Inflammasome

Negative regulation of inflammasome activation in bats

Previous studies have shown that bats have altered expression and function of inflammasome components, and it is suggested that their ability to dampen inflammatory pathways might contribute to their longevity and propensity for asymptomatic viral infections. Ahn et al. extend these findings by showing that bat ASC2 negatively regulates inflammasome activation in both sterile and infectious settings.

ASC2 was identified in the genomes of 13 bat species examined, in contrast to previous reports that it is a primate-specific gene. Whereas most human tissues have no or minimal expression of *ASC2* mRNA, bat myeloid cells constitutively expressed high levels of *ASC2*. Furthermore, levels of bat ASC2 protein were higher than levels of human ASC2 in cell lines with similar mRNA levels.

In human HEK293T cells reconstituted with the inflammasome sensors NLRP3 or AIM2 and the inflammasome adaptor ASC, overexpression of bat ASC2 inhibited inflammasome activation by forming long filaments that decreased ASC speck formation (aggregation of cytosolic ASC). Bat ASC2, but not human ASC2, was shown to colocalize with human ASC. As ASC2 lacks the caspase-recruitment domain that is present in ASC, this disrupted the downstream recruitment and activation of caspase-1 and processing of the pro-inflammatory cytokine IL-1β. In a human monocytic cell line or human keratinocytes primed with lipopolysaccharide and activated with stimuli of NLRP3, NLRP1 or AIM2 inflammasomes, bat ASC2 inhibited ASC speck formation, IL-1β release and pyroptosis more potently than human ASC2. Thus, expression of bat ASC2 inhibits activation of several types of inflammasome by different stimuli in human cells.

To investigate the role of ASC2 in inflammasome-associated disease, the authors generated a transgenic mouse model with doxycycline (Dox)-inducible expression of bat *ASC2* in the absence of endogenous ASC2 expression, which mice lack. Bone marrow-derived macrophages from transgenic mice treated with Dox had reduced inflammasome activation in response to NLRP3, AIM2 and NLRC4 stimuli. In a model of sterile peritonitis induced by NLRP3 or ASC activation, inflammation was significantly reduced in Dox-administered, ASC2-expressing mice, correlating with decreased IL-1β in peritoneal lavage fluid. In models of viral infection with influenza A virus, Zika virus or *Pteropine orthoreovirus 3*, IL-1β production was significantly reduced in mice expressing bat ASC2 in the presence of similar viral loads. Mortality of mice infected with H1N1 influenza A virus decreased from 100% to 50% when bat ASC2 was expressed.

The therapeutic potential of bat ASC2 to dampen inflammation was shown by fusing it to a cell-penetrating peptide for intracellular delivery, which inhibited human NLRP3 inflammasome activation in a dose-dependent manner. Treatment with the cell-penetrating ASC2 fusion protein reduced IL-1β production by primed human monocytes activated with SARS-CoV-2 immune complexes.

The authors identified 26 mismatches in the amino acid sequences of bat and human ASC2 and carried out site-directed mutagenesis of these sites in bat ASC2 to identify four mutations that disrupted its ability to inhibit inflammasome activation; these four key residues were almost entirely conserved in another five bat species analysed. Site-directed mutagenesis of human ASC2 to substitute these four sites with the bat residues led to increased protein expression and ability to inhibit NLRP3 inflammasome activation in human and mouse cells. Evolutionary analysis of *ASC2* and *ASC* sequences across mammals indicated that *ASC2* of placental mammals (including bats) share a common ancestor; in placental mammals, most ASC2 proteins have an inhibitory effect that seems to have been lost in primates, but most species except bats express very low levels of ASC2. Only in bats and the blind mole rat *Nannospalax galili*, both of which are very long-lived relative to body size, is ASC2 expressed and functional.

These results indicate the insights that can be gained from studying immunology in non-model organisms such as bats, and suggest that the unique properties of bat ASC2 could be exploited to inhibit inflammatory disease in humans.

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Related article: Wang, L.-F. et al. Decoding bat immunity: the need for a coordinated research approach. *Nat. Rev. Immunol.* **21**, 269–271 (2021)