AB0018 B CELL DISTURBANCE IN RHEUMATOID ARTHRITIS PATIENTS: COMPARATIVE STUDY BETWEEN TREATED AND NON-TREATED PATIENTS

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Background: B cells have been shown to play a key role in the pathogenesis of rheumatoid arthritis (RA). Alterations of B cell homeostasis as well as B cell activation markers, such as B-cell activating factor (BAFF) and A Proliferation-Inducing Ligand (APRIL) have been described in RA patients. Up to our knowledge, no data is currently available on differences between treatment naïve patients and those receiving disease modifying anti-rheumatic drugs (DMARDs), regarding B-cell surface expression as well as levels of circulating BAFF/APRIL.

Objectives: The aim of this study is to investigate disturbance of B cell as well as B cell activation markers namely BAFF and APRIL in patients with rheumatoid arthritis comparing treatment naïve patients with those receiving disease modifying anti-rheumatic drugs.

Methods: Sixty RA patients and 30 healthy controls were enrolled. Thirty patients receiving non biologic DMARDs and had not received prior biological treatment and 30 treatment naïve patients. Absolute number of blood CD19 B cells was determined by flow cytometry using the CD19-PE Kit (Immunotech, France). BAFF and APRIL blood concentration was measured using commercially available ELISA kits (Bosterbio, USA).

Results: There was statistically significant difference (p-value <0.05) between different study groups as regard B cell count with low mean among treated RA patients (137.2±60.5) and high mean among controls (243.7±22.5). The B cell count was diminished in the two groups of RA patients, however it is more pronounced in treated patients. Circulating BAFF levels were increased in RA compared to HC (p-value <0.05) with more increase in patients on treatment. Circulating APRIL levels were significantly lower (p-value <0.05) in treatment naïve rheumatoid arthritis patients (343.9±21.7) than the control group (371.5±24.3). However, there was no statistical significant difference (p-value >0.05) between each of treated rheumatoid arthritis group and controls or with non treated rheumatoid arthritis group.

Variables	B cell count		p-value
	Mean	SD	
G1: Treated RA patients	137.2	60.5	<0.001
G2: Non treated patients	179.3	59.1	
G3: Controls	243.7	22.5	
Variables	baff Concentration (pg/ml)		p-value
	Mean	SD	
G1: Treated RA patients	1419.4	417.3	<0.001
G2: Non treated patients	886.2	258.4	
G3: Controls	444.4	120.1	

Conclusions: More decrease in B cell count and more increase in BAFF level were observed in RA patients receiving non-biologic DMARDs. Conversely, APRIL levels were not affected by treatment.

The contribution of B lymphocyte to RA pathogenesis goes beyond autoantibody production. Disturbances in B cell homeostasis in RA are not only due to disease process itself but closely related to the use of anti-rheumatic drugs.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.2014

AB0019 HDL PROMOTES A REGULATORY PHENOTYPE OF THE IMMUNE SYSTEM BY INDUCING THE PROLIFERATION OF HUMAN CD4+ T CELLS WHILE INCREASING THE EXPRESSION OF FOXP3 IN REGULATOR T CELLS

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Background: High-density lipoproteins (HDL) are the plasma lipoproteins responsible for reverse cholesterol transport. Its protective effect on cardiovascular disease is attributed to the cholesterol efflux capacity as well as to its anti-oxidant and anti-inflammatory properties. HDL low levels are associated with immune dis-

eases, including systemic lupus erythematosus and rheumatoid arthritis, implying a potential link between HDL and immunity.

There is few evidence on the effects of HDL on human T cells. Animal studies suggested that HDL inhibits lymphocyte proliferation¹, but the only study in humans showed that HDL from young and healthy subjects increases the proliferation of lymphocytes². Previous studies also showed that HDL levels are positively correlated with the prevalence of regulator T cells in peripheral blood³. Furthermore, it has been shown that one of the main actions of HDL in lymphocytes is the disaggregation of lipid rafts provoked by cholesterol efflux from the membrane, but the final consequences of this effect are not known.

