CLINICAL IMPLICATIONS OF BASIC RESEARCH

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STING-Induced Inflammation — A Novel Therapeutic Target in ALS?

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Neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS), have a complex neurobiology that is still only partially mapped today. There is thus no effective treatment for patients with ALS. The neurodegenerative process mainly affects the motor system, with loss of the upper motor neurons in the motor cortex and the lower motor neurons in the brain stem and spinal cord. Progressive muscle weakness and atrophy, ultimately affecting the respiratory muscles as well, limits survival to 2 to 5 years after disease onset.

The development of effective treatments for ALS is particularly challenging: it is a heterogeneous disorder in terms of its causes, the diseased tissue is not accessible for direct examination, and the known biomarkers of disease do not sufficiently reflect the underlying biology or disease progression. However, knowledge of the genetic causes of ALS and the related proteins and disease cascades is steadily increasing. The diversity of disease mechanisms notwithstanding, most patients with ALS have cytoplasmic mislocalization and aggregation of the TAR DNA-binding protein (TDP-43) in the affected regions of the central nervous system. In addition, the process of neurodegeneration is accompanied the release of cytokines (a driver of inflammation), although the signs of inflammation, such as swelling and heat, are absent. In the context of ALS and for the purposes of discussion here, the inflammatory response is defined as the synthesis and release of type I interferons and other proinflammatory cytokines.

Yu and colleagues[1] recently showed how these common hallmarks of the pathology of ALS are linked: TDP-43 triggers an inflammatory response consisting of the activation of nuclear factor κB (NF-κB) and type I interferon signaling (Fig. 1). The authors identified the immune sensor that drives this inflammatory response and dissected the different steps of the pathway involved. As previously reported, cytoplasmic TDP-43 enters the mitochondria, where it induces mitochondrial toxicity and the production of reactive oxygen species.[2] This results in the release of mitochondrial DNA into the cytoplasm through mitochondrial permeability transition pores. Yu and colleagues showed that the cytosolic DNA sensor cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase (cGAS) is the link between cytosolic mitochondrial DNA and inflammation. cGAS drives the production of the intracellular messenger cGAMP, thereby activating the stimulator of interferon genes (STING), which drives the inflammatory gene responses by activating TANK binding kinase 1 (TBK1), which in turn phosphorylates (and thus activates) interferon regulatory factor 3 and the nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα), complex. Further investigation in mutant mice revealed that the genetic deletion or pharmacologic inhibition of STING had neuroprotective effects.

It is interesting that the inflammatory response triggered by TDP-43 may have connections with several genetic subtypes of ALS. The C9orf72 protein, levels of which are known to be reduced in patients with a *C9orf72* mutation, has been shown to suppress STING-induced inflammation, suggesting that an exaggerated immune response is a contributing disease mechanism.[3] It is perhaps paradoxical that heterozygous loss-of-function mutations in *TBK1* can cause ALS, whereas a reduction in STING-induced inflammation would be expected to be beneficial.

