Muscle fiber plays a critical role in therapeutic response to glucocorticoids during myositis

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Introduction. Myositis are autoimmune diseases characterized by chronic inflammation of skeletal muscle and muscle weakness. **Glucocorticoids (GC)**, the first line treatment of myositis, improve muscle strength, but recovery is partial and, chronically, they lead to steroid myopathy. Thus, myositis care has to be improved. GC effects are mediated by **glucocorticoid receptor (GR)**, which is expressed in various cell types including immune cells and myofibers, but it remains to be determined which cells are leading to therapeutic response.

Materials and Methods. Experimental myositis (EM) was induced in eight to ten week-old C57BL/6J female mice by a single intradermal injection of skeletal muscle fast-type C protein with Freund's adjuvant and an intraperitoneal (IP) injection of pertussis toxin, as previously described[1]. Prednisone (PDN) was administered 14 days (D) after the immunization at 1 mg/kg/day for 7 days by gavage. Mice were euthanized 21 days after myositis induction. Muscle strength was assessed by grip test at D 0, before the 1st PDN administration (D 14) and the day before sacrifice (D 20). To investigate whether the PDN effects are mediated by myofiber, we generated **transgenic mice** carrying two LoxP sites within the GR gene in muscle, expressing the tamoxifen-inducible Cre-ERT2 recombinase selectively in skeletal muscle fiber (Pre GR(i)skm-/- mice). Tamoxifen (1 mg/day for 5 days by IP injection) was administered 9 days after immunization to induce GR ablation selectively in skeletal muscle fiber (**GR(i)skm-/-** mice) (figure 1). Similar treatments were applied to **GR L2/L2** (control mice) that do not express Cre-ER(T2).

We compared 4 groups of EM mice, GR(i)skm$^{-/-}$ treated by PDN or vehicle (V) and control mice treated by PDN or V, by **grip test, histology** and **flow cytometry.**

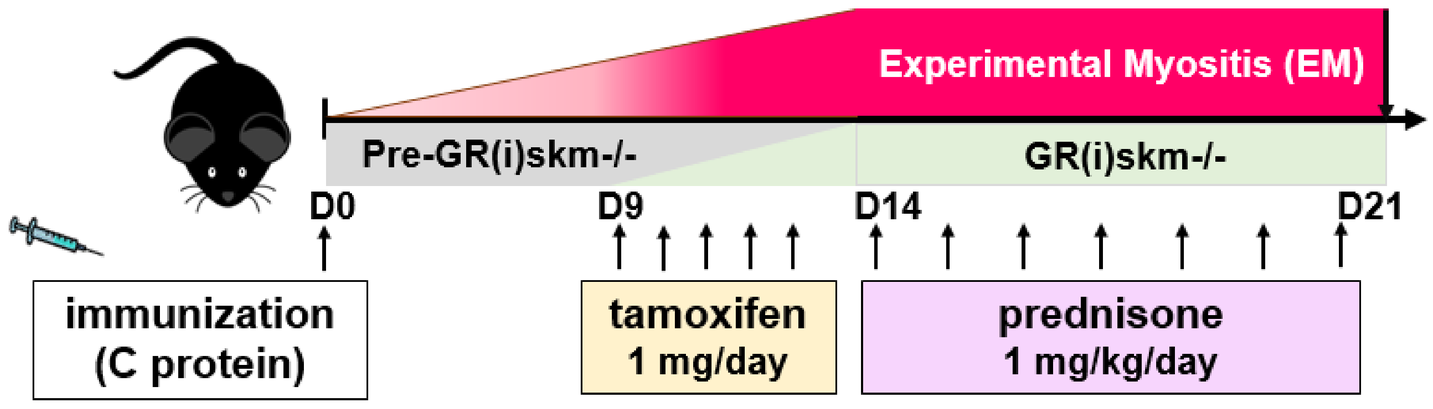

Figure 1. Timeline of the experiment. Immunization at day 0 (D0) was used to induce EM, whose peak occur at D14 with a plateau until D21. In Pre-GR(i)skm-/-, GR was ablated selectively in skeletal muscle fibers (GR(i)skm-/-) after a 5-days treatment by tamoxifen. Then, mice were treated with prednisone for 7 days from D14 until the day before the sacrifice.

Results. Muscle strength was decreased in GR(i)skm$^{-/-}$ and control mice from D14 to D20 in V groups. Control mice but not GR(i)skm$^{-/-}$ recovered muscle strength after PDN (figure 2).

At H&E and Gomori trichrome staining, no quantitative differences among the four conditions were found in inflammatory infiltrate and necrotic fibers. Nevertheless, in control mice, PDN induced a 3-fold decrease in **CD8+ cells** compared to vehicle group ($p<0,05$). This effect of PDN was abolished in GR(i)skm-/- mice (figure 3).

