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INSIGHTS

A20 and ABIN-1 team up against intestinal epithelial cell death

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A20 and its binding partner ABIN-1 are genetically linked to inflammatory diseases. In this issue of *JEM*, Kattah et al. (https://doi.org/10.1084/jem.20180198) demonstrate that simultaneous deletion in a mouse model leads to instantaneous cell death in the intestinal epithelium and mortality.

Genome-wide association studies have linked hundreds of variants with a variety of inflammatory and autoimmune disorders, launching efforts to characterize the immune function of the individual genes implicated by these large-scale population level studies. Many of these genes encode proteins that directly or indirectly interact with one another within pathways involved in negative feedback during immune signaling. Based on this information, investigators can surmise which pathways are dysregulated in a given disease and propose intervention strategies that restore balance through blocking signaling events. However, as demonstrated by numerous screens in yeast and classic synthetic lethality experiments with *Drosophila melanogaster*, mutation of two genes commonly leads to an outcome that is absent when genes are mutated on an individual basis (Nijman, 2011). Few studies have tested the epistatic relationship between susceptibility genes in models of inflammatory disease.

A20 (*TNFAIP3*) and ABIN-1 (*TNIP1*) are prime examples of disease susceptibility genes that function within the same pathway. Variants in the A20 and ABIN-1 loci are linked to several immune disorders including inflammatory bowel disease (IBD), a recurring and relapsing disease of unknown origin characterized by inflammation of the gastrointestinal tract (Jostins et al., 2012). A20 is a negative regulator of NF-κB downstream of the TNFα receptor (TNFR) and other immune receptors. The mechanisms by which A20 exerts this inhibitory function are incompletely understood, but likely include modification of ubiquitin chains on signaling scaffold molecules (Catrysse et al., 2014). ABIN-1 is a ubiquitin-binding protein identified as an A20-interacting protein and can serve as an adaptor (Heyninck et al., 1999). For instance, ABIN-1 recruits A20 to the TNFR signaling complex to deubiquitinate and deactivate RIPK1, a serine–threonine kinase that regulates NF-κB and cell death (Dziedzic et al., 2018). These observations are consistent with a model in which inhibition of either A20 or ABIN-1, such as through an inherited polymorphism, would lower the threshold for hyperinflammatory responses to TNFα. This model is further supported by numerous findings in the literature implicating TNFα in the dysfunction of the epithelial barrier that occurs during IBD.

In this issue, Kattah et al. generate a mouse model in which A20 and ABIN-1 can be deleted simultaneously from intestinal epithelium cells (IECs) in an inducible manner. Similar to a previous study examining A20 deletion in IECs (Vereecke et al., 2010), single knockouts ($A20^{-/-}$ and $ABIN\text{-}1^{-/-}$) did not develop spontaneous disease. In stark contrast, deleting both together ($A20^{-/-}ABIN\text{-}1^{-/-}$) resulted in extraordinarily rapid lethality accompanied by severe inflammation in the small intestine and colon. IECs in the double knockout mice displayed significant cell death compared with the individual deletion mutants. These findings are unexpected because A20 and ABIN-1 are binding partners considered to function together, in which case the predicted outcome would be that deleting one would be sufficient to ablate the activity of the other.

Another unexpected observation came from analysis of mice with heterozygous deletion of these susceptibility genes.

Insight from Ken Cadwell.

Mice with heterozygous deletion of A20 and homozygous deletion of ABIN-1 ($A20^{+/-}ABIN\text{-}1^{-/-}$) survive. Yet, mice with homozygous deletion of A20 and heterozygous deletion of ABIN-1 ($A20^{-/-}ABIN\text{-}1^{+/-}$) display 100% mortality, although not as rapid as the homozygous double-deletion mutants. These results suggest that A20 compensates for ABIN-1 deficiency better than ABIN-1 for A20. Deletion of TNFα restored survival in $A20^{-/-}ABIN\text{-}1^{+/-}$ but not $A20^{-/-}ABIN\text{-}1^{-/-}$ mice, thus revealing protective functions of these proteins in TNFα-dependent and -independent signals. Kattah et al. (2018) next considered the possibility that the TNFα-independent factor was downstream of TLRs that signal through MYD88. By generating bone marrow chimeras, they were able to show that removal of MYD88 in the hematopoietic compartment reversed lethality in $A20^{-/-}ABIN\text{-}1^{-/-}TNF\alpha^{-/-}$ mice, but not

