ABC transporters are involved in the eflux of MTX from cells. An increased expression and function of these transporters should decrease MTX concentrations in target cells, resulting in lack of therapeutic response. *ABCB1 3435* C/T (rs1045642) is a high frequency polymorphism, significantly associated with RA good responses, symptom remission and reduced adverse events, due to MTX treatment (3).
Thymidylate synthase (TYMS) is involved in thymidine synthesis. MTX decreases TYMS activity by inhibition and decreasing the access to tetrahydrofolate (THF) cofactors (1). The most common genetic variant of the TYMS gene consists of a 28 bp tandem repeat (rs34743033), with double and triple number of repeats (2R and 3R). The 3R allele genotype was associated with decreased efficacy and increased toxicity (4).
The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme is indirectly inhibited by MTX. The most common SNPs of the MTHFR gene are C677T (rs1801133) and A1298C (rs1801131). Both are associated with a decreased efficacy and an increased toxicity of MTX (5).
Conclusion: MTX response is affected by many gene variants; the effect of each variant separately is likely to be small. Additionally, gene-gene interaction, enhancing the potential role of linkage disequilibrium. This shows the emerging need for a better gene characterization and to improve the knowledge about variants distribution according to ethnicity, to explain different responses to MTX at an individual level.
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AB0015

STUDY OF VDR AND VDBP GENES AS CANDIDATE SUSCEPTIBILITY GENES FOR THE DEVELOPMENT OF IMMUNE-MEDIATED DISEASES IN THE PARAGUAYAN POPULATION

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Background: Immune Mediated Inflammatory Diseases (IMIDs) are complex diseases that are believed to have a strong interaction between the genome and the environment as part of their aetiology. In studies using the candidate gene strategy, genetic variation in a gene where functionality has been associated with the pathophysiology of the disease under study is being analyzed. In the last decade, polymorphisms of the vitamin D receptor (VDR) and VDBP genes have been more emphatically studied in IMIDs in different populations, but the results reported have not yet been conclusive.
Objectives: To identify an association between vitamin D receptor (VDR) and vitamin D-binding protein (VDBP) gene polymorphisms, and IMIDs in Paraguayan patients.
Methods: Association study of VDR (SNPs rs731236, rs7975232, rs2228570) and VDBP (rs4588) gene polymorphisms with susceptibility to IMIDs in Paraguayan population. A total of 399 patients with IMIDs (i.e. Systemic Lupus Erythematosus (SLE), Scleroderma (ES), Rheumatoid Arthritis (RA), and Cutaneous Psoriasis (CPS) and 100 hypernormal controls (HC) from the same population were included in this study. Genotyping was performed using Taqman real-time PCR-based technology (Life Technologies, USA). Statistical analysis was performed using Rv3.0.1 statistical language software (www.R-project.org). A p value ≤ 0.05 was used for statistical significance.
Results: A total of 399 individuals, 100 controls and 299 patients (99 RA, 100 SLE, 50 ES, and 50 PSO) were included. Seventy-six percent were female and 24% were male. The mean age was 43.7±14 years. Four SNPs were genotyped: rs731236, rs7975232, rs2228570, rs4588. The HWE test was not statistically significant for any of the 4 SNPs considered (P>0.05), confirming the quality of genotyping and the absence of technical bias. (Table 1).

Table 1. Genotyping of SNPs of the VDR and VDBP gene in Paraguayan population with IMIDs.

