Immunological characterization of a rat model of Duchenne's disease and increase in muscle strength after anti-CD45RC antibody treatment

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Introduction

Duchenne Muscular Dystrophy (DMD) is a severe genetic muscle-wasting disorder due to the lack of dystrophin characterized by a progressive muscle weakness and a cardiomyopathy leading to premature death. The dystrophin-deficient *Dmd*^{mdx} rats were generated using TALENs and offer a more reliable representation of human DMD, with marked muscle strength reduction, cardiomyopathy and muscle fibrosis that are higher that those observed in the mdx mouse model (1). A role for inflammation and autoimmune responses in muscle damages was shown both in DMD patients and the *mdx* mouse model (2).

In this study, we assessed by flow cytometry and immunohistochemistry the immune cell subsets infiltrating *Dmd*^{mdx} rat skeletal and cardiac muscles especially immunoregulatory and pro-inflammatory subsets (M1 and M2 macrophages, CD4$^+$ and CD8$^+$ Teff or Tregs…).

Then, we investigated the possibility of reducing disease in *Dmd*^{mdx} rats by administrating immunomodulatory treatments. The standard therapy for DMD patients is corticoids that decrease inflammation and immune responses but with variable responses, limited efficacy and important and numerous side effects. Therefore, there is need for new anti-inflammatory and pro-tolerogenic treatments that could replace or decrease the doses of corticoids. Anti-CD45RC monoclonal antibody (MAb) treatment has induced immune tolerance in models of organ transplantation and GVHD.

