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 (BH_4, 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 BH_4 ($p<0.02$) 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 patients[2]. 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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Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2019-eular.266

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).

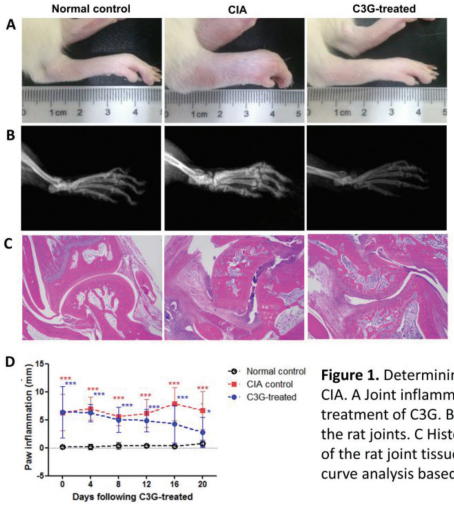

Figure 1. Determining effect of C3G on rat CIA. A Joint inflammation of CIA rats with treatment of C3G. B X-ray observation of the rat joints. C Histochemical observation of the rat joint tissues. D Inflammation curve analysis based on paw swelling.

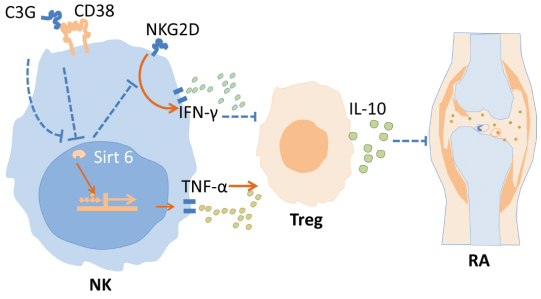

Figure 2. Pattern diagram explaining therapy mechanism of C3G to RA. C3G decreases CD38+ NK cells proportion and increases Sirt6 expression in CD38+ NK cells, which inhibits the NKG2D expression and results in increased TNF-α and decreased IFN-γ secretion. As a result, the proportion of IL-10+ Treg cells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

Acknowledgement: No applicable
Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2019-eular.1049