fined that COVID-19 has a higher case fatality rate in individuals over 60 years of age (*3*). Yet, most in vivo research models use young, healthy animals. Models that recapitulate this aging phenotype would be incredibly useful to examine the underlying biology behind this phenomenon. To address this, Shi *et al.* examined COVID-19 symptoms in kittens versus cats aged 6 to 9 months, but found the opposite phenotype. COVID-19 was more severe in kittens; one kitten died on day 3 after infection and had a larger viral distribution compared to older cats. This variation in viral dissemination in kittens may be reminiscent of the vast tissue tropism and variation in disease severity exhibited by SARS-CoV-2 in humans (*12*). By contrast, Rockx *et al.* tested SARS-CoV-2 in young and aged macaques and did not observe any age-dependent differences. Of note, the virus is not consistently lethal in any of the animals tested thus far, nor does SARS-CoV-2 infection in these animals recapitulate the severe clinical symptoms observed in humans. As these animal models continue to be developed, attention should be paid to the role of age and other health conditions; these factors may be critical parameters that are necessary to fully evaluate human disease.

Transmission of viruses between people, either through contact (direct or indirect) or virus-containing aerosols, is a key determinant of viral disease burden globally. An important aspect of the SARS-CoV-2 pandemic, and previous influenza pandemics, is efficient airborne transmission of the virus. Airborne transmission can include a wide range of aerosol sizes. At close contact ranges, the exposure to large and small aerosols containing viruses is high. Shi *et al.* examined airborne transmission of SARS-CoV-2 between kittens and aged cats and observed that in both scenarios, the virus could transmit through the air to 33% of naïve recipient cats or kittens. In these studies, animals were separated by perforated barriers that limit physical contact but allow for air to be shared between the experimentally infected donor and the susceptible recipient. Studies of influenza virus transmission have indicated that viral replication in the upper respiratory tract, and specifically the soft palate, play an important role in airborne transmission (*13*). Shi *et al.* found that SARS-CoV-2 replicated in the soft palate of cats, kittens, and ferrets. Although ferret transmission was not examined in this study, a report suggested a similar airborne transmission rate of 30% for SARS-CoV-2 in ferrets (*14*). No contact transmission between dogs and other animals (pigs, ducks, and chickens) was observed (*1*).

The transmission of SARS-CoV-2 between cats highlights the susceptibility of this animal model to infection. Consistent with this observation, transmission of SARS-CoV-2 from humans to tigers was recently documented, as was virus spread among big cat units in the Bronx Zoo (*15*). On the basis of data from Shi *et al.*, infected cats appear asymptomatic, so infections in cats may go undetected. Additional studies are needed into the seroprevalence of SARS-CoV-2–specific antibodies in cats and identification of coronaviruses from this animal source to ascertain the potential for cats to be an intermediate host for SARS-CoV-2.

As the pursuit of SARS-CoV-2 vaccines and antivirals surges on, animal models play the most important role to determine the effectiveness of potential therapeutic strategies. The available studies suggest that hamsters, ferrets, and cats may serve as attractive alternatives to nonhuman primate and transgenic mouse studies. Because hamsters and transgenic mice display the most severe clinical symptoms, such as weight loss, they may provide robust small-animal models for studying efficacy of various vaccine platforms. By contrast, cats and ferrets may provide a useful model system for studying transmissibility of the virus and the effectiveness of antivirals to limit spread. With robust reduction in viral load as presented by Gao *et al.* (*9*), nonhuman primates may offer the most relevant model to assess vaccine and antiviral effectiveness before rapid deployment to humans. Therefore, continued evaluation of mice to nonhuman primate models will provide critical data on the animals best suited to study the many open questions about COVID-19. ■

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IMMUNOLOGY

Killer cells add fire to fuel immunotherapy

Cytotoxic lymphocytes induce inflammatory target cell death, amplifying antitumor immunity

