Conclusion: To our knowledge this is the first experimental controlled study demonstrating rituximab may play a role in inducing granulomatous hepatitis with a sarcoidosis-like reaction.

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AB0111 THE ROLE OF KLOTRO IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic systemic and disabling disease affecting 0.5–1% of the general population. It is characterized by the presence of autoantibodies including rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA). Klotho is a membrane-bound protein predominantly expressed in the kidney, parathyroid gland and choroid plexus that displays enzymatic and co-receptor functions, being involved in the prevention of aging, in the calcium-phosphate balance and in the reparative and fibrotic processes. Klotho seems to play an essential role in the maintenance of vessel tone control, in tissue regeneration and reparation and in the electrolytic balance.

Objectives: We aimed to compare serum Klotho levels between RA patients and healthy controls and the possible association between soluble Klotho with different characteristic of the disease.

Methods: This cross-sectional study was performed in Vega-Baja Hospital, Orihuela (Spain) from November 2016 to May 2018. We prospectively enrolled 63 consecutive women patients affected by RA and followed at the Vega-Baja Hospital (Orihuela, Spain) and 65 matched healthy women controls. All patients included in this study had normal serum creatinine (Cr) levels and met the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for RA. ACPAs were detected using a second-generation ELISA (ACPAs) kit (ORGENTEC Diagnostika GmbH, Mainz, Germany) while IgM RF was determined as part of routine analysis by turbidimetric assay (Siemens Healthcare Diagnostic Inc. NY, USA) according to the manufacturers' instructions. Serum Klotho was analyzed using ELISA.

Results: The mean serum total ACPAs and RF were 571.22±1040.89 U/ml and 173.73±751.8 U/ml, respectively. Serum Klotho concentrations were significantly higher in the RA patients than those in the control group: [4.68 (1.4–7.8) vs. 3.5 (0.6–6.1), ng/mL; $P < 0.0001$]. They were positively associated with the presence of ACPA and RF ($p < 0.05$). Klotho serum levels were higher in RA patients treated with biologic agents than in those undergoing conventional therapy ($p = 0.008$).

Conclusion: The increased serum Klotho levels in RA patients, especially in those undergoing biologic therapy, may indicate a potential implication in the pathogenesis of the disease.

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AB0112 EVALUATION OF A NEW MULTIPLEX TECHNOLOGY ASSAY FOR ANTI-CITRULLINATED PEPTIDE ANTIBODIES DETERMINATION

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Background: Anti-citrullinated peptides antibodies (ACPA) are highly specific RA markers. In 2010, experts included them in the ACR/EULAR criteria for disease diagnosis, usually, measured by ELISA. However, a new assay has been developed, recently: Multiplex technology, which allows searching IgG ACPA directed against four citrullinated peptides: Human (HCP1 and 2) and Viral (VCP1 and 2).

Objectives: First, assess ACPA diagnostic performances using Multiplex technology and then compare them to those obtained with the reference technique (ELISA).

Methods: We studied sera of 273 RA patients versus 165 controls (64 healthy controls and 101 patients with other pathologies). We realized ACPA dosage by Multiplex technology and compared them with ELISA assay results. In the end, we looked for a correlation between ACPA production and shared epitope expression in RA patients.

Results: The overall agreement calculated between the two methods was about 91.3%. As to the comparison of diagnostic values of Multiplex technology vs. ELISA, it revealed a sensitivity of 80.2% vs. 82.4% and a specificity of 95.8% vs. 95.5%. Then, for a better evaluation, we plotted ROC curves for ACPA detected by Multiplex technology and ELISA ones. Therefore, we obtained the following areas under the curve (AUC) (p value < 0.0001): Anti-: HCP1 (0.775), HCP2 (0.798), VCP1 (0.715) and VCP2 (0.837) and ACPA ELISA (0.867). Finally, our results reveal higher serum levels of ACPA detected by Multiplex technology, in case of SE expression [SE (+) vs. SE (-)] (p value < 0.0001).

Conclusion: Diagnostic performance of ACPA detection methods is comparable with relatively good agreement. Our study demonstrated an association between elevated serum levels of anti-HCP and anti-VCP and SE expression, in RA patients.

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AB0113 ARTHRITIS IS ASSOCIATED WITH CEREBROVASCULAR ENDOTHELIAL DYSFUNCTION: MECHANISTIC INSIGHTS IN THE RAT ADJUVANT-INDUCED ARTHRITIS MODEL

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Background: Stroke is the second cause of premature mortality and sudden death in rheumatoid arthritis (RA) after myocardial infarction¹. The mechanisms involved in the high risk of stroke are currently unknown, but data from the general population argue for a contribution of cerebrovascular dysfunction.

Objectives: The aim of our study was to investigate cerebrovascular function in the rat adjuvant-induced arthritis (AIA) model and to unravel the mechanisms involved, with special emphasis on the pathways regulating nitric oxide (NO) vascular availability.

Methods: Arthritis was induced in 6 weeks-old male Lewis rats by a single injection at the base of the tail of a suspension of *Mycobacterium butyricum* in Freund's incomplete adjuvant. A control group received saline. Thirty-three days after induction, middle cerebral artery (MCA) were dissected and mounted on two glass micropipettes in a small vessel arteriograph before being pressurized at 80 mmHg. Endothelial function was evaluated in vessels pre-contracted with serotonin (10^{-6} M) by measuring the relaxant effect of bradykinin (BK, 10^{-6} M), adenosine diphosphate (ADP, 10^{-6} M) and cumulative concentrations of acetylcholine (ACh, 10^{-12} to 10^{-4} M) in the presence or not of a Nitric Oxide Synthase (NOS) inhibitor (L-NAME, 10^{-4} M), an arginase inhibitor (nor-NOHA, 10^{-4} M), a NOS co-factor (BH₄, 10^{-7} M), an analog of superoxide dismutase (Tempol, 10^{-4} M). The relaxant response of vascular smooth muscle cells to a NO-donor (SNP, 10^{-12} to 10^{-4} M) was also evaluated.

