DIVISION OF RHEUMATOLOGY

MEDIZINISCHE UNIVERSITÄT WIEN

# The role of microRNA-155 in autoimmune arthritis

Eliana Goncalves-Alves[1], Victoria Saferding[1], Antonia Puchner[1], Robert Benson[2], Mariola Kurowska-Stolarska[2], James Brewer[2], Christopher Schliehe[3], Andreas Bergthaler[3], Josef Smolen[1], Kurt Redlich[1], Stephan Blüml[1]

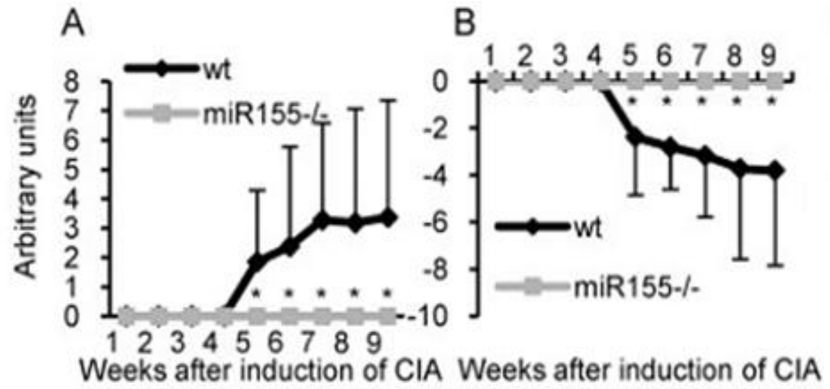
[1]Medical University Vienna, Dpt. of Rheumatology; [2]University of Glasgow, Institute of Infection, Immunity & Inflammation; [3]CeMM - Center for Molecular Medicine of the Austrian Academy of Sciences

## Background:

MicroRNA 155 (miR155) has been demonstrated to be essential for the development of collagen induced arthritis by controlling the generation of auto-reactive T and B cells. However, it is not fully understood which cells are responsible for this resistance.

## Methods:

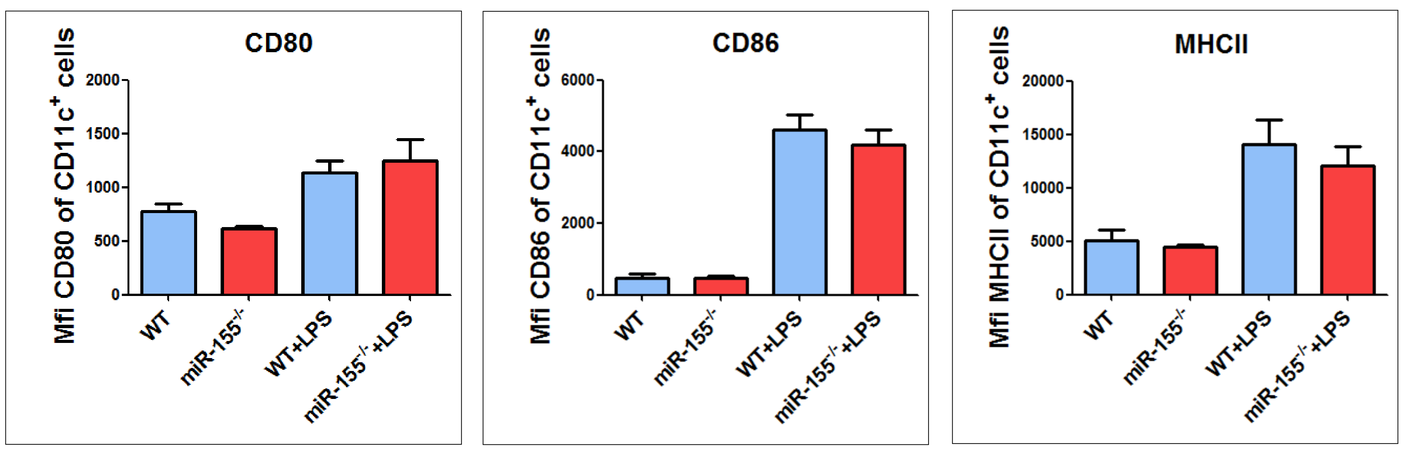
We analyzed activation and cytokine production of macrophages and dendritic cells (DCs) *in vitro* and *in vivo*, as well as their T-cells stimulatory capacity. MiR155 deficient mice were crossed into hTNFtg mice and arthritis development clinically as well as histologically was analyzed. OTII miR 155 deficient cells were obtained by crossing miR 155$^{-/-}$ and OTII mice.




