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INSIGHTS

Inflammasome-independent functions of AIM2

Junjie Zhao[1], William Miller-Little[1,2], and Xiaoxia Li[1]

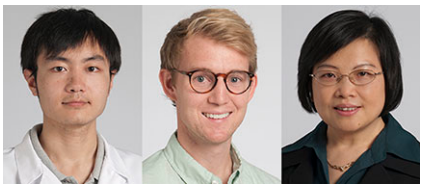
AIM2 is widely known for its role as a cytosolic dsDNA receptor that activates the inflammasome. In this issue of *JEM*, Ma et al. (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20201796) describe an inflammasome-independent function of AIM2 in microglia that restrains neuroinflammation via a novel crosstalk between AIM2 and cGAS signaling.

Absent in melanoma 2 (AIM2) was first cloned in 1997, but did not gain notoriety until 2009, when four groups independently identified AIM2 as the cytosolic receptor that recognizes double-stranded DNA (dsDNA) and relays the information to the inflammasome adaptor apoptosis-associated speck-like protein containing CARD (ASC; Bürckstümmer et al., 2009; Fernandes-Alnemri et al., 2009; Hornung et al., 2009; Roberts et al., 2009). Upon binding to dsDNA, AIM2 initiates the oligomerization of ASC, which further assembles pro-caspase 1 into a filamentous polymer that enables the proximity-induced activation of caspase 1. Assembly of the inflammasome complex leads to pyroptosis and the secretion of mature IL-1β, effectively triggering an inflammatory cascade.

Because AIM2 does not discriminate between host and microbial dsDNA, it is widely believed that activation of AIM2 inflammasome underlies the pathogenesis of many auto-inflammatory diseases (Kumari et al., 2020). In support of this notion, AIM2, along with other inflammasome components, has been shown to play a pathogenic role in various models of sterile inflammation, especially in the skin. Neuroinflammation is another setting where a preponderance of evidence points to a pathogenic role for inflammasomes. Genetic ablation of caspase 1 or ASC, or pharmacological inhibition of caspase 1, largely protects mice from neuroinflammation in experimental autoimmune encephalomyelitis (EAE), a widely used murine model of multiple sclerosis (MS; Gris et al., 2010; McKenzie et al., 2018). The wealth of data on the pro-inflammatory role of inflammasome in the EAE model have led to the expectation that abrogation of AIM2, should it be activated during EAE, would attenuate neuroinflammation.

Unexpectedly, in this issue of *JEM*, Ma et al. (2021) report a surprising finding of an inflammasome-independent function of AIM2 in restraining neuroinflammation. The authors demonstrated that AIM2 knockout mice exhibited a distinct phenotype in the EAE model when compared with ASC-deficient mice. While ASC deficiency reduced the symptoms induced by the autoimmune encephalomyelitis, loss of AIM2 dramatically exacerbated the disease. Importantly, AIM2 deficiency led to an increase, not reduction, of IL-1β in the sera of the EAE mice, further indicating these results are due to an inflammasome-independent function of AIM2 at play. Corroborating this finding, the authors showed that AIM2 deficiency aggravated the symptoms in an alternative EAE model that does not rely on robust inflammasome activation. Collectively, the evidence convincingly demonstrates a novel protective function for AIM2 in EAE, which is unrelated to its role as an inflammasome receptor.

To pinpoint the cell type responsible for the anti-inflammatory function of AIM2, the authors surveyed its expression in relevant immune and central nervous system (CNS) resident cells. AIM2 was highly expressed by myeloid-lineage cells, T cells, and microglia and was nearly undetectable in oligodendrocytes, astrocytes, and neurons. Further analysis showed that AIM2-deficient bone marrow did not exacerbate the disease. Rather, AIM2 expression in CNS resident cells conferred the heightened disease severity. Using conditional AIM2 knockout mice, the authors found that abrogation of AIM2 in the microglia was sufficient to induce the aggravated phenotype. The data highlight an inflammasome-independent function of AIM2 in microglia that restrains neuroinflammation.



