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THU0089 IL-17A+GM-CSF+ NEUTROPHILS ARE THE MAJOR INFILTRATING CELLS IN INTERSTITIAL LUNG DISEASE IN AN AUTOIMMUNE ARTHRITIS MODEL

O.C. Kwon¹, E.-J. Lee¹, E.-J. Chang², W.J. Seo³, J.S. Oh⁴, S. Hong¹, C.-K. Lee¹, B. Yoo¹, Y.-G. Kim¹. ¹Division of Rheumatology, Department of Medicine, University of Ulsan, College of Medicine, Asan Medical Center; ²Department of Biomedical Science, University of Ulsan, College of Medicine, Asan Medical Center; ³Division of Rheumatology, Department of Medicine, Seoul Veterans Hospital; ⁴Clinical Research Center, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea, Republic of Ireland

Background: Interstitial lung disease (ILD) is a common extra-articular manifestation of rheumatoid arthritis (RA). Discrepancy in the effect of biologic agents on synovial and lung inflammation exists, indicating that the nature of inflammation in the synovium and lung may be different in RA.

Objectives: To gain a better understanding of the pathogenesis of rheumatoid arthritis-associated interstitial lung disease (ILD), we sought to identify the characteristics of lung-infiltrating cells in SKG mice with ILD.

Methods: We injected curdlan in SKG mice at 8 weeks of age, and identified the presence of ILD by PET-MRI at 20 weeks post-injection and histological analysis at 22 weeks post-injection. Lung-infiltrating cells were examined by flow cytometry. Analysis of serum cytokines by the Luminex multiplex cytokine assay was performed at 14 and 22 weeks post-injection, and cytokine profiles before and after the development of ILD were compared. Opal multiplexed immunofluorescent staining of lung tissue was also performed.

Results: At 20 weeks post-injection, curdlan-treated SKG mice developed not only arthritis but also lung inflammation combined with fibrosis, which was identified by PET-MRI and histological analysis. The majority of inflammatory cells that accumulated in the lungs of curdlan-treated SKG mice were CD11b⁺Gr1⁺ neutrophils, which co-express IL-17A and GM-CSF, rather than TNF- α . Compared with 14 weeks post-injection, serum levels of GM-CSF, MCP1, IL-17A, IL-23, TSLP, and IL-17R α had increased at 22 weeks post-injection, whereas those of IFN- γ , IL-22, IL-6, and TNF- α remained unchanged. Furthermore, IL-23, CXCL5, IL-17A, and GM-CSF, but not TNF- α , were observed in immunofluorescent-stained lung tissue.

Conclusions: We found that IL-17A⁺GM-CSF⁺ neutrophils represented the major inflammatory cells in the lungs of curdlan-treated SKG mice. In addition, GM-CSF and IL-17A appear to play a more important role than TNF- α in ILD development.

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THU0090 PREVALENCE AND CLINICAL PHENOTYPE OF ANTI-CARBAMYLATED PROTEIN ANTIBODIES IN PALINDROMIC RHEUMATISM

R. Castellanos-Moreira¹, S. Cabrera-Villalba¹, M. José Gomara², V. Ruiz-Esqueda¹, G. Salvador³, S.D.C. Rodríguez-García¹, O. Camacho¹, J. Ramírez¹, M. V. Hernández¹, A. Cuervo¹, J.A. Gómez-Puerta¹, J.D. Cañete¹, I. Haro², R. Sanmarti¹. ¹Rheumatology, Hospital Clínic de Barcelona; ²Unit of Synthesis and Biomedical Applications of Peptides, IQAC-CSIC; ³Rheumatology, Hospital Universitario Mutua Terrassa, barcelona, Spain

Background: Anti-carbamylated protein antibodies (anti-CarP) are a newly-identified antibody family present in the sera of rheumatoid arthritis (RA) patients whose specificity is close to that of ACPA but whose sensitivity is lower. Palindromic rheumatism (PR) is an intermittent form of arthritis that may progress to RA. Patients with PR may present the characteristic autoantibody profile seen in RA, even in the absence of evolution to RA. There are no studies on the presence of anti-CarP antibodies in PR.

