Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Overall while the presence of shared barcodes supports a shared origin, their absence does not disprove it.

Feng et al.’s results suggest that pDCs are predominantly myeloid-derived in the steady state. With the caveat that physiological hematopoiesis is disrupted in disease, this is consistent with findings in pDC-associated pathology. The rare pDC disorder blastic plasmacytoid dendritic cell neoplasm (BPDCN) is associated with myeloid malignancies in 20% of cases. For example, although BPDCN and chronic monomyelocytic leukemia (CMML) have divergent mutational landscapes, they share BM TET2 mutations that skew hematopoiesis toward monocytes and DCs (Batta et al., 2021).

Similarly, in primary human DC immuno-deficiencies (e.g., GATA2, IRF8), both cDCs and pDCs are lost while lymphoid lineages are preserved (Bigley et al., 2016).

Collectively, Feng et al.’s study supports the notion that pDCs are closely related to cDCs in steady-state hematopoiesis and suggest that calls to exclude pDCs from the DC family may be premature.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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**The domiNO effect turns macrophage activation deadly**

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Macrophage activation is essential for effective immunity to infection but can also contribute to disease through incompletely understood mechanisms. In this issue of *Immunity,* Simpson et al. reveal that death of activated macrophages integrates extrinsic and intrinsic pathways of apoptosis that contribute to damaging host responses.

Interferon gamma (IFN-γ) coordination with Toll-like receptor (TLR) signaling is long established to drive the classical (M1) activation of macrophages (Schröder et al., 2006). Specialized in pathogen killing, M1 macrophages are defined as pro-inflammatory through expression of tumor necrosis factor (TNF) and interleukin-1β (IL-1β), as well as inducible nitric oxide synthase (iNOS) responsible for production of nitric oxide (NO). The eventual elimination of these cells through various forms of cell death is highly consequential to infection outcome, as the mode of death can determine a timely resolution of the response and eliminate infected cells or contribute to further inflammatory cytokine signaling and tissue damage. How cells die and their relative contributions to inflammation have become central questions in the current understanding of coronavirus disease 2019 (COVID-19) severity caused by infection with severe acute respiratory syndrome coronavirus
2 (SARS-CoV-2) (Diamond and Kanneganti, 2022). In this issue of Immunity, Simpson et al. reveal that the normally immunologically silent pathways of apoptosis can be rewired by IFNγ priming of macrophages and iNOS expression to drive an inflammatory caspase 8-dependent cell death that is detrimental to the host in the context of SARS-CoV-2 infection (Simpson et al., 2022).

Apoptosis is a form of programmed cell death that is critical to embryonic development and tissue homeostasis, including immune cell compartments (Anderton et al., 2020). The “extrinsic” pathway of apoptosis can be driven by ligation of specific members of the TNF receptor family (for example, TNFR and FAS) and activation of initiator caspase-8 or -10. Alternatively, the “intrinsic” pathway is initiated by mitochondria outer membrane permeability (MOMP) and loss of membrane potential, resulting in mitochondrial release of cytochrome c and activation of initiator caspase-9. Extrinsic and intrinsic apoptosis pathways converge at the level of downstream executioner caspases-3 and -7 that are ultimately responsible for stepwise cellular demise. The controlled nature of apoptosis is key to tissue homeostasis, wherein phagocytosis of dying cells by their neighbors prevents inflammatory responses. In contrast, programmed cell death pathways resulting in lysis, including pyroptosis and necroptosis, involve release of cytokines and cellular danger signals that recruit inflammatory cells and contribute to tissue damage. Despite their initial definitions, increasing evidence suggests that these pathways are not strictly partitioned and that cross-talk can occur between intrinsic and extrinsic apoptosis pathways or between apoptotic and lytic pathways (so-called PANoptosis). Understanding the molecular cascades that drive pro-inflammatory modes of cell death and their specific contributions to disease is important in developing countermeasures (Anderton et al., 2020), particularly in diseases like COVID-19 where dysregulated inflammation determines disease severity.