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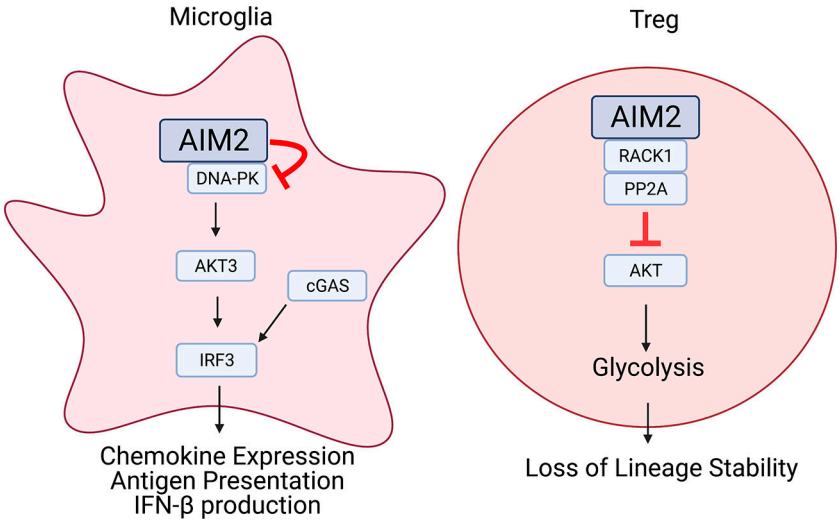
The unique function of AIM2 specifically in microglia, but not in infiltrating macrophages, is a particularly intriguing finding. Although phenotypically similar to infiltrating macrophages, microglia are functionally distinct. Unlike macrophages that are differentiated from circulating monocytes and infiltrate the CNS at the onset of neuroinflammation, microglia originate from progenitors in the yolk sac and enter the CNS during embryogenesis. As CNS-resident immune cells, microglia carry out

[1]Department of Inflammation and Immunity, Cleveland Clinic Lerner Research Institute, Cleveland, OH; [2]Medical Scientist Training Program, Case Western Reserve University, Cleveland, OH.

Xiaoxia Li: lix@ccf.org.



Inflammasome-independent functions of AIM2 in microglia and T reg cells. Two divergent mechanisms have been reported for the regulatory roles of AIM2 during neuroinflammation. In microglia, AIM2 forms a complex with DNA-PK, suppressing its activity and thereby abrogating the activation of AKT3. Inhibition of AKT3 reduces the phosphorylation of the master transcriptional factor for cGAS signaling, IRF3, ultimately attenuating the production of chemokines, type I interferons, and the expression of antigen presentation molecules by microglia. In T reg cells, AIM2 forms a complex with RACK1 and the phosphatase PP2A, which reduces the activation of AKT. Consequently, blunted AKT signaling leads to diminished mTOR and Myc activation and shift the metabolic program toward oxidative phosphorylation, which helps to maintain the lineage stability of T reg cells.

an array of homeostatic activities by actively surveilling the brain parenchyma. In the setting of human neuroinflammatory disease, activated microglia are readily detected in MS lesions. In murine models, targeted inhibition of microglia activation suppresses the development of EAE. Of note, microglia cells express functional NLRP3 inflammasome components and produce IL-1β; inhibition of inflammasome activation by ablating ASC expression specifically in microglia substantially attenuates the severity of EAE (Zhang et al., 2018). Because activated microglia exhibit increased expression of antigen presentation and costimulatory molecules, they were thought to participate in presenting autoantigen to encephalitogenic T cells (Zrzavy et al., 2017). But there has been limited evidence to support this idea. In the current study, Ma et al. (2021) show that AIM2 deficiency in microglia leads to increased expression of antigen presentation molecules, accompanied by increased infiltration of proinflammatory T cells in both CNS and the peripheral lymphoid organs. The data suggest that, at least under certain pathological conditions, microglia may, either directly or indirectly, contribute to activating the adaptive autoimmune response.

Notably, as the work of Ma et al. (2021) went to press, Jenny Ting's group reported similar observations that also demonstrate an inflammasome-independent function of AIM2 in restraining inflammation (Chou et al., 2021). In contrast to the study by Ma et al., Chou et al. show that AIM2 expression in regulatory T (T reg) cells contributes to the anti-inflammatory response (see figure). AIM2 expression programs T reg cells to favor the oxidative phosphorylation over glycolysis and stabilizes them during tissue inflammation. These data suggest that the inflammasome-independent function of AIM2 may be important for regulating other nonmyeloid cell types.

