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The Kiss of Death

The programmed cell death (PCD) of neurons is generally thought to be cell autonomous and not to require a death signal from other cells. A recent study by Marin-Teva et al., in this issue of *Neuron*, brings this theory into question and suggests that neighboring microglia actively participate in the PCD of Purkinje cells in the cerebellum.

The question of whether developmental PCD of neurons is cell autonomous versus mediated by death signals derived from adjacent cells has, until recently, been largely unresolved. Like most cell-physiological processes, the death and scavenging of cells requires flawless orchestration of numerous players. The conductor of this cellular symphony leading to the climactic death and removal of cells is largely unknown. Previous studies suggest that it is the apoptotic cell that initiates phagocytosis; however, recent work presented in this issue of *Neuron* suggests that microglia may be the impetus for death (Marin-Teva et al., 2004). One might imagine a number of cellular "verses" that must be accurately executed between these two cell populations for complete engulfment of the apoptotic cell to occur. Verses played by the apoptotic cell include "come hither," triggering the recruitment of phagocytic cells, and "eat me," indicating the need of the dying cell to be engulfed. The phagocyte responds by stimulating its mobilization to the target area, then voices "eat it" signals causing the phagocyte to tether itself to the apoptotic cell, followed by reorganizing "commands" that alter the cytoskeletal architecture necessary for engulfment, and finally an "activate" song resulting in the release of proinflammatory cytokines, often induced by lysis of the dying cell.

Although microglia have long been appreciated as the resident scavenger cells of the nervous system, little thought has been given to them as active killers of other cells. The laboratory of Michel Mallat has used an innovative strategy to selectively ablate microglia in a slice preparation of the early postnatal cerebellum. This microglial destruction results in the dramatic rescue of Purkinje neurons that otherwise would die within 24 hr in vitro. Furthermore, the authors postulate a mechanism by which Purkinje cells are compromised by the release of superoxide ($O_2^{\cdot -}$) following the respiratory burst elicited by the engulfing microglia. This burst, a key feature of most engulfing phagocytes, is the result of the conversion of oxygen to $O_2^{\cdot -}$ by activated NADPH oxidase. Additionally, $O_2^{\cdot -}$ dismutates, resulting in the formation of H_2O_2 as well as other potentially toxic molecules. These results raise the question of whether the microglial respiratory burst is orchestrating Purkinje cell death or whether the dying Purkinje cells initiate the microglial respiratory burst. Although further studies are required to definitively answer this question, these authors have made great strides toward understanding this issue.

When one attempts to understand the complex schedule of events associated with PCD, past experience tells us that the nematode *C. elegans* may be a helpful informant, whereas the immune system may play a similar role for understanding the potential involvement of microglia in this process. Using the powerful genetic tools provided by *C. elegans*, several "engulfment" genes have been identified that are thought to be part of two discrete transduction pathways, which exhibit some functional redundancy. The first group, *ced-1*, *ced-6*, and *ced-7*, encode homologs of a low-density lipoprotein receptor-related protein (LRP), the adaptor protein GULP, and a 12 transmembrane ATP binding cassette transporter protein, respectively. The second group, *ced-2*, *ced-5*, *ced-10*, and *ced-12*, encode homologs of CrkII, DOCK180, Rac, and ELMO. The first set of proteins is thought to function in corpse recognition, whereas the second is postulated to control the cytoskeletal rearrangements necessary for engulfment to occur.

In an effort to understand the role of these engulfment genes in programmed cell death, two recent studies investigated the effect of mutating these genes in concert with *ced-3*, a caspase-3 homolog. When engulfment genes were mutated alone, no alterations in cell number were observed, suggesting that these genes alone cannot actively induce death. However, in weak *ced-3(op149)* mutants, where death is only partially suppressed and a basal level of caspase activation occurs, some cells advanced through the early morphological stages of cell death but then reverted to their normal appearance and survived. These observations suggest that, under reduced caspase activation, cells have the ability to elude death, even after the initiation of an apoptotic program (Hoeppner et al., 2001). Furthermore, these studies support a threshold mechanism by which sufficient caspase-3 activation is required for the cell to undergo a complete death, which includes its engulfment and subsequent digestion.

Accordingly, a critical question is what stimulates caspase activation above this theorized threshold? Some might argue that it is the physical process of cell engulfment that is the final trigger for death, as mutants

of engulfment genes in a weakly active capase-3 background further increase the number of viable cells; however, the current study suggests that it is not the engulfment process itself, but damage imparted by the engulfing cell, in this case, the microglial respiratory burst. Furthermore, although free radical damage is known to be deleterious to neurons, other receptor-ligand interactions are present between the dying cell and the engulfing cell that may be implicated in translating or enhancing this death signal. Even though these pathways are not directly implicated as being critical for tipping the balance toward death in this current study, the types of signals that they transmit suggest that they are prime candidates to integrate a death signal. Two such pathways are involved in phospholipid signaling, one of which can be initiated by activation of caspase-3.

