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COMMENT

Defining lytic IFNγ for deciphering the CTL mechanism

Jaewoo Park[1] and SangJoon Lee[1,2]✉

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Since the discovery of the "viral interference factor" by Isaacs and Lindenmann in 1957 and its subsequent identification as an antiviral substance in phytohemagglutinin (PHA)-treated human leukocytes by Wheelock in 1965, interferon-gamma (IFNγ) has been a focal point of immunological research. Following the elucidation of the JAK-STAT pathway in the 1990s, the role of IFNγ in tumor elimination and adaptive immunity became indisputable. However, despite decades of research, the mechanisms by which IFNγ functions within the highly specialized cytotoxic T lymphocyte (CTL) killing machinery remain incompletely understood.

By addressing three fundamental mysteries that have challenged the canonical understanding of IFNγ, Li et al. initiated their investigation [1]. The first mystery concerns the packaging and delivery paradox. If granzyme B (GzmB) and IFNγ are secreted via distinct pathways, specifically GzmB via lytic granules and IFNγ via the constitutive Golgi-derived secretory pathway [2, 3], how could they reach the target cell simultaneously and at sufficiently high concentrations to be effective during the rapid phase of synaptic engagement? While classical views hold that cytokines are released multidirectionally, conflicting evidence suggesting that the synaptic polarization of IFNγ has created confusion in the field [4]. The second mystery involves the mechanistic gap. While IFNγ is rapidly produced upon T-cell activation, a defined molecular mechanism explaining its direct contribution to acute cytotoxicity has not been defined. Emerging evidence has suggested a direct role [5], but how IFNγ, traditionally viewed as a slow-acting modulator, could influence the rapid kinetics of CTL killing remains unclear. The third mystery is the spatial paradox. The cytotoxic synapse is designed to restrict lethal hits to a specific antigen-bearing target cell, thereby preventing collateral damage [4]. Conversely, IFNγ is known for its widespread, pleiotropic effects on the surrounding tissue [6]. Explaining how IFNγ can simultaneously fulfill the roles of a lytic, precision-guided weapon and a nonlytic, broad-spectrum modulator requires a new biological model.

Li et al. systematically resolved these mysteries through a rigorous experimental design combining high-resolution imaging with functional genetics [1]. To address the first mystery, packaging, the authors employed structured illumination microscopy (SIM) and correlative light and electron microscopy (CLEM). These techniques revealed that endogenous IFNγ is physically stored within GzmB-positive compartments. CLEM analysis further classified these compartments into single-core granules (SCGs) and multicore granules (MCGs), confirming that IFNγ is not merely in the vicinity but is structurally copackaged with lytic effector molecules. Total internal reflection fluorescence microscopy (TIRFM) provided real-time evidence that IFNγ and GzmB are coreleased at immunological synapses with identical kinetics. This effectively resolved the packaging paradox; they arrived together because they traveled together. To elucidate the underlying mechanism, a combination of killing assays, antibody neutralization, and specific pathway inhibitors were used. The data demonstrated that neutralizing IFNγ significantly reduced the killing efficiency of wild-type CTLs. Importantly, the authors showed that while recombinant IFNγ alone is nontoxic, it acts synergistically with GzmB and perforin to enhance cytotoxicity. Mechanistically, this process was shown to depend on the JAK-STAT1 pathway in target cells, leading to caspase-3 activation. These findings proved that synaptic IFNγ serves as an immediate sensitizer, priming target cells for GzmB-mediated apoptosis. Finally, addressing the third mystery, the spatial paradox, required looking beyond the synapse. By extending the observation time to more than one hour, the researchers captured a distinct phenomenon: the secretion of IFNγ from the distal membrane away from the synapse. Crucially, experiments using Munc13-4 knockout mice, which lack the machinery for granule exocytosis, revealed that although early synaptic release was abolished, late distal secretion persisted. These findings confirmed that the broad immunomodulatory function of IFNγ is mediated by a separate, nonlytic pool of IFNγ originating from multivesicular bodies (MVBs) rather than from cytotoxic granules.

The most significant conceptual advance of this study lies in the definition and categorization of 'lytic' and 'nonlytic' IFNγ pools, a distinction grounded in four key criteria: storage location, secretion site, timing, and function. The lytic pool is stored within cytotoxic granules (SCGs and MCGs) alongside GzmB and is polarized toward the immunological synapse during the early phase of engagement, a process strictly dependent on the priming factor Munc13-4, thereby driving acute cytotoxicity via direct synergy with perforin and granzymes. In contrast, the nonlytic pool resides in MVBs or small vesicles segregated from lethal effectors and is released from distal membrane sites or multidirectionally during prolonged stimulation. This release occurs independently of Munc13-4 and is likely to facilitate long-range immunomodulation and intercellular signaling (Fig. 1). This "dual-pool model" elegantly reconciles the conflicting observations of previous decades, explaining how a single cytokine can act as both a precise killer and a systemic regulator.