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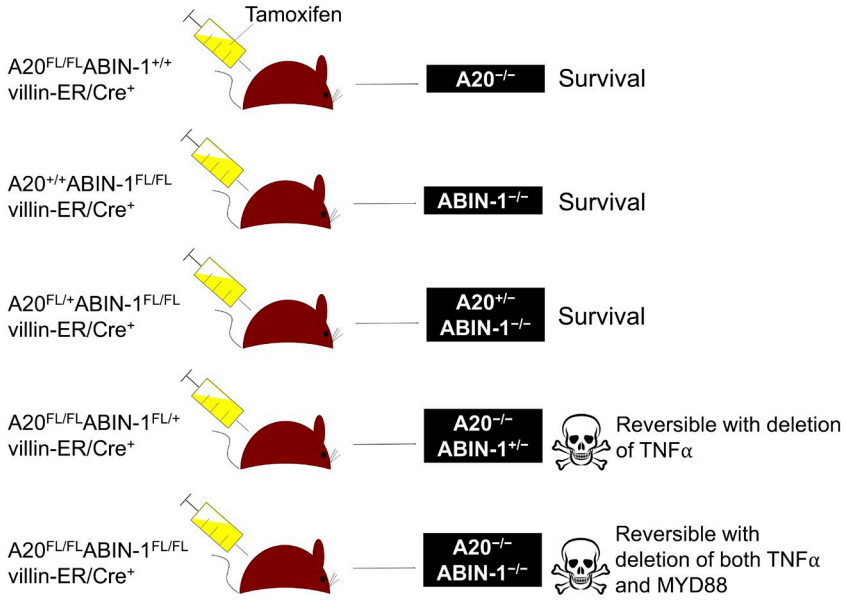

Dual deletion of A20 and ABIN-1 leads to rapid lethality. A20 and ABIN-1 are binding partners that are genetically associated with susceptibility to immune disorders such as IBD. Mice were generated harboring a tamoxifen-inducible Cre recombinase under the IEC-specific *villin* promoter along with combinations of loxP flanked (flox; FL) A20 and ABIN-1 alleles. Tamoxifen injection of adult mice led to deletion of A20 and/or ABIN-1 in IECs. Deletion of A20 ($A20^{-/-}$) or ABIN-1 ($ABIN\text{-}1^{-/-}$) alone did not affect viability. Also, mice with heterozygous deletion of A20 and homozygous deletion of ABIN in IECs ($A20^{+/-}ABIN\text{-}1^{-/-}$) survived. In contrast, mice with homozygous deletion of A20 and heterozygous deletion of ABIN ($A20^{-/-}ABIN\text{-}1^{+/-}$) displayed 100% mortality that was accompanied by epithelial cell death and inflammatory disease, which was reversible by deletion of TNFα. Deletion of both alleles of A20 and ABIN-1 together ($A20^{-/-}ABIN\text{-}1^{-/-}$) accelerated death further. In this case, deletion of TNFα together with transplantation with MYD88-deficient bone marrow cells was required to restore survival. These sophisticated genetic approaches show that A20 and ABIN-1 have critical functions in protecting the intestinal epithelial barrier that compensate for one another and highlight the need for examination of interactions between disease susceptibility genes.

$A20^{-/-}ABIN\text{-}1^{-/-}TNF\alpha^{+/+}$ mice. Therefore, inhibition of both TNFα and MYD88 is required to restore viability when A20 and ABIN-1 are deleted together. Although it is unclear whether this MYD88-dependent signal is originally of microbial origin, these experiments show that IEC-extrinsic factors contribute to mortality. Finding the MYD88-dependent factor is potentially of great interest because TNFα blockade is effective in only a subset of IBD patients. A combination therapy that inhibits both TNFα and this elusive factor would theoretically ameliorate disease in a wider range of patients.

Further mechanistic insight came from experiments with enteroids (small intestinal organoids), a primary 3D culture system in which IECs are differentiated from epithelial stem cells. The effect of deleting A20 and ABIN-1 in enteroids was strikingly similar to the observations in mice. Inducible deletion of both A20 and ABIN-1 in enteroids led to spontaneous cell death that was reversible upon deletion of TNFα, indicating that TNFα produced by $A20^{-/-}ABIN\text{-}1^{-/-}$ IECs is responsible for their own death. The fact that TNFα deletion alone is sufficient to reverse death and does not require additional MYD88 deletion makes sense. Enteroids are cultured in sterile conditions in the absence of leukocytes and microbes, whereas an intact animal harbors trillions of microbes in the intestine along with dynamic myeloid and lymphoid cell populations. Therefore, IEC-intrinsic and -extrinsic factors contribute to the heightened sensitivity of the $A20^{-/-}ABIN\text{-}1^{-/-}$ epithelium.