By **Christopher J. Nicolai** *and* **David H. Raulet**

Cytotoxic lymphocytes, including natural killer (NK) cells and cytotoxic T lymphocytes (CTLs or $CD8^+$ T cells), mediate antiviral and antitumoral immunity. Target cell killing by CTLs and NK cells is primarily mediated through the release of cytotoxic granules that contain serine proteases called granzymes and a pore-forming protein, perforin. Perforin delivers granzyme B (GZMB) into target cells, where it initiates apoptosis, a noninflammatory form of programmed cell death (*1*, *2*). On page 965 of this issue, Zhou *et al.* (*3*) report their discovery of a new mechanism of cytotoxicity in which granzyme A (GZMA), delivered to certain target cells by NK cells and CTLs, activates gasdermin B (GSDMB), a pore-forming protein, which causes a proinflammatory form of cell death called pyroptosis (*4*). Expression of GSDMB by mouse tumor cells conferred better tumor control in response to immune checkpoint therapy.

Programmed cell death can occur through a variety of pathways that result in distinct biological outcomes. The best studied programmed cell death pathway is apoptosis, in which cytoplasmic and nuclear material condense into membrane-bound fragments, called apoptotic bodies, which are phagocytosed and destroyed by macrophages (*2*). Cell death through apoptosis is considered noninflammatory and is necessary for organismal development and homeostasis. By contrast, pyroptosis ("fiery death") is a proinflammatory form of cell death, usually activated by multiprotein oligomers called inflammasomes, which are triggered by a variety of stimuli. Inflammasomes activate inflammatory caspase-1, -4, -5, or -11, leading to lytic cell death and the release of proinflammatory

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molecules, such as the cytokines interleukin-1β (IL-1β) and IL-18, and eicosanoids (*4*, *5*). Two pivotal studies identified gasdermin D (GSDMD) as a target of inflammatory caspases and the key executor of pyroptosis (*6*, *7*). Upon cleavage, the amino-terminal domain of GSDMD oligomerizes to form pores in the cell membrane, leading to cell permeabilization and cytokine release. GSDMD is one of a family of gasdermin proteins (*8*) that have pore-forming domains but differ in the proteases that activate them. The roles of some members of the gasdermin family are unknown.

Zhou *et al.* provide compelling evidence that cytolysis by CTLs and NK cells is sometimes pyroptotic instead of apoptotic. In addition to GZMB, granules released by cytotoxic lymphocytes contain GZMA, which directly cleaves GSDMB in the cytosol, unleashing its amino-terminal pore-forming domain and initiating pyroptosis. Because this can be faster than apoptosis, cells that expressed GSDMB underwent pyroptosis rather than apoptosis. GSDMB expression was rare in a large panel of human tumor cell lines, but its expression could be induced in ~30% of the lines upon stimulation with cytokines, especially interferon-γ (IFN-γ), a pleiotropic cytokine secreted by activated lymphocytes, including CTLs and NK cells. Mice do not express a GSDMB homolog yet still express GZMA. Mice bearing tumors engineered to express GSDMB showed greater tumor rejection than did mice bearing GSDMB-deficient tumors when combined with anti–programmed cell death protein 1 (PD-1) immunotherapy. These findings suggest that PD-1 blockade was necessary to unleash antitumor immune responses that GZMA-dependent GSDMB activation helped to amplify. The results support the view that cytotoxic lymphocytes both kill target cells and in some cases activate additional inflammatory signals through pyroptosis that amplify the immune response.

GSDMB was more frequently expressed in human tumors that arose in mucosal and gastric tissues than in other types of tumors, although less often than in corresponding normal tissues, possibly reflecting selective loss of GSDMB in advanced cancers. In some types of human cancer, notably bladder carcinoma and skin cutaneous melanoma, there was a correlation between GSDMB expression and higher patient survival, although GSDMB expression in renal clear cell carcinomas was associated with lower patient survival.

Pyroptotic killing induced by cytotoxic lymphocytes has been observed previously, although mediated by other gasdermin family members. For example, GZMB from NK cells directly cleaved and activated GSDME in target cells and concomitantly activated caspase 3, which also activates GSDME (*9*). Cleaved GSDME initiated pyroptosis and amplified immune infiltration of mouse mammary tumors grafted in mice, macrophage phagocytosis of tumor cells, and tumor rejection. Disruption of the *Gsdme* gene in mouse tumor cell lines resulted in faster-growing tumors and reduced survival in vivo, building on previous evidence that GSDME is a tumor suppressor that is frequently mutated or repressed in tumors.

