present more than 4 infections. The significance of heterozygous HYLX promoter was observed only for the presence of infection. Table 1.

Table 1. Association between MEX-SLEDAI and Infections with MBL structural and promoter genotypes in SLE patients

	MEX-SLEDAI median (IQR)	p*	Patients with infections events ≥4, n (%)	Total patients with infections events ≥4, n (%)	p**	OR
Codon 52, n=71		0.68		12 (16.9)	0.44	
A/A	3 (6)		10 (17.5)			
A/D	3 (8)		0 (0)			
D/D	1 (7)		2 (25)			
Codon 57, n=73		0.02		13 (17.8)	0.03	8.7 (1.2–58)
A/A	2 (6)		10 (14.7)			
A/C	0 (0)		0 (0)			
C/C	9 (0)		3 (60)			
Promoter, n=74		0.35		13 (17.6)	0.01	
HYHY	3 (7)		2 (18.2)			
LYLY	2 (8)		5 (21.7)			
LXLX	1.5 (6)		1 (4.8)			
LXLY	2.5 (5)		2 (13.3)			
HYLY	5 (0)		1 (50)			
HYLX	0 (0)		2 (100)			

MEX-SLEDAI: Mexican Systemic Lupus Erythematosus Disease Activity Index, IQR: interquartile range, OR: Odds ratio. *Kruskal–Wallis H test, ** Chi-square test.

Conclusions: MBL2 gene polymorphisms of the homozygous C/C in codon 57 of the structural region and heterozygous HYLX of the promoter region are associated with increased risk of a higher number of infections. Also, we observed that homozygous C/C in codon 57 was asociated to a higher MEX-SLEDAI.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.3304

AB0024 A NOVEL REAL-TIME IMAGING TECHNIQUE TO CHARACTERIZE MECHANISMS OF CELL DEATH IN NEUTROPHILS

S. Gupta[1], D.W. Chan[1], K.J. Zaal[2], E. Ralston[2], M.J. Kaplan[1]. *[1]Systemic Autoimmunity Branch; [2]Light Imaging Section, NIAMS/NIH, Bethesda, United States*

Background: Neutrophils play a key role in the pathogenesis of autoimmune diseases through various mechanisms including the formation of neutrophil extracellular traps (NETs). NETosis, a recently described distinct form of program neutrophil cell death, is characterized by reactive oxygen species generation, chromatin and nuclear decondensation, membrane rupture and extrusion of a meshwork of chromatin bound to granule peptides.
Objectives: Techniques to assess and quantitate NETosis in an unbiased, reproducible and efficient way are lacking. We developed a new method to automatically quantify the percentage of neutrophils undergoing NETosis using real-time quantitative live-cell analysis with IncuCyte ZOOM™ (Essen BioScience, Inc.) platform and a dual-dye system dependent on membrane integrity to stain DNA, to image neutrophils and characterize their mechanisms of cell death.
Methods: Neutrophils were isolated from healthy controls using density gradient methods and their DNA was stained with a membrane permeable NUCLEAR-ID Red DNA dye. Neutrophils were plated and incubated with various stimuli to induce NETosis (PMA, ionomycin and/or SLE sera), apoptosis (Staurosporin) or necroptosis (TNF with a pan-caspase inhibitor, Z-VAD) and with Sytox, a membrane-impermeable DNA dye. Three 20x magnification images from different areas per well were captured at 10-minute intervals. A processing definition was set and optimized to count all neutrophils (NUCLEAR-ID stained) at baseline and neutrophils undergoing cell death (Sytox stained) at three hours using fluorescence intensity and stained area size (Figure 1).