Although the precise mechanisms of these phospholipid pathways are not completely known, they represent critical points of potential intersection with the apoptotic machinery, including caspase-3. The display of phosphatidylserine (PS) on the surface of a dying cell has for many years been recognized as a hallmark of apoptosis. Only recently has a more mechanistic understanding of this lipid expression been gained. Two differing strategies were used to elucidate the importance of the interaction between PS and its receptor (PSR). The first, a mutant study in *C. elegans*, suggests that cell corpse engulfment is mediated by the interaction of PS with PSR and that the signal initiated by this interaction is facilitated by direct binding of PSR with CED-5 and CED-12 (Wang et al., 2003). Although not directly addressed, these data imply that the receptor-ligand interaction between PS and PSR triggers the cytoskeletal rearrangements necessary for the engulfment of a dying cell. Concurrently, studies of PSR knockout mice further underscore the importance of the PS-PSR interaction. This mouse, which is viable for only a few hours after birth, is unable to breathe, cyanotic, and displays severe lung and brain malformations. In the brain of PSR-deficient mice, a hyperplasic phenotype was noted, along with the increased presence of TUNEL- and caspase-3-positive cells. These results are consistent with the role for PSR in the engulfment and removal of apoptotic cells in the CNS (Li et al., 2003).

A second recently identified lipid mediator of the engulfment signal is lysophosphatidylcholine (LPC). LPC can be formed following the cleavage of calcium-dependent phospholipase A (PLA) by caspase-3. LPC then can be released by the dying cell, where it serves as a chemoattractant to phagocytic cells (Lauber et al., 2003). Although the vast majority of the studies investigating the chemotaxic properties of LPC have been performed on T cells, macrophages, and monocytes, these mechanisms are likely to be conserved in the brain. The recent identification of G2A as the putative receptor target of LPC on the engulfing cell provides a further understanding of how the dynamic process of chemoattraction occurs (Kabarowski et al., 2001).

It is likely that LPC signaling may be critically involved in monocyte and macrophage recruitment in the immune system, and the release of this signal may be a "threshold trigger" for caspase activation leading to death; however, not all mechanisms observed in the immune system are conserved in the nervous system, an "immune privileged" region. Furthermore, although $O_2^{\cdot -}$ release by microglia appears to be critical in Purkinje cell death, neuronal populations are heterogeneous. This is illustrated by the way that unique neuronal populations respond differently to diverse growth and survival stimuli, as well as to different inducers of apoptosis. This is further illustrated by the incapacity of Purkinje cells to respond to the inactivation of NGF, which has been shown in the developing retina to be critical for microglia-assisted death (Frade and Barde, 1998).

Cerebellar apoptosis is still incompletely understood and is inferred in vivo based on the fact that the overexpression of *Bcl-2*, an antiapoptotic gene, results in an increase in the number of viable cells during development (reviewed in Ghoumari et al., 2000). Pathologically, cell death of Purkinje cells has been noted in the *lurcher* mutant mouse, as well as in patients affected by Borna disease virus and Creutzfeldt-Jakob disease. Though it is likely that the developmental PCD of Purkinje cells in many ways mimics the death of other developing neuronal populations, critical differences may also exist since these neurons in *lurcher* mouse die independently of the proapoptotic molecule Bax (Selimi et al., 2000). Additionally, chick motoneurons, in vivo, appear to degenerate and fragment into apoptotic bodies prior to engulfment, and these degenerating cells are rarely, if ever, engulfed by phagocytes at earlier stages of degeneration (Chu-Wang and Oppenheim, 1978). Accordingly, one must be cautious in extending these findings to other neuronal systems and embrace the possibility that both microglia and neurons may serve as conductors in the symphony of death. Despite these caveats, the report of Marin-Teva et al. is significant for providing a new perspective on the regulation of vertebrate PCD, a perspective that broadens our understanding of this basic biological process and that also suggests novel targets for modulating pathological neurodegeneration.

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Local Gating of Information Processing through the Thalamus

Inhibitory sculpting of afferent signals in the thalamus is exerted by two types of neurons using γ-amino butyric acid (GABA) as neurotransmitter. Of them, local-circuit neurons exert their functions via two outputs: axons and presynaptic dendrites. In this issue of *Neuron*, Govindaiah and Cox reveal that synaptic activation of metabotropic glutamate receptors selectively increases the output of presynaptic dendrites of local interneurons in rat visual thalamus, without affecting the axonal output.

