A92 A20 (TNFAIP3) DEFICIENCY IN MYELOID CELLS TRIGGERS RHEUMATOID ARTHRITIS

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Background and objectives Rheumatoid arthritis is an inflammatory autoimmune disease characterised by chronic inflammation of the joints associated with progressive destruction of cartilage and bone. Deregulated cytokine production is known to contribute to the aetiology of rheumatoid arthritis but the underlying molecular mechanism is still unclear. A20 (also known as TNFAIP3) is a protein that is involved in the negative feedback regulation of nuclear factor-κB (NF-κB) signalling in response to specific proinflammatory stimuli in different cell types. To define the contribution of A20 to rheumatoid arthritis pathology, the authors generated mice deficient in A20 in myeloid cells, B cells or T cells.

Materials and methods Conditional A20/*tnfaip3* knockout mice were generated. $A20^{FL/FL}$ mice were crossed with LysM-Cre transgenic mice to generate a myeloid-specific A20 knockout mouse. T and B cell-specific A20 knockout mice were obtained by crossing with CD4-Cre and CD19-Cre transgenic mice, respectively. Mice were scored twice a week for development of peripheral arthritis, until they were killed. Histological analysis was performed on paraffin embedded mouse paws. Immunofluorescence stainings for B220, CD3, F4/80 were performed and analysed by confocal microscopy. In separate experiments, in vivo monitoring of inflammation by positron emission tomography-CT was conducted using fluorodeoxyglucose. Peritoneal and bone marrow-derived macrophages were isolated and stimulated with various toll-like receptor (TLR) ligands. Cell lysates were subject to Western blot analysis for IκBα, phospho-IκBα, phospho-JNK, phospho-Erk and phospho-p38, A20 and actin. Quantitative real-time PCR for interleukin 1β (IL-1β), IL-6, IL-23 was conducted on joint tissue. In vitro induction of osteoclast formation was done by macrophage colony-stimulating factor and receptor activator of NF-κB. Tartrate resistant acid phosphatase staining and Pit-forming assays were conducted.

Results Specific ablation of A20 in myeloid cells, but not in B or T cells, resulted in spontaneous development of a severe destructive polyarthritis with many features of rheumatoid arthritis. Myeloid A20 deficient mice have high levels of tumour necrosis factor (TNF) and other inflammatory cytokines in their serum, consistent with a sustained NF-κB activation and higher TNF production by cultured macrophages. Remarkably, the authors observed no histological evidence of inflammation or damage in other tissues of myeloid A20 deficient mice. Secondary lymphoid organs were markedly enlarged and the number of myeloid cells in the spleen was increased. Destructive polyarthritis in myeloid A20 knockout mice was TLR4/MyD88 dependent. Myeloid A20 deficiency also promoted osteoclastogenesis in mice.

Conclusions These observations indicate a critical and cell-specific function for A20 in the aetiology of rheumatoid arthritis, supporting the concept of developing A20 modulatory drugs as cell targeted therapies.