$A20^{-/-}ABIN\text{-}1^{-/-}$ enteroids displayed hallmarks of apoptosis, including activation of caspase-3 and caspase-8, but viability could not be restored by treatment with a pan-caspase inhibitor. Such a finding is common when RIPK1 signaling is triggered aberrantly downstream of the TNFR. RIPK1 typically has a protective function in IECs (Dannappel et al., 2014; Takahashi et al., 2014). However, RIPK1 can form a complex with FADD and caspase-8 to induce apoptosis, and in conditions where caspase-8 is inactivated, RIPK1 functions together with RIPK3 and MLKL instead to execute a form of programmed necrosis known as necroptosis (Günther et al., 2011; Vlantis et al., 2016). Similar to caspase inhibition, Kattah et al. (2018) show that inactivation of RIPK3 is ineffective. In contrast, inhibiting RIPK3 and caspases together, or inhibiting RIPK1 alone, restores survival of $A20^{-/-}ABIN\text{-}1^{-/-}$ enteroids. These well-designed experiments in the enteroid system indicate that A20 and ABIN-1 deletion primarily leads to apoptosis through TNFR-RIPK1 signaling, but inhibiting apoptosis will force IECs to undergo necroptosis.

When taken together, the findings in Kattah et al. (2018) support a critical protective role for A20 and ABIN-1 in IECs beyond their better-known functions in immune cell types, and these molecules have at least some nonredundant features relevant to cell death signaling. An important clue revealed by this current study is that the synergistic effect of deleting A20 and ABIN-1 seems to still involve RIPK1. A20 and ABIN-1 could potentially regulate overlapping but distinct ubiquitination events of RIPK1 that influence the formation of complexes that integrate signals between the TNFR and other immune receptors. Another possibility suggested by the authors is that A20 may be regulating autophagy, a pathway that is also genetically associated with IBD susceptibility that is critical for maintaining cellular homeostasis and epithelial barrier integrity (Matsuzawa-Ishimoto et al., 2018). A20 deletion leads to reduced $CD4^+$ T cell survival due to unrestricted mTOR activation, resulting in impaired autophagy (Matsuzawa et al., 2015). Recent studies show that inhibition of the autophagy gene *Atg16l1* in IECs leads to TNFα-dependent necroptosis and apoptosis in the small intestine and colon, respectively, upon exposure to infectious agents that are otherwise innocuous (Matsuzawa-Ishimoto et al., 2017; Pott et al., 2018). Of relevance, dual deletion of *Atg16l1* together with the ER stress gene *Xbp1* has a greater effect than single mutants and causes inflammation similar to Crohn's disease, a type of IBD (Adolph et al., 2013). A key feature of this *Atg16l1*/*Xbp1* (both susceptibility genes) dual deletion model is that the inflammation is due to loss of Paneth cells, antimicrobial IECs in the small intestine. A future direction would be to explore the possibility that A20 and ABIN-1 have functions that are specific to a region of the

intestine or an IEC subset such as Paneth cells, which are potentially subject to different cell death modalities. These concepts are clinically relevant because the way IBD presents itself can differ significantly between patients. RIPK1 inhibitors are being considered for treatment of IBD and can ameliorate mortality and Paneth cell loss in *Atg16l1* mutant mice (Matsuzawa-Ishimoto et al., 2017). Perhaps prior knowledge of the pathophysiology and genotype of patients may be necessary to identify individuals who will benefit most from RIPK1 inhibition.

In conclusion, the authors deserve credit for performing the challenging genetic crosses that ultimately revealed cryptic functions of two genes of great interest to human immune disorders. It is notable that enteroids recreated the functional relationship between A20 and ABIN-1 that was initially observed in vivo. It may be possible to screen for synthetic lethal interactions using enteroids, or even use this culture model to test precision therapies designed for an individual that harbors specific genetic variants. Understanding gene–gene interactions, as well as gene–environment interactions, may lead to the discovery of hidden functions of susceptibility genes that are otherwise buffered by compensatory pathways. These hidden functions may be effective therapeutic targets.

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