In fact, the first evidence for an inflammasome-independent function of AIM2 was reported by the Ting laboratory. They found that AIM2 suppressed colon tumorigenesis via the inhibition of DNA-dependent protein kinase (DNA-PK), a PI3K-related family member that promotes Akt phosphorylation (Wilson et al., 2015). Building on this premise, Ma et al. (2021) found that AIM2 formed a complex with and inhibits DNA-PK in microglia. Hence, deletion of AIM2 enhanced the phosphorylation of AKT3, leading to increased activation of IRF3, the cardinal transcription factor for cGAS (cyclic guanosine monophosphate–adenosine monophosphate synthase) signaling. With these results, the authors proposed that AIM2 tames the activation of microglia by negatively regulating cGAS activation via the DNA-PK–AKT3 axis. This proposed pathway differs from the regulatory mechanism described for AIM2 in T reg cells, where the evidence implicates a RACK1–PP2A phosphatase axis in suppressing AKT, mTOR and Myc signaling. Taken together, the evidence suggests that AIM2 exerts its regulatory function via cell-type specific mechanisms.

AIM2 contains a DNA-binding hematopoietic expression, interferon-inducible nature, and nuclear localization (HIN) domain and a pyrin domain, which binds ASC. The structural information for dsDNA-activated AIM2 inflammasome suggests that, in the absence of dsDNA, the HIN domain sequesters the pyrin domain in an autoinhibitory conformation (Jin et al., 2012). Findings reported by Ma et al. and Chou et al. raise a key question: is dsDNA required for AIM2 to engage DNA-PK or RACK1? The prevailing view on AIM2 inflammasome would favor a model where dsDNA-free AIM2 exerts a regulatory function and dsDNA-bound AIM2 triggers inflammasome activation. Nonetheless, kinetic analysis indicates that AIM2 cannot efficiently oligomerize around dsDNA fragments shorter than a minimum length. Since AIM2 oligomerization and subsequent assembly of ASC disk is required for the activation of inflammasome, it is possible that binding to short dsDNA licenses the regulatory role of AIM2. In addition, while there is no sequence specificity to the recognition of dsDNA by AIM2, the immunosuppressive DNA motif (i.e., TTAGGG repeat, commonly found in mammalian telomeric DNA) has been shown to suppress AIM2 inflammasome activation (Kaminski et al., 2013). Thus, it is also plausible for these endogenous DNA fragments to stimulate an anti-inflammatory function of AIM2.

The potential requirement of dsDNA ligand for the regulatory function of AIM2 carries significant mechanistic and therapeutic implications. Ma et al. (2021) postulated that phagocytosed DNA activated AIM2 to restrain microglia activation. However, there are hints for an alternative scenario. AIM2 has been shown to translocate into the nucleus in response to

genotoxic stress (Hu et al., 2016). Evidence presented in the current study cannot rule out a possible nuclear, noninflammasome AIM2 that prevents microglia hyperactivation, especially given the connection with DNA-PK, which can bind to DNA double-strand breaks. Curiously, nuclear cGAS has also been linked to DNA double-strand breaks (Liu et al., 2018). These "coincidences" warrant further exploration for a possible role of AIM2 in protecting the genome integrity of microglia, thereby taming their activation during neuroinflammation.

Understanding whether dsDNA ligand is required for AIM2 to engage its regulatory function will also inform therapeutic design. If DNA binding is required for the anti-inflammatory effect of AIM2, pharmaceutical agents that inhibit the DNA binding may have the unintended side effect of activating microglia. Notably, there are a number of active drug development pipelines for inhibitors against DNA-PK and cGAS. The work by Ma et al. (2021) suggests that targeting DNA-PK or cGAS may confer therapeutic benefit to MS patients who suffer from a severe form of the disease that is driven by diminished activity of AIM2 in microglia and/or T reg cells. Furthermore, microglial AIM2 also attenuated disease severity in an EAE model that is resistant to IFN-β, the first line treatment for MS patients. Considering the prevalence of IFN-β resistance among MS patients, the current work illuminates a new therapeutic opportunity for refractory disease.

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