**microRNA 155 deficient mice are resistant to collagen induced arthritis (animal model dependent on adaptive immune system)**

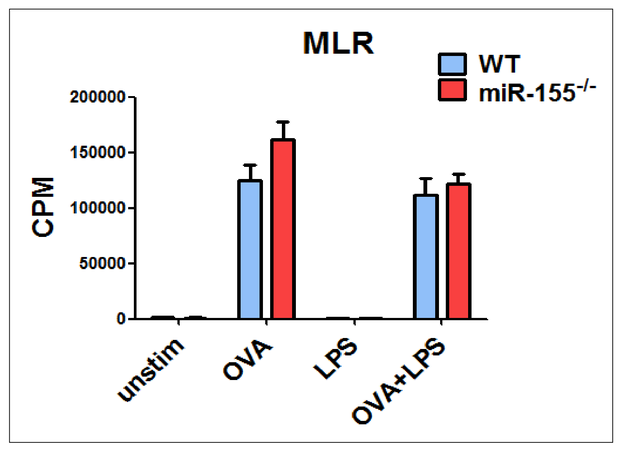
## The importance of miR-155 in innate immunity

A



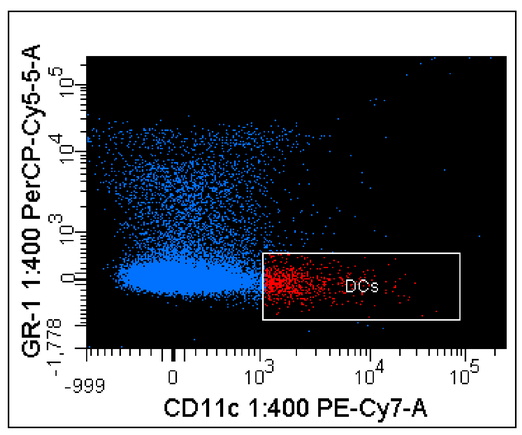

A, Flow cytometry analysis of bone marrow derived CD11c$^{+}$ cells, from wild type and miR-155$^{-/-}$ mice, treated without or with LPS (100µg/mouse) over night. Expression level of CD80, CD86 and MHCII of CD11c$^{+}$ cells (mean fluorescence intensity).

