SATURDAY, 17 JUNE 2017

Innate immunity in rheumatic diseases

SAT0001 LACK OF OBESITY-RELATED FEATURES IN ADIPOCYTES AND INFLAMMATORY CELLS IN THE INFRAPATELLAR FAT PAD (IFP)

A. Ioan-Facsinay[1], A. de Jong[1], I. Klein-Wieringa[1], S. Andersen[1], J. Kwekkeboom[1], L. Herb-van Toorn[1], B. de Lange-Brokaar[1], D. van Delft[2], J. Garcia[3], W. Wei[3], H. van der Heide[2], Y. Bastiaansen-Jenniskens[3], G. van Osch[3], A.-M. Zuurmond[4], V. Stojanovic-Susulic[5], R. Nelissen[2], R. Toes[2], M. Kloppenburg[2]. *[1]Rheumatology; [2]Leiden University Medical Center, Leiden; [3]Erasmus MC, University Medical Center, Rotterdam; [4]TNO, Leiden, Netherlands; [5]Janssen, Pharmaceutical Companies Johnson & Johnson, Springhouse, United States*

Background: Obesity is associated with the development and progression of osteoarthritis (OA), both for weight-bearing and non-weight bearing joints. Several lines of research indicate that obesity-related systemic factors, such as adipose tissue-derived factors, could be involved in this association. The infrapatellar fat pad (IFP) is an adipose tissue depot localized in the knee joint. and could mediate obesity-associated effects. However, it is currently unknown whether and how obesity affects IFP.
Objectives: To investigate the presence of obesity-related features in adipocytes and infiltrating immune cells in the IFP of OA patients.
Methods: Knee OA patients (N=155: 68% women, mean age 65 years, mean (SD) BMI 29.9 kg/m^2 (5.7)) were recruited: IFP volume was determined by MRI in 79 knee OA patients, while IFP and subcutaneous adipose tissue (SCAT) were obtained from 106 patients undergoing arthroplasty. Crown-like structures (CLS) were determined using immunohistochemistry. Adipocyte size was determined by light microscopy and histology. Stromal vascular fraction (SVF) cells were characterized by flow cytometry.
Results: IFP volume (mean (SD) 23.6 (5.4) mm^3) was associated with height, but not with BMI or other obesity-related features such as waist circumference, fat percentage and waist to hip ratio. The volume of IFP adipocytes did not correlate with BMI, in contrast to SCAT adipocytes. Few CLS were observed in IFP and their number did not differ between individuals with high and low BMI. Moreover, high BMI was not associated with higher infiltrating immune cell numbers in IFP, nor with changes in immune cell populations. Likewise, no molecular differences were observed in FCM-secreted factors between high and low BMI, except for an increased TNFa secretion in obesity. Since obesity is usually associated with a shift towards pro-inflammatory macrophages in conventional adipose tissue, we have extensively characterized IFP macrophages. Surprisingly, CD206 and CD163, usually associated with an anti-inflammatory phenotype were the most abundantly expressed surface markers on macrophages (81% and 41% respectively). In contrast, cytokine profiles revealed a pro-inflammatory phenotype of the total macrophage population, with cells producing predominantly IL-6 and TNFα, but little IL-10. Interestingly, the CD163+ macrophages were bigger and had a more activated and pro-inflammatory phenotype than their CD163- counterparts. However, no association with BMI could be observed for different macrophage populations or their cytokines.
Conclusions: BMI-related features usually observed in SCAT and visceral adipose tissue could not be detected in IFP of OA patients, a fat depot implicated in OA pathogenesis.
Disclosure of Interest: None declared
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SAT0002 INNATE LYMPHOID CELLS (ILCS) ARE DIFFERENTIALLY DISTRIBUTED IN INFLAMMATORY AND NON-INFLAMMATORY JOINT DISEASES

I. Meunier[1], I. Marois[2], M. Richter[3], G. Boire[4]. *[1]Meakins-Christie Laboratories, McGill University, Montréal; [2]Université de Sherbrooke, Sherbrooke, Canada; [3]Medicine; [4]Medicine, Division of Rheumatology, Université de Sherbrooke, Sherbrooke, Canada*