Immunophenotyping

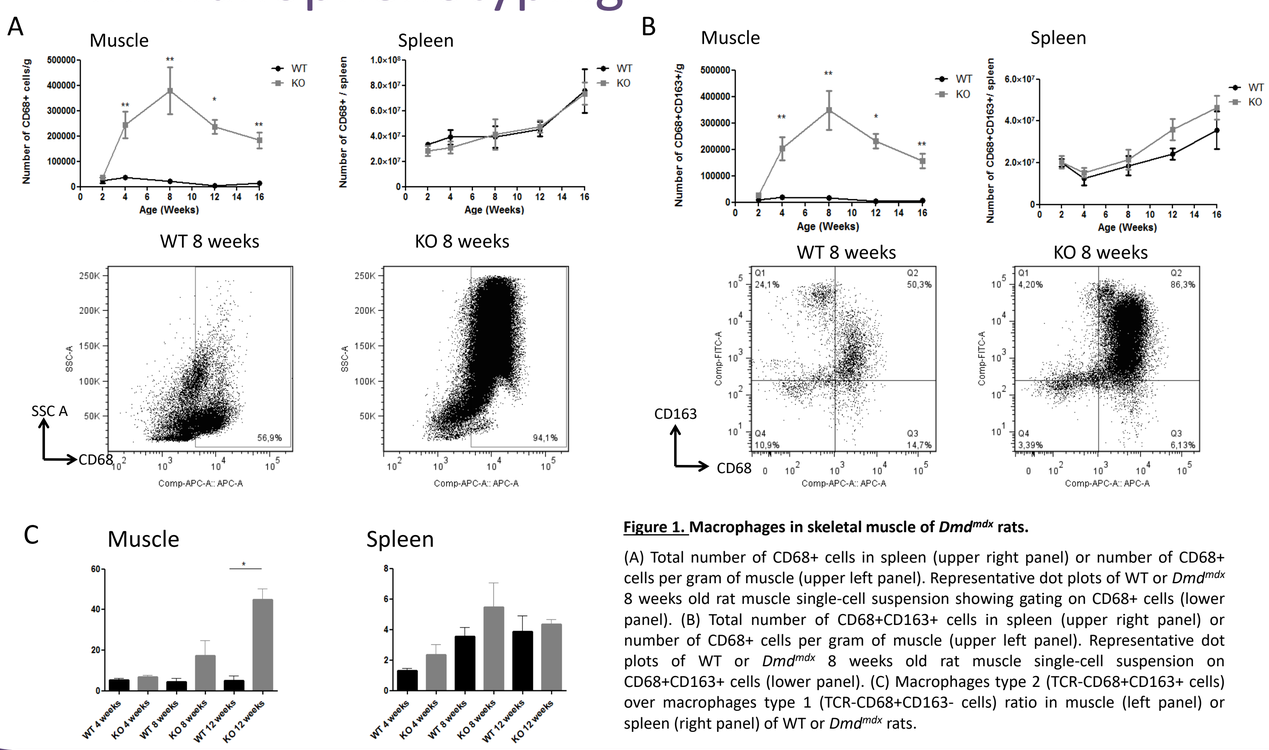

Figure 1. Macrophages in skeletal muscle of *Dmd*^{mdx} rats.

(A) Total number of CD68+ cells in spleen (upper right panel) or number of CD68+ cells per gram of muscle (upper left panel). Representative dot plots of WT or *Dmd*^{mdx} 8 weeks old rat muscle single-cell suspension showing gating on CD68+ cells (lower panel). (B) Total number of CD68+CD163+ cells in spleen (upper right panel) or number of CD68+ cells per gram of muscle (upper left panel). Representative dot plots of WT or *Dmd*^{mdx} 8 weeks old rat muscle single-cell suspension on CD68+CD163+ cells (lower panel). (C) Macrophages type 2 (TCR-CD68+CD163+ cells) over macrophages type 1 (TCR-CD68+CD163- cells) ratio in muscle (left panel) or spleen (right panel) of WT or *Dmd*^{mdx} rats.