Simpson et al. used the power of mouse genetics to determine how classic activation pathways of inflammatory macrophages determine cellular fate (Figure 1). Stimulation with IFNγ prior to addition of toll-like receptor 4 (TLR4), TLR2, or TLR3 agonists resulted in an apoptotic death dependent on expression of the death receptors, TNFR or FAS, and initiator caspase 8. In mouse bone marrow-derived macrophages (BMDMs), caspase 8 was found to co-regulate IFNγ- and TLR-induced upregulation of genes encoding proinflammatory cytokines TNF as well as iNOS. Caspase 8 also transcriptionally repressed expression of the pro-survival protein BCL-2 (Simpson et al., 2022). BCL-2 represents a major checkpoint governing the cellular decision to undergo apoptosis where its ratio of expression relative to that of the pro-apoptotic BCL2-associated X protein (BAX) and BCL2-antagonist/killer 1 (BAK) determines cell fate at the level of mitochondria. BAX and BAK are responsible for pore formation in the outer mitochondrial membrane, MOMP, release of cytochrome c and downstream activation of the caspase-9 cascade. The canonical function of caspase 8 in BAX and BAK activation is through cleavage of BH3-interacting domain death agonist (BID) that drives oligomerization of BAX and BAK into pores (Korsmeyer et al., 2000). However, while Bax−/−Bak−/− BMDMs were partially resistant to IFNγ- and TLR-induced death, BID was not required. In addition, a clinically relevant BCL-2 inhibitor (ABT-199) restored IFNγ- and TLR-induced cell death in the absence of caspase 8 (Simpson et al., 2022). Thus, caspase 8-dependent MOMP by BAX and BAK is non-canonical and may be determined by reduced BCL-2 expression and loss of apoptotic suppression.

Interestingly, in a curious feedback loop, activation of caspase 8 appeared to be further regulated by iNOS expression. Consistent with the transcriptional...
role of caspase 8 in promoting iNOS expression, Simpson et al. showed that caspase 8 was required for maximal nitrite (NO₂⁻; product of NO oxidation) in cell supernatants. However, genetic or pharmacological inhibition of iNOS prevented optimal caspase 8 processing and blunted all downstream activation cascades required for cell death. Loss of iNOS and NO also decreased protein expression of two additional pro-survival proteins, myeloid cell leukemia-1 (MCL-1) and Bcl-2-related protein A1. As cell survival is determined by the relative expression levels of multiple pro-survival proteins rather than a single protein (Carrington et al., 2017), it is likely that the coordination of iNOS-mediated production of NO with caspase 8 to suppress BCL-2 transcription and downregulate MCL-1 and A1 protein expression is responsible for sensitizing macrophages to MOMP and apoptotic death.

What is the consequence of this domino effect? In contrast to classical apoptosis, it appears that macrophages primed by IFNγ and TLR ligands and responding to death receptor ligation further promoted inflammatory responses. Increases in inflammation most likely resulted from the combination of transcriptional upregulation of inflammatory genes (including TNF and IL-6) together with mitochondrial damage (Simpson et al., 2022). The machinery of cell lysis pathways traditionally linked to inflammasome activation (pyroptosis), including the pore forming protein GSDMD, NLRP3 inflammasomes, and caspase-1 or caspase-11, was not required for caspase8-dependent cell death. However, mitochondrial stress has direct implications to activation of inflammasomes, as well as release of mitochondrial DNA through BAX and BAK pores that is sensed by the cGAS-STING pathway recently implicated as a driver of SARS-CoV-2 pathogenesis (Di Domizio et al., 2022; McArthur et al., 2018). Indeed, mice deficient for either iNOS or caspase 8 and infected with SARS-CoV-2 did not experience the weight loss observed in virus-infected wildtype mice during the early stages of infection. Deficiency in iNOS or caspase 8 did not result in notable effects on virus burden in the mouse lung, suggesting that the iNOS-caspase 8 cascade contributes to a damaging host response rather than direct pathogen control (Simpson et al., 2022). As innate immune cells, early events in macrophage responses likely have ripple effects to amplify pathogenic inflammation in susceptible individuals.

A major unresolved question arising from this work is the specific role of catalytically active caspase 8. Multiple experiments implicated the catalytic activity as being important for downstream events including BAX and BAK activation, but a specific catalytic substrate was not identified (Simpson et al., 2022). It is possible that caspase 8 enzymatic activity was required for its roles in transcriptional regulation and separately in activation of execution caspase-3 and -7. However, caspase 8 has a catalytic-independent role as a scaffolding protein that promotes NFκB activation (Henry and Martin, 2017), which is a key transcription factor downstream of the TLRs, IFNγ, and death receptors. Therefore, the precise roles of caspase 8 remain to be determined. Furthermore, a recent study also implicated IFNγ-mediated sensitization of macrophages to TNF-induced cell death as an important driver of inflammation and tissue damage in a mouse model of COVID-19 (Karki et al., 2021). Here, the mechanism was also dependent on crosstalk between IFNγ signaling (requiring STAT1 and IRF-1), caspase 8, and iNOS but was associated with hallmarks of PANoptosis (that includes markers of pyroptosis, apoptosis, and necroptosis). Activation of multiple effectors of cell death that likely contribute to inflammation were observed in the studies by both Simpson et al. and Karki et al., respectively. The precise identification of drivers and executioners of the caspase 8-iNOS cascade of cell death identified using mouse genetics have provided a clearer picture of therapeutic targets that could further limit damaging inflammation in the context of COVID-19 and other inflammatory disorders (Simpson et al., 2022). Importantly, removal of a few key dominos could disrupt the rippling cascades primed by IFNγ and accelerated by iNOS and caspase 8.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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