Where immunology meets genetics: A snapshot of immunophenotypes contributing to LAMA2-deficient congenital muscular dystrophy pathophysiology

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The importance of immune cells in skeletal muscle regeneration & diseases

- Skeletal muscle repair and regeneration are highly dependent on the inflammatory responses. Furthermore, understanding the immune response to diseased skeletal muscle, such as in muscular dystrophy, would be essential for the development of therapeutic strategies.
- **LAMA2-deficient Congenital Muscular Dystrophy (LAMA2-CMD)** is an autosomal recessive disorder caused by mutations in the *LAMA2* gene, which encode the laminin-α2, an extracellular protein crucial for skeletal muscle myogenesis and maintenance of healthy muscle. Individuals affected by LAMA2-CMD have low muscle tone (hypotonia) and impaired mobility. There is no treatment available and the immune cell signatures in LAMA2-CMD remains understudied.

Goal: To investigate the immune cell dynamics across disease progression in a LAMA2-CMD mouse model (dy^W/dy^W)

Hypothesis: There is a distinct profile of immune cells and their inflammatory mediators between early vs. advanced disease stages in the dy^W/dy^W mice.

Aim 1: Identify the composition of immune cells that are present in dy^W/dy^W tissues.

Aim 2: Quantify the circulating inflammatory mediators in the dy^W/dy^W mice.

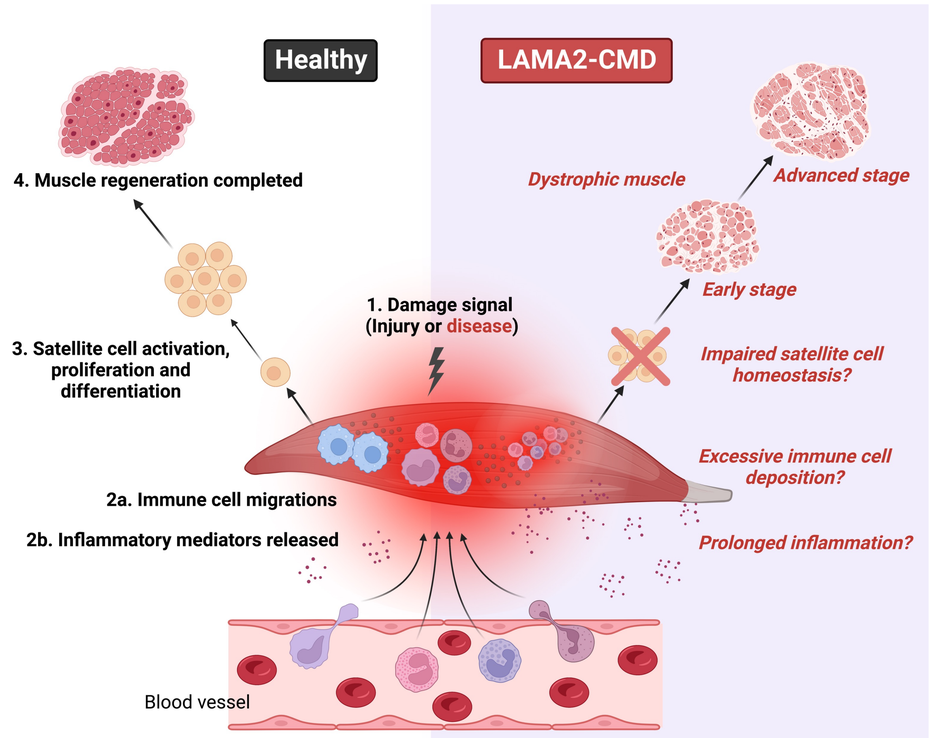
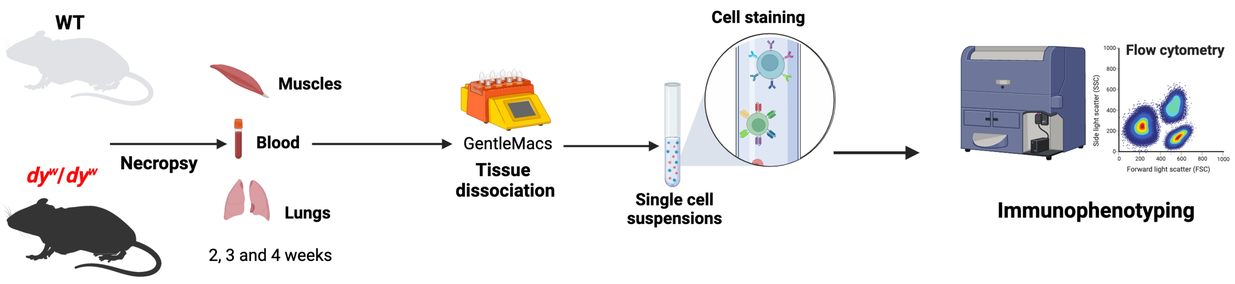