Objectives: To analyse the prevalence of anti-CarP in patients with PR and evaluate their clinical phenotype and relationship with ACPA.

Methods: Patients diagnosed with PR attended by the Arthritis Unit, Rheumatology Service, Hospital Clínic de Barcelona were included according to the criteria of Guerne et al. Only patients considered as pure PR (patients without evidence of RA or other chronic rheumatic disease at the time of the first determination of anti-CarP) were analysed. An established RA control group was included. Anti-CarP were analysed by a home-made ELISA using a fibrin/chimeric flaggrin homocitrullinated peptide as antigen. IgG, IgA and IgM isotypes were measured. ACPA were analysed with a commercial CCP2 test. The clinical and therapeutic characteristics were compared according to anti-

CarP positivity/negativity and to the clinical evolution, including the progression to RA during the follow-up.

Results: Anti-CarP were analysed in 54 patients with pure PR and 53 RA patients. Anti-CarP were detected in 16.7% and 37.7% (p: NS) of patients with PR and RA, respectively. All PR patients positive for anti-CarP were ACPA positive and had higher ACPA titers. IgG was the predominant isotype (100%), only one patient presented IgA (11.1%) and none IgM. In the RA group, the percentages of IgG, IgA and IgM were 55%, 50% and 40%, respectively, in anti-CarP positive patients. More anti-CarP positive patients developed RA (33.3% vs. 13.6%), although the difference was not significant, during a mean follow-up of 3.8 $\pm$ 1.1 years. Remission at the last follow-up visit was more frequent in patients who were anti-CarP negative (22% vs. 60.5% p<0.05). Anti-CarP positive patients were receiving DMARD more frequently than anti-CarP negative patients (100% vs. 60% p<0.05).

Conclusions: This is the first study to demonstrate the presence of anti-CarP in patients with pure PR. Anti-CarP was associated with ACPA positivity and higher ACPA titers. Anti-CarP isotypes differed between PR and RA, with lower isotype use in PR. Anti-CarP positivity in PR patients could indicate a more refractory disease, with lower rates of remission and greater predisposition to evolve to RA.

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THU0091 TIE2 INDUCES AN INFLAMMATORY PHENOTYPE IN RHEUMATOID ARTHRITIS AND PSORIATIC ARTHRITIS PATIENTS DRIVEN BY ANGIOPOIETIN-2

S. García Pérez¹, P.A. Kabala¹, B. Malvar Fernández¹, M.W. Tang², S. A. Hartgring¹, C. Conde³, M. Sleeman⁴, D.L. Baeten², P.P. Tak⁵, J. Connor⁶, K. A. Reedquist¹, T.R. Radstake¹. ¹Laboratory for Translational Immunology (LTI) and Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht; ²Department of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; ³Laboratorio de Investigación 8 y Servicio de Reumatología, Instituto de Investigación Sanitaria (IDIS), Hospital Clínico Universitario de Santiago de Compostela (CHUS), Santiago de Compostela, Spain; ⁴Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA; ⁵GlaxoSmithKline, Stevenage, UK; ⁶MedImmune LCC, Maryland, USA

Background: Several studies have shown that angiopoietin signalling to Tie2 may play a role in the initiation and perpetuation of disease rheumatoid arthritis (RA) and psoriatic arthritis (PsA). However, the cell type(s) involved in this process, as well as the specific role of Tie2 signalling in the synovium of arthritis patients, remain unclear.

Objectives: To examine the role Tie2 signalling in macrophages and fibroblast-like synoviocytes (FLS) within the context of the synovial microenvironment of arthritic patients.

