Perforin-dependent cytotoxicity: ‘Kiss of death’ or prolonged embrace with darker consequences?

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As both NK cells and CTL can kill successive targets in rapid succession (‘serial killing’), the interaction is transient and the killer detaches from its ‘victim’ before engaging another. Decades ago, researchers observing this series of events aptly attached the term ‘kiss of death’ to this fatal interaction. Our study commenced out of our curiosity for how cell detachment occurs: we were aware that the literature on how CTL and NK cells form a stable conjugate boasts hundreds of papers, but there is virtually none that deals specifically with dis-engagement.

CTL and NK cells utilize very different receptors and co-receptors to recognize virus-infected or tumor target cells, but both need to form a stable conjugate to generate membrane signals that culminate in exocytosis of the potent granule-bound toxins, perforin and the serine protease granzymes (particularly granzyme B, GzmB) into the immune synapse.3 Perforin forms pore-like transmembrane channels that permit GzmB access to the target cell cytosol where caspase activation takes place. In humans and many types of outbred/wild mice, this occurs principally through the mitochondrial pathway due to direct truncation of Bid by GzmB; by contrast, the allotYPE of GzmB present in inbred mouse strains, GzmB predominantly processes pro-caspases directly. 4, 5

Our recent work is the first to shed some light on how CTL/NK cells terminate their contact with a target cell. Our findings arose from two sets of observations that we ultimately linked. Two years ago we developed live cell microscopy methodologies that pinpoint the precise timing of the calcium flux that signals the CTL/NK cell to mobilize its secretory granules to deliver perforin and GzmB to the immune synapse via exocytosis, a process also known as ‘degranulation’.6 By adding a higher than usual concentration (100 uM) of the RNA-binding dye propidium iodide (PI) to the culture medium, we observed intense red fluorescence in the target cell some 60–80 s after degranulation in the killer. This signified that PI had entered the target cell cytosol via perforin pores that had rapidly assembled in the plasma membrane, a process we have shown to be unidirectional. 7 Starting just 2-15 min later, the target cell started to show obvious apoptotic morphology, and detachment of the killer cell then followed.6

The second observation sprang from our interest in congenital perforin deficiency. Infants born with familial hemophagocytic lymphohistiocytosis Type 2 (FHL2) have bi-allelic mutations in the perforin (PFN1) gene, and die from an overwhelming cytokine storm soon after birth.8 To model the disorder, we allowed Prf1 gene-deficient mouse NK or TCR-transgenic CTL to form bona fide immune synapses under the time-lapse microscope, while also assaying the culture supernatant for inflammatory cytokines.

As we expected, the killer cells formed normal synapses, but failed to kill their targets. To our surprise, we also found that the two cells remained in contact for far longer (on average around five times as long) than when apoptosis was induced with wild type CTL/NK cells. Furthermore, calcium signaling in the Prf1-deficient effector lymphocytes did not terminate, with calcium levels continuing to oscillate indefinitely. As a result, secretion of the key cytokine interferon-γ and various chemokines was greatly enhanced in comparison to cultures that included immune-competent killers. The cytokine cocktail was able to induce naïve syngeneic macrophages to secrete large quantities of interleukin (IL)-6. These findings explained how the absence of perforin from lymphoid killer cells is linked to hyperactivation of the myeloid compartment, a deadly hallmark of FHL2 and related conditions.9, 10

Studying rare but important diseases can be rewarding, but our study then took an unexpected twist with potentially far broader significance. NK and CTLs that expressed normal perforin but lacked granzymes also failed to killed target cells efficiently and showed a very similar phenotype to Prf1-null killers: delayed detachment, persistent calcium signaling.

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Failure of natural killer (NK) cells or cytotoxic T-lymphocytes (CTL) to killognate target cells results in cytokine/chemokine hypersecretion and markedly delayed killer/target cell detachment. With congenital perforin deficiency, fatal cytokine storm results. In cancer cells, where corrupted apoptotic signaling frequently delays apoptosis, we propose that failed death may alter the tumor microenvironment and skew immune infiltrates, even when perforin is delivered normally.

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and elevated interferon-γ secretion. Collectively, the results clearly indicated that detachment depended on the occurrence of target cell death, rather than the specific presence of either perforin or the granzymes.

In turn, this raised a key question: how is the signal for detachment generated? Does the killer cell possess a ‘molecular timer’ and detach after ‘sufficient’ time has elapsed for the target to be irreversibly damaged? Alternatively, does detachment occur only when the target cell ‘makes it known’ to the killer that cell death is proceeding, and its mission accomplished?

A very simple experiment showed the latter to be the case. When we reverted to wild type CTL or NK cells and delayed target cell death by blocking caspase activity (either chemically or by co-overexpressing Bcl–2 and XIAP), we were able to phenocopy perforin or granzyme deficiency. We are yet to precisely identify the signal for detachment, but our experiments show that a caspase-dependent event/s in the target cell is essential. Importantly, all our observations were reproduced in human NK and primary CTL clones as well as mouse CTL/NK cells, indicating the mechanism is conserved in evolution.

Primary immune deficiencies are all rare, but inhibition of cell death pathways is extremely common, both in cancer cells and cells infected with certain viruses. If our observations are valid in vivo, the array of cytokines and chemokines expressed by activated CTLs or NK cells in a tumor (or an infected organ) should depend on the degree to which target cell caspases can be activated (Fig. 1). It is easy to envisage that the composition of immune infiltrates might vary considerably (even in tumors of similar histological type), most obviously the degree and type of myeloid cell infiltration and activation.

After 40 years, our work has shown that the ‘kiss of death’ can take different forms. Typically, a quick ‘peck’ is sufficient to dispatch the target cell. But if there is significant resistance on the part of the victim, the ‘kiss of death’ can transform into a prolonged involuntary embrace with sinister sequelae.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.