Results: Vasodilation induced by BK, ADP and ACh were significantly reduced in AIA compared to controls rats ($p < 0.0001$). L-NAME decreased ACh-induced relaxation in controls but not in AIA rats. By contrast, nor-NOHA ($p < 0.0001$), Tempol ($p < 0.0001$) and BH₄ ($p < 0.02$) significantly improved ACh-induced relaxation in AIA but not in controls. The response to SNP was not different between the 2 groups.

Conclusion: Arthritis is associated with endothelial dysfunction in MCA. These results indicate that this model is relevant for mimicking the cerebrovascular impairments recently observed in RA patients². Cerebrovascular endothelial dysfunction relies on a low NOS activity, a high arginase activity and excessive superoxide anions production. Overall data suggest that therapies able to reverse the imbalance in NOS/arginase pathways would be efficient for reducing the incidence of cerebrovascular events in RA.

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AB0114

POTENTIAL THERAPEUTIC EFFECTS OF CENTAUREA CYANUS L. ON RHEUMATOID ARTHRITIS THROUGH CD38+ NK CELLS

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Background: CD38 catalyzes nicotinamide adenine dinucleotide (Coenzyme I, NAD+) to cyclic ADP ribose (cADPR). We previously reported that CD38 was specifically overexpressed in synovial membrane during rheumatoid arthritis (RA), and CD38+ natural killer (NK) cells are not only highly proportionate in the peripheral blood of RA patients but are also closely related to the disease severity. Cyanidin-3-O-glucoside (C3G) is an inhibitor of CD38 that has anti-inflammatory effects.

Objectives: This study aimed to determine the roles and mechanisms of CD38 and its inhibitor C3G on RA and provides a basis for C3G to become a potential therapeutic agent for RA.

Methods: This study employed bovine type II collagen-induced arthritis (CIA) rats, in vitro cultured RA synovial fibroblasts (RASFs) and mononuclear cells (MNCs) as models to explore the potential therapeutic effects and mechanisms of C3G on RA.

Results: Rats following C3G injections showed significantly alleviated CIA, while the concentrations of inflammatory interleukin (IL)-6 and interferon (IFN)- γ and the proportion of CD38+ NK cells decreased, the levels of anti-inflammatory IL-10 and the proportion of regulatory T (Treg) cells increased, in the peripheral blood and synovial fluids (Figure 1). After C3G treatment, the RASF proliferation and the level of IL-6 in the culture medium decreased, apoptosis increased. C3G also increased IL-2 and IL-10 secretion, and decreased IL-6 and IFN- γ level and

the proportion of CD38+ NK in MNCs. The coculture of CD38+ NK cells with MNCs depleted of CD38+ NK cells decreased the proportion of Treg cells and the IL-10 levels, meanwhile the coculture in the presence of C3G showed increased the proportion of Treg cells and the IL-10 levels and decreased IL-6 and IFN- γ level. However, C3G did not directly affect the Treg cell proliferation and their cytokine production. C3G treatment also increased sirtuin (Sirt)6 expression, while decreasing the expression of the NK activation receptor natural killer group 2D (NKG2D) in CD38+ NK cells. The expression level of NKG2D in CD38+ NK cells transfected with Sirt6 siRNA was not significantly changed in the presence of C3G. After CIA rats were injected with both C3G and OSS_128167, a Sirt6 inhibitor, the rats remained joint inflammation and the low proportion of Treg cells, although the proportions of CD38+ NK cells decreased in these CIA rats. After C3G treatment, the concentration of tumor necrosis factor (TNF)- α in the CD38+ NK culture medium increased and the concentration of IFN- γ decreased. When cocultured MNC and CD38+ NK cells were treated with C3G and TNF- α or C3G and anti-IFN- γ antibody, the proportion of IL-10+ Treg cells increased significantly, while the proportion decreased when cocultured MNC and CD38+ NK cells were treated with C3G and IFN- γ or C3G and anti-TNF- α antibody. The secretion level of TNF- α decreased sharply, and the concentration of IFN- γ increased significantly in the CD38+ NK cell culture treated with both Sirt6 siRNA and C3G.

Conclusion: C3G has therapeutic effects for CIA and RA. C3G decreases the proportion of CD38+ NK cells, and increases the expression of Sirt6 in CD38+ NK cells, which increases TNF- α secretion and decreases IFN- γ secretion and sequentially stimulates MNC differentiation into IL-10+ Treg cells and the secretion of IL-10. This study also suggests that the inhibition of MNC differentiation into Treg cells by CD38+ NK cells is an important cause of immune imbalance in RA and CIA (Figure 2).

Figure 1. Determining effect of C3G on rat CIA. A Joint inflammation of CIA rats with treatment of C3G. B X-ray observation of the rat joints. C Histochemical observation of the rat joint tissues. D Inflammation curve analysis based on paw swelling.

Figure 2. Pattern diagram explaining therapy mechanism of C3G to RA. C3G decreases CD38+ NK cells proportion and increases Sirt6 expression in CD38+ NK cells, which inhibits the NKG2D expression and results in increased TNF- α and decreased IFN- γ secretion. As a result, the proportion of IL-10+ Treg cells and IL-10 secretion elevates to exert therapy to RA

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