27 CONDITIONAL MODEL TO STUDY THE TISSUE- AND TIME SPECIFIC EFFECTS OF NADPH OXIDASE 2 -DERIVED REACTIVE OXYGEN SPECIES DURING ARTHRITIS

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Background and objectives Neutrophil cytosolic factor 1 (NCF1) is the key regulatory component of the phagocytic NADPH oxidase 2 (NOX2) complex, and mutations in this gene lead to lowered production of reactive oxygen species (ROS). *Ncf1* has been linked to rheumatoid arthritis, and naturally occurring mutations in this gene have shown to increase arthritis severity in rodents. Interestingly, ROS production protected rodents from autoimmunity which is against the dogma that ROS are solely harmful in inflammation. In this study, the authors aimed at generating targeted *Ncf1* knock-in mice in which *Ncf1* expression can be induced in preferred cell type or at a desired time during the disease progression. This enables us to identify the critical cell type and the time window during which the *Ncf1* expression would protect the mice from arthritis.

Materials and methods The authors generated the targeted *Ncf1* knock-in mice on a pure C57BL/6N genetic background. Those mice were compared with B10Q.$Ncf1^{m1J}$ mice which have an intronic SNP in their *Ncf1* gene, leading to lowered ROS production by the NOX2 complex. In the *Ncf1* knock-in mice *Ncf1* expression was induced in vivo by Cre or FLP recombinase, and the gene activation was followed by analysing a simultaneously activated EGFP expression, used as a reporter gene. NCF1 protein variants were generated by in vitro mutagenesis and their activity assessed by luminometric ROS measurements.

Results The targeting silenced the *Ncf1* gene as intended. The authors observed that, in terms of ROS production, the B10Q $Ncf1^{m1J}$ mice and the knock-in mice were similar to each other. The observation was supported by the data indicating that the NCF1 protein variants expressed at low levels in the B10Q $Ncf1^{m1J}$ mice were completely defective in activating the NOX2 complex to produce ROS. After activating the targeted knock-in gene by recombination, using universally expressed Cre or FLP recombinase, the cell specific expression pattern of NCF1 was similar to that of the wild type mice, mostly showing expression in granulocytes and other cells known to make oxidative burst.

Conclusions The data indicated that the knock-in mice produced do not express functional *Ncf1*, while the NCF1 expression can be activated by universally expressed Cre of FLP recombinase, inducing a physiological expression pattern of the protein. The model can, thus, be utilised to study the spatiotemporal effects of reactive oxygen species generated by the NOX2 complex.