Objectives: To determine the *in vitro* effects of HDL in human T cell proliferation and in the frequency of FoxP3+ regulator T cells.

Methods: Peripheral blood mononuclear cells from six healthy donors were cultured with and without HDL to measure membrane cholesterol and proliferative response to CD3/CD28 stimulation. Membrane cholesterol was measured by filipin binding, assessed by flow cytometry. T cell proliferation was studied by Ki-67 expression and cell trace staining for multiple generations. T cell phenotyping and FoxP3 fluorescence were analyzed by flow cytometry.

Results: HDL significantly reduced the amount of membrane cholesterol in CD4+ T cells, after 24 hours in culture. When added in the absence of stimulation, HDL did not induce any proliferation in CD4+ T cells. When added in conjunction with CD3/CD28 stimulation, HDL significantly increased the proliferation of CD4+ T cells. T cell phenotyping showed a significant increase in CD4+CD25+ FoxP3 fluorescence, after culture with HDL.

Conclusions: This study shows that HDL promotes cholesterol efflux from the membrane of CD4+ T cells *in vitro* and increases CD4+ T cell proliferation. However, the increase in T cell numbers seem to favor the expression of FoxP3 in regulator in T cells, which is associated with suppression of inflammation. With the development of therapies to increase HDL levels, the knowledge of the HDL effects on T cell subsets is very important for the future management of cardiovascular and rheumatic diseases.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.6681

AB0020 EXPRESSION LEVELS OF SIGNALING LYMPHOCYTE ACTIVATION MOLECULE FAMILY 6 (SLAMF6) ON CIRCULATING FOLLICULAR HELPER T CELLS ARE ASSOCIATED WITH DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: T follicular helper cells (Tfh) are necessary for B-cell maturation and differentiation in the germinal centers (GC). The signaling lymphocyte activation molecule family (SLAMF) receptors on the cell surface mediate T cell:B cell interaction for formation and maintenance of GC. But it remains to be elucidated whether SLAMF is expressed in circulating T follicular helper cells (cTfh).

Objectives: The aim of this study was to investigate the levels of SLAMF3 and SLAMF6 on cTfh and to analyze their association with disease activity in systemic lupus erythematosus (SLE) patients.

Methods: Blood samples were collected from 50 SLE patients, 24 Sjogren's syndrome (SS) patients and 25 age- and sex-matched healthy controls (HCs). In this study, CXCR5, programmed cell death protein 1 (PD-1), and CD4 were used as markers to define cTfh in peripheral blood mononuclear cells (PBMCs). The expression levels of SLAMF3 and SLAMF6 on cTfh were compared to that of circulating B cell subsets by flow cytometry. Clinical features including disease activity and laboratory tests were analyzed according to expression of SLAMF3 and SLAMF6.

Results: Surface expression of SLAMF6 on CD4 T cells was significantly increased in SLE patients compared to SS patients (mean fluorescence intensity [MFI], mean ± SD: 1291±319 vs 1098±147, p=0.015) and HCs (1291±319 vs 1094±243, p=0.011). But there was no difference in expression of SLAMF3 on CD4 T cells between SLE patients and SS patients or HCs. SLAMF6 expressions on cTfh cells, identified as CD4+CXCR5+PD-1+, were significantly increased in SLE patients than in SS patients (1500±270 vs 1296±140, p=0.001) and HCs (1500±270 vs 1295±161, p=0.001). SLAMF6 expressions on cTfh cells had correlation with systemic lupus erythematosus disease activity index (SLEDAI) (Spearman's rho=0.477, p=0.001), proteinuria (rho=0.321, p=0.030) and double strand DNA (rho=0.340, p=0.026) in SLE patients. Moreover, expression levels of SLAMF6 on cTfh cells were correlated with those of several B cell subsets including total B cells (CD19+), naïve B cells (CD19+CD27-IgD+) and class-

switched memory B cells (CD19⁺CD27⁺IgD⁻). But SLAMF6 expressions of B cell subsets were not correlated with disease activity.