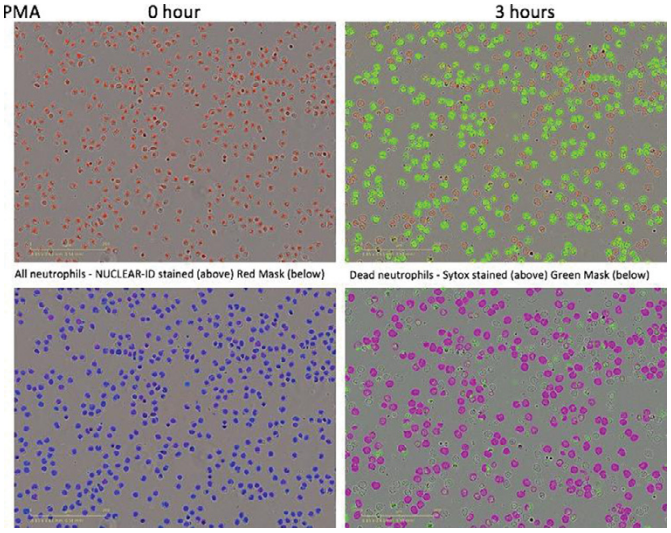


Figure 1

Results: Percentage of neutrophils undergoing cell death using various stimuli was highly reproducible. Characterization of changes in nuclear morphology, quantified by the processing definition, distinguished between NETosis, apoptosis and necroptosis. Findings were confirmed and counts correlated with previously established method using immunofluorescence microscopy.
Conclusions: This novel real time assay distinguishes types of neutrophil cell death and quantifies NETosis in a rapid, accurate and reproducible way. This technique may facilitate studies in neutrophil biology.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.3593

AB0025 PREGNANCY RESULTS IN THE SELECTIVE MODULATION OF PLATELET TISSUE FACTOR EXPRESSION INDEPENDENTLY OF THE PRESENCE OF ANTIPHOSPHOLIPID ANTIBODIES OR OF OTHER AUTOIMMUNE FEATURES

V. Canti[1], M.T. Castiglioni[2], M. Angelo A[2], P. Rovere-Querini[2], N. Maugeri[2]. *[1]San Raffaele Scientific Institute, Milano, Italy; [2]via Olgettina 58, San Raffaele Scientific Institute, Milano, Italy*

Background: Neutrophils and platelets are key innate immune cells that productively interact upon activation, generating/releasing moieties that can damage the bystander tissue and prompt vasculogenesis. Successful pregnancy critically depends on a tight regulation of the latter events. Conversely pregnancy complications are associated with alteration/damage of the vasculature associated to the placenta. Blood-born tissue factor (TF) due to the expression of the moiety by platelets and leukocytes has been involved in the pro-thrombotic diathesis associated with sustained human autoimmunity, including that associated with anti-phospholipid syndrome (APS).
Objectives: To test the modulation of parameters related to blood-born TF during normal and pathological pregnancy
Methods: The expression of TF by platelets, monocytes and neutrophils has been studied in 40 women at the 12th week of gestation (wg) including twelve healthy women, 14 patients with insulin-dependent diabetes mellitus (IDDM) and 14 patients with a previous history of pregnancy complications, six of them with APS. 30 healthy age-matched non-pregnant women served as controls. When possible, patients were studied again at least one year after the pregnancy completion. Blood samples were collected and processed as described[1,2]. Other features reflecting cell activation were assessed in parallel[1,2].
Results: The expression of platelet TF was significantly higher in pregnant women compared with age-matched controls. Platelet P-selectin was as well significantly up-regulated. Neutrophils circulating in all pregnant women were mildly degranulated. The content of the neutrophil secondary granules was depleted in particular in subiects with previous pregnancy complications *sine causa*.
Conclusions: Our data support the contention that the activation of the innate immune system is a key feature of pregnancy, regardless of the presence of features of systemic or organ-specific autoimmunity. This implies an important modulation of the machinery involved in the reciprocal activation of platelets and neutrophils and in the pro-thrombotic phenotype of circulating cells. The analysis of the potential modulation of these parameters by ongoing treatment is currently being carried out.
References:
[1] Manfredi A & al. Ann Rheum Dis. 2016;75(8):1511–20. doi: 10.1136/annrheumdis-2015-208442.
[2] Maugeri N et al. Blood. 2011 Sep 22;118(12):3359–66. doi: 10.1182/blood-2011-02-337337.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.6429