B

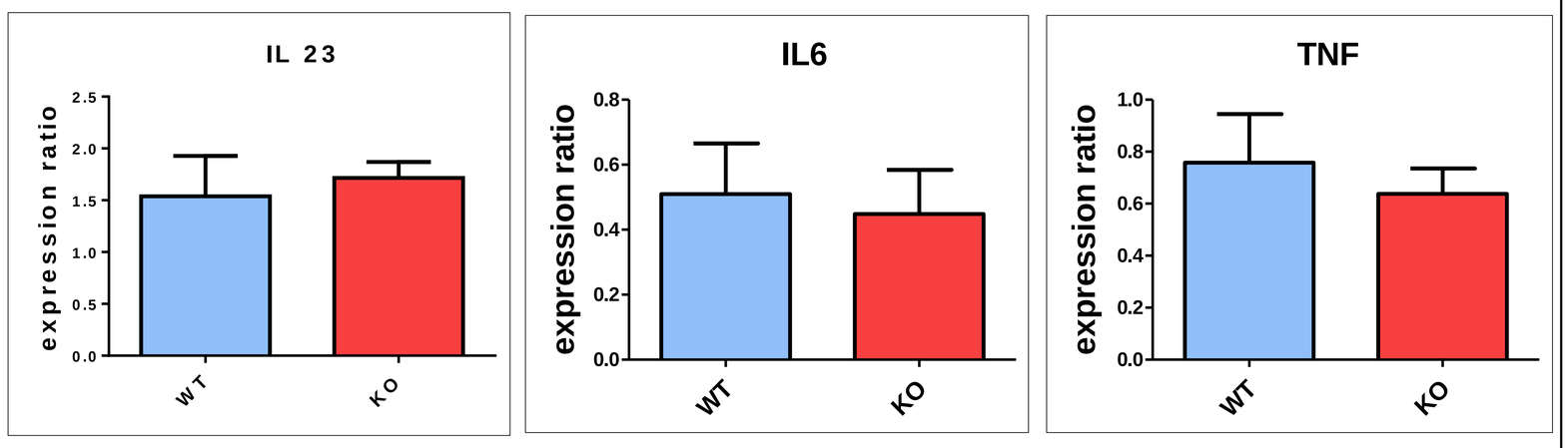



B, Mixed lymphocyte reaction to measure OTII cell stimulatory capacity of CD11c$^{+}$ cells from miR-155$^{-/-}$ and wild type mice. Cells were stimulated with Ovalbumin and LPS, proliferation was measured by $^{3}$H-Thymidine uptake.

C

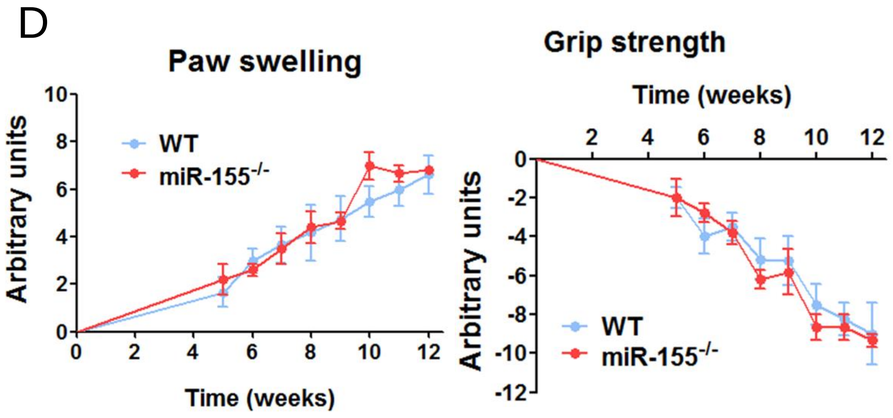



Analysis of mRNA expression



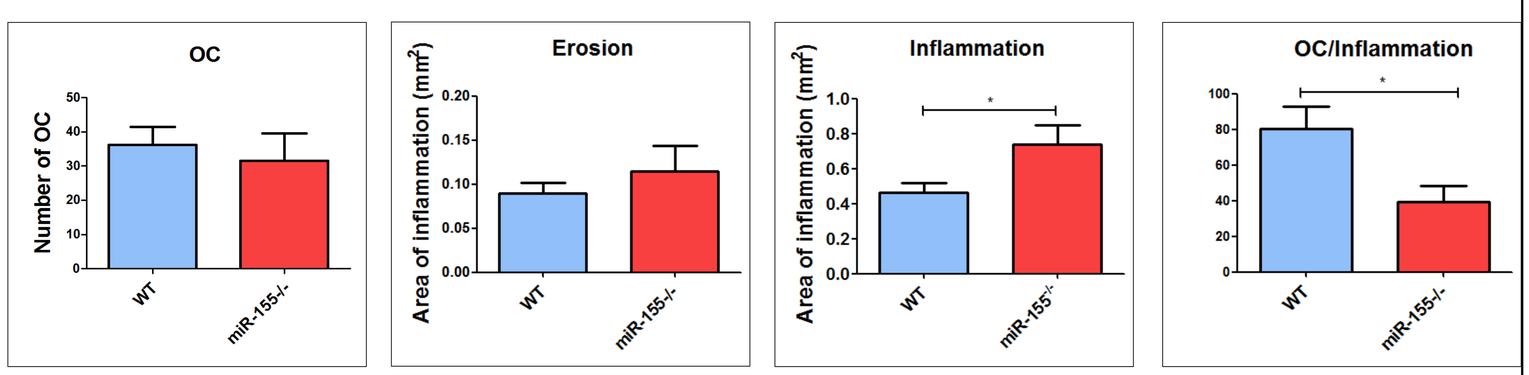

C, Spleens from LPS treated wild type and miR-155$^{-/-}$ mice were FACS-sorted for CD11c$^{+}$ cells. mRNA expression levels of proinflammatory cytokines were measured with quantitative real-time PCR.

