the "Slow Progressor" (3303 ± 6065 pg/ml vs 13300 ± 10308 pg/ml; $p=0.052$) without reaching a significant value, due probably to the low number of cases.

Conclusion: Our study confirms that the presence of specific autoantibodies and capillaroscopic abnormalities correlate to an increased risk of developing SSc in patients with UCTD [6]. Beside we found significantly higher levels of CXCL4 in our 27 VEDOSS patients respect to controls, in agreement with other authors showing the association of this chemokine with early stages and specific organ involvement [4][5][7]. The finding of CXCL4 lower levels in "fast progressor" cases is consistent with our recent report of anti-CXCL4 antibodies in patients with early SSc, determining lower levels of this antigen [7]. We need deeper investigations to better evaluate the role of CXCL4 in the different stages of SSc.

REFERENCES:

- [1] J. Avouac et al., Ann. Rheum. Dis., 2011.
- [2] F. van den Hoogen et al., Ann. Rheum. Dis., 2013.
- [3] G. Valentini et al., Clin. Exp. Med., 2017.
- [4] R. Lande et al., Nat. Commun., 2019.
- [5] L. van Bon et al., N. Engl. J. Med., 2014.
- [6] M. Vasile et al., Clin. Exp. Rheumatol., 2018.
- [7] R. Lande et al., Int. J. Mol. Sci., 2020.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2188

OP0247

IN MYOSITIS MUSCLE FIBRE PLAYS A DIRECT AND CRITICAL ROLE IN THERAPEUTIC RESPONSE TO GLUCOCORTICIDS

M. Giannini¹, G. Laverny², D. Duteil², D. Rovito², A. L. Charles³, B. Geny⁴, D. Metzger², A. Meyer⁵. ¹University of Strasbourg, Service de Physiologie et d'explorations fonctionnelles, Nouvel Hôpital Civil, Centre de Référence des Maladies Autoimmunes Rares, Fédération de Médecine Translationnelle, UR3072, Strasbourg, Strasbourg, France; ²University of Strasbourg, CNRS UMR7104, INSERM U1258, IGBMC, F-67400 Illkirch, Illkirch, France; ³University of Strasbourg, Fédération de Médecine translationnelle, UR3072, Strasbourg, Strasbourg, France; ⁴University of Strasbourg, Service de Physiologie et d'explorations fonctionnelles, Nouvel Hôpital Civil, Fédération de Médecine Translationnelle, UR3072, Strasbourg, Strasbourg, France; ⁵University of Strasbourg, Service de Physiologie et d'explorations fonctionnelles, Nouvel Hôpital Civil, Centre de Référence des Maladies Autoimmunes Rares, Fédération de Médecine Translationnelle, UR3072, Strasbourg, Strasbourg, France

Background: Myositis are rare autoimmune diseases, affecting more women than men, characterized by chronic inflammation of skeletal muscle causing muscle weakness, decreased quality of life and increased mortality.

Glucocorticoids (GC) are potent anti-inflammatory drugs, and are the first line treatment of myositis. They improve muscle strength of myositis patients (therapeutic effect), yet muscle recovery is generally only partial. Moreover, GC have an iatrogenic effect on skeletal muscle fibre leading to steroid myopathy. Thus myositis care has to be improved. Despite the autoimmune terrain of myositis, our team has recently shown that muscle fibres themselves develop immuno-metabolic modifications that participate to muscle weakness and perpetuation of the disease¹. GC effects are mediated by the glucocorticoid receptor (GR), which is expressed in various cell types including immune cells and myofibres, but the cells mediating therapeutic responses remain to be determined.

Objectives: Unravel the mechanisms underlying the therapeutic effect of GC in myositis, particularly elucidate the role of skeletal muscle fibres.

Methods: Experimental myositis was induced in eight to ten week-old C57BL/6J female mice by a single intradermal injection of part of skeletal muscle fast-type C protein along with Freund's adjuvant and an intraperitoneal (IP) injection of pertussis toxin, as previously described². 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 myofibre, 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 fibre (HSA-CreERT2/GR L2/L2). Tamoxifen (1 mg/day for 5 days by IP injection) was administered 9 days after immunization to induce GR ablation selectively in skeletal muscle fibres (GR(i)skm^{-/-} mice). Similar treatments were applied to GR L2/L2 that do not express Cre-ERT2, and served as controls.

