A20-binding inhibitor of NF-κB (ABIN1) controls Toll-like receptor-mediated CCAAT/enhancer-binding protein β activation and protects from inflammatory disease

Jingran Zhoua, Ruiqiong Wu, Anthony A. High, Clive A. Slaughterb,1, David Finkelsteinc, Jerold E. Rhegc, Vanessa Redecke, and Hans Häckerb,2

Departments of aInfectious Diseases and bPathology and cHartwell Center for Bioinformatics and Biotechnology, St. Jude Children’s Research Hospital, Memphis, TN 38105

AUTHOR SUMMARY

Inflammation is initiated by immune cells upon exposure to noxious stimuli, such as infectious agents and tissue injury, and is characterized by immune cell infiltration, the release of molecules that promote inflammation (i.e., proinflammatory mediators), and leakage of plasma (i.e., blood fluid) into tissues. These changes eventually result in the hallmark clinical symptoms of inflammation, such as redness and swelling with heat and pain, and possibly disturbed organ function (1). Different receptor systems, including Toll-like receptors (TLRs), are used to recognize noxious stimuli and activate diverse signaling pathways that control proinflammatory mechanisms, such as the production of cytokines (molecules that stimulate the immune system) and cytotoxic mediators, including nitric oxide species. As a physiological part of an immune response (e.g., upon pathogen challenge), inflammation is essential for immune defense; however, exaggerated or persistent inflammation leads to tissue injury and possibly to organ failure. Specific Toll-like receptors (i.e., TLR9 and TLR7) and their signaling pathways have been implicated in the persistent inflammatory diseases systemic lupus erythematosus and psoriasis, but the etiology or causes of these diseases remain largely unknown. In this study, we used a biochemical approach and identified the protein A20-binding inhibitor of NF-κB (ABIN1) as a component of Toll-like receptor signaling pathways. We evaluated the role of ABIN1 in inflammation by generating ABIN1-deficient mice. These mice developed an inflammatory disease that shared many characteristics with human systemic lupus erythematosus, consistent with ABIN1’s function as an anti-inflammatory molecule in normal mice. Moreover, we found that ABIN1 negatively regulates CCAAT/enhancer-binding protein β (C/EBPβ), a protein that has been shown to control a select repertoire of immune functions. These findings may have important implications for the development of novel and more selective therapeutic strategies for these diseases.

Persistent, chronic inflammatory diseases, such as systemic lupus erythematosus and psoriasis, are thought to involve different types of immune cells whose specific effector functions contribute to the development of disease. Innate immune cells, such as macrophages and granulocytes, produce a variety of factors upon Toll-like receptor activation, including cytokotoxic substances and antimicrobial peptides, which they normally use to combat infectious agents but which may damage host tissue as bystander effect. Other immune cells, known as “B-lymphocytes,” produce molecules called “antibodies,” which bind and inactivate pathogens but under certain circumstances may be directed against host tissue, including DNA, a phenomenon typically found in patients who have systemic lupus erythematosus.

Systemic lupus erythematosus can affect almost any organ, including skin, brain, joints, and kidney. Psoriasis is limited initially to the skin but progresses toward arthritis in as many as 10–30% of cases. The two diseases seem to involve similar mechanisms. First, tissue injury, e.g., during an infectious challenge, leads to release of host factors, including DNA and/or RNA, that form complexes with autoreactive antibodies (systemic lupus erythematosus) or antimicrobial


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

1Present address: Georgia Health Sciences University-University of Georgia at Athens Medical Partnership, Athens, GA 30602.

2To whom correspondence should be addressed. E-mail: hans.haecker@stjude.org.

See full research article on page E998 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1106232108.

www.pnas.org/cgi/doi/10.1073/pnas.1106232108
pathways induced by TNF-α, an important inflammatory cytokine (5); however, the function of ABIN1 in the Toll-like receptor pathway had not been investigated. After we identified ABIN1 biochemically as a component in Toll-like receptor signaling pathways, we generated ABIN1-deficient mice to study the function of ABIN1 in vivo and during Toll-like receptor activation. We found that ABIN1-deficient mice developed a progressive, lupus-like inflammatory disease characterized by expansion of myeloid cells, the infiltration of immune cells into different organs such as the kidneys, liver, and lung, the activation of lymphocytes, and the appearance of autoreactive antibodies. Kidneys developed disease, reflected by inflammation (glomerulonephritis) and proteinuria. Surprisingly, ABIN1-deficient macrophages exhibited normal regulation of major proinflammatory signaling pathways, i.e., the NF-κB and MAPK pathways. These mice also showed normal, if not reduced, expression of respective target genes encoding TNF-α, IL-12, and IL-6. However, ABIN1-deficient macrophages exhibited a selective increase in the expression of the transcription factor C/EBPβ and its target genes, such as colony-stimulating factor 3 (Csf3), nitric oxide synthase 2, inducible (Nos2), and S100 calcium binding protein A8 (S100a8), whose gene products are linked intimately to innate immune cell proliferation (G-CSF), cytotoxicity (nitric oxide species), and host factor-derived inflammation (S100A8). Although the TLR-dependent signal-transduction pathway that controls C/EBPβ activity is largely undefined, it appears that ABIN1 controls the ratio of the two major forms of the mature C/EBPβ protein, i.e., liver-enriched transcriptional activator protein (LAP) and liver-enriched inhibitory protein (LIP), favoring expression of the transcriptionally active LAP form. Active C/EBPβ also regulates its own promoter via a characterized feed-forward loop, possibly accounting for the sustained increase of C/EBPβ activity observed in ABIN1-deficient cells.

Together, our data reveal that ABIN1 is an essential anti-inflammatory component of the Toll-like receptor signaling pathways controlling the activity of C/EBPβ, whose target genes may explain, at least in part, the chronic inflammatory response in ABIN1-deficient mice. Based on these data, we propose a model for inflammatory disease development depicted in Fig. P1. In this model, we propose the deregulation of Toll-like receptor signaling pathways in innate immune cells as an initiating factor. Importantly, in contrast to other models based on mutations in other negative regulatory molecules and the suspected role of major proinflammatory signaling pathways (e.g., the NF-κB and MAPK pathways), this model emphasizes the role of the C/EBPβ pathway and a rather select set of Toll-like receptor–induced functions as potential culprits in inflammatory disease development. Given the genetic link between ABIN1 (TNIP1) and human systemic lupus erythematosus, the observations made in ABIN1-deficient mice may have important implications for human disease and the development of novel, possibly more selective therapeutic strategies.