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INSIGHTS INTO THE PATHOGENESIS OF PERIPHERAL NERVE LESIONS IN A MODIFIED VIRUS-INDUCED ANIMAL MODEL OF MULTIPLE SCLEROSIS

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Introduction

Viral infections induce devastating and partly life threatening diseases in the peripheral (PNS) and central nervous system (CNS) of humans and animals. Demyelinating diseases such as multiple sclerosis (MS) or Guillain-Barré syndrome (GBS) are pathologically linked to antecedent infections [1, 2]. Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) represents a well-established animal model for MS. CNS lesions evolving after experimental intracerebral infection are well described [3]. While highly virulent strains induce lytic neuronal infection with lethal outcome, low virulent strains allow the establishment of virus persistence within glial cells and macrophages of susceptible (e.g. SJL) but not resistant (e.g. C57BL/6) mice [3, 4]. PNS involvement is considered a minor phenomenon following intracerebral TMEV-infection [5, 6]. Interestingly, shifting the site of injection from cerebrum to the spinal cord (SC), leads to the induction of demyelinating lesions in the SC in both susceptible and resistant mouse strains, and subsequently to the emergence of a demyelinating peripheral neuropathy resembling features of GBS [7, 8]. Within the PNS, NG2 is expressed by fibroblast-like cells and vascular pericytes as well as potentially by non-myelinating and immature Schwann cells, which could be a potential target for the virus [10]. Knowledge on the nature of TMEV-induced PNS lesions is scarce [7-9]. The objective of this study is to provide a more detailed characterization of peripheral nerve lesions (PNL) after intraspinal TMEV infection.

Materials and Methods:

Animals:

- 4-8, young-adult, female C57BL/6 (B6)- and SJL.NG2CreERT2xRosa26.floxed.stop-tdTomato mice;
- Intraspinal (i.s.) inoculation with TMEV BeAn strain or vehicle;
- Necropsies at 14, 28 (B6) or 28, 63 (SJL) days post inoculation (dpi);

Methods:

- Morphometric analysis of toluidine-blue stained semi-thin sections (Fig. 1);
- Ultrastructural analysis using transmission electron microscopy (Fig. 1);
- Immunofluorescence staining targeting TMEV, Schwann cells, axons, macrophages and cells of nerve/glial antigen 2 (NG2) lineage (Fig. 2);