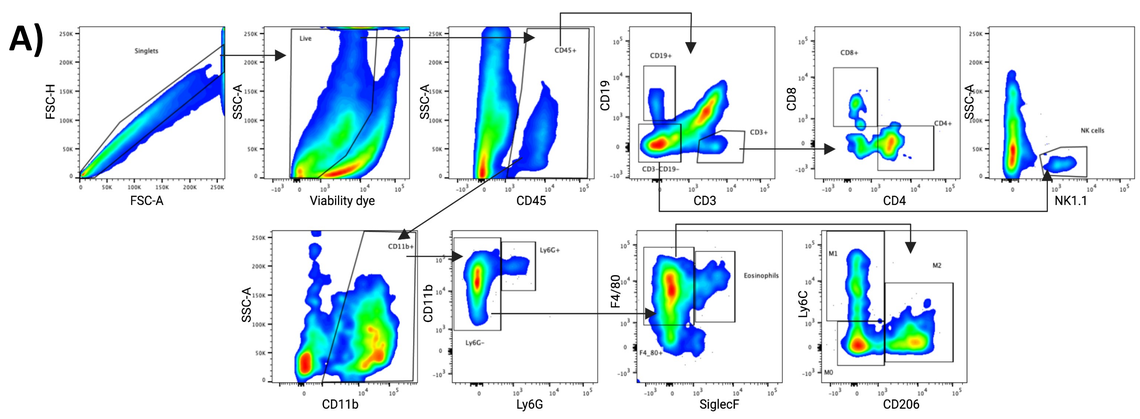
Figure 1: Schematic representing the immune responses in healthy and dystrophic muscles

Indication of an early surge of the majority of immune cells in the dy^W/dy^W skeletal muscles, which are distinct from blood & lungs

Approach



Inflammatory cells in the dy^W/dy^W muscles



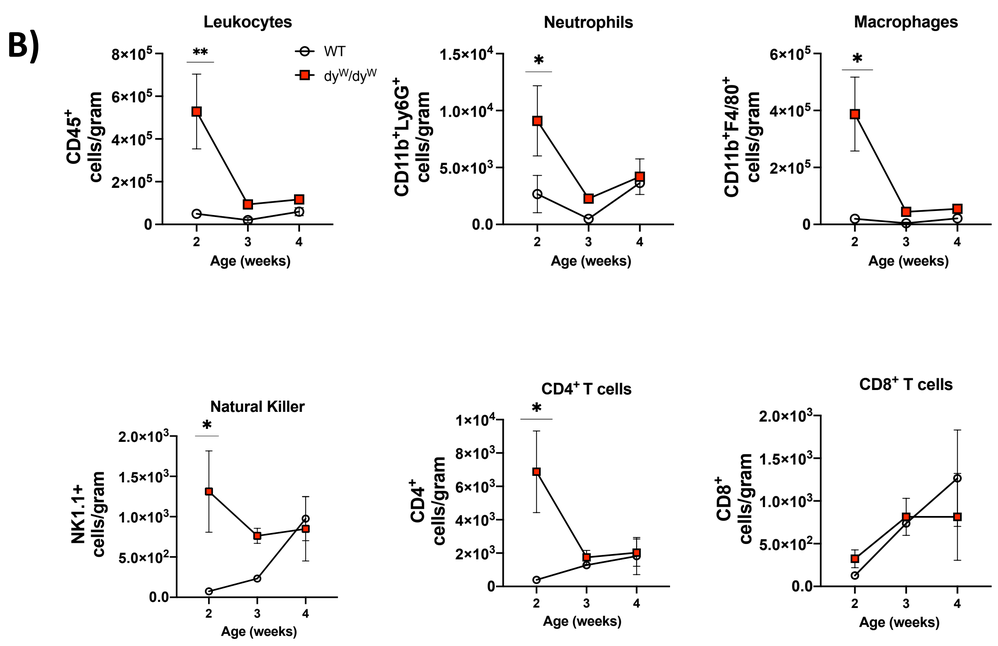


Figure 2: Composition of inflammatory infiltrate in dystrophic muscles. Pools of hindlimb and forelimb muscles (Tibialis anterior, triceps, quadriceps, and gastrocnemius) were analyzed by flow cytometry at different time points. (A) Gate strategy used to identify immune population in the muscles. (B) Absolute cell counts of immune cells at 2 weeks (WT n=4, dy^W/dy^W n=4), 3 weeks (WT n=4, dy^W/dy^W n=3), 4 weeks (WT n=3, dy^W/dy^W n=4). *P<0.05, **P<0.01 by two-way ANOVA Tukey´s multiple comparisons.

