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SPOTLIGHT

IFNγ: Priming for death

James E. Vince[1,2]

TNF signaling does not result in cell death unless multiple inhibitory signals are overcome, which can be accomplished by simultaneous signaling through IFNγ. In this issue, Deng and colleagues (http://doi.org/10.1083/jcb.202305026) dissect the mechanisms by which IFNγ signaling combines with TNF to mediate cell death through caspase-8, discussed by James E. Vince.

Cell death aficionados know that the prototypical death ligand, tumor necrosis factor (TNF), actually isn't very good at inducing cellular necrosis, let alone apoptosis. In fact, in the majority of cells, TNF will generate a strong pro-survival signal and trigger cells to churn out a plethora of inflammatory mediators. It could be said that the cell is certainly excited by TNF, but at no stage does TNF appear to sway the cell into committing suicide despite its name suggesting it should do just this, in spades.

So, what does the cell death scientist do should they want to uncover a new TNF-induced cell death mechanism and study its potential role in disease? Like our laboratory and many others have done, we chemically or genetically intervene. We block the TNF-induced pro-survival response by genetic targeting or by small molecules, which remove the TNF receptor 1 (TNFR1) signaling components responsible for restricting self-immolation (e.g., the inhibitor of apoptosis [IAP] proteins). While this approach shows us the potential of TNF to turn deadly and allows us to uncover interesting biology, it could hardly be claimed that this represents a common physiological scenario.

However, it has been known for decades that IFNγ can sweet-talk cells with active TNF or Toll-like receptor (TLR) signaling into killing themselves, including innate immune cells and cancer cells (1). Despite this well-known two-hit approach for eliciting a physiological TNF-induced cell death program in a variety of cell types, details are still being uncovered on the mechanisms by which IFNγ allows TNF and TNFR1 to live up to their status as a death ligand and death receptor. This is important to understand, because cell death induced by IFNγ and TNF (or TLR ligands) has been implicated in cytokine shock syndromes associated with infections (2, 3) and in tumor cell susceptibility to cancer immunotherapy (4, 5). Moreover, these cytokines can co-mingle in a number of conditions associated with exacerbated immune responses and cell death, such as inflammatory bowel disease where anti-TNF and JAK inhibitors are used therapeutically.

In this issue, Buhao Deng and colleagues aimed to identify IFNγ responsive genes that license the TNF-induced cell death response (6). RNA sequencing, and subsequent qPCR and western blot validation of IFNγ-treated cancer cell lines, identified increased expression of the cell death regulators caspase-8, caspase-7, and cylindromatosis (CYLD). Moreover, melanoma patients that responded favorably to anti-PD-1 therapy showed higher expression of IFNγ and TNF, as well as caspase-7, caspase-8, and CYLD. This suggested that the in vitro IFNγ and TNF killing mechanism might be one means of tumor cell elimination in patients following immune checkpoint blockade.

Caspase-8 is a death receptor apoptotic initiator caspase that undergoes proximity-induced activation upon recruitment to death receptor signaling complexes (7). When activated by TNFR1, caspase-8 can cleave the apoptotic effector caspases, caspase-3 and -7, which leads to cell death. CYLD is a de-ubiquitylating enzyme that has been reported to remove ubiquitin chains from TNFR1 complexes that are important for the TNFR1 pro-survival signal (8), and therefore its increased activity can favor a TNFR1-driven death response (Fig. 1).

To examine the functional significance of IFNγ-induced caspase-8 and CYLD expression, the authors performed genetic knockout, knockdown, and overexpression studies in order to mimic or prevent IFNγ-induced caspase-8 and CYLD. Importantly, these gene dosage titrations confirmed that cancer cell lines are exquisitely sensitive to caspase-8 and CYLD levels when it comes to TNF signaling responses: reduced amounts protected from IFNγ and TNF killing, while increased amounts sensitized to TNF-induced cell death, with co-depletion or co-expression of caspase-8 and CYLD having an additive impact.

Next, the authors asked how IFNγ induced caspase-8 and CYLD expression. Both increased caspase-8 and CYLD expression, and IFNγ and TNF killing, were abolished by genetic loss of the transcription factor IFN regulatory factor 1 (IRF1). Meanwhile IRF1 overexpression alone sufficed to induce caspase-8 and CYLD and sensitize cells to TNF, indicating that IFNγ-induced production of IRF1 drives caspase-8 and CYLD production. Consistent with this, IRF1 bound

[1]The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia; [2]The Department of Medical Biology, University of Melbourne, Parkville, Australia.

Correspondence to James E. Vince: vince@wehi.edu.au.