Results

Morphometric analysis and Transmission electron microscopy

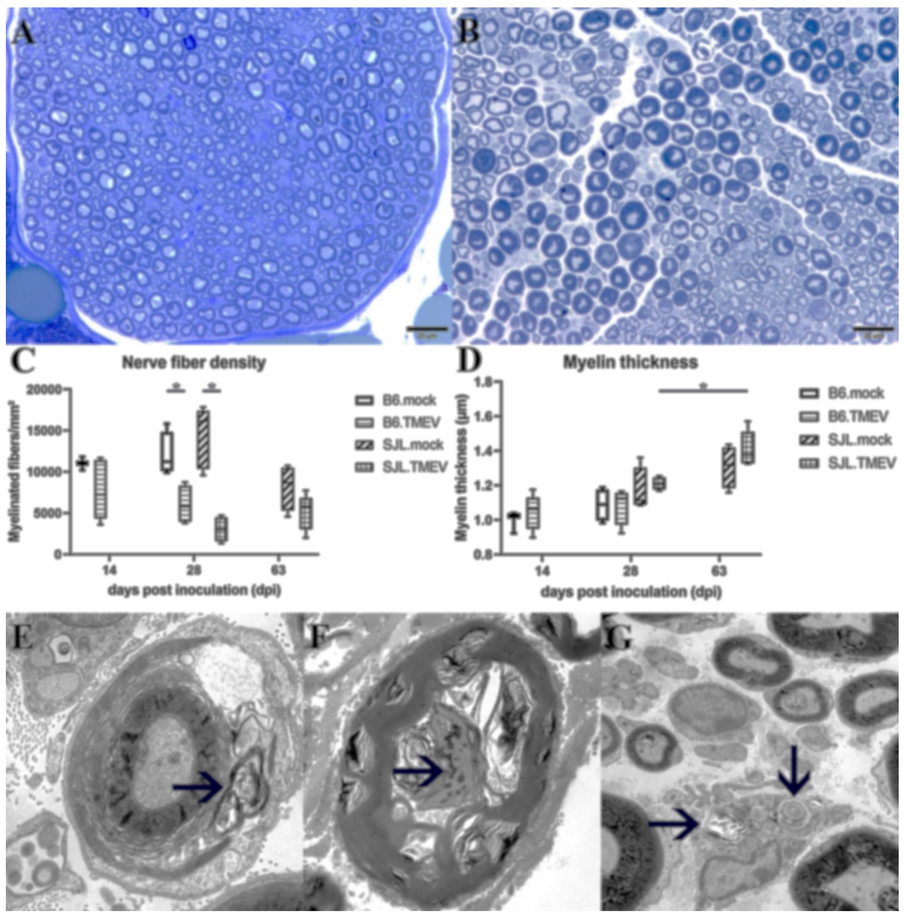

Figure 1: A, B: Toluidine-blue stained semi-thin sections of peripheral nerves of a mock (A) and virus infected (B) SJL mouse at 63 days post infection (dpi) showing swollen myelin sheaths within the infected animal. C,D: Statistical analysis revealed a decrease in nerve fiber density at 28 dpi in both, SJL and C57BL/6 mice and an increase in myelin thickness between 28 and 63 dpi in SJL mice. Significant differences as detected by Kruskal Wallis and Dunn's-Bonferroni testing; * = $p < 0.05$. E-F: Transmission electron microscopy showed vacuolization of myelin (E; arrow; 25000x), accumulation of electron dense structures within axons (F; arrow; 10000x) and intracytoplasmic myelin debris within macrophages (G; arrows; 6300x) in a peripheral nerve of a SJL mouse at 28 dpi.

