Regulated cell death represents an important biological response with major roles during development and in tissue homeostasis. Apoptosis, the prototypic and best-known cell suicide program, is often considered a silent form of cell death due to its lack of inflammation and efficient clearance mechanisms. This well-defined mode of destruction is orchestrated by a signaling cascade led by caspases, and is characterized by the integrity of the cell membrane until engulfment by phagocytes, ensuring minimal cytoplasmic release during the cell death process. Similarly ordered forms of cell death, albeit with opposite proinflammatory consequences, are now being recognized in numerous pathophysiologic settings. Morphologically similar to accidental necrosis, most of these forms of cell death share the capacity to release, often as a direct consequence of loss of membrane integrity, intracellular molecules endowed with the capacity to alert the immune system and therefore to initiate an inflammatory response. The signaling cascades regulating these types of necrotic cell death are exceedingly diverse, often ascribed to a particular and well-defined stimulus or cell type, and their identification and description have been abundantly discussed in the literature. Cell demise as a result of metabolic stress deserves particular attention, based notably on its pathologic consequences when proceeding uncontrolled. In particular, death secondary to DNA strand breaks has been ascribed to the excessive activity of poly-ADP ribose polymerase 1 (PARP1), an enzyme that catalyzes the transfer of successive units of nicotinamide adenine dinucleotide (NAD+) -derived ADP-ribose to several nuclear proteins, including PARP1 itself. This post-translational modification has been shown to facilitate DNA repair and protect against moderate genotoxic stress. High levels of DNA damage can, however, lead to a severe depletion of intracellular NAD+ pools, a key step that, depending on cell type and metabolic status, can lead to further loss of ATP, reduced mitochondrial function, and release of cell death-inducing factors. Despite some diversity in the signaling pathways at work, loss of intracellular NAD+ appears to represent a key step in a growing number of modes of cell death (termed parthanatos, oncosis, or regulated necrosis, depending on cell origin, intracellular mediators, and stimuli). Remarkably, a sharp reduction in the intracellular content of an important cell metabolite does not merely cause cell death by “sabotage” (i.e., the inability to sustain basic cellular functions), but rather sets in motion a series of signaling cascades leading to the execution of a well-controlled, regulated cell death response. These observations have led to the concept of NAD+ as a “survival factor,” endowing cells with the capacity to resist environmental stress in response to oxidative damage (such as increased endogenous reactive oxygen species production) or upon exposure to natural (i.e., ultraviolet irradiation) or pharmacologic alkylating agents. This conclusion has been further strengthened by the discovery that exogenous and endogenous NAD+ can also oppose other forms of cell death, including apoptosis.

With these notions in mind, we have undertaken a series of experiments to further assess the putative survival properties of NAD+ during necroptosis, a recently uncovered mode of cell death that is of particular interest for human pathology. Necroptosis displays all the hallmarks of regulated cell death characterized by a well-described, caspase-independent, signaling cascade, with a prominent role for the receptor interacting protein kinase 3 (RIPK3) and its substrate mixed lineage kinase like (MLKL) in mediating a form of regulated cell necrosis. Of note, a member of the caspase family (caspase 8) has been identified as a negative regulator of necroptosis, indicating a possible antagonism between apoptosis and this form of regulated cell death. Accordingly, in several experimental settings pharmacologic inhibition of caspase activity shifts the predominant mode of cell death from a non-inflammatory apoptotic mode to necroptosis. This observation has led many authors to propose that necroptosis may represent a back-up cell death mechanism to counteract the antiapoptotic, prosurvival strategy devised by many pathogens, as suggested by several in vivo...
CAUSES AN EFFECTIVE REDUCTION IN INTRACELLULAR NAD+ CONCENTRATION, SENSITIZING CELLS TO NECTROTIC CELL DEATH. NAD+ CAN BE REPLENISHED VIA MULTIPLE BIOSYNTHETIC PATHWAYS USING NICOTINIC ACID (NA), NICOTINAMIDE RIBOSIDE (NR), AND NICOTINAMIDE (Nam) AS STARTING PRECURSORS. IN MOST CELLS, THE CONVERSION OF Nam INTO NICOTINAMIDE MONONUCLEOTIDE (NMN) CATALYZED BY NICOTINAMIDE PHOSPHOBUSTRANSFERASE (NAMPT) REPRESENTS A MAJOR NAD+ BIOSYNTHETIC STEP THAT CAN BE SPECIFICALLY TAIKED BY NAMPT INHIBITORS SUCH AS FK866 OR CH5828. NAMPT INHIBITION OFTEN CAUSES AN EFFECTIVE REDUCTION IN INTRACELLULAR NAD+ CONCENTRATION, SENSITIZING CELLS TO MOST FORMS OF REGULATED CELL DEATH WITH THE NOTABLE EXCEPTION OF NECROPTOSIS.

STUDIES. ALTERNATIVELY, NECROPTOSIS MAY REPRESENT A PREFERRED RESPONSE THAT ENABLES Dying CELLS TO ALERT THE IMMUNE SYSTEM. USING A WELL-DESCIBED IN VITRO CELL LINE ABLE TO UNDERGO BOTH APOPTOSIS AND NECROPTOSIS IN RESPONSE TO EXTRACELLULAR AGONISTS—ANTIBODIES TO FAS (ALSO KNOWN AS CD95 OR TNFRSF6) AND TUMOR NECROSIS FACTOR (TNF), RESPECTIVELY—WE HAVE RECENTLY DEMONSTRATED THAT ALTHOUGH INTRACELLULAR NAD+ PROTECTS CELLS FROM APOPTOSIS AND PARP1-DEPENDENT NECROSIS, IT IS REQUIRED FOR AND PROMOTES THE NECROTIC RESPONSE (FIG. 1).4

Thus, and in marked contrast to the widely held view, intracellular NAD+ does not act as a "universal" cell survival factor, but rather acts as a key metabolite regulating the choice of cell demise. Intracellular NAD+ appears to modulate necroptosis by acting on members of the sirtuin family, a group of NAD+-dependent deacylases with previously described roles in many biological responses including apoptosis.

These observations may bear important consequences for cancer therapy. Indeed, the recently developed concept of "immunogenic cell death" posits that cells dying in a "non-silent" fashion possess the capacity to inform the immune system of the level of threat caused by cell damage.5 Shifting the tumor cell death program from a silent (mainly apoptotic) process to a proinflammatory form therefore represents a promising avenue for improving conventional chemotherapy. In this context, the contrasting effects of intracellular NAD+ (promoting cell survival in response to genotoxic damage but favoring RIPK3-dependent cell death) suggest caution in devising chemotherapeutic approaches targeting NAD+ metabolism. Pharmacologic agents inhibiting NAD+biosynthesis have indeed been shown to synergize with alkylating agents in reducing tumor cell viability.6 In contrast, pharmacologic and nutritional compounds able to increase NAD+ biosynthesis may best support antitumor therapies relying on RIPK3-dependent cell demise.7-10 Further studies are therefore warranted to better understand the intricate regulatory pathways linking NAD+ to cell survival and death.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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AUTHOR CONTRIBUTION STATEMENT

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