T_{reg}-ABLATED FOXP3^{DTR} MICE ARE A MODEL OF INFLAMMATORY MYOPATHY AND AUTOIMMUNE MYOCARDITIS

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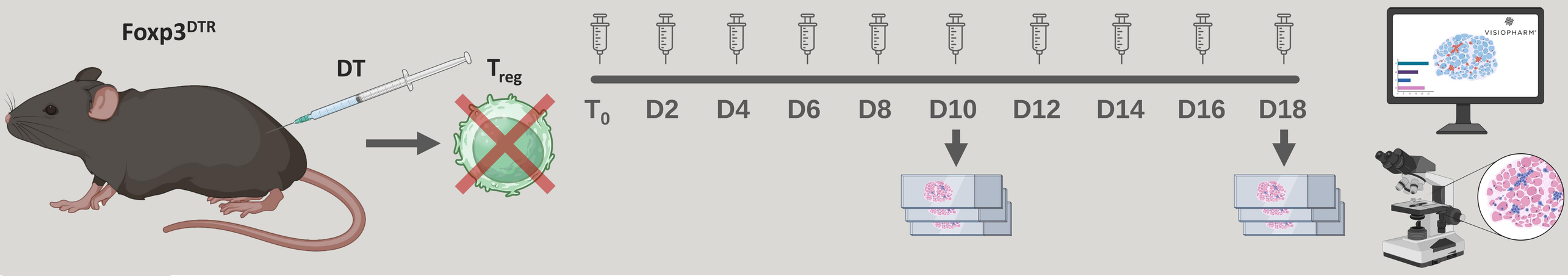
Introduction

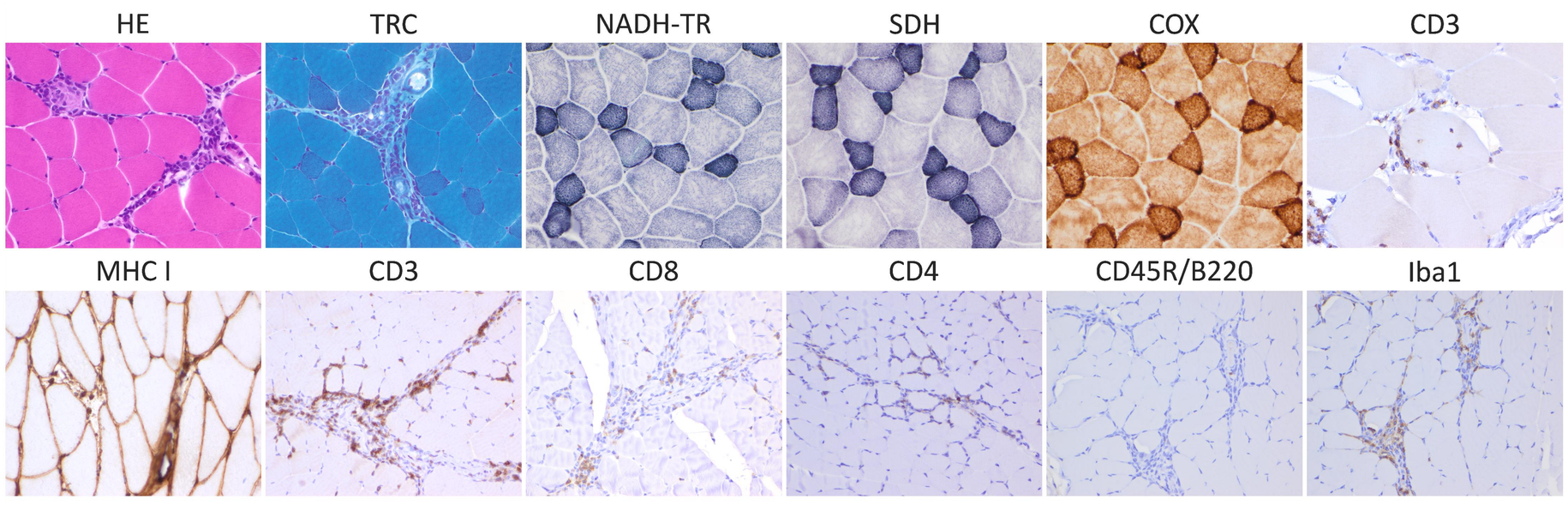
Inflammatory myopathy (IM) stands for a heterogeneous group of acquired immune-mediated disorders in which the skeletal muscle is targeted by the immune system.[1] Since self-reactive T cells play a central role in IM [1] we hypothesised that inhibition of their suppression by regulatory T (T_{reg}) cells could trigger IM. The aim of the project was to establish a mouse model of IM that results from specific loss of T_{reg} cell activity to suppress self-reactive T cells.