Double immunofluorescence stainings

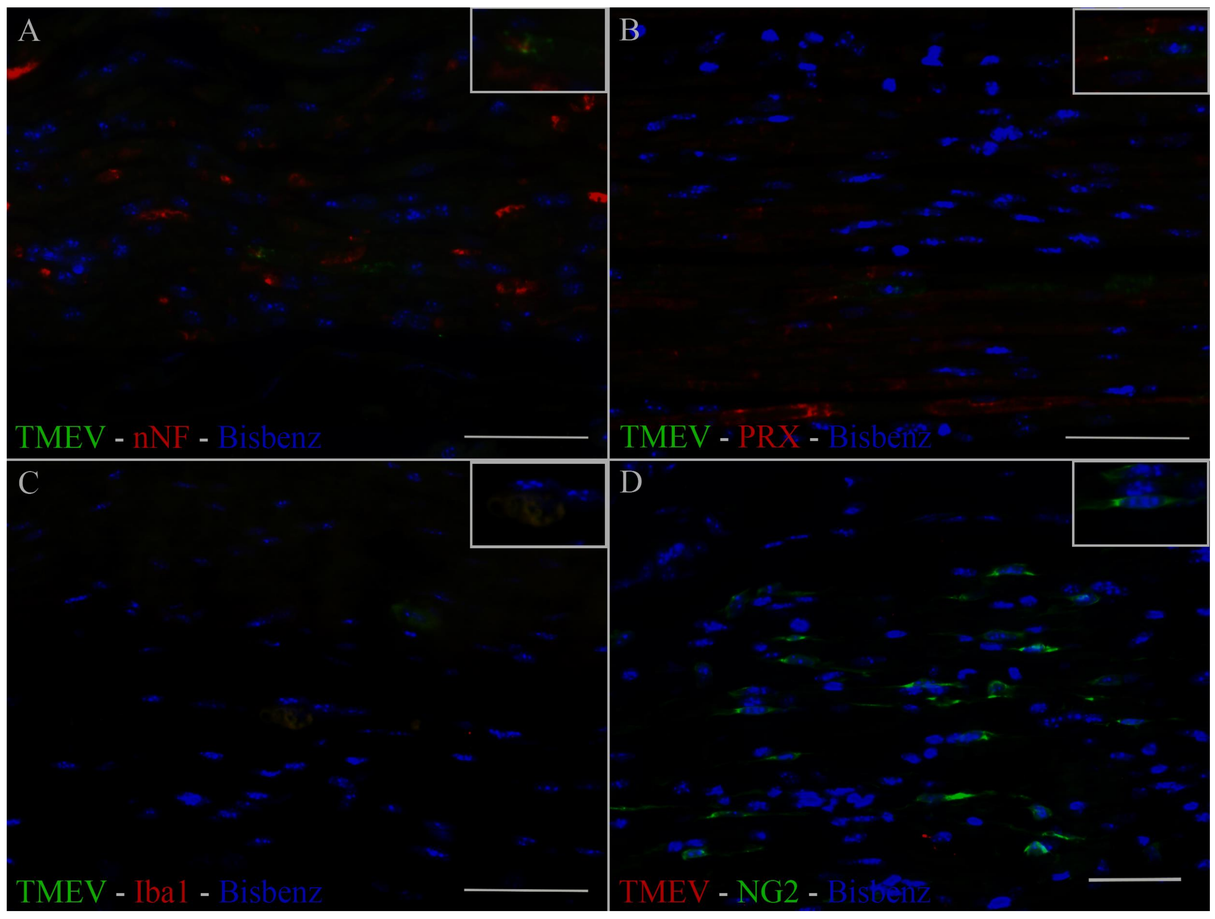

Figure 2: A-C: Double immunofluorescence (IF) using antibodies targeting Theiler's murine encephalomyelitis virus (TMEV; green) as well as non-phosphorylated neurofilament (A: nNF, axonal damage; red), Periaxin (B: PRX, peripheral myelin; red) and ionized calcium binding adaptor molecule 1 (C: Iba1, macrophages; red). IF-staining revealed co-localization of TMEV with nerve damage in a C57BL/6 mouse at 14 days post infection (dpi; A), with myelin vacuolization within nerve sheaths showing loss of periaxin staining intensity in a SJL mouse at 28 dpi (B; demyelination) and with macrophages exhibiting vacuolated cytoplasm in the peripheral nerve of an SJL mouse at 63 dpi (C). D: Double IF using antibodies targeting TMEV (red) as well as cells of NG2 lineage (NG2, green). IF-staining revealed no co-localization of TMEV with NG2 positive cells.

Conclusions:

After i.s. TMEV infection, inflammatory and degenerative changes (decreased fiber density, swollen myelin sheaths, macrophage infiltration) were observed in peripheral nerves of TMEV-infected mice, especially within persistently infected SJL-mice. There were no obvious signs of attempted regeneration, suggesting persistent and/or continuous nerve damage. Viral capsid protein was detected in macrophages as well as in close proximity to axonal and myelin damage within PNLs. These results provide new insights into cell tropism of TMEV in the PNS and show for the first time a direct link between lesion development and virus localization. The occurrence of virus in the CNS after intranerval injection [9] and, conversely, the detection of virus in the PNS after intraspinal injection [7, 8] suggest antero- and retrograde spread of TMEV, most likely along axons, although spread via macrophages cannot be completely excluded.
Co-localization of TMEV and cells of NG2 lineage was not observed. Although NG2 cells do not seem to be a cellular target of TMEV in peripheral nerves, it has been hypothesized that they may differentiate into myelinating Schwann cells after PNS damage [11], and thus may be involved in regeneration and repair. Therefore, further investigations are needed to determine whether or not cells of NG2 lineage contribute to remyelination of peripheral nerves after TMEV induced damage.

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