TNF-α load and excess in MS white matter lesions originates from astrocytes rather than TSPO expressing microglia/macrophages.

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INTRODUCTION

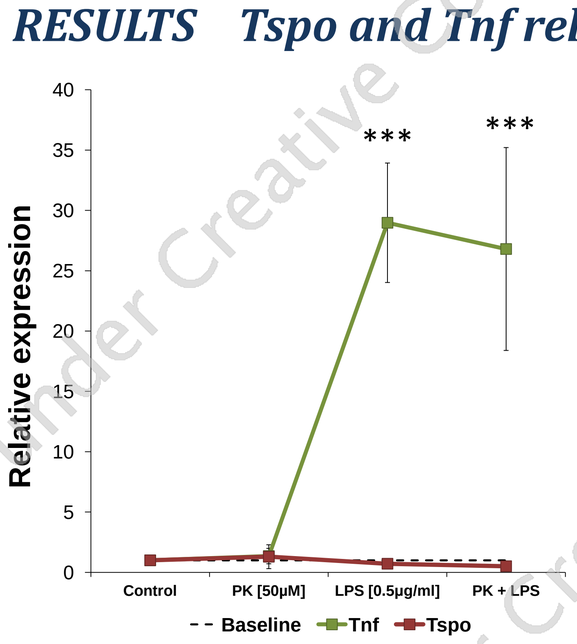
Multiple sclerosis (MS) is a well characterised inflammatory demyelinating autoimmune disease with a robust immune and microglial component (Magliozzi et al., 2010) and TSPO has been shown to be a useful biomarker to detect lesion in vivo (Oh et al., 2011). TSPO, a translocator protein, is expressed on the outer membrane of mitochondria and its up-regulation has been mostly associated with microglial activation at primary sites of pathology in brain disorders (Owen and Matthews, 2011). Nevertheless, no direct evidence has to date shown that the presence of TSPO in microglia suggests a microglia of a classical M1 phenotype with the release of TNF as the main pro-inflammatory cytokine.

METHOD

The transformed C57BL/6 mouse microglial cell line (BV2) were used as in vitro model of inflammation. Standard cell culture protocols were used.

Active white matter lesions from 5 MS cases were investigated and compared to aged-matched 4 non-MS white matter (controls). Total RNA was extracted from the lesions as well as control white matter and sections of the lesion were available for in situ protein labelling using standard immunohistochemistry.

RESULTS Tspo and Tnf relationship – in vitro model

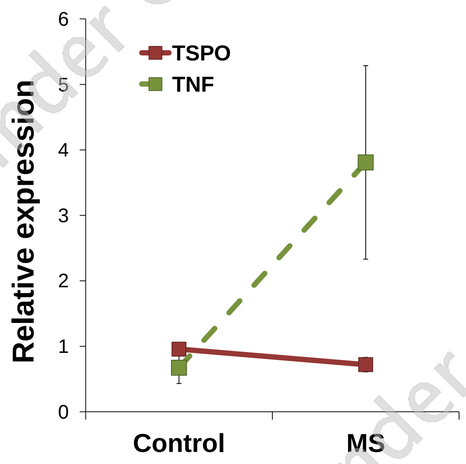


The Tspo antagonist PK11195 was previously found to reduce Tnf in a model of human embryonic microglia (Choi et al. 2002). We therefore aimed to investigate the effect of PK11195 on Tnf expression in BV2 cells. Expression levels increased following LPS stimulation however Tspo ligand did not significantly reduce Tnf mRNA expression levels in our model. PK11195 did not have any modulatory effect on Tspo nor on Tnf mRNA expression in BV2 murine microglia.

Additional data compiled in our lab suggests that Tspo had little to do with Tnf signalling or expression since our attempts to modulate Tspo whether with Tspo ligand PK11195 or with Tspo antisense oligonucleotides, the in vitro model showed no significant changes in TNF levels. We investigated further the Tspo/Tnf relationship in human tissue, i.e. MS white matter lesions.

TSPO and TNF relationship in MS - mRNA

In the first instance, TSPO and TNF mRNA levels were compared in MS and control white matter.
No significant difference in TSPO mRNA expression was found between control and MS. Similarly, TNF mRNA also did not reach significance (p = 0.06) using a Man-Whitney comparison test even with a mean TNF mRNA expression much higher in MS (3.81 ± 1.48) compared to controls (0.67 ± 0.24).

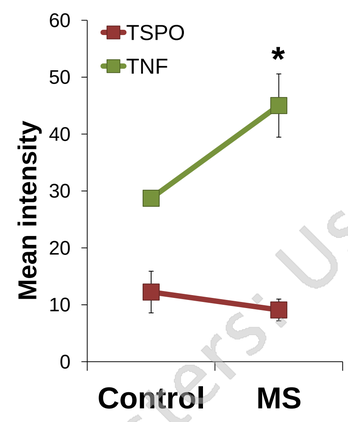


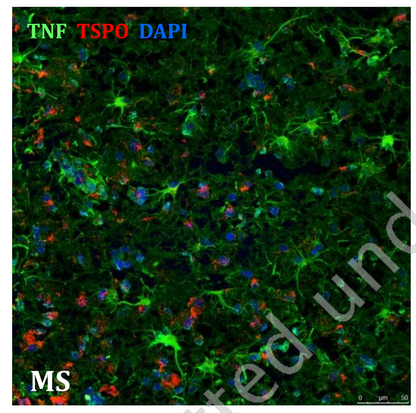
In at least 3 MS cases, the increases of mRNA TNF observed in the white matter lesion did appear independent from TSPO expression.

