

A20 Protects Against Arthritis by Regulation of the NLRP3 Inflammasome

Zahid Manzoor and Young-Sang Koh*

Department of Microbiology and Immunology, School of Medicine and Brain Korea 21 PLUS Program, and Institute of Medical Science, Jeju National University, Jeju, Korea

Rheumatoid arthritis is an autoinflammatory disease that primarily affects joints and is characterized by pervasive joint inflammation. A20/Tumor necrosis factor, alpha-induced protein 3 (Tnfaip3) inhibits activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and has been associated with rheumatoid arthritis. However, the precise role of A20 in rheumatoid arthritis remains unclear. Deletion of *A20/Tnfaip3* gene in mice elicits impulsive erosive polyarthritis that is similar to rheumatoid arthritis in patients. Recently, it has been shown that A20 protects against arthritis by regulating the NLRP3 inflammasome.

Key Words: A20, Nod-like receptor pyrin domain-containing protein 3, Inflammasome, Rheumatoid arthritis

In Nature on 7th August 2014, Vande Walle *et al.* reported that negative regulation of the NLRP3 inflammasome by A20 protects against arthritis (1). Various pattern recognition receptors including C-type lectin receptors, Toll-like receptors, Retinoic acid inducible gene (RIG-I)-like receptors and Nod-like receptors (NLRs) are responsible for recognition of molecular patterns highly conserved in invading pathogens (2). NLRP3 is one of the members of NLR family of intracellular proteins that plays critical role in innate immunity (3). Inflammasome is a complex multiprotein structure comprising of NALP, PYCARD and caspase-1. The precise composition of an inflammasome relies on the stimuli which recruit inflammasome assembly (3, 4). Activation of inflammasome leads to proteolytic processing and release of inflammatory cytokines interleukin (IL)-1 β and IL-18. It

results in activation of inflammatory processes and induces a programmed cell death called as pyroptosis (4).

A20/Tnfaip3 is a deubiquitinating protein and it negatively regulates nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) by deubiquitinating specific NF- κ B signaling molecules (5). A20-deficient mice die within 2 weeks of birth due to spontaneous development of multiorgan inflammation and cachexia (6). Recently, it has been shown that several single nucleotide polymorphism (SNPs) in the human TNFAIP3 loci are associated with higher susceptibility to systemic lupus, Crohn's disease, psoriasis, rheumatoid arthritis and type 1 diabetes, indicating that lack of A20 expression could be associated to specific autoimmune diseases (7). Nevertheless, the precise role of A20 expression in various diseases is still unidentified.

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*Corresponding author: Young-Sang Koh. Department of Microbiology and Immunology, Jeju National University School of Medicine, 102 Jejudaehakno, Jeju 690-756, Korea.

Phone: +82-64-754-3851, Fax: +82-64-702-2687, e-mail: yskoh7@jejunu.ac.kr

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Here, we summarize the results of Vande Walle *et al.* that A20 negatively regulates the NLRP3 inflammasome and protects against arthritis (1).

Vande Walle *et al.* generated mice carrying A20 deletion in myeloid cells which is susceptibility gene for rheumatoid arthritis (1). Lipopolysaccharide (LPS) stimulation in $A20^{myel-KO}$ macrophages was unable to induce A20 messenger RNA, confirming the efficacy of A20 deletion in myeloid cells (1). As MyD88 functions downstream of both interleukin-1 receptor (IL-1R) and TLRs, they investigated the role of IL-1 signalling in rheumatoid arthritis pathogenesis (1). All $A20^{myel-KO}Il1r1^{+/+}$ mice developed spontaneous arthritis whereas $A20^{myel-KO}Il1r1^{-/-}$ mice were significantly protected against clinical signs of arthritis (1). $A20^{myel-KO}Il1r1^{+/-}$ (heterozygous for IL-1R1) mice showed transitional arthritic phenotype and these results were further confirmed by histological examination of ankle joints (1). Histological examination of ankle joint sections of $A20^{myel-KO}Il1r1^{+/-}$ mice exhibited substantial synovial and periarticular inflammation along with infiltrated mononuclear cells (1). In comparison the haematoxylin and eosin staining of ankle joints of $A20^{myel-KO}Il1r1^{-/-}$ mice showed less number of infiltrating inflammatory cells (1). Collectively, these results suggest that production of IL-1 is injurious for arthritis pathogenesis in $A20^{myel-KO}$ mice (1).