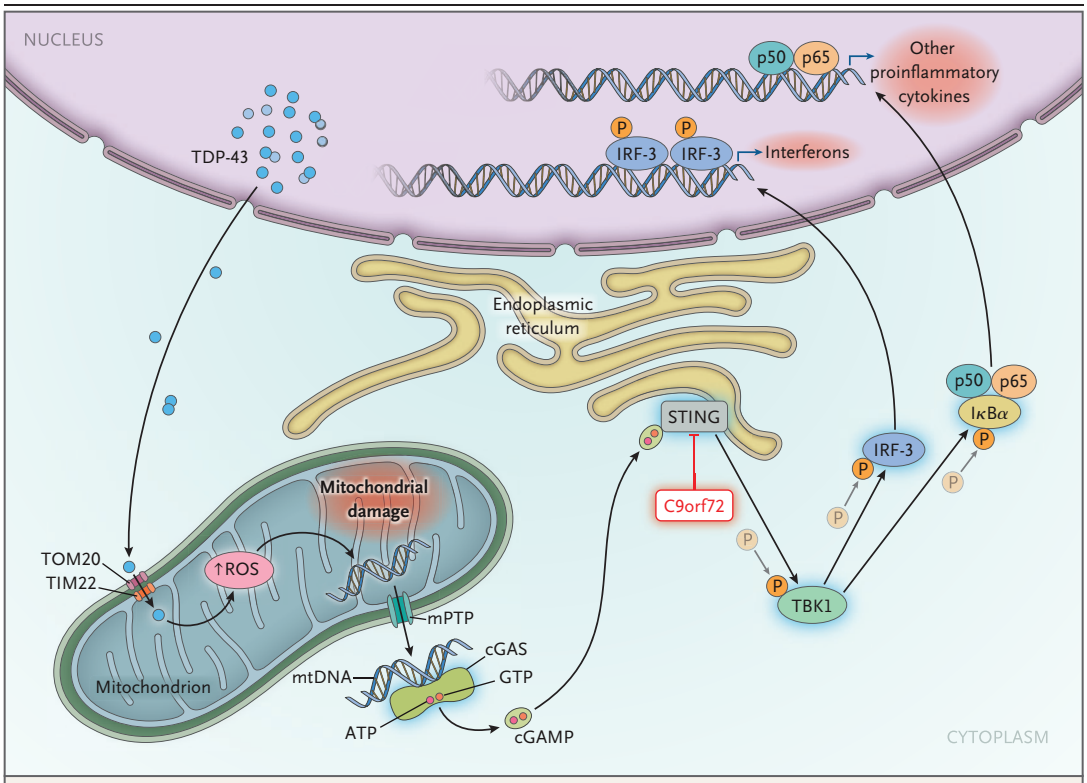

Figure 1. Activating STING in ALS.

Yu and colleagues recently reported evidence that underscores the importance of the cytoplasmic DNA sensor cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase (cGAS) as an etiologic factor in amyotrophic lateral sclerosis (ALS).[1] Cytoplasmic TAR DNA-binding protein 43 (TDP-43) is transported across the outer and inner mitochondrial membranes by the translocases TOM20 (translocase of outer mitochondrial membrane 20) and TIM22 (translocase of inner mitochondrial membrane 22), causing mitochondrial damage through the production of reactive oxygen species (ROS), which results in the release of mitochondrial DNA. cGAS triggers an innate immune response in which cytosolic DNA sensor cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase (cGAS), a stimulator of genes encoding interferon, is released, driving inflammatory gene responses by activating the stimulator of interferon genes (STING), which in turn activates TANK binding kinase 1 (TBK1). The phosphorylation of interferon regulatory factor 3 (IRF-3) and the nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα), then generates transcription of type I interferons and proinflammatory cytokines. C9orf72 suppresses STING-induced inflammation. GTP denotes guanosine triphosphate, mPTP mitochondrial permeability transition pore, mtDNA mitochondrial DNA, P phosphorylation, and TNF-α tumor necrosis factor alpha.

Alternatively, *TBK1* mutations could cause neurodegeneration by limiting another function of TBK1, such as the regulation of autophagosome maturation. It is interesting that mutations in *OPTN*, the gene encoding optineurin, a protein required for the activation of TBK1, also cause ALS.

Taken together, these findings provide a clear framework for further research. Discovery of the inflammatory pathway triggered by TDP-43 offers great potential for therapeutic intervention at different levels: inhibition of TDP-43 entry into mitochondria, of mitochondrial DNA release, of the cGAS response, and of STING activation. Some of these inhibitors already exist, and gene silencing of important players with the use of antisense oligonucleotides[4] is within reach as well. Nonetheless, evidence of the activation of STING-induced inflammation in patients with ALS remains limited. Several steps need to be taken before clinical trials can be started. First, it would be useful to study the different steps of the pathway in postmortem samples of the brain

and the spinal cord, and culture systems derived from induced pluripotent stem cells might help to assess the generalizability of the findings and to determine the contributions of different cell types. Second, reliable tools for measuring the activation of the STING pathway in living patients are needed to provide further evidence of its relevance to ALS and to show target engagement of new therapeutic interventions. Type I interferons and tumor necrosis factor (TNF) α can be assayed in the cerebrospinal fluid and blood, and previous work indicates that levels of TNF-α are elevated in peripheral blood obtained from patients with ALS.[5] In addition, cGAMP, which is up-regulated by cGAS activation upon cytoplasmic DNA sensing, may be an important upstream biomarker of this pathway. In conclusion, in identifying STING as a potential therapeutic target, Yu and colleagues have opened an exciting new avenue for further translational studies in ALS.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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1. Yu C-H, Davidson S, Harapas CR, et al. TDP-43 triggers mitochondrial DNA release via mPTP to activate cGAS/STING in ALS. Cell 2020;183(3):636-649.e18.
2. Wang W, Wang L, Lu J, et al. The inhibition of mitochondrial localization blocks its neuronal toxicity. Nat Med 2016;22:869-78.
3. McCauley ME, O'Rourke JG, Yáñez A, et al. C9orf72 in myeloid cells suppresses STING-induced inflammation. Nature 2020;585:96-101.
4. Miller T, Cudkowicz M, Shaw PJ, et al. Phase 1–2 trial of antisense oligonucleotide tofersen for *SOD1* ALS. N Engl J Med 2020;383:109-19.
5. Hu Y, Cao C, Qin X-Y, et al. Increased peripheral blood inflammatory cytokine levels in amyotrophic lateral sclerosis: a meta-analysis study. Sci Rep 2017;7:9094.

DOI: 10.1056/NEJMcibr2031048