Methods: Peripheral blood (PB) monocytes from healthy donors (HD) were differentiated with synovial fluid (SF) of RA and PsA patients. PB and SF monocytes from RA and PsA patients were differentiated into pro-inflammatory macrophages with IFN- γ . Tie2 expression was analysed by flow cytometry and quantitative PCR. Macrophages, RA FLS and synovial tissue explants were stimulated with Ang-1 or Ang-2 (200 ng/ml) alone or in combination with TNF (10 ng/ml) for 4 hour or 24 hour. mRNA and protein expression of inflammatory mediators was analysed by quantitative PCR and ELISA and luminex, respectively. Arthritis was induced in wild type (WT) and Tie2 over-expressing (Tie2-TG) mice by intraperitoneal injection of 100 ml of K/BxN serum on day 0 and day 2. Mice were sacrificed on day 14 after serum transfer.

Results: Tie2 expression was observed IFN- γ -differentiated macrophages, from RA and PsA patients, as well as HD macrophages differentiated with RA and PsA SF. In all cases, both Ang-1 and Ang-2 stimulation significantly enhanced TNF-induced expression of pro-inflammatory cytokines (IL-6, IL-12B) and chemokines (IL-8, CCL-3 and CXCL-6). Tie2 activation also enhanced TNF-mediated production of these inflammatory mediators in RA FLS. The clinical severity of serum-induced arthritis was significantly higher in Tie2-TG mice compared to WT mice, associated with enhanced synovial expression of IL-6, IL12B, iNOS, CCL-2 and CXCL-10.

Finally, we found that Ang-2, and to a lower extent Ang-1, induced the production of IL-6, IL-12B, IL-8, and CCL-3 in the synovial tissue explants of RA and PsA patients. Importantly, Ang-2 blockade with a specific neutralising anti-Ang2 antibody suppressed the production of IL-6 and IL-8 in synovial tissue of RA patients.

Conclusions: These results suggest that Tie2 signalling, even within the complex microenvironment of affected synovial tissue, has an important pro-

inflammatory role in the pathology of RA and PsA. As this effect can be reversed by Ang-2 neutralisation, interfering with Tie2 activity may be a promising therapeutic target in arthritic diseases.

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THU0092 INTRA-CYTOPLASMIC CYTOKINES IN CD20 POSITIVE LYMPHOCYTE SUBPOPULATIONS IN RHEUMATOID ARTHRITIS (RA) PATIENTS

S. Amar¹, A. Kerpel², R. Rabinowitz², P. Levy², O. Zelig¹, N. Schlesinger³, M. Schlesinger². ¹Hadassah hospital; ²Hebrew University Medical School, Jerusalem, Israel; ³Rutgers Robert Wood Johnson Medical School, New Brunswick, USA

Background: The administration of biological drugs inactivating various elements of the immune system such as cytokines and immune cells, induces a significant ameliorating effect on rheumatoid arthritis (RA) patients. The mechanisms involved in the pathogenesis of RA are unclear, and the precise role of different cellular elements of the immune system in its pathogenesis remain to be elucidated.

Objectives: In the present study we concentrated on one cellular target for a biologic treatment in RA (Rituximab), the CD20 positive lymphocytes. Various types of lymphocytes are positive to CD20. Based on its expression, these lymphocytes include dim and bright CD20 +types. These cells' capacity to secrete various cytokines was determined among lymphocytes of healthy controls (HC) and RA patients.

Methods: Peripheral blood lymphocytes (PBL) of RA patients (n=20) and HC (n=15) were isolated using Ficoll Hypaque gradient columns. To 5×10^5 cells in 0.5 ml RPMI-1640 medium, 1.25 ng PMA, 0.5 μ g Ionomycin and 5 μ g Brefeldin were added for 5 hours of activation after which cells were incubated for 30 min at 4°C with Phycoerythrin-(PE) conjugated anti-CD20 monoclonal antibody (mab). Thereafter, suspensions were incubated in fixation buffer for 45 min, washed and re-suspended in 100 μ l of permeabilization buffer and 20 μ l of fluorescent (FITC) conjugated anti-cytokine mabs, and were incubated at 4°C for 30 min. A t-test for independent samples, was performed.