Caspase-1 is a cytosolic enzyme, which proteolytically cleaves precursor forms of cytokines IL-1 β and IL-18 into active mature peptides (4). To investigate the contribution of A20 in inflammasome signalling, caspase-1 processing was assessed in macrophages of $A20^{myel-KO}$ and wild-type mice (1). Upon adenosine triphosphate (ATP) or nigericin treatment, LPS-primed $A20^{myel-KO}$ macrophages showed increased activation of caspase-1 compared to wild-type macrophages (1). Secretion of IL-1 β in ATP or nigericin-treated $A20^{myel-KO}$ macrophages was significantly higher as compared to wild-type cells (1). Immediately after ATP or nigericin treatment, higher caspase-1 autoprocessing, IL-1 β secretion and the initiation of caspase-1-dependent pyroptosis was evident in $A20^{myel-KO}$ macrophages (1). The amplified sensitivity of $A20^{myel-KO}$ macrophages towards activation of inflammasome was limited to the Nlrp3 inflammasome (1).

AIM2 or Nlrp4 inflammasome showed similar levels of caspase-1 processing and induction of pyroptosis in $A20^{myel-KO}$ and wild-type macrophages in response to cytosolic double-stranded DNA stimulation or infection with *Salmonella enterica serovar Typhimurium* respectively (1). These results suggest that A20 negatively regulates caspase-1 activation by the Nlrp3 inflammasome and there is no role of AIM2 and Nlrp4 inflammasome (1).

TLRs signalling activate transcription factor NF- κ B which results in upregulation of the *Nlrp3* expression and production of proIL- β as well as proIL-18 (4). It has been shown that A20 is essential for cessation of Toll-like receptor responses by interfering with NF- κ B translocation (5). In wild-type macrophages Nlrp3 inflammasome activation is snugly regulated at various levels (4). LPS-stimulation in A20-deficient bone-marrow-derived macrophages resulted in increased expression of Nlrp3 mRNA (1). In agreement to mRNA expression level, production of Nlrp3 and proIL-1 β was also increased in LPS-induced $A20^{myel-KO}$ macrophages as compared to wild-type cells (1). The pharmacological inhibition of inhibitor of kappa B kinase (IKK) resulted in reduced Nlrp3 levels in LPS-induced $A20^{myel-KO}$ macrophages (1). Furthermore, TCPA-1 which is selective inhibitor of IKK2, considerably reduced ATP-induced caspase-1 auto processing and IL-1 β secretion in LPS-primed $A20^{myel-KO}$ macrophages (1). Collectively, these data suggest that A20 negatively regulates activation of Nlrp3 inflammasome (1).

Authors speculated that undue activation of Nlrp3 might drive rheumatoid arthritis pathology in $A20^{myel-KO}$ mice upstream of IL-1R1 (1). $A20^{myel-KO}Nlrp3^{+/+}$ mice showed redness and swelling of the hind paws at the age of 11 weeks and become more prominent with the increasing age (1). $A20^{myel-KO}Nlrp3^{-/-}$ mice showed marked protection from rheumatoid arthritis as confirmed by their normal hind paws (1). $A20^{myel-KO}Nlrp3^{-/-}$ mice showed reduced periarticular inflammation and less infiltration of mononuclear cells compared with $A20^{myel-KO}Nlrp3^{+/+}$ mice tissue (1). In comparison to $A20^{myel-KO}Nlrp3^{-/-}$ mice, the hind paws of $A20^{myel-KO}Nlrp3^{+/+}$ mice showed sever loss of bone thickness establishing the central role for Nlrp3 in the development of rheumatoid arthritis (1).

Authors also investigated the effect of caspase-1/11 deficiency on development of rheumatoid arthritis in *A20^{myel-KO}* mice (1). *A20^{myel-KO}Casp1/11^{-/-}* mice showed reduced circulation of IL-1 β as compared to *A20^{myel-KO}Casp1/11^{+/+}* mice (1). Like *A20^{myel-KO}Nlrp3^{-/-}* mice, production of IL-6 in serum was significantly reduced in *A20^{myel-KO}Casp1/11^{-/-}* mice (1). Furthermore, hind paws of *A20^{myel-KO}Casp1/11^{+/+}* mice showed clear inflammation (1). In comparison, *A20^{myel-KO}Casp1/11^{-/-}* mice exhibited very mild or no clinical signs of arthritis (1). Likewise, haematoxylin and eosin staining study of joint sections in arthritic *A20^{myel-KO}Casp1/11^{+/+}* mice revealed substantial mononuclear cell infiltration related with marked articular and synovial inflammation (1). In disparity, joints of *A20^{myel-KO}Casp1/11^{-/-}* mice showed significant protection from the mononuclear cell infiltration and synovial inflammation (1).

Vande Walle *et al.* showed that A20 negatively regulates the Nlrp3 inflammasome and protects against arthritis (1). Furthermore, the authors revealed that undue activation of Nlrp3 inflammasome leads to development of arthritis in *A20^{myel-KO}* mice (1). *A20^{myel-KO}* mouse model might be appropriate for studying the efficacy of therapies for targeting inflammasomes and/or IL-1 signalling in rheumatoid arthritis.

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