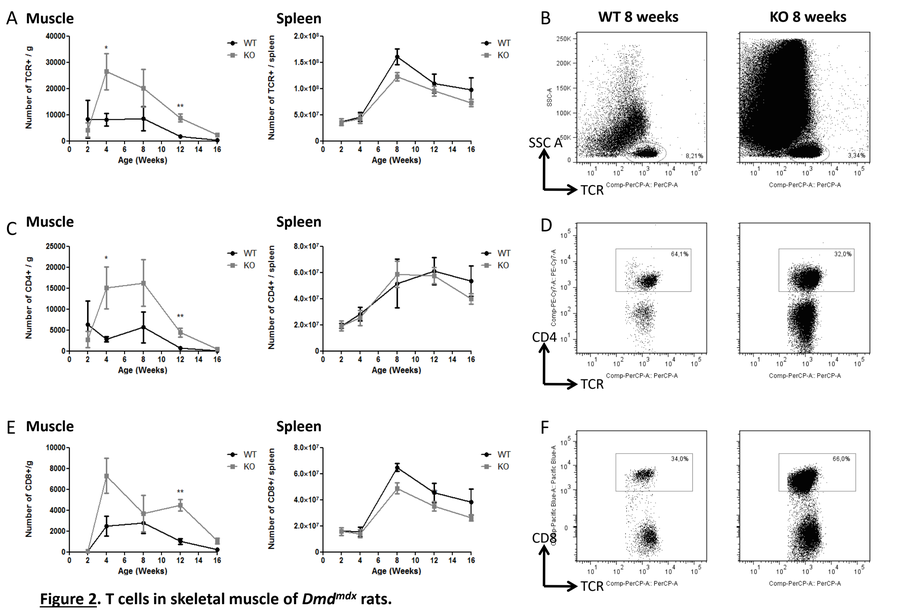

Figure 2. T cells in skeletal muscle of *Dmd*^{mdx} rats.

(A) Total number of TCR+ cells in spleen (right panel) or number of TCR+ cells per gram of muscle (left panel). (B) Representative dot plots of WT or *Dmd*^{mdx} 8 weeks old rat muscle single-cell suspension showing gating on TCR positive cells. (C) Total number of TCR+CD4+ cells in spleen (right panel) or number of TCR+CD4+ cells per gram of muscle (left panel). (D) Representative dot plots of WT or *Dmd*^{mdx} 8 weeks old rat muscle single-cell suspension showing gating on TCR+CD4+ cells. (E) Total number of TCR+CD8+ cells in spleen (right panel) or number of TCR+CD4+ cells per gram of muscle (left panel). (F) Representative dot plots of WT or *Dmd*^{mdx} 8 weeks old rat muscle single-cell suspension showing gating on TCR+CD8+ cells.