SNP	IMID	Minor Allele	Major Allele	MAF Control	MAF Case	OR	IC.L	IC.H	p allelic	P.Genetic
rs731236	SLE	G	A	0.5	0.4	0.64	0.42	0.97	0.035	0.08
rs731236	RA	G	A	0.5	0.41	0.69	0.46	1.05	0.071	0.12
rs731236	SS	G	A	0.5	0.42	0.71	0.42	1.18	0.18	0.37
rs731236	CPS	G	A	0.5	0.38	0.6	0.36	1.01	**0.049**	**0.042**
rs2228570	SLE	A	G	0.36	0.38	1.14	0.74	1.74	0.6	0.45
rs2228570	RA	A	G	0.36	0.31	0.83	0.53	1.28	0.4	0.56
rs2228570	SS	A	G	0.36	0.36	1.02	0.6	1.73	1	0.057
rs2228570	CPS	A	G	0.36	0.39	1.16	0.68	1.96	0.61	0.83
rs7975232	SLE	C	A	0.36	0.32	0.82	0.53	1.26	0.4	0.072
rs7975232	RA	C	A	0.36	0.29	0.72	0.46	1.12	0.14	0.064
rs7975232	SS	C	A	0.36	0.22	0.49	0.27	0.88	**0.012**	**0.0064**
rs7975232	CPS	C	A	0.36	0.41	1.21	0.72	2.03	**0.45**	**0.016**
rs4588	SLE	T	G	0.23	0.27	1.24	0.77	2	0.42	0.48
rs4588	RA	T	G	0.23	0.22	0.93	0.56	1.53	0.81	0.84
rs4588	SS	T	G	0.23	0.21	0.89	0.47	1.65	0.77	0.76
rs4588	CPS	T	G	0.23	0.29	1.37	0.76	2.43	0.26	0.53

Conclusion: There is evidence of nominal association between VDR SNPs: rs731236 (in SLE and CPS), and rs7975232 (in SS and CPS) and the presence of IMIDs disease in Paraguayan patients.
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Adaptive immunity (T cells and B cells) in rheumatic diseases

AB0016

THE IMPACT OF IL-17A THERAPY ON IGG SIALYLATION IN HUMANS

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Background: Rheumatoid arthritis (RA) is characterized by autoreactive B- and T cells. Autoantibodies are a hallmark of RA and contribute to synovial inflammation. We have recently demonstrated that Th17 cells suppress the enzyme ST6 a-galactoside b-2,6-sialyltransferase (ST6GAL1) in developing plasma cells. Thereby, Th17 cells regulate the degree of autoantibody sialylation leading to the increased inflammatory activity of autoantibodies. These events correlate with the onset of RA, arguing for a crucial role of the IL-23/Th17 axis during the transition of asymptomatic autoimmunity into active RA. Therefore, treatment against the IL-23/TH17-axis might present an attractive therapeutic approach to halt or delay RA's onset. However, the effects of Th17 cytokines like IL-17 on IgG glycosylation in humans are so far poorly studied.
Objectives: To explore whether anti-IL17A treatment can inhibit pro-inflammatory IgG glycosylation patterns in humans.
Methods: Total IgG from patient cohorts suffering from psoriatic arthritis (PsA) treated with Secukinumab (anti-IL-17 blockade, n=26) or Ustekinumab (anti-IL12/23 blockade, n=14) was compared with patients treated with anti-TNFa blockade as a control (n=20). The cohorts were age- and sex-matched and included patients being on therapy for at least six months. Total IgG was isolated using Protein G columns, and IgG glycopeptides of IgG1, IgG2, and IgG4 were analyzed using the LC-MS technique. The effect of IL-17 depletion on IgG glycosylation was analyzed in psoriatic arthritis patients who have been treated with secukinumab for at least six months. Furthermore, in a longitudinal approach, IgG1, IgG2, and IgG4 glycosylation were analyzed from samples, isolated before the beginning of anti-IL-17 blockade and after at least six months of therapy (n=16).
Results: Cross-sectional comparison of cohorts treated with Ustekinumab, Sekukinumab, and anti-TNFa therapy did not show any significant differences in sialylation, galactosylation, or fucosylation of IgG1 and IgG2. IgG4 from anti-TNFa treated patients displayed a small increase of sialylation when compared to the Ustekinumab treated cohort.
Longitudinal analyses, however, showed that IL-17A blockade during Secukinumab therapy caused a significant increase of sialic acid-rich IgG glycoforms on IgG1, IgG2 IgG4 patients, while the galactosylation, fucosylation remained unaffected.
Conclusion: This data indicates that IL-17A blockade specifically affects IgG sialylation, while other Fc-glycan modifications remain unaltered. This data confirms our recent findings in mice, where cytokines of the IL-23/Th17 axis specifically induce the production of hypo-sialylated, proinflammatory autoantibodies in