Cytokines and inflammatory mediators

AB0026 CHEMOKINE SIGNALS ARE CRITICAL FOR HOMING AND ENHANCED DIFFERENTIATION OF CIRCULATING OSTEOCLAST PROGENITOR CELLS

A. Sucur[1], Z. Jajic[2], M. Artukovic[3], M. Ikic Matijasevic[3], F. Grubisic[2], B. Anic[4], S. Ivcevic[1], D. Flegar[1], D. Grcevic[1]. *[1]Department of Physiology and Immunology, University of Zagreb School of Medicine; [2]Department of Rheumatology, Physical Medicine and Rehabilitation, Clinical Hospital Center "Sestre Milosrdnice", University of Zagreb School of Medicine; [3]Department of Clinical Immunology and Pulmology, Clinical Hospital "Sveti Duh"; [4]Department of Clinical Immunology and Rheumatology, Clinical Hospital Center "Zagreb", Zagreb, Croatia*

Background: Peripheral blood (PB) monocyte pool contains cells capable of differentiating into osteoclasts (OCs). These osteoclast progenitors (OCPs) contribute to osteoresorption in inflammatory arthritides under influence of the cytokine milieu and chemokine mediated trafficking.

Objectives: Our study aimed to define chemokine receptor profile of peripheral OCPs in rheumatoid arthritis (RA), with comparison to psoriatic arthritis (PSA), as well as their susceptibility to chemotactic signals.
Methods: 129 RA, 53 PSA and 110 control patients were enrolled after Ethical approval. PB samples and synovial fluid (SF) samples, with clinical data of disease activity, inflammation and autoantibody levels were collected. Patients starting anti-TNF therapy were followed up 6 months. TNF-α and CTX serum levels were measured by ELISA. Frequency of OCP-rich subpopulation (CD3-CD19-CD56-CD11b+CD14+), expression of OC differentiation (CD115, RANK) and chemokine (CCR1, CCR2, CCR4, CXCR4) receptors was assessed by flow cytometry. OCPs were sorted using FACS, cultured with M-CSF and RANKL, stained for TRAP enzyme and mature OCs counted. Levels of CCL2, CCL3, CCL4, CCL5, CXCL9 and CXCL10 were measured using cytometric bead array, and of CXCL12 by ELISA. Osteoclastogenic effects of CCL2, CCL5 and CXCL10 were analyzed in cell culture, and chemotactic effects on OCPs were studied by cell migration assay using Transwell, with count of number of migrated cells and subsequently differentiated mature osteoclasts.
Results: OCP population was moderately enlarged in PB, further expanded in SF and correlated with TNF-α and rheumatoid factor (RF) levels in patients with RA. However, sorted OCPs generated similar number of mature OCs as control. RANK+ subpopulation was enlarged in SF vs PB and correlated with number of tender joints. In PSA, the OCP population was not enlarged, but had a higher RANK expression. OCPs in RA and PSA had higher expression of CCR1, CCR2, CCR4, CXCR4, and all except CCR4 showed positive PB-to-SF gradient. RA had higher levels of CCL2, CCL3, CCL4, CCL5, CXCL9 and CXCL10, with a positive PB-to-SF gradient for all except CCL5 and CXCL9. OCP frequency correlated with levels of CCL2 and CCL5. Subset expressing CXCR4 was associated with TNF-α, CTX and RF levels and was lower in patients treated with DMARD, who at the same time had lower osteoresorption (CTX). Subset expressing CCR4 showed significant negative trend during anti-TNF treatment. CCL2, CCL5 and CXCL10 showed significant osteoclastogenic effect. CCL5 showed greatest chemotactic effect, attracting the highest number of cells in the migration assay. At the same time, attracted cells possessed greater osteoclastogenic potential.
Conclusions: Our study provides evidence of the specific importance of certain chemokine signals for stimulation of OCP mobilization, subsequent tissue homing, and maturation, explaining local as well as generalized bone loss seen in RA. Novel insights in regards to migratory behavior and functional properties of PB OCPs in response to chemotactic signals could open way to new therapeutic targets in RA.
Acknowledgements: This work was supported by a grant from the Croatian Science Foundation (project number 5699).
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.2317