Inflammatory cells in the dy^W/dy^W blood

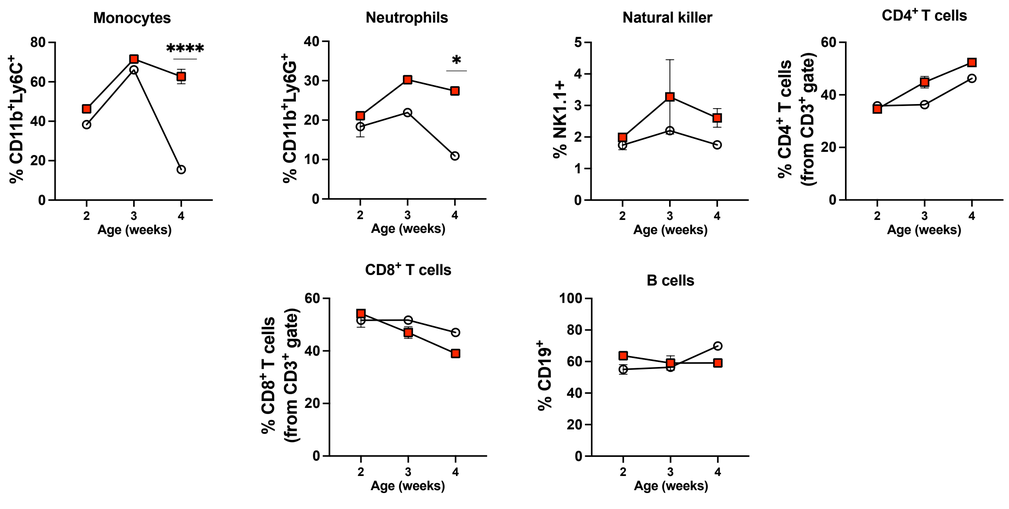
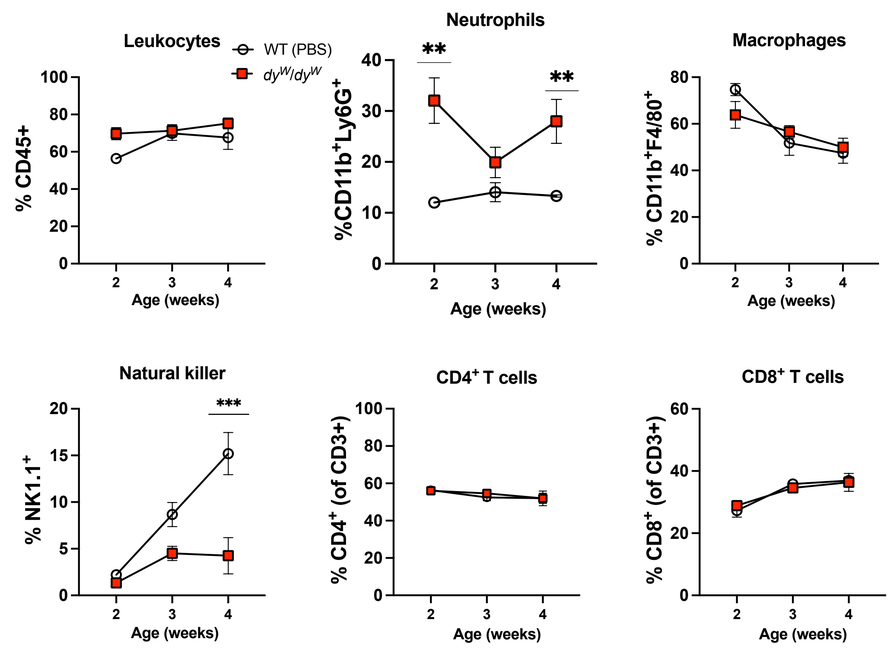


Figure 3: Frequency of Immune cells in the blood. Whole blood from dy^W/dy^W and wild-type mice were stained and analyzed by flow cytometry at 2 weeks (WT n=4, dy^W/dy^W n=2), 3 weeks (WT n=1, dy^W/dy^W n=3), and 4 weeks (WT n=1, dy^W/dy^W n=2).