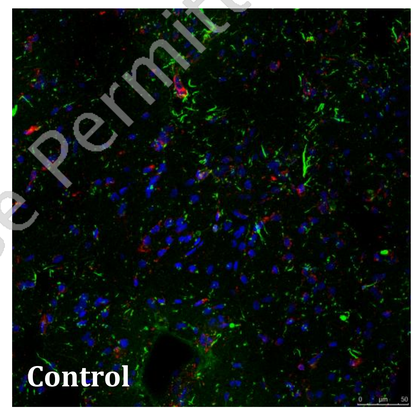
TSPO and TNF relationship in MS - Protein

We investigated further TNF and TSPO expression in situ using standard immunostaining. TNF protein expression appeared in the first instance more prominent in WML compared to controls. Analysis of mean intensity TNF and TSPO signal per image was assessed in a series of 8 images from 2 controls and 4 MS lesions. A total of 8 images were taken per case and each image was constructed from a stack of 12 images taken from a minimum of 4 µm of thickness within the section. Same settings were used across cases.

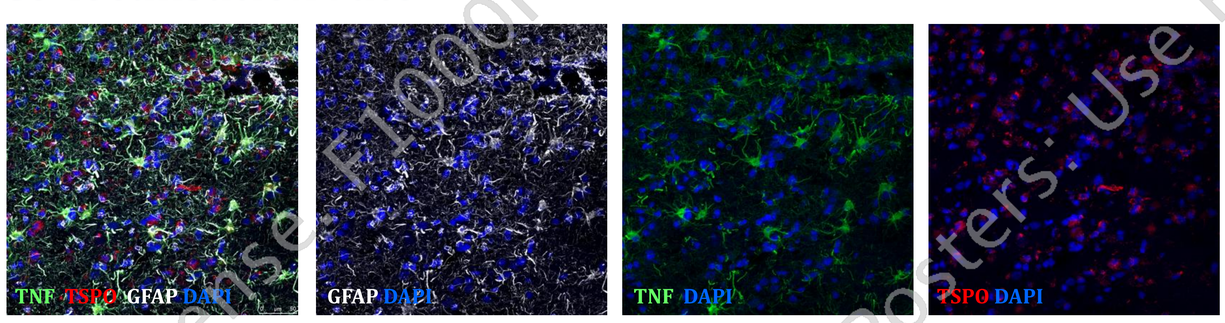
First we observed a statistical significant (p = 0.03) increase in mean intensity of TNF protein in MS WML (45.01 ± 5.56) compared to controls (28.72 ± 0.64). TSPO protein remained unchanged across conditions.



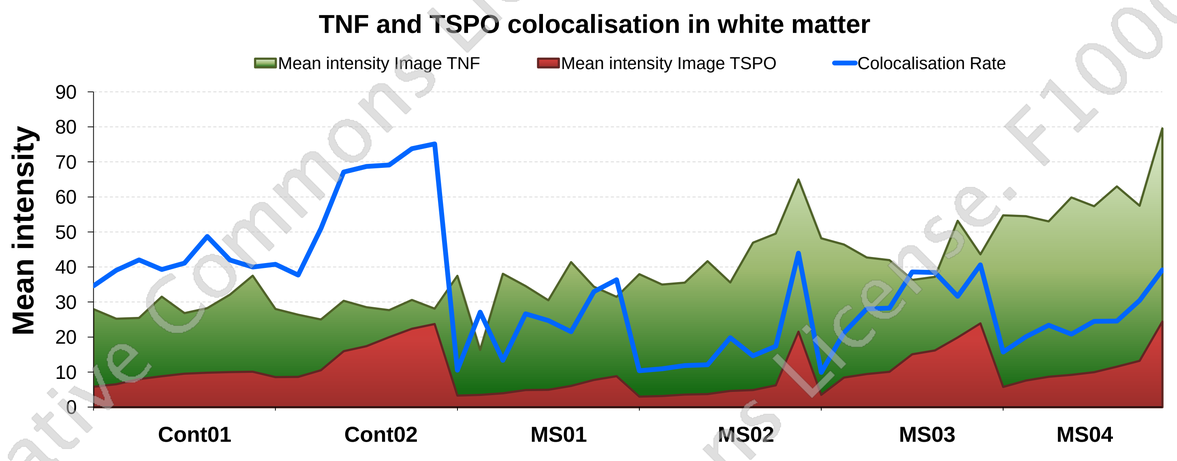




Co-localisation rate



TNF expression was not restricted solely to TSPO expressing cells but also to non-TSPO expressing cells of a different morphology, mostly of astrocytic or dendritic nature. Most star-shaped TNF positive cells were GFAP positive (above). A co-localisation rate between TSPO and TNF for each image was determined (see graph below). The controls showed much higher co-localisation rate as the MS cases where the co-localisation rate dropped dramatically. It was observed that contribution of TNF originated from cells other than microglia resulting for the lower co-localisation rate in MS WML.



CONCLUSION

This preliminary data would suggest that the de novo TNF load and excess in WML appears to originate mostly from astrocytes rather than microglia TSPO positive. Incidentally, in human post-mortem tissue, no TSPO expression has been co-localised with astrocytes. Direct molecular pathways between the presence of TSPO expressing microglia and TNF-expressing astrocytes in MS WML and whether de novo expression of TNF in astrocytes is protective or detrimental remains to be fully determined as yet and worth further investigations.

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