

● PERSPECTIVE

Neurodegeneration and neuroinflammation: two processes, one target

The etiology of neurodegenerative diseases is diverse, however most of them share common characteristics: accumulation of misfolded proteins, chronic and sustained neuroinflammation, and the dysfunction and death of certain populations of neurons. The brain of Alzheimer's disease (AD) patients presents amyloid plaques and aggregation of hyperphosphorylated tau. The latter is also present in neurodegenerative tauopathies and in Parkinson's disease (PD). Aggregates of α -synuclein is the characteristic hallmark of PD. In amyotrophic lateral sclerosis (ALS) the mutation of SOD1 promotes its accumulation; and the polyglutamine expansion in huntingtin protein favors its aggregation in Huntington's disease (HD). Thus, the initial proteinopathy could be responsible for triggering the activation of the immunological defenses in the nervous system, as it has been demonstrated in some cases. Microglia and astrocytes are the main glial cells involved in the innate inflammatory response in the central nervous system (CNS). These cells are capable of detecting danger signals, and when activated they secrete inflammatory mediators to try to protect or prevent

damage. However, in some cases the inflammatory response becomes sustained by an amplified feedback of release of factors between microglia and astrocytes that further activate these cells. This promotes the recruitment of more glial cells that prolongs and up-regulates the neuroinflammatory response contributing to the progression of the disease (the review of Glass et al. (2010) outlines in detail the contribution of glial cells in neurodegenerative diseases). Neurodegeneration has a remarkable apoptotic component; sustained neuroinflammatory response along with the deregulation of protective mechanisms trigger neuronal death.

Apoptosis and inflammation are physiological processes that can act in coordination as occurs at the end of an inflammatory episode, when the number of lymphocytes is regulated by apoptosis. In the brain, apoptosis mediates the precise and programmed natural death of neurons that is a relevant process in the maturation of the CNS. Apoptosis can be initiated by an internal stimulus that affects the mitochondria (intrinsic pathway); or by the activation of specific membrane receptors, death receptors (DRs) (extrinsic pathway). DRs contain death domains able to initiate the apoptotic cascade after ligand binding. They belong to the tumor necrosis factor receptor superfamily. Among the different members, the best characterized are Fas (CD95 or Apo1) and TNFR1 (p55 or CD120a). Their ligands belong to the tumor necrosis factor family, FasL for Fas and TNF α for TNFR1. In normal conditions, neurons do not trigger apoptosis by DRs activation. Apoptosis is a highly regulated homeostatic mechanism controlled at several levels by different

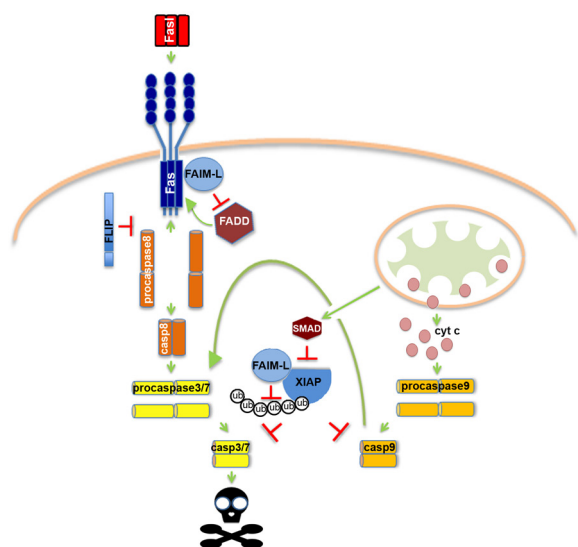


Figure 1 Fas apoptotic inhibitory molecule (FAIM)-L in Fas-induced death.

Simplified representation of the two levels in which FAIM-L acts in the apoptotic cascade induced by Fas engagement. The ligation of FasL to Fas receptor promotes receptor trimerization that results in the recruitment of the adaptor Fas-associated death domain protein (FADD) and the consequent activation of the apical procaspase-8. At the level of the receptor, FAIM-L interacts with Fas preventing the recruitment and binding of FADD and therefore the activation of caspases-8. The initiation of the extrinsic apoptotic cascade triggers the activation of the mitochondrial pathway. When the intrinsic apoptotic cascade is turned on, then the mitochondria release second mitochondria-derived activator of caspase (SMAC) and cytochrome c (cyt c) into the cytosol. Cyt c activates caspase-9 and subsequently caspases-3/7. XIAP is able to inhibit caspase-9 and executors caspase-3/7. SMAC antagonizes XIAP effects. Expression of XIAP is regulated by auto-ubiquitinylation and degradation by the proteasome. FAIM-L stabilizes XIAP inhibiting its auto-ubiquitinylation and preventing cell death.

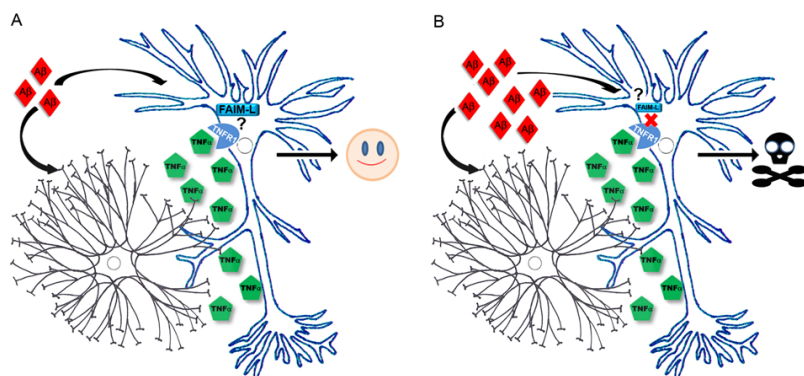


Figure 2 Schematic representation of the proposed model for the role of Fas apoptotic inhibitory molecule (FAIM)-L in Alzheimer's disease (AD).