Inflammatory cells in the dy^W/dy^W lungs



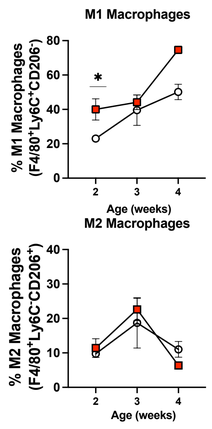
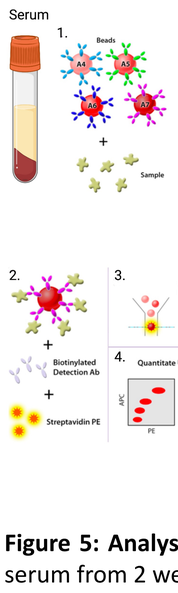


Figure 4: identification of immune cells in lungs. Lungs were analyzed by flow cytometry at 2 weeks (WT n=4, dy^W/dy^W n=4), 3 weeks (WT n=4, dy^W/dy^W n=3),), 4 weeks (WT n=3, dy^W/dy^W n=4). *P<0.05, **P<0.01 by two-way ANOVA Tukey´s multiple comparisons.

Increased circulating cytokines & chemokines in dy^W/dy^W mice: A possible driver of pathophysiology?

Approach



Cytokines in the dy^W/dy^W blood (preliminary)

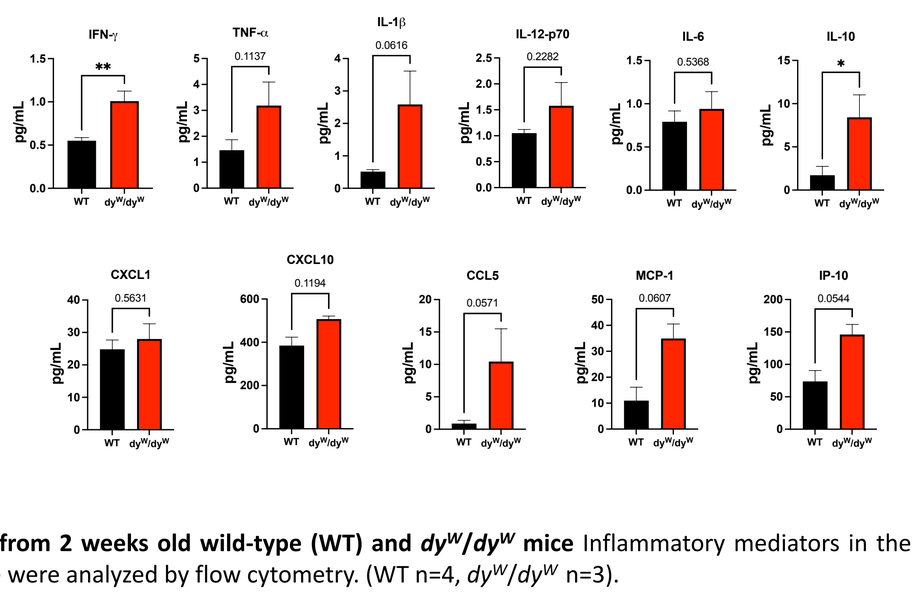


Figure 5: Analysis of serum from 2 weeks old wild-type (WT) and dy^W/dy^W mice Inflammatory mediators in the serum from 2 weeks old mice were analyzed by flow cytometry. (WT n=4, dy^W/dy^W n=3).

Conclusion and future research

- Comprehensive profiling shows the most abundant transient and resident immune cell population in Muscles (M), Lungs (L), and Blood (B) comprising neutrophils (M, L, B), $CD4^+$ (M), NK cells (M, L), macrophages (M), and M1 macrophages (L).
- Inflammatory cells and mediators (cytokines & chemokines) are elevated in 2 weeks old dy^W/dy^W muscles and serum, respectively.

Ongoing work:

- Analyze older/symptomatic dy^W/dy^W mice for their inflammatory cells and mediators.
- Study the role of neutrophils in LAMA2-CMD mice and the impact of modulating neutrophil activity in LAMA2-CMD mice.
- Evaluate the implications of CMD-associated immune profiles on genetic therapy.

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