Background: Innate lymphoid cells (ILC) are immune cells of the lymphoid lineage not expressing specific antigen receptors.They are classified into three subsets: *ILC1* (including Natural Killer (NK) cells and ILC1) secrete IFN-γ and TNF-α; *ILC2* secrete IL-4, IL-5, IL-9, IL-13, and amphiregulin; *ILC3* (including Lymphoid Tissue Inducer (LTi) and NK cell activating receptor (NCR+ ILC3) secrete IL-17A and IL-22.
Objectives: To enumerate the different subsets of ILCs in peripheral blood and synovial fluids of patients with inflammatory and non-inflammatory joint effusions.
Methods: Patients with confirmed synovial effusion presenting at the Centre hospitalier universitaire de Sherbrooke (CHUS) signed an informed consent form. Cells from joint effusions were separated by Ficoll-density gradient centrifugation, stained for ILC, and analysed using a BD FACS Aria III flow cytometer. For cytokine detection, cells were stimulated for 6h with PMA/ionomycin prior to cell staining. The distribution of ILC subtypes according to various diagnoses is presented in cells/ml and ratios of ILC subtypes per ml of synovial fluid relative to peripheral blood. The protocol was approved by the CRC-CHUS ethics committee.
Results: Synovial fluids and blood from 57 patients with various diagnoses were analyzed. The highest concentrations (cells/mL) of ILC cell subtypes found in the synovial fluids/peripheral blood were: ILC1: 7/4; ILC2: 70/3; LTi: 4x10^4 (in RA)/8x10^4 (in OA); NCR+ ILC3: 1.3x10^5 (in JIA)/5.8x10^4; NK: 5/1.
Synovial fluids relative to peripheral blood frequently presented ratios of ILC cell subtypes $\leq$1, suggesting little preferential homing in the joints. However, synovial fluids (relative to blood) were enriched 45 and 7 times for NCR+ ILC3 in Juvenile Idiopathic Arthritis (JIA) and spondylarthropathy patients, respectively, and 8 and 6 times for LTi in Psoriatic Arthritis and Rheumatoid Arthritis (RA) patients, respectively. We observed marked heterogeneity in ILC numbers within patients with the same inflammatory joint diseases. Part of this heterogeneity was associated with the presence of concomitant joint degenerative disease and low cell numbers in the synovial fluids.
Conclusions: 1. LTi and NCR+ ILC3 subtypes are the ILC most abundant in synovial fluids.
ILC1 and NK cells are rare in synovial fluids and unlikely to be involved in pathogenesis; ILC2 remain infrequent, even when enriched in synovial fluid relative to peripheral blood (e.g. in JIA, RA and gout).
2. Relative to their concentrations in peripheral blood, LTi and NCR+ ILC3 subtypes are markedly enriched in synovial fluids of patients with autoimmune-mediated diseases, notably LTi in RA and Psoriatic Arthritis, and NCR+ ILC3 in spondylarthropathy and JIA. The abundance of these IL-17 secreting cells in synovial fluids from these diseases is especially intriguing.
3. We observed significant heterogeneity within patients with the same clinical diagnosis.
4. The pathophysiological implications of the differential distribution of subtypes of ILC cells across diseases and within clinical diagnoses remain unclear.
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SAT0003 SINGLE-NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH P2X7R FUNCTION REGULATE THE ONSET OF GOUTY ARTHRITIS

J. Tao, M. Cheng. *Department of Rheumatology & Immunology, Anhui Provincial Hospital Affiliated to Anhui Medical University, hefei, China*

