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

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Background: Behçhet 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 Nautral 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 K526 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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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 $\geq$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 $\geq$4 events. The associations between the codons, clinical activity, and having $\geq$4 infection events were by odds ratio.
Results: There were 13/73 (17.8%) SLE patients with $\geq$4 infections. The presence of homozygous C/C codon 57 was observed to be greater risk for SLE activity and

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

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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).

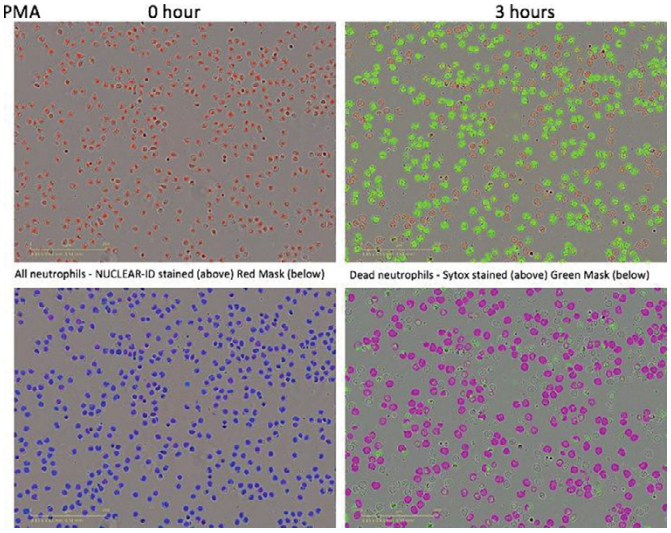

Figure 1

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

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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.
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Cytokines and inflammatory mediators

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

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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.