rheumatoid arthritis (RA) [2]. Therefore, neutralizing IL-17 might be a therapeutic option during the asymptomatic autoimmune prodromal phase in autoimmune diseases like RA, where TH17 cytokines orchestrate the emergence of a pro-inflammatory autoantibody response and the transition into active RA.

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AB0017 IMMUNE CHARACTERISTICS OF PERIPHERAL BLOOD IN SECONDARY SJOGREN'S SYNDROME PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Secondary Sjogren's Syndrome (sSS) is diagnosed when symptoms of SS coexist with other systemic connective tissue disease, often secondary to rheumatoid arthritis(RA).The occurrence of SS secondary with RA will worsen the course of disease and increase the high incidence and mortality of RA. At present, the immune characteristics of peripheral blood of sSS with RA are not clear.

Objectives: To observe the difference of immune Immune characteristics in peripheral blood between sSS secondary to RA, primary Sjogren's syndrome(pSS) and RA patients.

Methods: 20 sSS with RA patients, 20 pSS paients and 20 RA pateints hospitalized in ShanXi medical university the second Hospital were enrolled. The percentage and absolute numbers of lymphocyte phenotypes and CD4+ T subsets in peripheral blood were examined by flow cytometry.

Results: As for the percentage and absolute number of total T, B, NK, CD4+T,CD8+ T and the ratio of CD4 + T to CD8+ T cells, there was no significant difference between the sSS with RA, RA, and SS group. There was also no statistical difference in the percentage of CD4+T subsets(Th1,Th2,Th17 and Treg) between the three groups. But the ratio of Th17 and Treg in sSS with RA group was increased than pSS group.About comparison of absolute number of CD4+T subsets, there was no statistical difference among the three groups except that the Th1 cells in RA group was significantly higher than SS group.

Conclusion: Elevated Th17/Treg may be an immunological feature that differentiates sSS with RA patients from pSS patients. In addition, in general, peripheral blood of patients with RA and SS have similar immune characteristics.

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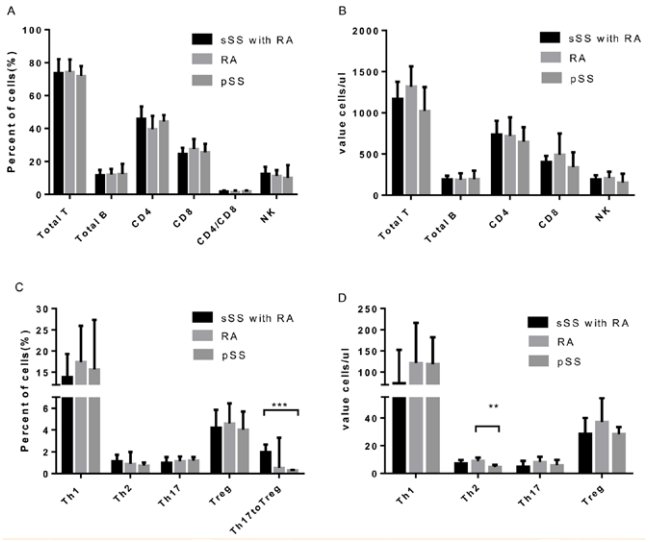

Figure 1. The comparsion about the lymphocyte phenotypes and CD4+ T subsets in peripheral blood of sSS with RA(n=20), pSS(n=20) and RA patients(n=20).(*p<0.05,**p<0.001,*p<0.0001)

