## Blocking TNF (using cV1q) reduces the severity of stretch-induced muscle damage in dystrophic, mdx mice

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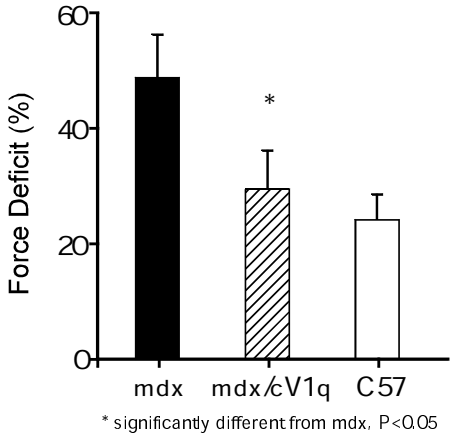
Duchenne muscular dystrophy (DMD) is a disorder characterized by progressive loss of muscle mass and function due to damage, necrosis and incomplete regeneration. The lack of dystrophin in skeletal muscle of DMD patients and in the mdx mouse model of DMD, render these muscles highly susceptible to contraction induced damage leading to an exacerbated inflammatory response which may further increase muscle necrosis. It has been suggested that excessive inflammation in dystrophic muscle, in particular the pro-inflammatory cytokine tumor necrosis factor (TNF), is a major contributor to the dystrophic pathology. This study investigated the effect of *in vivo* blockade of TNF using cV1q, a mouse specific TNF antibody, on the extent of muscle damage and subsequent necrosis following a damaging eccentric exercise protocol.

Experiments were performed using 10-13 week old male mdx and C57Bl/10 (C57) mice. Mice were anaesthetized by inhalation of a gaseous mixture of isoflurane (isoflurane, 0.4 L/min $N_20$, 0.4 L/min $O_2$) and the right hindlimb attached to an *in vivo* mouse dynamometer to quantify the contractile properties of the anterior crural muscles and to induce eccentric muscle damage (Ridgley *et al.*, 2008). Anterior crural muscles were activated by stimulation of the common peroneal nerve and the peak torque and optimal joint angle for torque production were determined. A damaging eccentric exercise protocol was performed consisting of 20 lengthening contractions (15°-55° plantar flexion at 1000°$s^{-1}$). Mice were sacrificed by cervical dislocation 48 h after the dynamometer protocol and the tibialis anterior (TA) muscle excised, snap frozen in isopentane cooled by liquid nitrogen and sectioned on a cryostat for histological analysis. Muscle damage was quantified by the decrease in joint torque immediately after the dynamometer exercise and the area of necrosis was determined from H&E staining. The effect of blocking the pro-inflammatory cytokine TNF on the extent of muscle damage following eccentric exercise was evaluated in mdx mice using the mouse specific anti-TNF antibody cV1q (4.0 mg/ml; Centocor U.S.A.), injected at 1 week and one day before the dynamometer exercise.

The peak isometric torque was significantly lower ($p < 0.05$) in mdx (60.5 ± 5.0 $Nm^2$/kg) compared to C57 mice (77.5 ± 5.4 $Nm^2$/kg). The peak torque in cV1q treated mdx mice (67.2 ± 3.3 $Nm^2$/kg) was not significantly different from mdx. The deficit in peak joint torque induced by the eccentric exercise protocol was two-fold greater in mdx compared to C57 mice. However, blocking TNF significantly reduced this force deficit in cV1q treated mdx mice (see figure). Furthermore, cV1q treatment significantly reduced the amount of necrosis in mdx mice (mdx 13.6 ± 2.4%, mdx/cV1q 5.0 ± 1.8%). There was no detectable necrosis in C57 mice.




The ability of cV1q to reduce the force deficit and subsequent necrosis following exercise-induced muscle damage raises interesting questions about the early events in dystrophic muscle that occur in response to such exercise and the precise mechanism responsible for the adverse effects of TNF on force production. The results further support the proposal that increased TNF contributes to the dystrophic pathology and that blockade of TNF is a potential protective therapy to reduce the severity of DMD.

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