Introduction Systemic sclerosis (SSc) is a rare intractable autoimmune disease, with unmet medical need. Cell therapy using mesenchymal stem cells (MSC) is a promising approach, and we recently reported its efficacy in a murine model of SSc induced by hypochlorite (HOCI). Since MSC act primarily through the secretion of soluble factors released within extracellular vesicles (EV), the use of EV instead of cells seems an attractive alternative. Herein, we compared the effects of two types of EV, exosomes and microparticles, in HOCI-induced SSc.

Objectives Herein, we compared the effects of two types of EV, exosomes and microparticles, in HOCI-induced SSc.

Methods BALB/c mice were challenged with daily intradermal HOCI injections during 6 weeks to induce SSc. Each group was treated at mid-experiment with infusions of 2.5×10⁵ murine MSC, 250 ng of exosomes or microparticles isolated from IFNγ-activated or non-activated (NA) MSC. We measured skin thickness every week. At euthanasia (d42), we analysed the effects of remodelling parameters in skin and lung tissues. The observed effects were similar to those obtained with MSC. No difference was noted between NA and IFNγ-activated EV.

Results Mice treated with each subtype of EV displayed lower clinical scores, less histological lesions, lower expression of fibrotic and inflammatory markers, with enhanced expression of remodelling parameters in skin and lung tissues. The observed effects were similar to those obtained with MSC. No difference was noted between NA and IFNγ-activated EV.

Conclusions MSC-derived EV display potent antifibrotic properties in murine SSc. This new acellular therapy represents a promising approach in this disease.

Disclosure of interest None declared

**Abstracts**

**0022** FOR EACH HLA-DRB1 GENOTYPE, THE LIKELIHOOD TO DEVELOP RA CORRELATES WITH THE PROBABILITY OF BINDING AT LEAST A PEPTIDE FROM PAD4

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**Introduction** The production of IgG ACPA can be helped by T lymphocytes specific for PADs, the enzymes that transform argin in citrullin. Thus, the molecular basis for the HLA-DRB1 association with RA might be the capability for the two alleles encoded by a given HLA-DRB1 genotype to bind PAD4 derived peptides. We recently published a table showing that the relative risk to develop ACPA positive RA for the 106 most common genotypes. French HLA-DRB1 genotypes vary from 28 to 0.2. For a given HLA-DRB1 genotype, the risk to develop RA should be correlated with the probability for the two HLA-DRB1 molecules encoded by this genotype to bind peptides from PAD4 peptides.

**Objectives** To test whether the risk of developing ACPA positive RA for each HLA-DRB1 genotypes correlates with the probability for the 2 HLA-DR alleles encoded by each genotype to bind at least one PAD4 peptide.

**Methods** 65 Synthetic peptides (20 mers) of human PAD4 and 167 peptides encompassing the A and B chains of human fibrinogen were synthesised in solid phase. Whenever there was an arginine residue, both the arginine and the citrullin variant were synthesised. In the end, we had 25 fibrinogen peptides containing neither arginin nor citrulline, 71 citrullinated and 71 arginine peptides from fibrinogen.

**Results** HLA-DRB1 peptide binding studies were performed by adding one microgram of purified HLA-DRB1 to ELISA wells coated with 10 micrograms PAD peptide. Bound HLA-DR was revealed by biotinylated anti HLA-DR antibody followed by peroxidase conjugated avidin.

**Statistical analyses** Correlation between HLA-DRB1 genotypic risk for RA and Likelihood to bind PAD4 for a given genotype was evaluated by Spearman’s.

**Conclusions** HLA-DRB1 genotypes are associated with a risk to develop RA and a likelihood to bind at least one of 65 overlapping PAD4 peptides. The strong correlation between these two parameters suggest that PAD4 peptide binding to HLA-DRB1 may be the basis of the HLA-DRB1 RA association. Such correlation is not observed when testing the binding of citrullinated or native peptides from Fibrinogen to HLA-DRB1 molecules.
RARE SERONEGATIVE DESTRUCTIVE RA: IDENTIFICATION OF SOMATIC MUTATIONS IN THE EXPANDED CD8+ LYMPHOCYTES

Introduction Clonally expanded CD8+ lymphocytes harbour somatic mutations in RA patients. Seronegative RA, that often displays milder symptoms and slower disease progression, does not share the genetic linkage to the MHC II locus. Thus, we hypothesise that somatic mutations in the clonal CD8+ lymphocytes could modulate inflammation in these patients.

Objectives Our aim was to validate the concept of somatic mutations as regulators of chronic inflammation.

Methods We collected blood samples from seronegative RA patients (n=8) who displayed unusually aggressive, progressive disease that was refractory to treatment and had led to severe joint erosions. Flow cytometry was used to screen lymphocyte clonality in both CD4 and CD8 lymphocytes and to enrich the mutated clone. TCR-repertoire analysis was performed by TCRB-deep sequencing. Somatic variants were called from deep sequencing data (panel of 1000 immunological genes and exome sequencing) and the data was confirmed by capillary sequencing.

Results Similarly as in our earlier work, there were more suggestive clonal expansions in CD8+ lymphocytes than in CD4+ lymphocytes. The overall clonality of CD8+ cells did not statistically differ between our seronegative destructive RA cohort and age-matched healthy controls (n=19), seropositive (n=53) or seronegative diagnostic phase patients (n=12).

Sequencing of one of the seronegative RA patients' CD4 and CD8 T cells with the targeted gene panel, revealed novel somatic mutations in the CD8+ cell pool. The expanded T cell clone was enriched using flow cytometry and from this clone, 22 mutations were identified in genes that are expressed in healthy CD8+ cells. Eight of these mutations were selected and confirmed using capillary sequencing. Based on computational prediction algorithms, we hypothesise that the identified mutations (in PIK3CG, KPNA1, PLCG2, ITGAE, NFKAP genes) may regulate the clonal properties, immunological phenotype and even contribute to the regulation of chronic inflammation.

Conclusions We identified somatic mutations in the clonally expanded CD8 lymphocytes from a patient with unusual, aggressive seronegative RA. More work is warranted to elucidate the biological and clinical consequences of the identified mutations.

Disclosure of interest None declared