBP-specific T cells in MBP-NP-treated mice



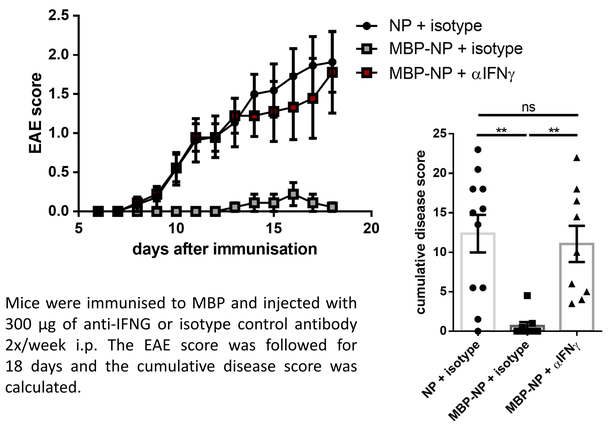
On day seven after immunisation, expression of CTLA-4 was analysed on MBP-specific T cells in the liver via flow cytometry.

In vivo inhibition of IFNG prevented migration of MBP-specific T cells into the liver and decreased their expression of CTLA-4



MBP-NP-treated mice were immunised to MBP and injected with 300 µg of anti-IFNG or isotype control antibody 2x/week i.p. On day seven after immunisation, the number of MBP-specific T cells was determined in the liver (left). Expression of *Cxcl9* was determined in whole liver tissue (middle). CTLA-4 expression was determined on MBP-specific T cells in the liver (right).

In vivo inhibition of IFNG abolished tolerance to MBP in MBP-NP-treated mice and induced clinical EAE



Mice were immunised to MBP and injected with 300 µg of anti-IFNG or isotype control antibody 2x/week i.p. The EAE score was followed for 18 days and the cumulative disease score was calculated.