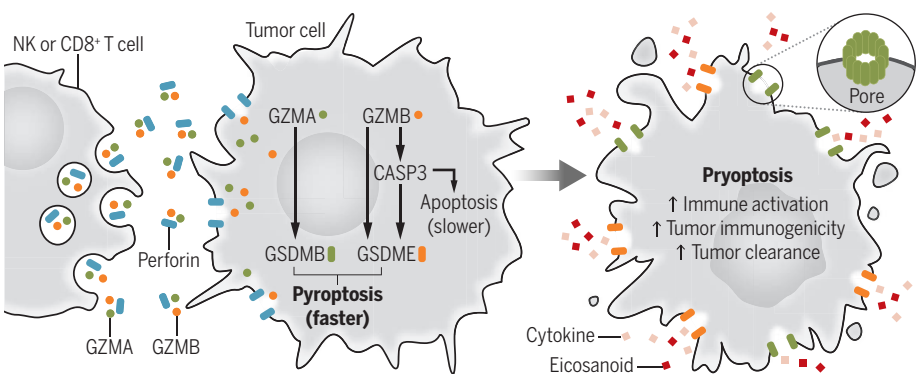
Killing of human CD19$^+$ leukemia cells in vitro by CD19-targeting chimeric-antigen receptor (CAR) T cells initiates tumor cell pyroptosis (*10*). In this case, pyroptosis was associated with systemic inflammation, known as cytokine release syndrome, after CAR-T cell treatments in mouse models. The authors reported that GSDME-dependent pyroptosis was induced only when the T cells encountered the strong activating signals mediated by CAR engagement with CD19 and not through typical T cell antigen receptor–mediated signaling. Furthermore, another study showed that introducing nanoparticles containing active GSDMA into tumor cells mobilizes powerful antitumor T cell responses (*11*). It was shown that IL-1β, and to a lesser extent IL-18, were necessary for this amplified antitumor response.

Together with the study of Zhou *et al.*, these studies suggest that pyroptosis is a common outcome of encounters between cytotoxic lymphocytes and susceptible target cells and that pyroptosis provides a feed-forward mechanism to amplify cellular immunity and tumor rejection (see the figure). These studies do not yet provide a detailed understanding of how pyroptosis amplifies the antitumor response. Inflammatory signaling coupled with the release of target cell antigens are likely important, but the relevant inflammatory signals remain poorly defined. In one case, IL-1β and possibly IL-18 were shown to play a role in pyroptosis-induced immune enhancement (*11*), whereas another report argued that IL-1β plays no role (*9*). If IL-1β and/or IL-18 are important, it remains unclear exactly how they mediate their amplifying effects.

Moreover, it is not clear how the cytokines are elaborated in these circumstances. In inflammasome-induced pyroptosis, "priming" of cells by pattern-recognition receptors (which recognize pathogens) is often required for synthesis of pro–IL-1β and pro–IL-18, and the cytokines are processed by caspases to generate the active forms (*5*). How priming might occur in tumor cells is unclear, but activation of stimulator of interferon genes (STING; a sensor of cytoplasmic DNA) is an intriguing possibility (*12*). Although much remains to be learned, these findings require a revision of how cell-mediated cytotoxicity occurs and its consequences. ■

Pyroptosis amplifies cellular immunity

Granzyme B (GZMB) and GZMA released by cytotoxic lymphocytes [natural killer (NK) and CD8+ T cells] penetrate target cells with the help of perforin. Recent evidence reveals that GZMA directly cleaves gasdermin B (GSDMB), whereas GSDME can be activated directly by GZMB but also indirectly by caspase 3 (CASP3). Activation of GSDMB or GSDME initiates pyroptosis, which promotes inflammation that amplifies cellular immunity.



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Killer cells add fire to fuel immunotherapy

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