Conclusions: Surface expression of SLAMF6 was increased in cTfh cells in patients of SLE and had correlation with disease activity.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.6078

Innate immunity in rheumatic diseases

AB0021 HUMAN T CELL LEUKEMIA VIRUS TYPE 1 (HTLV-1) EXACERBATES RHEUMATOID ARTHRITIS; EXOSOMES AND IFN-GAMMA DERIVED FROM HTLV-1 INFECTED CELLS ENHANCE THE INFLAMMATORY RESPONSE OF RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS VIA PATTERN RECOGNITION RECEPTOR, RIG-I

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Background: Human T cell leukemia type 1 (HTLV-1) positive rheumatoid arthritis (RA) patients show severe inflammatory state and resistance to anti-rheumatic therapy, including biologic agents (1). HTLV-1 infected T cells was increased in the synovial fluid and tissue from an HTLV-1 positive RA patients (2). However the mechanism of worsening RA by HTLV-1 infection remains unclear. We focused on the role of HTLV-1 infected T cells as a key player in the exacerbation of RA.

Objectives: To clarify the role of HTLV-1 infected T cells in the pathogenesis of RA. We investigate inflammatory mediators derived from HTLV-1 infected cells.

Methods: Peripheral blood mononuclear cells (PBMCs) were collected from asymptomatic HTLV-1 carriers (AC) (n=5) and healthy subjects (HS) (n=5). Rheumatoid arthritis synovial fibroblasts (RASFs) were co-cultured with PBMCs for 5 days. Cytokine profiles of supernatants were analyzed by multiplex. Exosomes were isolated and purified from cultured medium of HTLV-1 infected cell line (MT2). RASF was cultured with MT2 derived exosomes with and without IFN-gamma for 24hours. Total RNA was extracted using TRIZOL method. The expression of RIG-I, IL-6, CXCL10, and CCL5 mRNA in RASF was measured using real-time quantitative PCR. The expression of pattern recognition receptor, RIG-I was determined by immune blotting. Silencing of RIG-I in RASF was performed by transfection of siRNA against RIG-I.

Results: The levels of cytokine, including IFN-gamma, IL-2, IL-9, IL-13, IL-6, and CCL20, were higher in supernatants co-cultured with HTLV-1 positive PBMCs than in those of negative PBMC (p<0.05). The expression of CXCL10 and IL-6 mRNA was increased in RASF co-cultured with HTLV-1 positive PBMCs compared to those of negative PBMCs. IFN-gamma is well known to be an important cytokine in the pathogenesis of HTLV-1 associated inflammatory diseases. IFN-gamma induced the expression of IL-6, CCL5, and CXCL10 mRNA in RASF. HTLV-1 infected cell line, MT2, autonomously released a large amount of exosomes which contain nucleic acids such as RNA and DNA. MT2 derived exosomes significantly enhanced the expression of CXCL10 mRNA, but not IL-6 and CCL5, in RASF activated by IFN-gamma. Therefore, we hypothesized that exosomes play the role of ligand for pattern recognition receptors. IFN-gamma increased the expression of RIG-I protein in RASF in a dose-dependent manner. The expression of RIG-I protein also increased in RASF co-cultured with HTLV-1 positive PBMCs compared to those of negative PBMCs. Finally, the silencing of RIG-I suppressed the expression of CXCL10 in RASF induced by co-stimulation of both exosomes and IFN-gamma.

Conclusions: It is possible that HTLV-1 infected T cells exacerbate the inflammatory responses of RASFs. Exosomes derived from HTLV-1 infected cells enhance the expression of CXCL10 in RASF induced by IFN-gamma via pattern recognition receptor, RIG-I.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.2036

AB0022 CYTOTOXIC PROFILE CHARACTERIZATION OF NK AND NKT CELLS IN PATIENTS WITH BEHÇET DISEASE

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Background: Behçet disease (BD) is a rare inflammatory small vessel vasculitis. It is a chronic systemic disorder with multiorgan damage and various clinical manifestations such as oral ulcers, genital ulcers and uveitis. Etiology is still unknown. Some gene polymorphisms have been associated with BD [1]. In addition, a high frequency of circulating Natural Killer T cells (NKT cells) has been found in BD patients respect to patients with other inflammatory uveitis such as Vogt-Koyanagi-Harada disease (VKH) [2].