We compared 4 groups of myositis mice, GR L2/L2 treated by PDN (n=9) or vehicle (n=9) and GR(i)skm^{-/-} treated by PDN (n=10) or vehicle (n=10), by grip test and at the histological level (hematoxylin-eosin (HE) and Gomori trichrome (GT) staining). Moreover, LC3 expression was studied by RTqPCR and western blot.

Results: Muscle strength was decreased in both GR L2/L2 and GR(i)skm^{-/-} myositis mice from D 14 to D 20. GR L2/L2 myositis mice recovered muscle strength after PDN treatment; no significant difference compared to D 0 was detected. In contrast, PDN did not improve muscle strength in GR(i)skm^{-/-} myositis mice (Figure 1).

HE and GT staining did not reveal quantitative differences in inflammatory infiltrate. Necrotic and degenerative fibres were detected in the 4 groups. At RTqPCR, LC3, an autophagy marker, was upregulated in PDN-treated GR L2/L2 myositis mice compared to untreated GR L2/L2 myositis mice; moreover it was 2-fold more expressed in PDN-treated GR L2/L2 myositis mice compared to PDN-treated GR(i)skm^{-/-} mice.

Conclusion: GR in skeletal muscle fibre is crucial to mediate the therapeutic response to GC in a murine model of myositis. Autophagy is one of the candidate pathways controlled by myofibre GR underlying this effect.

REFERENCES:

- [1] Meyer A et al. IFN- β -induced reactive oxygen species and mitochondrial damage contribute to muscle impairment and inflammation maintenance in

Figure 1. Grip test analysis in GR L2/L2 and GR(i) skm^{-/-} myositis mice. ns, not significant; * $p<0.01$; ** $p<0.001$; *** $p<0.0001$

dermatomyositis. Acta Neuropathol. 2017 Oct;134(4):655-666.

- [2] Sugihara T et al. A new murine model to define the critical pathologic and therapeutic mediators of polymyositis. Arthritis Rheum. 2007 Apr;56(4):1304-14.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2863

OP0248

DEVELOPMENT OF A 3D SKIN MICROTISSUE MODEL FOR FIBROTIC DISEASES

E. Pachera¹, G. Kania¹, A. Juengel^{1,2}, M. Calcagni³, O. Distler^{1,2}. ¹Department of Rheumatology, Center of Experimental Rheumatology, University Hospital Zurich, University of Zurich, Zurich, Switzerland; ²Department of Physical Medicine and Rheumatology, Balgrist University Hospital, University of Zurich, Zurich, Switzerland; ³Department of Hand Surgery, Balgrist University Hospital, University of Zurich, Zurich, Switzerland

Background: Traditional preclinical approaches, such as two-dimensional cell culture and animal models, are often inadequate to mimic the pathophysiological features of complex diseases such as systemic sclerosis (SSc). Human specific targets, such as the recently described pro-fibrotic long non coding RNA (lncRNA) H19X¹, are becoming increasingly relevant in preclinical research, creating the need of new strategies and tools in translational medicine. The employment of novel three-dimensional (3D) culture systems, where multiple cell types are included, is filling an important gap left by the traditional preclinical methods.

Objectives: To develop an easy to produce 3D fibrotic skin microtissues model for translational proof of concept studies.

Methods: Two thousand five hundred dermal fibroblasts isolated from skin of SSc patients were seeded in ultra-low attachment 96-well plates. Fibroblast were let to aggregate into spheres for 48h. Two thousand five hundred primary normal human keratinocytes were added to the culture and let to layer onto the fibroblast spheres for 72h. H19X silencing experiments were used as proof of concept studies. H19X silencing with antisense oligonucleotides or transfections with a scrambled control were performed in fibroblasts prior to the sphere formation for 24h. TGF β (10 ng/ml) was added to microtissue to exacerbate the fibrotic phenotype. Haematoxylin eosin staining as well as immunohistochemistry staining for vimentin and cytokeratin 10 was performed. Skin microtissues were processed for RNA and protein isolation. Pro-collagen I α 1 and fibronectin were quantified in the supernatants with ELISA.