Immunomodulatory treatments

Prednisolone treatment

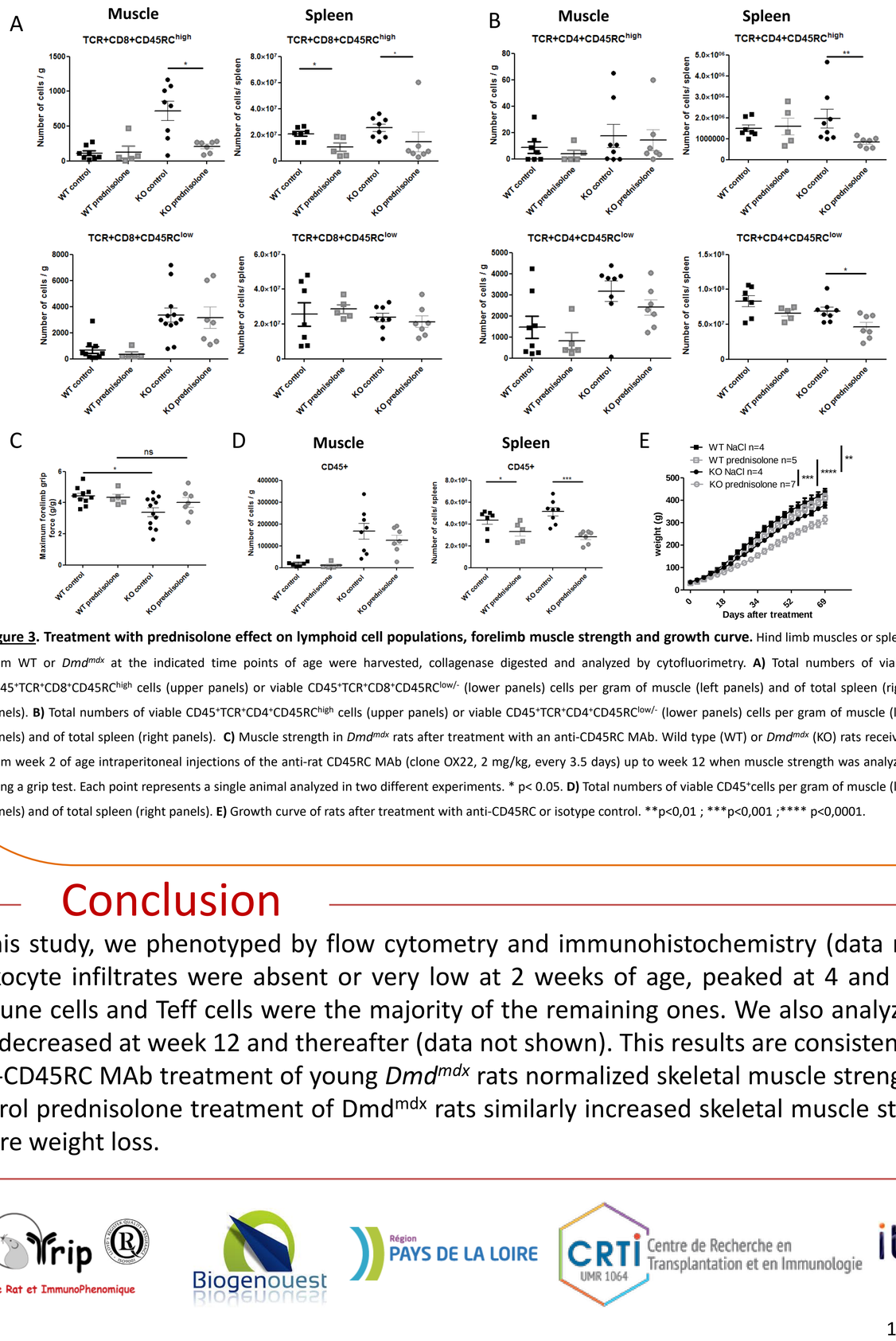

Figure 3. Treatment with prednisolone effect on lymphoid cell populations, forelimb muscle strength and growth curve. Hind limb muscles or spleen from WT or *Dmd*^{mdx} at the indicated time points of age were harvested, collagenase digested and analyzed by cytofluorimetry. **A)** Total numbers of viable CD45$^+$TCR$^+$CD8$^+$CD45RC^{high} cells (upper panels) or viable CD45$^+$TCR$^+$CD8$^+$CD45RC$^{low/-}$ (lower panels) cells per gram of muscle (left panels) and of total spleen (right panels). **B)** Total numbers of viable CD45$^+$TCR$^+$CD4$^+$CD45RC^{high} cells (upper panels) or viable CD45$^+$TCR$^+$CD4$^+$CD45RC$^{low/-}$ (lower panels) cells per gram of muscle (left panels) and of total spleen (right panels). **C)** Muscle strength in *Dmd*^{mdx} rats after treatment with an anti-CD45RC MAb. Wild type (WT) or *Dmd*^{mdx} (KO) rats received from week 2 of age intraperitoneal injections of the anti-rat CD45RC MAb (clone OX22, 2 mg/kg, every 3.5 days) up to week 12 when muscle strength was analyzed using a grip test. Each point represents a single animal analyzed in two different experiments. * p< 0.05. **D)** Total numbers of viable CD45$^+$cells per gram of muscle (left panels) and of total spleen (right panels). **E)** Growth curve of rats after treatment with anti-CD45RC or isotype control. **p<0,01 ; ***p<0,001 ;**** p<0,0001.