Results: Intra-cytoplasmic detection of various key-role cytokines, such as interleukins 4 (IL-4), 17 (IL-17), 10 (IL-10) and interferon gamma (INF- γ) was determined. The percentage of IL-4 positive lymphocytes among CD20 +cells in HC was higher in CD20 +dim cells (4.5%) than in CD20 +bright cells (1.2%) (p value 0.0001). Among lymphocytes of RA patients the proportion of IL-4 positive cells was much higher than in HC (p<0.0002) and was comparable between dim (54.5%) and bright (48.6%) CD20 +lymphocytes. The proportion of IL-10 positive cells among both dim and bright CD20 +lymphocyte subpopulations was significantly lower in RA patients than in HC (p<0.0002). While in HC, 56% of CD20 +dim and 61% of CD20 +bright lymphocytes, showed IL-10 positivity, among RA lymphocytes it constituted only 17.8% and 9.3% of the CD20 +dim and bright lymphocytes, respectively.

Staining with anti-IL-17 mab yielded in low proportions of IL-17 positive cells in all CD20 +populations tested, in both HC and RA. Among dim CD20 +lymphocytes, IL-17 positive cells constituted 1.4% (HC) and 3.6% (RA). IL-17 staining appeared on only 0.74% of CD20 +bright lymphocytes in HC and similarly in RA (0.54%). While the proportion of INF- γ positive lymphocytes was similar in HC and RA, it was significantly higher among dim (11.8% HC, 9.5% RA) than in bright CD20+ (1% HC, 1.4% RA) indicating a different cell pattern (p=0.0001).

Conclusions: The present study demonstrates that CD20 +lymphocytes, targeted in RA treatment, expressing different levels of CD20, in RA differ from HC in their capacity to produce various cytokines. Although there was no difference in IL-17 and INF- γ they may modulate the immune response in RA due to higher levels of IL-4 and reduced IL-10 levels produced by both CD20 +cell populations; dim and bright lymphocytes.

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THU0093

NADPH OXIDASES ASSOCIATED PRODUCTION OF REACTIVE OXYGEN SPECIES IN RHEUMATOID ARTHRITIS

S.-J. Yoo, S.W. Kang, J. Kim, I.S. Yoo, C.K. Park. Internal Medicine, Chungnam National University School of Medicine, Daejeon, Korea, Republic of Ireland

Background: Reactive oxygen species (ROS) is produced during metabolism of Oxygen. ROS is important in cell signalling and homeostasis. Production of ROS can be elevated in stressful condition. Oxidative stress has been known to be related with the disease like infection and malignancy. NADPH oxidases (Nox) are membrane proteins which produce ROS.

Objectives: In this study we aimed to investigate the role of Nox in rheumatoid arthritis (RA) associated with production of ROS.

Methods: Nox and Granulocyte macrophage colony-stimulating factor (GM-CSF) messenger ribonucleic acid (mRNA) were analysed in fibroblast like synovocyte (FLS) of patients with RA and osteoarthritis (OA) by reverse transcription polymerase chain reaction. Amount of ROS which is produced in FLS of patients with RA and OA is determined using the cell permeant fluorophore 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA) by flow cytometry. Same experiments were performed after treatment with cytokine, interleukin (IL)-17 and tumour necrosis factor- α (TNF- α).

Results: Among Nox subunits (Nox1, Nox2, Nox4, Nox5, DUOX1, DUOX2, NOXA1, NOXO1), NOXA1 and NOXO1 mRNA were expressed higher in RA FLS than in OA FLS. After treatment with IL-17 and TNF- α for 24 hours GM-CSF, Nox1 and NOXO1 mRNA in RA FLS were elevated. Amount of ROS production was also elevated after treatment with IL-17 and TNF- α . When RA FLS were treated with bromopyruvic acid (BrPa), glucolysis inhibitor by inhibition of hexokinase II, GM-CSF mRNA and ROS were decreased and Nox1 and Nox4 mRNA showed no difference.

Conclusions: Several factors may be involved between ROS and Nox in RA FLS. Both ROS and Nox were elevated in inflammatory condition in RA FLS. From this result we expect that Nox-targeted therapy may be effective for treatment with RA.

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