D




D, Clinical assessment of paw swelling and grip strength was started at the age of 4 weeks up to 12 weeks old wild type and miR-155$^{-/-}$ mice.

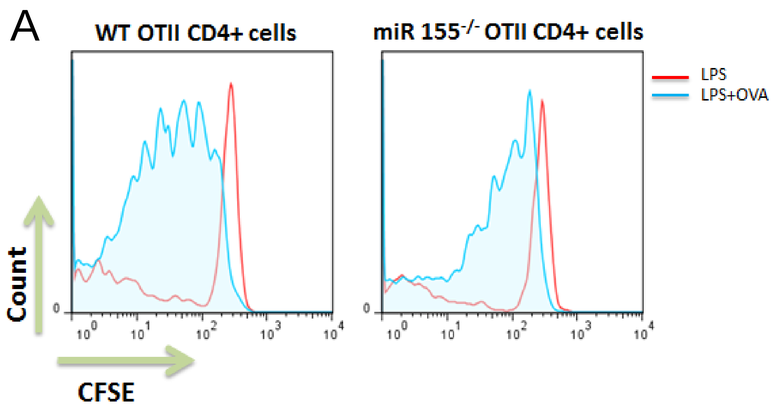
E




E, Histological assessment of inflammation, erosion, number of osteoclasts (OC) and OC per inflammation, in the tarsal area of the hind paws from hTNFtg/ miR155$^{-/-}$ compared to wild type mice.

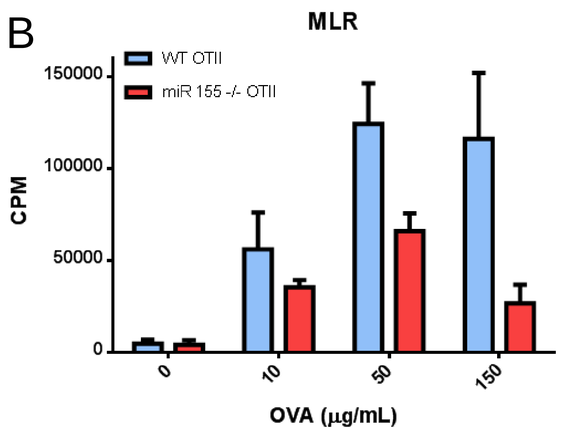
## Effect of miR 155 deficiency in T helper cells

A



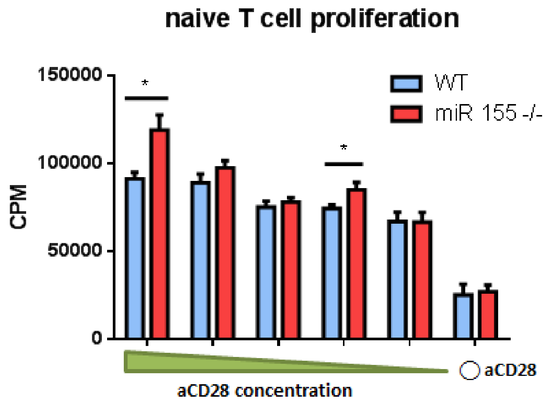

A, Proliferative capacity of wild type and miR-155 deficient OTII T helper cells *in vivo*. CD4+ cells were MACS isolated, label with CFSE and transferred intravenously into wild type Mice were immunized with LPS or Ovalbumin (OVA)+LPS. Evaluation of proliferation was done by flow cytometry.

B




B, Proliferative capacity of wild type and miR-155 deficient OTII T helper cells *in vitro*. CD4+ cells were MACS isolated and cultured together with bone marrow derived dendritic cells and different dosages of Ovalbumin. Proliferation was measured by $^{3}$H-Thymidine uptake.

C




C, Proliferative capacity of wild type and miR-155 deficient naive T cells. Sorted naïve T cells were stimulated with different concentrations of anti-CD28 together with anti-CD3 or CD3 alone. Proliferation was measured by $^{3}$H-Thymidine uptake.

## Conclusion:

In conclusion, contrasting with its limited influence in innate immunity dependent arthritis, miR-155 plays a central role in adaptive autoimmune arthritis. Therefore miR-155 might represent an interesting therapy target for autoimmune diseases such as rheumatoid arthritis.