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The inflammatory response to sciatic nerve injury in a familial amyloidotic polyneuropathy mouse model

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ABSTRACT

Inflammation is a hallmark of several neurodegenerative disorders including familial amyloidotic polyneuropathy (FAP). FAP is associated with extracellular deposition of mutant transthyretin (TTR), leading to degeneration of cells and tissues, particularly in the peripheral nervous system (PNS). With this work, our goal was to characterize the expression/deposition of TTR and the associated inflammatory immune response, induced by nerve injury, in WT mice and in a mouse model carrying the most common TTR mutation in FAP (V30M). Our results indicate that upon nerve injury TTR is significantly produced by Schwann cells and is dynamically regulated over time in V30M mice, accompanying a peak of inflammation. Strikingly, V30M TTR deposition in nerve tissue occurred, suggesting that inflammation contributes to TTR polymerization. In response to nerve injury, V30M mice display a downregulated innate immune response when compared to WT mice. More specifically, we saw decreased expression of cytokines and chemokines important for the recruitment of immune cells like macrophages and neutrophils, known to be important for the tissue regenerative process which was found impaired in V30M mice. In conclusion, with this work we were able to characterize the biology of TTR both in WT and V30M animals, upon nerve injury, and found that V30M TTR impairs the inflammatory response necessary for nerve regeneration. Taken together, our findings suggest that inflammation is an important target to be considered in therapeutic strategies for FAP.

Introduction

Amyloidoses constitute a large group of acquired and hereditary disorders characterized by the extracellular accumulation of misfolded proteins, sharing a crossed β-sheet structure (Westermark, 2005). Among them, transthyretin (TTR) amyloidosis is the most common form of hereditary autosomic dominant systemic amyloidosis, in which TTR point mutations result in deposition of amyloidogenic species in different tissues (Benson and Kincaid, 2007). The TTR mutation most commonly associated with familial amyloidotic polyneuropathy (FAP) is V30M (Saraiva et al., 1984) that leads to extracellular TTR deposition particularly at the peripheral and autonomic nervous systems, with neuropathy as a cardinal feature. TTR reaches the peripheral nervous system (PNS) through the cerebrospinal fluid, the blood–nerve barrier and over contact with peripheral nerve roots (Saraiva and Sousa, 2003) accumulating in the endoneurium, near Schwann cells, blood vessels and collagen fibrils, ultimately leading to neurodegeneration and cell death (Coimbra and Andrade, 1971).

TTR functions as a carrier for thyroxin and retinol (Kanai et al., 1968; Woeber and Ingbar, 1986) and is synthesized mainly by the liver and the choroid plexus of the brain (Soprano et al., 1985). Recently, minute levels of *TTR* expression were also found in Schwann cells of the sciatic nerve (Murakami et al., 2010). The mechanism leading to TTR deposition in tissues include a decrease in its conformational stability with dissociation of the mutant homotetrameric form into unfolded monomers that self-assemble forming aggregates, oligomers and amyloid fibers (Quintas et al., 2001).

The inflammatory response has been shown to be important for the development of peripheral neuropathies either by leading to deterioration or to amelioration of the disease process (Ydens et al., 2013). In the case of FAP, previous work with nerve biopsies from FAP patients shows the close association between production of inflammatory mediators and TTR pre-fibrillar aggregates or amyloid fiber deposition suggesting a prominent role for inflammatory pathways in the progress of the disease (Sousa et al., 2001a). Additionally, infiltration of inflammatory cells in response to TTR aggregates or amyloid fibers deposition in tissues of FAP patients would be expected. However, despite cytokine production by axons, no influx of such cells is found in FAP nerve biopsies (Misu et al., 1999; Nyhlin et al., 2000; Sousa et al., 2001a), suggesting that mechanisms must operate to prevent or inhibit the correct innate immune response. TTR aggregates or amyloid material were never found