Material and Methods

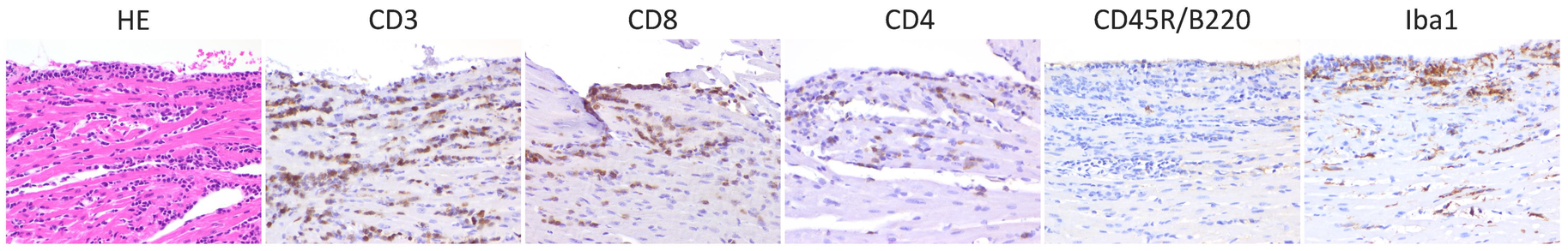
Adult Foxp3^{DTR} mice were depleted of T_{reg} by intraperitoneal injection of diphtheria toxin (DT) every 2 days within up to 18 days. Mice were sacrificed either 10 days (n=5) and 18 days (n=4) of T_{reg} ablation. Control (C57BL/6) mice received the same DT injections. A defined set of skeletal muscles and the heart were sampled and processed for histology. Consecutive sections were stained with haematoxylin and eosin (HE), with a standard panel of stains for skeletal muscle and by immunohistochemistry for T cell subsets, B cells and macrophages, followed by a morphometric analysis.



Results

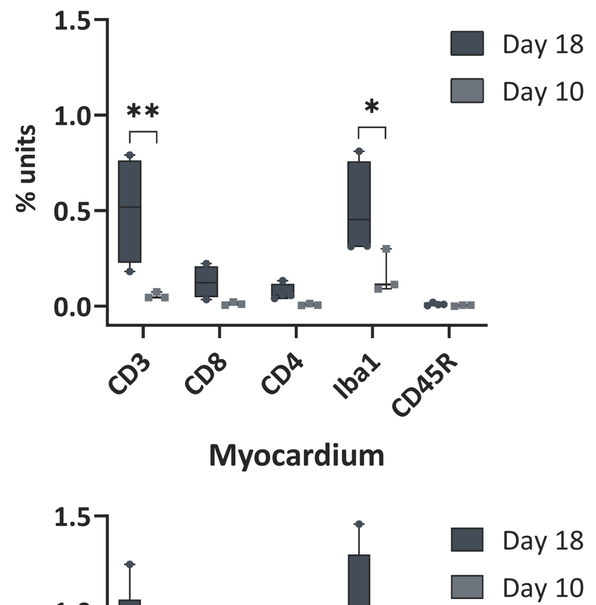


M. quadriceps femoris, T_{reg} depleted Foxp3^{DTR} mice, day 10 and 18. All examined muscles exhibit moderate multifocal endomysial, perimysial and often perivascular mononuclear infiltrates with myonecrosis and myophagocytosis. The infiltrates also arranged around and occasionally invaded unaltered muscle fibres. Oxidative enzyme reactions (NADH-RT, SDH and COX) show disruption of the intermyofibrillar network with irregular patchy negative areas ("moth-eaten" appearance). There is overexpression of the skeletal muscle inflammatory marker MHC I. The inflammatory infiltrate is comprised of CD3-positive T cells (mainly CD8+) and Iba1-positive macrophages, with rare CD45R/B220-positive B cells. Gomori trichrome (TRC), Nicotinamide adenine dinucleotide tetrazolium reductase stain (NADH-TR), Succinate Dehydrogenase (SDH), Cytochrome c oxidase (COX).

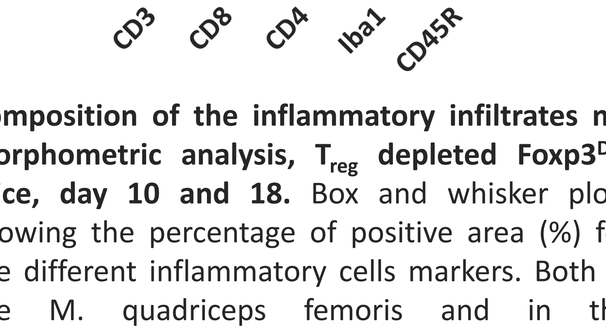


Myocardium, T_{reg} depleted Foxp3^{DTR} mice, day 10 and 18. A concurrent mild mononuclear myocarditis is also observed. The inflammatory infiltrate is comprised of CD3-positive T cells (mainly CD8+) and Iba1-positive macrophages, with rare CD45R/B220-positive B cells.

M. quadriceps femoris



Myocardium



Composition of the inflammatory infiltrates my morphometric analysis, T_{reg} depleted Foxp3^{DTR} mice, day 10 and 18. Box and whisker plots showing the percentage of positive area (%) for the different inflammatory cells markers. Both in the M. quadriceps femoris and in the myocardium; the infiltrate is predominantly composed of T lymphocytes and macrophages with rare B lymphocytes.

Discussion and Conclusions

- The histologic findings are consistent with immune-mediated polymyositis and myocarditis.[1]
- We hypothesize that the IM results from activation of self-reactive T cells due to the lack of suppression of the immune response, normally operated by T_{reg} cells.[2,3]
- Since this model results from the ablation of T_{reg} cells and without immunisation towards a specific muscle protein, it might be useful to study the role of T_{reg} in the spontaneous breakdown of tolerance underlying IM.
- This model will also be of help to study therapeutic effect of in vitro expanded polyclonal T_{reg} and T cells modulators.[4]
- Future research is required to explore whether T_{reg} ablation results in a humoral response with circulating autoantibodies.

References

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