Objectives: The objective of this study was to characterize the cytotoxic profile of circulating Natural Killer (NK) and NKT cells in BD patients.

Methods: Peripheral Blood Mononuclear Cells (PBMCs) were collected from 23 BD patients (according to 1990 ISGB criteria), 7 VKH patients (according to 2001 Revised Diagnostic Criteria) and 9 healthy subjects [3,4]. BD activity was evaluated with BD Current Activity Form 2003. Anti-CD56 and anti-CD3 antibodies were used to identify NK (CD56⁺CD3⁻) and NKT (CD56⁺CD3⁺) cells by flow-cytometry. Expression of one inhibiting receptor (NKG2A) and five activating receptors (CD16, CD69, NKG2D, Nkp30 and Nkp46) was determined on the surface of NK and NKT cells. Cytotoxic potential of NK and NKT cells was assessed through incubation of PBMCs with K562 cells in presence or absence of IL-15 followed by flow-cytometry detection of the surface marker CD107a on NK and NKT cells [5].

Results: A higher frequency of NKT cells was detected in peripheral blood of BD patients than VKH patients. Compared to healthy subjects, an increased proportion of CD16 positive NKT cells was found in BD patients. Furthermore it was observed a higher percentage of NKG2D positive cells in both NK and NKT lymphocytes. No difference in the other markers was detected. In BD patients, the incubation of PBMCs with K562 cells in absence of IL-15 induced a higher percentage of NK cells expressing CD107a compared to VKH patients. Frequency of CD107a positive NKT cells was <1% and similar between groups. Finally, no differences were found between BD patients with active and inactive phase of the disease.

Conclusions: Our study confirms previous reports about an increased level of NKT cells in peripheral blood of BD patients, but we additionally identified a cytotoxic profile of NK and NKT cells characteristic of BD patients when compared to healthy subjects and patients with VKH. Our data revealed for the first time a potential involvement of NKG2D in the pathogenesis of BD. We can speculate that NK and NKT cells of BD patients are more prone to respond to stress/danger signals when exposed on target cells leading to cyclic auto-inflammation.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.3493

AB0023 ASSOCIATION BETWEEN MEX-SLEDAI AND INFECTIONS WITH MBL STRUCTURAL AND PROMOTER GENOTYPES IN MEXICAN-MESTIZO PATIENTS

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Background: The mannose-binding lectin protein (MBL) is a multimeric molecule with a structure that is the analogue to the C1q protein. Deficient and low MBL concentrations in serum are due to the presence of mutations in the structural or promoter region

Objectives: To investigate the role of alleles and haplotypes of MBL2 gene in the clinical expression of systemic lupus erythematosus (SLE) and its association with infections in Mexican-mestizo patients.

Methods: An observational, cross-sectional, retrospective study. We included 74 SLE patients and 75 matched controls. All ≥16 years-old who met at least four 1982 or revised 1997 ACR criteria for SLE were included. The association of MBL locus haplotypes with disease activity and past history of infection was studied in those patients. Allele and haplotype determinations in the promoter and structural regions of the MBL2 gene were performed from genomic DNA isolated peripheral blood. Probes were sent to Invitrogen (Carlsbad, California) for synthesis. The disease activity was determined by MEX-SLEDAI. Infections were categorized arbitrarily if patients had ≥4 events. The associations between the codons, clinical activity, and having ≥4 infection events were by odds ratio.

Results: There were 13/73 (17.8%) SLE patients with ≥4 infections. The presence of homozygous C/C codon 57 was observed to be greater risk for SLE activity and