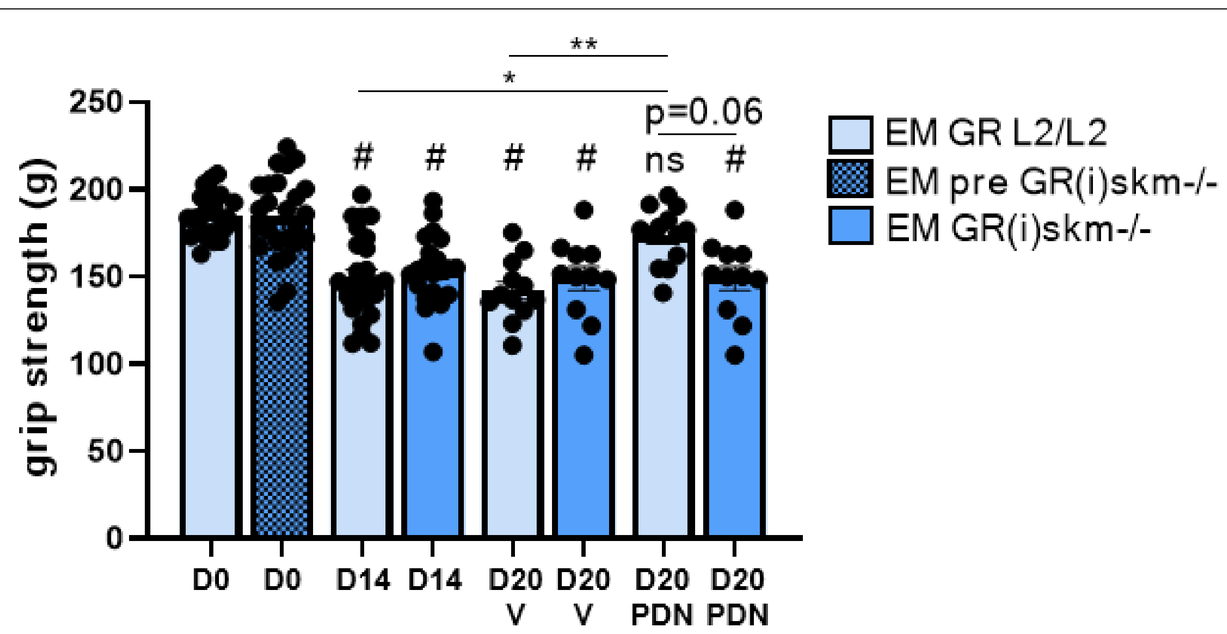

Figure 2. **Grip strength in EM mice.** Grip strength was evaluated in experimental myositis (EM) mice the day before the immunization (D0), 14 days (D14) and 20 days (D20) after the immunization, upon vehicle (V) or prednisone (PDN), Ns: not significant; *: $p<0.05$, **: $p<0.005$, vs. comparator indicated by the line; #: $p< <0.0001$ vs. D0. The bars represent mean ± SEM. N>10 per group.

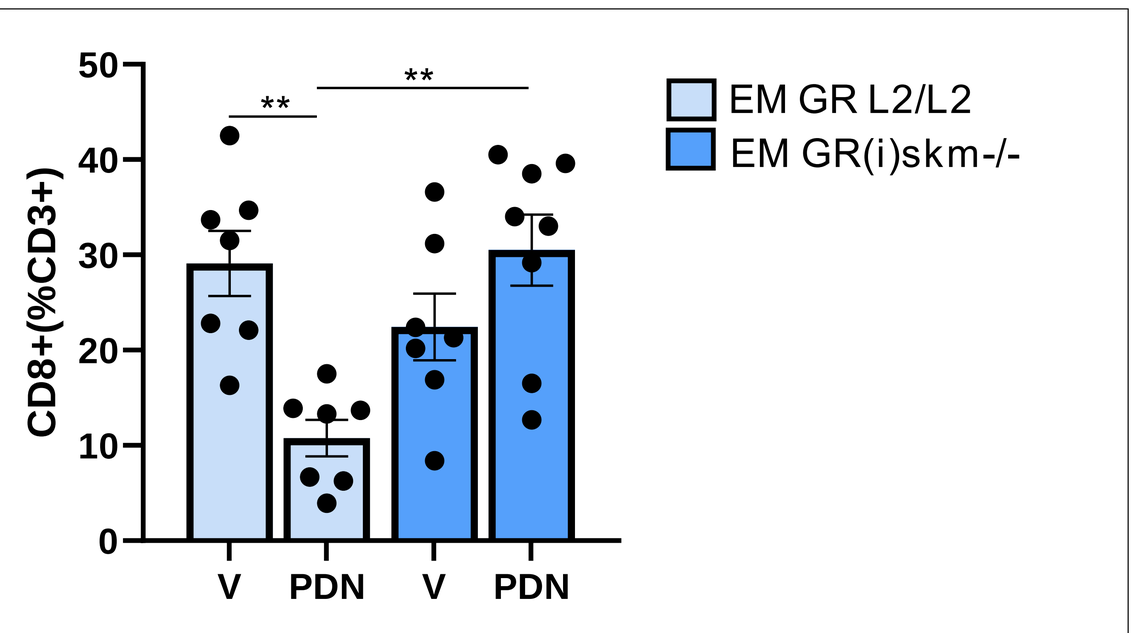

Figure 3. Flow cytometry on both sides gastrocnemius, soleus, quadriceps, anterior tibialis from experimental myositis (EM) mice, CD45 marker was used to gather inflammatory cells. Among lymphocytes, CD3+ were selected; then CD8+ were expressed as percentage of CD3+. The bars represent the mean ±SEM. N>7 per group. **p<0.01.

Conclusions. GR in myofiber is crucial for GC therapeutic response in myositis. Particularly, GC polarize inflammatory infiltrate toward an anti-inflammatory response, through a specific effect on myofiber.

[1]Sugihara T et al. Arthritis Rheum. 2007 Apr;56(4):1304-14.