It is now firmly established that the thalamus is not just an anteroom for relaying signals to the cerebral cortex, but a structure actively implicated in shaping afferent information through inhibitory processes, thus participating in highly integrative functions during adaptive states of behavior. A large set of data using extra- and intracellular recordings from thalamic and neocortical neurons in vivo revealed that, although long-lasting inhibitory periods are reduced or erased upon awakening from sleep, the short-lasting inhibition is preserved or even enhanced (see Steriade, 2003). This reinforcement in short-lasting inhibitory processes during waking, compared to states of sleep, provides a mechanism subserving accurate discrimination of incoming signals and leads to improvement in directional selectivity (Livingstone and Hubel, 1981). Two types of inhibitory GABAergic neurons operate in the thalamus: reticular and local-circuit neurons. Both have been implicated in discrimination functions. Although thalamic reticular neurons may play a role in attention, which is impaired following large lesions of the reticular nucleus (Weese et al., 1999), and activation of some thalamic reticular sectors is observed following exploration of a novel environment (Montero, 1997), the cellular mechanisms underlying the attentive function of thalamic reticular neurons are not elucidated. Moreover, thalamic reticular neurons are mainly implicated in the generation of global oscillations in thalamocortical systems, which characteristically define the states of slow-wave sleep and some types of paroxysmal discharges during which conscious processes are suspended (Steriade, 2003). On the other hand, local-circuit interneurons have been implicated in processes related to focused attention and local discrimination processes. The latter neuronal type is confined within virtually all dorsal thalamic nuclei of felines and primates as well as the dorsal lateral geniculate nucleus (dLGN) of rats but is absent in other thalamic nuclei of rodents. The output of local interneurons arises from axon terminals that form inhibitory synapses onto somata and dendrites of thalamocortical neurons but also, importantly, from the dendritic appendages of interneurons that are equipped with presynaptic vesicles, known as F2 terminals, which contact the dendrites of thalamocortical neurons and form symmetrical (inhibitory) profiles within the triadic circuitry of synaptic aggregations called glomeruli (Jones, 1985).

In this issue of *Neuron*, Govindaiah and Cox (2004) used parasagittal slices from rat dLGN to preserve the optic tract (OT) input, recorded intracellularly from relay cells and local interneurons, and revealed that OT tetanic stimulation activated metabotropic glutamate receptors (mGluRs) located on presumed presynaptic GABA-containing dendrites of interneurons, which led to increased inhibition in target thalamocortical neurons. The contrast between these very interesting results and some previous data indicating that OT stimulation does not produce mGluRs-mediated synaptic responses in thalamocortical neurons might be explained by differences between the recording technique in the present paper (whole-cell configuration) and the sharp-electrode recordings used previously. In about 25% of thalamic relay cells, the increased incidence of inhibitory postsynaptic potentials (IPSPs) resulting from strong tetanic OT stimulation was independent of the slow depolarization. Using a series of pharmacological manipulations in the bath, the authors concluded that the increased IPSPs in thalamic relay cells was not due to suprathreshold depolarization of the interneurons at the somatic level but to OT-induced activation of mGluRs that are presumably localized on presynaptic dendrites of dLGN interneurons. The reason behind this assumption was that the OT-elicited increase in IPSPs recorded from thalamic relay cells was independent of action potentials fired by local interneurons, as would have been the case within the conceptual frame of feed-forward inhibition. Also, changing the site of stimuli showed that the increased IPSPs were selectively due to OT stimulation, since this increase was not obtained using stimuli applied to optic radiation that contains corticothalamic axons. Govindaiah and Cox hypothesized that synaptic activation of mGluRs on presynaptic dendrites of dLGN interneurons increases the release of GABA from these dendrites, without influencing the axonal output, and may modulate synaptic transmission at retino-dLGN synapses, thus representing a focal form of information integration.

Of course, any attempt toward deciphering the function of presynaptic dendrites of local thalamic interneurons is of tremendous interest since direct recordings from presynaptic dendrites in thalamic glomeruli are not yet feasible. This technical difficulty (say impossibility, at least nowadays) is combined with the fact that axons arising from thalamic reticular neurons have access not only to thalamocortical cells, but also to local interneurons, in both dLGN (Montero and Singer, 1985) and ventroposterior nuclear complex (Liu et al., 1995). Although this connection to interneurons arising in the thalamic reticular nucleus is numerically less important than that contacting relay cells, its effects may be dramatic. Indeed, following interruption of the input from thalamic reticular neurons, there is a release from inhibition of local interneurons, which results in greatly increased incidence of IPSPs in thalamic relay cells (Steriade et al., 1985). To safely circumvent the possible interven-