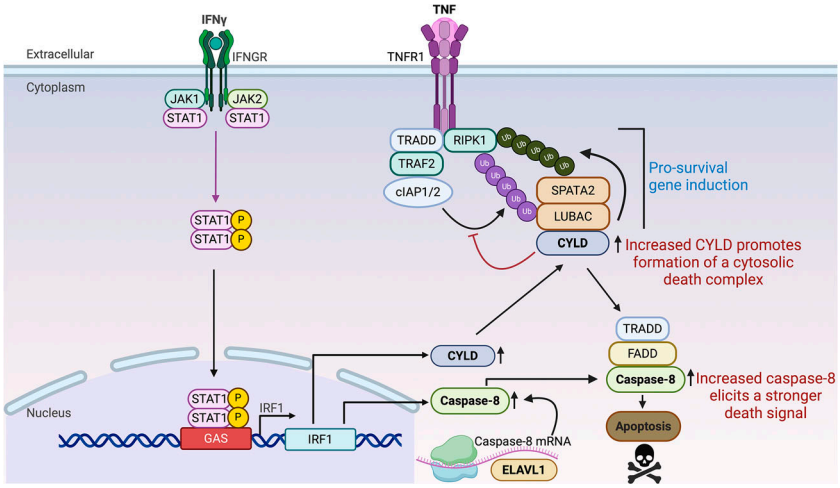

Figure 1. **IFNγ licenses TNF-induced cell death through increased CYLD and caspase-8 expression.** IFNγ signaling activates STAT1, which induces production of the transcription factor IRF1, capable of binding ISREs in the capase-8 and CYLD promoters. Consequently, CYLD and caspase-8 expression is increased and, in the presence of TNF signaling, heightened CYLD levels and activity remove ubiquitin chains from the pro-survival TNFR1 complex. This de-ubiquitination of TNFR1 complex components, such as RIPK1, promotes formation of a cytosolic death signaling complex containing apoptotic caspase-8. Increased caspase-8 not only results from IRF1-driven de novo gene transcription, but also via ELAVL1 binding caspase-8 mRNA to stabilize it and enhance its translation. IFNGR, IFNγ receptor; GAS, γ IFN activation site; TRADD, TNFR1-associated death domain protein; FADD, FAS-associated death domain protein; LUBAC, linear ubiquitin chain assembly complex; SPATA2, spermatogenesis associated protein 2; TRAF2, TNF receptor associated factor 2; cIAP, cellular IAP; Ub, ubiquitin.

to the promoter regions of caspase-8 and CYLD, and IFN-stimulated response elements (ISREs) were identified whereby CRISPR/Cas9-mediated mutation of these caspase-8 and CYLD ISREs prevented IFNγ-induced caspase-8 and CYLD expression (Fig. 1).

Having defined key IFNγ-responsive cell death regulators that sensitize cells to TNF, the authors focused on the role of ELAV-like RNA binding protein 1 (ELAVL1, also known as human antigen R, HuR)—an mRNA binding protein that the authors identified from a CRISPR/Cas9 screen as being required for IFNγ and TNF killing. Although ELAVL1 expression was not induced by IFNγ treatment, ELAVL1 binding to caspase-8 mRNA was critical for its pro–cell death functions (Fig. 1). The loss of ELAVL1 specifically de-stabilized caspase-8 mRNA, not other important TNFR1 complex cell death regulators, and also prevented increased caspase-8 levels when cells were treated with IFNγ. In fact, the levels of caspase-8 protein in ELAVL1-deleted cells were markedly reduced and, consequently, this conferred some protection from cell death induced by other activators of TNFR1 killing (TNF co-treatment with IAP antagonists or cycloheximide).

The discoveries from this study have broad relevance to our understanding of the physiological scenarios by which TNF's capacity for inducing cell death is unleashed. Although findings provide one explanation for how IFNγ can prime cells for TNF killing, via increasing caspase-8 and CYLD expression, the authors also observed apoptotic caspase-7 induction, and the significance of this was not further explored. Similarly, it will be of interest to examine the relevance of the other important death receptor initiator caspase, caspase-10. This is because in other cancer cell lines, such as HT29 cells, the expression of caspase-10 was induced by IFNγ treatment to a higher level than caspase-8, and cell death caused by IFNγ and IAP protein antagonist treatment could only be blocked when both death receptor initiator caspases, caspase-8 and caspase-10, were co-deleted (on a necroptotic deficient background) (9). On the other hand, recent research has implicated non-enzymatic caspase-8 activity in the cell death caused by IFNγ and TNF treatment of intestinal epithelial cells, although this conclusion requires genetic testing (10).

The circumstances and cellular context of IFNγ challenge will influence the genes that are expressed, and the mode of cell death subsequently engaged, following TLR or TNFR1 activation or pathogen sensing. For example, while the current study primarily focused on cancer cell lines, primary cells can behave differently. As shown by the authors themselves and other labs (2, 3), in mouse macrophages, but not cancer cell lines, IFNγ primes for TNF and/or TLR killing via the production of inducible nitric oxide synthase (iNOS). Why these cell type–specific discrepancies in killing mechanisms occur remains unknown—although the critical anti-pathogen roles of innate immune cells may have endowed them with unique cytokine responses and sensitivities to free radicals, such as iNOS generated nitric oxide. Similarly, ELAVL1 can act to limit cell death in some circumstances by, for example, repressing caspase-2 levels in cancer cell lines (11), while in bone marrow progenitor cells ELAVL1 deletion increases levels of pro-apoptotic proteins, including caspase-8, caspase-9, NOXA, and PUMA (12). Therefore, how broadly ELAVL1 acts to stabilize caspase-8 mRNA and increase its translation across diverse cell types to allow for efficient death receptor killing will be important to define.

Collectively, building on the discoveries from Buhao Deng et al., further explorations are warranted into the differential mechanisms of IFNγ- and TNF-induced cell death in primary cell types versus cancer cells. Such findings may expose cancer cell vulnerabilities that can be exploited to induce selective tumor cell death or identify targets for therapeutic intervention in autoinflammatory conditions.

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