Disclosure of Interests: None declared

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AB0018 ACCUMULATION OF FUNCTIONALLY MATURE CD1C+ DENDRITIC CELLS CONTRIBUTES TO SYNOVIAL INFLAMMATION IN INFLAMMATORY ARTHRITIS

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Background: Myeloid Dendritic Cells (DC) are potent antigen presenting cells that can be subdivided into CD141 and CD1c$^+$ DC. We have previously reported an unacknowledged role for CD141$^+$DC in the IA synovium. However, the identification and function of CD1c$^+$ DC in the IA synovium has yet to be fully elucidated.

Objectives: To investigate if CD1c$^+$DC reside in the IA synovium and ascertain if they represent a unique population, distinct from peripheral CD1c$^+$DC and if they contribute to synovial inflammation.

Methods: Synovial tissue (ST) biopsies and synovial fluid mononuclear cells (SFMC) were obtained via arthroscopy and healthy control (HC) ST was obtained during ACL surgery. Synovial tissue single cells suspensions were generated following enzymatic and mechanical digestion. Single cell analysis of synovial tissue cell suspensions, along with PBMC and SFMC was performed by multicolour flow cytometry. CD1c$^+$DC were sorted from IA synovial fluid and peripheral blood and bulk RNA sequencing was performed. CD1c$^+$DC functionality and maturation was assessed using OVA DQ phagocytosis assays, multiplex ELISA and DC: T cell cocultures.

Results: Within the circulation the frequency of CD1c$^+$DC are significantly decreased in IA peripheral blood compared to HC ($p<0.01$) in addition to expressing significantly higher levels of the maturation markers CD80 ($p<0.01$) and CD40 ($p=0.08$). IA peripheral blood DC also express significantly higher levels of CXCR3 ($p<0.01$) and CCR7 ($p<0.05$) compared to HC - suggestive of DC migration from the periphery to the synovium. Following RNA-seq analysis, IPA and differentially expressed gene (DEG) analysis revealed an enrichment in genes involved in DC maturation, TLR signalling and chemokine signalling in IA peripheral blood compared to HC. In support of the hypothesis that DC migrate and accumulate in the IA synovium, CD1c$^+$ DC were identified in IA ST and were significantly enriched compared to IA peripheral blood ($p<0.01$). IA ST CD1c$^+$DC express significantly higher levels of the activation marker CD80 compared to IA peripheral blood ($p<0.05$) or HC ST ($p<0.05$). Upon examination of IA synovial fluid, we report similar findings to ST, whereby CD1c$^+$DC are enriched in synovial fluid compared to PB ($p<0.001$). Moreover, RNA sequencing and PCA analysis of synovial versus blood CD1c$^+$DC revealed distinct transcriptional variation between both sites. Functionally, synovial CD1c$^+$DC express higher levels of the maturation markers CD80, CD83, CD40, PD-L1 and BTLA (all $p<0.05$) and have distinct coexpression of these maturation markers which is unique to the synovium. Synovial CD1c$^+$DC are less phagocytic compared to peripheral blood DC, have decreased production of MMP1 and MMP9 and importantly are still capable of additional activation *in-vitro*. Finally, synovial CD1c$^+$DC induce the proinflammatory cytokines TNFα, GMCSF, IL-17a and IFNγ from CD4$^+$ T-cells in allogeneic DC: T cells cocultures.

Conclusion: Mature circulatory CD1c$^+$DC migrate and accumulate in the IA synovium. Synovial DC are present in the IA synovium in a mature state, have distinct tissue specific characteristics and can induce proinflammatory CD4$^+$T cell responses.

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AB0019 TREATMENT OF AUTOIMMUNE AND INFLAMMATORY SKIN DISEASES USING SKIN-TARGETING BIFUNCTIONAL ANTIBODIES: A LOCALIZED IMMUNOMODULATION APPROACH

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