Background: Gout is an inflammatory disease, considered to be caused by the increased production of IL-1β stimulated by monosodium urate (MSU) crystals. However, some hyperuricemia patients, even gouty patients with tophi in the joints, never have gout attack, indicating some other pathogenic pathways participating in the secretion of IL-1β rather than MSU in the pathogenesis of acute gouty arthritis. ATP-P2X7R-IL-1β axis may be one of them.
Objectives: The purpose of this study is to explore the role of ATP in the pathogenesis of gout, and the association between ATP receptor (P2X7R) function associated single nucleotide polymorphisms and gout arthritis.
Methods: The non-synonymous SNPs loci of P2X7R in Chinese people were screened, to compare the frequencies of different alleles and genotype distribution of selective SNPs in 117 gouty patients and 95 hyperuricemia patients. Then peripheral white blood cells were purified from the peripheral blood of randomly selected 43 gout patients and 36 hyperuricemia patients from the total. After culturing the cells with MSU or MSU+ATP, supernatants were collected and the concentrations of IL-1β were detected by enzyme linked immunosorbent assay (ELISA).
Results: 1. Eight SNPs loci including rs1653624, rs10160951, rs1718119, rs7958316, rs1621388, rs16950860, rs208294, rs17525809 and rs2230912 were screened and detected, and rs1653624, rs7958316 and rs17525809 were demonstrated associated with gout arthritis. 2. After the stimulation with MSU+ATP, the concentrations of IL-1β in supernatants of gouty patients were significantly higher than that in hyperuricemia groups [(131.08±176.11)pg/ml vs (50.84±86.10)pg/ml]. Furthermore, gouty patients carrying susceptibility genotype AA or AT of rs1653624 had significant higher concentration than that carrying non-susceptibility genotype TT [(104.20±164.25)pg/ml vs (21.90±12.14)pg/ml]. However, no differences were found while stimulated with MSU alone.
Conclusions: ATP promotes the pathogenesis of gouty arthritis by increasing the secretion of IL-1 β, and its receptor (2X7R) function associated single nucleotide polymorphisms may be related to gouty arthritis, which indicates that ATP-P2X7R signaling pathway plays a significant regulatory role in the pathogenesis of gout.
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SAT0004 NEW AUTOANTIGEN (JKTBP) PART OF STRESS GRANULES CLOSES THE SENSITIVITY GAP IN RHEUMATOID ARTHRITIS

B. Marklein, M. Jenning, K. Muenzer, G. Burmester, K. Skriner. *Department of Rheumatology and Clinical Immunology, Charité University Medicine, Berlin, Germany*

Background: Rheumatoid arthritis triggers the formation of prion-like stress granules. To investigate which members of the heterogeneous nuclear ribonucleoprotein (hnRNP)-family, components of functionally important subcellular

particles are targeted by autoantibodies from RA and other systemic rheumatic diseases.

Methods: Using a protein macroarray we identified JKTBP in humans and animal models of inflammatory rheumatic diseases. Bacterially expressed recombinant JKDBP proteins were used to confirm the obtained data. Epitope, TLR7/9 and MyD88 dependency was determined by ELISA. JKTBP expression in cultivated cells and synovial tissue was analysed by indirect immunofluorescence, immunoblot and immunohistochemistry.

Results: Anti-JKTBP autoantibodies were detected in 46% of the patients with systemic lupus erythematosus (n=103), in 20–30% of the patients with rheumatoid arthritis (n=286), in 10% of the patients with mixed connective tissue disease (n=20) or spondyloathropathy (n=20), and in <10% of patients with other autoimmune disorders (n=382). Sera positive to JKTBP as well as hnRNP-B1, revealed nearly two thirds of the RF IgM/ CCP2-seronegative patients as early RA patients. Combining sensitivities to all autoantigens tested (JKTBP, AUF1, hnRNP-B1), it was possible to identify 92% of the early RA patients (n=91). In the MRL/lpr mouse model of SLE, mice deficient of MyD88 or TLR7/9 lacked anti-JKTBP autoantibodies, whereas mice deficient of SIGIRR/TIR8 showed enhanced anti-JKTBP autoantibody production. These results show that autoantibody generation against JKTBP, AUF1, hnRNP-B1 is dependent on TLR 7 and TLR9 like rheumatoid factor different to TLR 7 dependent generation of snRNPs. For all tested autoantigens either their titter or generation are dependent on the activation of innate immunity genes MyD88 and SIGIRR/TIR8 gene.