AB0027 EXPRESSION DENSITY OF RECEPTORS TO TNF-ALPHA IS ASSOCIATED WITH DAS-28 SCORE IN RHEUMATOID ARTHRITIS

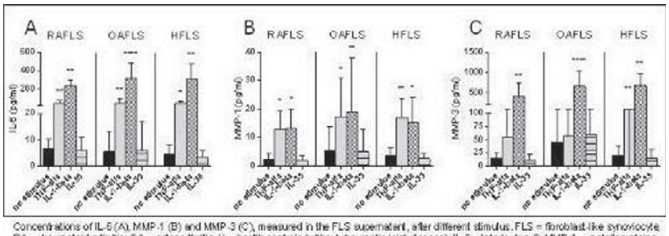
A.A. Alshevskaya[1], J. Lopatnikova[1], N. Shkaruba[1], O. Chumasova[1], A. Sizikov[1], V. Lukinov[2], A. Moskalev[2,3], S. Sennikov[1]. [1]*Federal State Budgetary Scientific Institution "Research Institute of Fundamental and Clinical Immunology"; [2]EOL Labs Ltd; [3]Biostatistics and Clinical Trials Center, Novosibirsk, Russian Federation*

Background: For a number of cytokines and growth factors density of receptors expression has been shown to be important in regulation of action intensity and variation. Although TNF-alpha is actively involved in the development and progression of chronic inflammation in rheumatoid arthritis (RA) role of membrane receptors and their regulatory function in RA remains unclear.
Objectives: To assess associations of membrane-bound receptors expression and soluble receptors and cytokine content with disease activity score in patients with rheumatoid arthritis.
Methods: To reveal linear relationships among integrated score DAS-28 and studied parameters of intact T-cells, B-cells and monocytes (evaluated by flow cytometry) and parameters of mediators soluble content (evaluated by ELISA) building of multiple linear regression model (MLRM) with a standard assessment of the regression coefficients by least squares method were used. To determine receptor number on the cells QuantibritePE Beads (BD) were used.
Results: For the group of patients with acute stage of RA MLRMs with best statistical quality characteristics revealed that for lymphocytes both TNFR1 and TNFR2 number had significant associations with DAS-28; for monocytes only number of TNFR2 was significant. Lower DAS-28 index is associated with higher number of TNFR2 per cell and lower number of TNFR1.
Comparison of models with different combinations of studied TNFR-parameters revealed that percentages of cells expressing receptors for TNFα validated not more than 20% of DAS-28 variation, while the number of receptors indicators on cells validated 40–50% of DAS-28 variation. Full MLRM with all 15 studied parameters validated about 70% of DAS-28 variation.
For the group of patients with acute stage of RA MLRMs with best statistical quality characteristics revealed that for lymphocytes both TNFR1 and TNFR2 number had significant associations with DAS-28; for monocytes only number of TNFR2 was significant. Lower DAS-28 index is associated with higher number of TNFR2 per cell and lower number of TNFR1. Comparison of models with different combinations of studied TNFR-parameters revealed that percentages of cells expressing receptors for TNFα validated not more than 20% of DAS-28 variation, while the number of receptors indicators on cells validated 40–50% of DAS-28 variation. Full MLRM with all 15 studied parameters validated about 70% of DAS-28 variation.
Conclusions: Number of receptors to TNF-alpha is more associated with RA activity score as compared with soluble receptors or cytokine. Number of receptors type 1 and type 2 to TNF-alpha per cell have opposite influence on disease activity score. Our findings indicate the involvement of receptors expression density changes in the pathological process in RA.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.2643