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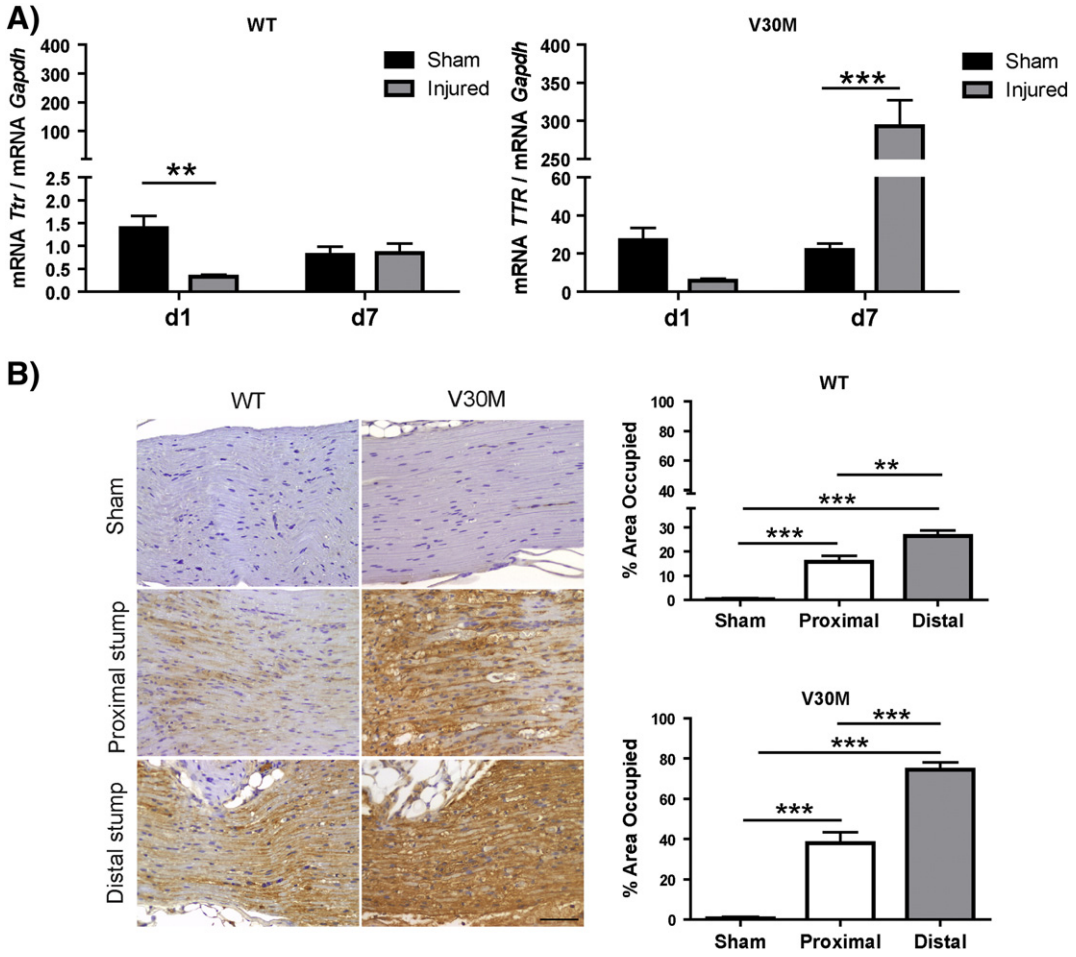

Fig. 1. Sciatic nerve injury leads to local upregulation of V30M *TTR* expression. A) Histograms represents *Ttr* mRNA levels in the sciatic nerve of WT (left chart) and V30M mice (right chart), 1 and 7 days after injury ($n = 6$), as compared with sham nerves ($n = 6$). Data were analyzed with two-way ANOVA followed by Bonferroni post-test and is represented as mean ± SEM (**$p < 0.01$ and ***$p < 0.001$). B) Representative SQ-IHC against mouse (left panels) and human TTR (right panels) in the proximal and distal stumps, 7 days after sciatic nerve injury, as compared with respective sham nerves. Charts represent quantification of immunohistochemical images ($n = 5$ V30M and $n = 5$ WT mice, scale bar 100 μm), data was analyzed with one-way ANOVA followed by Bonferroni post-test and is represented as mean ± SEM (**$p < 0.01$ and ***$p < 0.001$).

in the PNS of the classical FAP animal models carrying the V30M mutation (Nagata et al., 1995; Sasaki et al., 1986; Yamamura et al., 1987) which led us to hypothesize whether neuroinflammation might be a contributor for TTR production and deposition in the PNS. To address this possibility we induced nerve injury, in both wild-type (WT) and in a well-described transgenic mouse for human TTR V30M in a TTR null background (V30M), and tested in vivo the local effect on TTR synthesis, deposition and the inflammatory response.

Materials and methods

Animals

Six months-old male and female WT and V30M mice, in the 129/Sv background, were used for the experiments. This was the chosen age since by this period, V30M mice have TTR non-fibrillar deposition in both the gastrointestinal tract and skin but not in the PNS (Kohno et al., 1997). Animals were housed in pathogen-free conditions, in a controlled-temperature room, maintained under a 12 h light/dark period, with water and food ad libitum. One or 7 days after injury, mice were sacrificed with a lethal injection of a premixed solution containing ketamine and medetomidine.

All animal experiments were carried out in accordance with the European Community Council Directive (2010/63/EU) and the number of total animals for this research was approved by ethical committee and by National General Veterinarian Board.

Surgical procedures: Sciatic nerve injury

Anesthesia was induced with a premixed solution containing ketamine (75 mg/kg) plus medetomidine (1 mg/kg) and animals received a subcutaneous injection of butorphanol (1 mg/kg) prior to surgery. The thigh and legs were shaved, disinfected, the eyes coated to protect from drying and animals were then placed in abdominal position on a 37 °C heating pad. A skin incision of approximately 1 cm was made over the gluteal region exposing the left sciatic nerve from the sciatic notch to the point of trifurcation. The ischiocrural musculature was prepared with minimal tissue damage to ensure optimal conditions for functional recovery. Sciatic nerve ligation, adapted from the method described by Brumovsky et al. (2004) was performed using one ligature placed around the sciatic nerve at the mid-thigh level, using 5.0 silk. A sham operation was performed similarly at the contralateral side, except that the sciatic nerve was not constricted. The skin incision was closed with 5.0 silk suture and physiological saline solution was injected subcutaneously. Mice were kept in a recovery room with infrared heating lamp for 1–2 h. Post-surgical pain treatment consisted of supplying mice subcutaneously with butorphanol every 12 h for the first 48 h.