In localization, experiments anti-JKTBP autoantibodies specifically stained stress granules (SG) in the cytoplasm. Immunohistochemical studies revealed JKTBP to be highly expressed in SG in the cytoplasm of RA synovial tissue different from OA and normal control tissue

Conclusions: These data identify SG as targeted particle in RA and JKTBP as a novel autoantigen in RA, SLE patients and mouse models of inflammatory rheumatic diseases. In combination with the hnRNPs AUF1 and hnRNP-B1, JKTBP autoantibodies close the sensitivity gap in RA left by rheumatoid factor and anti-CCP2 antibodies.

Disclosure of Interest: None declared

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SAT0005 DNA METHYLATION INHIBITORS PRODUCE SUSTAINED REMISSION OF ARTHRITIS IN MICE AND PROMOTE REGULATORY T CELL RESPONSES

W. Tseng [1,2], I.-S. Huang [1], F. Clanchy [1], K. McNamee [1], D. Perocheau [1], P. Ericsson [3], H.-O. Sjogren [3], Z. Xue [3], L. Salford [3], A. Sundstedt [4], R.O. Williams [1]. *[1] Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Oxford University, The Kennedy Institute of Rheumatology, Oxford, United Kingdom; [2] Division of Rheumatology, Allergy and Immunology, Chang Gung Memorial Hospital, Keelung, Taiwan, Province of China; [3] Department of Clinical Sciences, Lund University, The Rausing Laboratory; [4] Idogen, Lund, Sweden*

Background: Dysfunction of Tregs results in a breakdown of immunological tolerance and has been implicated in the pathogenesis of autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis (RA) and type 1 diabetes. Treg function is regulated by epigenetic factors and we have previously reported the presence of Tregs expressing an aberrant DNA methylation profile in RA.

Objectives: The aim of this study was to assess the potential utility of DNA methylation inhibitors for the treatment of RA, using collagen-induced arthritis (CIA) as an animal model.

Methods: DBA/1 mice were immunised with bovine type II collagen emulsified in complete Freund's adjuvant. The mice were treated with zebularine (400 mg/kg), decitabine (1 mg/kg) or psammaplin A (10 mg/kg) for 4 days, starting on the day of arthritis onset. Treatment was then stopped and the disease was monitored up to day 10 of arthritis. The expression of Treg genes was measured in lymph nodes on day 10 by qPCR. To assess the effect of DNA methylation inhibitors on generation of Tregs, naïve $CD4^+CD25^-$ T cells were cultured with mitomycin C treated APCs plus IL-2, TGFβ and anti-CD3 in the presence or absence of DNA demethylating agents and numbers of $CD4^+FoxP3^+$ Tregs were determined by FACS after 72h.

Results: Treatment with zebularine resulted in a sustained reduction of arthritis severity, accompanied by an increase in the expression of Treg associated genes, *Foxp3, Ctla4 and Tgfb1*, in draining lymph nodes. Treatment with decitabine produced a more profound reduction in disease severity whereas the therapeutic effect of psammaplin A was more transient. All three DNA methylation inhibitors could convert $CD4^+CD25^-$ T cells into $CD4^+FoxP3^+$ Tregs in a dose-dependent manner *in vitro*.

Conclusions: This study has shown that pulse treatment with DNA demethylating drugs produces a sustained reduction in the severity of arthritis and promotes the generation of Tregs. The findings raise the possibility that epigenetic drugs can be used on a short-term basis for re-setting tolerance and boosting Treg responses in human autoimmune disease.

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SAT0006 ANTI-TNF AGENTS INDUCE MACROPHAGES WITH PRO-RESOLVING PHENOTYPE

Y. Degboe [1,2], B. Rauwel [2], M. Baron [2], J.-F. Boyer [1,2], A. Cantagrel [1,2], A. Constantin [1,2], J.-L. Davignon [1,2]. *[1] Centre de Rhumatologie, CHU Purpan; [2] CPTP, INSERM UMR 1043, Toulouse, France*

Background: Macrophages contribute to the rheumatoid arthritis (RA) pathogenesis. They can display various states of activation or " polarization ", characterized by distinct functions in inflammation, and reversibility depending on their environment [1]. M1 polarization corresponds to the "classical", pro-inflammatory activation as identified in RA. M2 "alternative" polarizations display pro-resolving or wound-healing properties.