AB0028 FIBROBLAST-LIKE SYNOVIOCYTES MAY NOT BE THE TARGET OF IL-33 IN THE JOINT PHISIOPATHOLOGY

C.R.L. Machado[1], G.G. Resende[2], R.B.V. Macedo[2], V.C. Nascimento[2], T.P. Silva[2], A.M. Kakehasi[2], M.V.M. Andrade[2]. [1]*Universidade Federal Minas Gerais; [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Background: Rheumatoid arthritis (RA) and osteoarthritis (OA) are chronic joint diseases in which fibroblast-like synoviocytes (FLS) actively participate in the synovitis- damage cycle, trough production of cytokines such as IL-6 and metalloproteinases (MMPs)[(1)]. It has already been showed that serum IL-33 levels correlated with disease activity in RA[(2)]. IL-33 is capable to enhance TNF-α effects in FLS[(3)]. In the collagen-induced arthritis (CIA) model, the injection of IL-33 exacerbated the disease[(4)]. Since ST2 receptor is expressed in FLS, it is hypothesized that IL-33 could activate FLS and increase downstream production of inflammatory cytokines.
Objectives: To evaluate the production of IL-6, MMP-1, and MMP-3 by FLS stimulated with IL-33, TNF-α, and IL-1β.
Methods: FLS were cultured from samples of synovial fluid and tissue of OA patients (OAFLS n=8), RA patients (RAFLS n=3), and patients without rheumatic disease (health) (HFLS n=4). FLS were stimulate with TNF-α at concentrations of 1; 5; 10 and 50 ng/ml, IL-1β at concentrations of 0.1; 0.2; 0.3; 0.5 and 1 ng/ml and IL-33 at concentrations of 1; 3; 10; 30; 100 ng/ml, soon after, IL-6, MMP-1, and MMP-3 levels were evaluated by ELISA, in the cell supernatant.
Results: MMP-1, MMP-3 and IL-6 were constitutively expressed by FLS at baseline in all groups. Both TNF-α and IL-1β stimulated the production of IL-6 and MMP-1 with statistical significance in a dose-dependent manner in all three groups. Only IL-1β increased the production of MMP-3. TNF-α stimulated the production of MMP-3 only on HFLS. There was no difference between the concentration of MMP-1, MMP-3 and IL6 in the supernatant of OAFLS, ARFLS and HFLS when IL-33 stimulated and non-stimulated were compared.



Concentrations of IL-6 (A), MMP-1 (B) and MMP-3 (C), measured in the FLS supernatant, after different stimulus. FLS = fibroblast-like synoviocyte; RA = rheumatoid arthritis; OA = osteoarthritis; H = health controls (without rheumatic joint disease); IL-6 = Interleukin-6; MMP-1 = metalloproteinase-1; MMP-3 = metaloproteinase-3; TNF-alpha = Tumor Necrosis Factor-alpha; IL-1-beta = Interleukin-1-beta; IL-33 = Interleukin-33. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.

Conclusions: This study demonstrated that IL-33 failed to induce the production of IL-6, MMP-1 and MMP-3 by FLS of different diseases sources, suggesting that must be another cell type that plays the role of target to IL-33 in physiopathology of joint inflammation. The absence MMP-3 in response to TNF-α stimulus in RAFLS and OAFLS could be explain by saturation of this cytokine in synovial cells from these diseases.
References:
[1] Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. Immunological reviews. 2010;233(1):233–55.
[2] Matsuyama Y, Okazaki H, Tamemoto H, Kimura H, Kamata Y, Nagatani K, et al. Increased levels of interleukin 33 in sera and synovial fluid from patients with active rheumatoid arthritis. The Journal of rheumatology. 2010;37(1):18–25.
[3] Kunisch E, Chakilam S, Gandesiri M, Kinne RW. IL-33 regulates TNF-alpha dependent effects in synovial fibroblasts. International journal of molecular medicine. 2012;29(4):530–40.
[4] Xu D, Jiang HR, Kewin P, Li Y, Mu R, Fraser AR, et al. IL-33 exacerbates antigen-induced arthritis by activating mast cells. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(31):10913–8.
Acknowledgements: Capes, Fapemig and Fundo de Apoio a Pesquisa e Ensino da Sociedade Brasileira de Reumatologia - FAPE-SBR.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.6673