Li et al. demonstrated that lytic IFNγ synergizes with GzmB to accelerate the cleavage of Caspase-3, thereby driving the canonical apoptotic pathway in target cells [1]. While the authors focus on this established mechanism of cell death, the observation of a locally high-concentration "Lytic IFNγ" at the synapse raises the possibility that additional programmed cell death pathways,

[1]Department of Biological Science, Ulsan National Institute of Science and Technology (UNIST), Ulsan, Republic of Korea. [2]Graduate School of Health Science and Technology, Ulsan National Institute of Science and Technology (UNIST), Ulsan, Republic of Korea. ✉email: sangjoon.lee@unist.ac.kr

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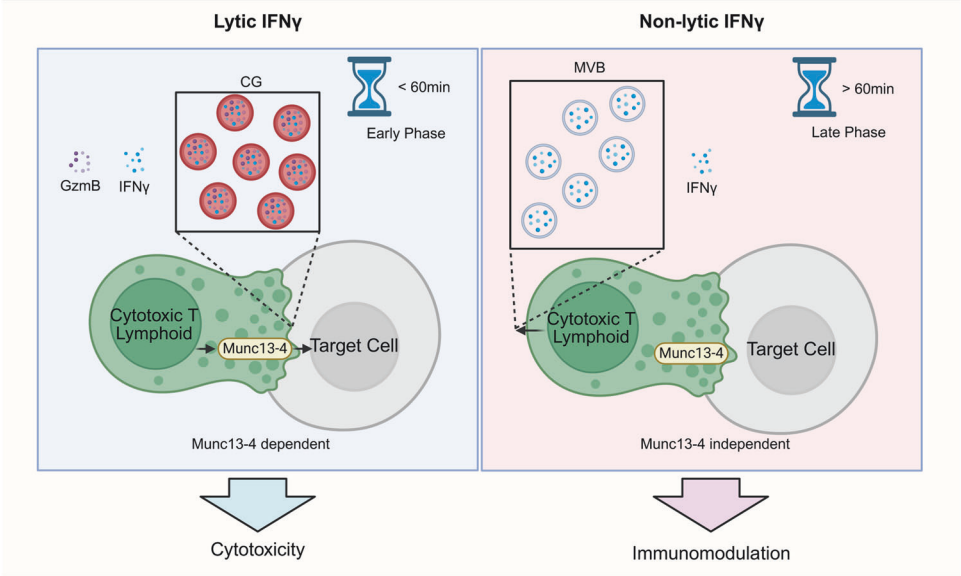

Fig. 1 Lytic IFNγ and Nonlytic IFNγ. Munc13-4-dependent lytic IFNγ is released during the early phase, whereas Munc13-4-independent nonlytic IFNγ is released during the late phase. GzmB: Granzyme B, CG Cytotoxic Granule, MVB Multivesicular Bodies

specifically PANoptosis, may be engaged. PANoptosis is an inflammatory cell death pathway regulated by the PANoptosome complex, which integrates components of pyroptosis, apoptosis, and necroptosis [7]. Recent studies beyond the scope of this paper have suggested that IFNγ, often acting in concert with TNFα, can trigger this pathway [8]. Specifically, IFNγ signaling via the JAK-STAT1 axis activates interferon regulatory factor 1 (IRF1). IRF1 is a critical transcription factor that drives the expression of ZBP1 and other components necessary for the assembly of the PANoptosome [8, 9]. The findings of Li et al. provide a plausible delivery mechanism for this phenomenon. The "lytic IFNγ" released at the synapse creates an immediate, localized "cytokine storm" microenvironment. It is highly conceivable that this intense burst of synaptic IFNγ could rapidly upregulate IRF1 in target cells, thereby initiating PANoptosis alongside canonical GzmB-mediated mitochondrial damage. This potential connection between the lytic IFNγ mechanism described by Li et al. and the broader concept of cytokine-induced PANoptosis provides fertile ground for future investigations. If lytic IFNγ acts as a trigger for the PANoptosome, it would further explain the potent synergistic lethality observed in the study. In addition, the confirmation of lytic IFNγ in vivo using the LL/2 tumor model underscores the physiological relevance of these findings. These findings open new avenues for therapeutic intervention. For instance, in the engineering of CAR-T-cell therapies, strategies could be devised to differentially regulate these two pools. Armized CAR-T cells could be designed to increase the loading of IFNγ into lytic granules, maximizing tumor killing (the lytic pool) while fine-tuning distal release (the nonlytic pool) to manage systemic toxicity or cytokine release syndrome (CRS).

In summary, Li et al. provide a conceptual redefinition of IFNγ biology. By identifying the "lytic" subset of IFNγ, this study solves long-standing paradoxes regarding its packaging and mechanism. The proposal of the dual-pool model not only clarifies the multifaceted nature of T-cell effector functions but also provides a crucial theoretical framework for understanding the spatial efficiency of cytotoxicity. In the future of immunotherapy, understanding and manipulating these distinct cytokine pools will likely prove essential for optimizing the delicate balance between effective tumor eradication and immune homeostasis.

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AUTHOR CONTRIBUTIONS

JP and SL conceived this study, prepared the manuscript, and critically revised and approved the final submitted version of the manuscript. All the authors contributed to the manuscript and approved the submitted version.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to SangJoon Lee.