Anti-CD45RC treatment

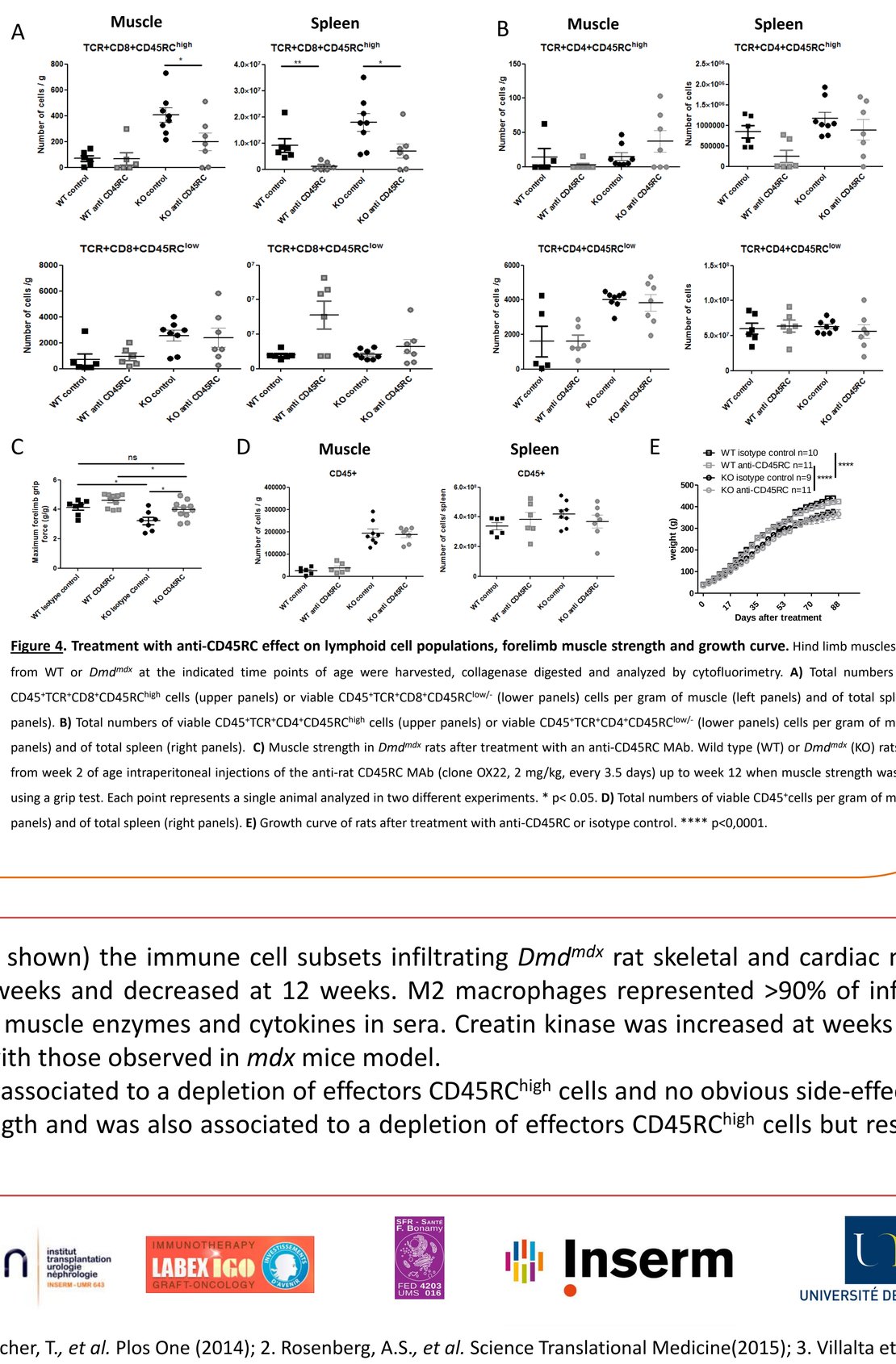

Figure 4. Treatment with anti-CD45RC effect on lymphoid cell populations, forelimb muscle strength and growth curve. Hind limb muscles or spleen from WT or *Dmd*^{mdx} at the indicated time points of age were harvested, collagenase digested and analyzed by cytofluorimetry. **A)** Total numbers of viable CD45$^+$TCR$^+$CD8$^+$CD45RC^{high} cells (upper panels) or viable CD45$^+$TCR$^+$CD8$^+$CD45RC$^{low/-}$ (lower panels) cells per gram of muscle (left panels) and of total spleen (right panels). **B)** Total numbers of viable CD45$^+$TCR$^+$CD4$^+$CD45RC^{high} cells (upper panels) or viable CD45$^+$TCR$^+$CD4$^+$CD45RC$^{low/-}$ (lower panels) cells per gram of muscle (left panels) and of total spleen (right panels). **C)** Muscle strength in *Dmd*^{mdx} rats after treatment with an anti-CD45RC MAb. Wild type (WT) or *Dmd*^{mdx} (KO) rats received from week 2 of age intraperitoneal injections of the anti-rat CD45RC MAb (clone OX22, 2 mg/kg, every 3.5 days) up to week 12 when muscle strength was analyzed using a grip test. Each point represents a single animal analyzed in two different experiments. * p< 0.05. **D)** Total numbers of viable CD45$^+$cells per gram of muscle (left panels) and of total spleen (right panels). **E)** Growth curve of rats after treatment with anti-CD45RC or isotype control. **** p<0,0001.

Conclusion

In this study, we phenotyped by flow cytometry and immunohistochemistry (data not shown) the immune cell subsets infiltrating *Dmd*^{mdx} rat skeletal and cardiac muscles. Leukocyte infiltrates were absent or very low at 2 weeks of age, peaked at 4 and 8 weeks and decreased at 12 weeks. M2 macrophages represented >90% of infiltrating immune cells and Teff cells were the majority of the remaining ones. We also analyzed muscle enzymes and cytokines in sera. Creatin kinase was increased at weeks 4 and 8 and decreased at week 12 and thereafter (data not shown). This results are consistent with those observed in *mdx* mice model.

Anti-CD45RC MAb treatment of young *Dmd*^{mdx} rats normalized skeletal muscle strength associated to a depletion of effectors CD45RC^{high} cells and no obvious side-effects. As a control prednisolone treatment of Dmd^{mdx} rats similarly increased skeletal muscle strength and was also associated to a depletion of effectors CD45RC^{high} cells but resulted in severe weight loss.

1. Larcher, T.*, et al.* Plos One (2014); 2. Rosenberg, A.S.*, et al.* Science Translational Medicine(2015); 3. Villalta et al. JI(2011)