Data concerning the effects of RA biological drugs (bDMARDs) on macrophage polarization are scarce.

Objectives: To assess in vitro modulation of macrophage polarization by RA bDMARDs.

Methods: Blood monocytes from 14 healthy donors were positively sorted by CD14+ magnetic selection. Macrophages were Derived from Monocytes (MDM) by 5 days of culture in the presence of MCSF, and activated or not for 24h as M1 pro-inflammatory MDM (by LPS + IFNγ) or as M2 alternative MDM (by IL10 or IL4). MDM were cultured with or without bDMARDs.

We evaluated 2 anti-TNF agents (etanercept (ETA), adalimumab (ADA)), 1 anti-IL6R agent (tocilizumab (TCZ)), and 1 anti-CD20 agent (rituximab (RTX)) used as control monoclonal antibody. bDMARDs effects were assessed separately on differentiation and activation phases by flow cytometric analysis of membrane markers. Functional aspects of polarization were assessed by analysis of cytokine production in supernatants (cytometric bead array) and phagocytosis (flow cytometry).

MDM cultured in the presence of bDMARDs were compared to untreated MDM by a Wilcoxon matched pairs test.

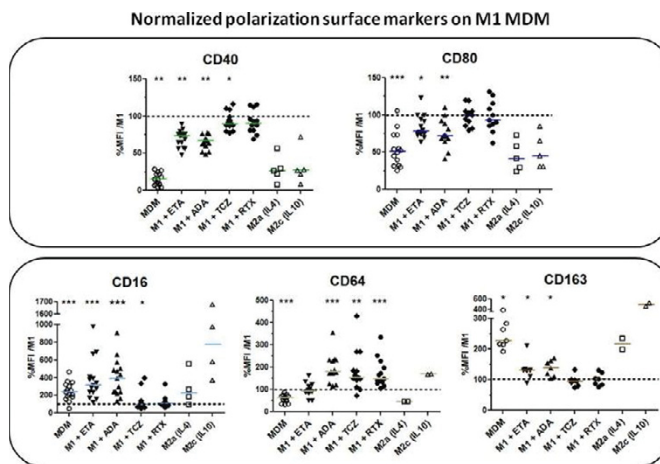
Results: We first validated *membranous polarization markers* in our culture model: CD40 and CD80 as M1 (LPS + IFNγ) markers; CD16, CD163 and CD64 as M2 (IL10) markers, CD206 and CD200R as M2 (IL4) markers.

Anti-TNF significantly modulated surface polarization markers of *MDM* obtained after differentiation. CD40 decreased with both anti-TNFs. CD16 increased with ADA, whereas opposite effects on CD64, decrease with ETA, increase with ADA, were observed. CD206 increased with ADA. CD200R increased with both anti-TNF. We observed a significant increase in CD64 with TCZ. RTX induced no modulation except an increase in CD64.

Concerning the effect of bDMARDs on *M1 activation* (M1 MDM) shown in Figure, anti-TNF agents induced a significant decrease in M1 markers and a significant modulation in M2 (IL10) markers. We observed a decrease in CD40 and CD80, an increase in CD16, CD163, a decrease in CD64 with ETA and an increase with ADA. TCZ induced a slight but significant decrease in CD40 and an increase in CD64. RTX only affected CD64 as observed for differentiation.

In *M2 (IL4) et M2 (IL10) activated MDM*, anti-TNF agents induced a similar trend toward M2 (IL10) phenotype.

Anti-TNF agents led to a significant decrease in *TNFα, IL6, IL12 and IL10 levels* measured in cell culture supernatant after M1 MDM. TCZ mainly affected IL6 and TNFα productions. No significant effect was observed with RTX.



Data were normalized to M1 MDM (represented as a dotted line) for each donor. Statistical comparison of M1 MDM without bDMARD (dotted line) versus M1 MDM with bDMARD, using a Wilcoxon matched pairs test → * p < 0.05 ; ** p<0.005 ; *** p<0.0005