support from: Bayer, O. Distler Grant/research support from: Actelion, Bayer, BiogenIdec, Boehringer Ingelheim, ChemomAb, espeRare foundation, Genentech/Roche, GSK, Inventiva, Lilly, medac, MedImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Sinova and UCB, 2, Actelion, Bayer, BiogenIdec, Boehringer Ingelheim, ChemomAb, espeRare foundation, Genentech/Roche, GSK, Inventiva, Lilly, medac, MedImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Sinova and UCB

0021

MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES: A NOVEL THERAPEUTIC OPTION IN SYSTEMIC SCLEROSIS

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10.1136/annrheumdis-2018-EWRR2018.21

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 (HOCl). 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 HOCl-induced SSc.

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

Methods BALB/c mice were challenged with daily intradermal HOCl injections during 6 weeks to induce SSc. Each group was treated at mid-experiment with infusions of 2.5×10^5 murine MSC, 250 ng of exosomes or microparticles isolated from IFN γ -activated or non-activated (NA) MSC. We measured skin thickness every week. At euthanasia (d42), we analysed the expression of fibrotic and inflammatory markers (collagens 1 and 3, α Sma, TGF β , MMP 1 and 9, TIMP1, IL1 β , IL6, TNF α) in lungs and skin samples using RT-qPCR.

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 IFN γ -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

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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10.1136/annrheumdis-2018-EWRR2018.22

Introduction The production of IgG ACPA can be helped by T lymphocytes specific for PADs, the enzymes that transform arginin into 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.¹ We recently published a table showing that the relative risk to develop ACPA positive RA for the 106 most common genotypes.² French HLA-DRB1 genotypes varies 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 PAD 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.

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

Results HLA-DRB1 genotypic risks to develop RA correlate with likelihood to bind PAD4 peptides ($p=0.06$, Pearson's), not citrullinated Fibrinogen peptides ($p>0.6$ and $p>0.9$).

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.

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Disclosure of interest None declared

0023

RARE SERONEGATIVE DESTRUCTIVE RA: IDENTIFICATION OF SOMATIC MUTATIONS IN THE EXPANDED CD8+ LYMPHOCYTES

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10.1136/annrheumdis-2018-EWRR2018.23

Introduction Clonally expanded CD8 +lymphocytes harbour somatic mutations in RA patients.¹ 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.² 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*, *NKAP* 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.

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Disclosure of interest None declared

0024

HUMAN MEMORY TH17 CELL POPULATIONS CHANGE INTO ANTI-INFLAMMATORY CELLS WITH REGULATORY CAPACITY UPON EXPOSURE TO ACTIVE VITAMIN D

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10.1136/annrheumdis-2018-EWRR2018.24

Introduction In autoimmune diseases such as rheumatoid arthritis (RA), an important therapeutic goal is to normalise the imbalance between pro- and anti-inflammatory cells. This imbalance is illustrated by elevated levels and activity of memory CCR6 +Th17 cell populations, such as Th17 and Th17.1 cells, in RA patients compared to healthy controls. Therefore, modulating these cells to become anti-inflammatory could contribute to restoring the immunological balance.

Objectives The active vitamin D metabolite 1,25(OH)₂D₃ is known to inhibit pro-inflammatory cytokine production by CCR6 +Th memory cells. Therefore, we investigated whether 1,25(OH)₂D₃ can induce an anti-inflammatory phenotype in these memory T cells.

Methods CCR6 +Th memory cells, excluding Tregs, were sorted from treatment-naïve early RA patients or healthy controls and cultured with or without 1,25(OH)₂D₃. Effects were analysed using microarray, RT-PCR, ELISA or flow cytometry. Functional properties were assessed via suppression and chemotaxis assays.

Results 1,25(OH)₂D₃ inhibits pro-inflammatory cytokines such as IL-17A, IL-17F and IL-22 in CCR6 +Th memory cells from both healthy controls and RA patients. This is accompanied by induction of anti-inflammatory factors, including IL-10 and CTLA4. Interestingly, these formerly pathogenic cells suppress proliferation of autologous CD3 +T cells, similar to classical Tregs. Importantly, the modulated memory cells still migrate towards the site of inflammation, modelled by synovial fluid, and retain their suppressive capacity in this environment.

Conclusions Committed pro-inflammatory IL-17A-producing CCR6 +Th memory cells can become anti-inflammatory cells with functional regulatory capacities upon exposure to active vitamin D. This process can contribute to restoring the immunological balance and inhibiting synovial inflammation in RA.

Disclosure of interest None declared

0025

UNOPPOSED INTERLEUKIN 18 SIGNALLING LEADS TO SEVERE TOLL LIKE RECEPTOR 9-INDUCED MACROPHAGE ACTIVATION SYNDROME IN MICE

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10.1136/annrheumdis-2018-EWRR2018.25