(A) In physiological conditions, endogenous levels of FAIM-L allow tumor necrosis factor- α (TNF α) to be protective against amyloid beta ($A\beta$)-induced neuronal death, resulting in a beneficial inflammatory response. (B) However, chronic accumulation of $A\beta$ induces a decrease in the FAIM-L levels by an uncharacterized mechanism. Without the protective effect of FAIM-L, TNF α is unable to protect from $A\beta$ toxicity, thus further contributing to neurodegeneration.



sets of proteins. In the extrinsic pathway, decoy receptors (DcRs) regulate ligand binding to the receptor. DcRs are receptors homologues to TNFRs but without death domains. They are able to bind the same ligands as DRs but they do not transduce the death signaling, thus they antagonize DR-induced cell death. The activation of DR by the ligand induces its trimerization and the recruitment of several proteins forming a complex called. It is possible to antagonize DR signaling by blocking the DISC formation. The best-characterized DISC antagonist is FLIP, a non-active caspase-8-like protein that competes with caspase-8 for binding to DISC. Inhibitors of apoptosis (IAPs), such as XIAP, bind and inhibit caspases. Other proteins have been described are able to antagonize the induction of death mediated by DRs, but the molecular characterization of their mechanism is not fully understood. One of these proteins is Fas apoptotic inhibitory molecule (FAIM), which was initially described as an antagonist of Fas in B lymphocytes (Schneider et al., 1999). Two isoforms of the FAIM have been described and are produced by splicing, the short (S) and the long (L) form, the latter contains 22 extra amino acids in the N-terminal. FAIM-S is widely expressed in all body tissues, while FAIM-L only is expressed in post-mitotic neurons in brain and in some cells of testis (Zhong et al., 2001; Segura et al., 2007). We have demonstrated that FAIM-L functions as an antagonist of death induced by the activation of Fas and TNFR1 in neuronal cells (Segura et al., 2007).

The levels of inflammatory mediators such as FasL or TNF α can increase as a physiological response to certain pathological conditions in the nervous system. It has even been suggested that modulation of the signaling pathways of these proteins could be a potential therapeutic approach. However, the pleiotropic effects that these proteins exert limit their potential pharmacological applicability. In this scenario, proteins such as FAIM-L, which is able to prevent cell death induced by activation of Fas and TNFR1, emerge as exceptional and powerful target. The full knowledge of FAIM-L signaling will allow the design of the best strategy to use it pharmacologically. In this regard, in Fas-induced apoptosis we have demonstrated that FAIM-L is able to act at two levels in the apoptotic cascade. FAIM-L can bind Fas competing or impeding the binding to Fas of Fas-associated protein with death domain (FADD), one of the proteins that form the DISC complex, and therefore preventing the activation of the apical caspase-8 (Segura et al., 2007). In addition, FAIM-L can act downstream of the mitochondria through direct interaction with XIAP (Moubarak et al., 2013). This explains why its expression is not restricted to membrane domains and it presents a cytosolic distribution. We have proven that FAIM-L interacts with XIAP throughout an IAP-binding domain (IBM) in its neuronal specific sequence in the N-terminal. This interaction is necessary for the antiapoptotic function of FAIM-L in Fas-induced apoptosis. Two evidences of this are the fact that FAIM-L is unable to protect from Fas-induced death when: 1) XIAP is knocked down, and 2) when the IBM of FAIM-L is mutated preventing the interaction with XIAP. Moreover, the binding of FAIM-L to XIAP inhibits its auto-ubiquitination and degradation by the proteasome. All together it generates a positive antiapoptotic signaling in which the binding of FAIM-L to XIAP maintains its stability allowing the protection from Fas-induced apoptosis (Moubarak et al., 2013). Deregulation in the quantity of XIAP has been described in some neuropathologies including AD, HD, ALS and in motor neurons after axotomy. Moreover, some evidences suggest that XIAP may also be involved in PD and dementia with Lewy bodies. In these pathological conditions, it has been reported that the Fas/FasL system could also be involved. Therefore, the

ability of FAIM-L to stabilize XIAP expression and confer protection against FasL-induced cell death highlights this neuronal protein as a promising pharmacological target for those disorders. **Figure 1** shows a simplified scheme of how FAIM-L acts in Fas-induced cell death.

In addition to its function as an antiapoptotic molecule in Fas-induced cell death, FAIM-L is also able to prevent neuronal death mediated by the engagement of the TNFR1 by TNF α (Segura et al., 2007). The TNF system is the paradigm of neuroinflammatory signaling pathway considered to be implicated in neurodegenerative diseases. However, in healthy conditions, neurons are insensitive to death induced by activation of TNFR1. In the nervous system, TNF α has several and different functions. It plays an important role during neuronal development (Golan et al., 2004) and in the control of synaptic transmission (Beattie et al., 2002) and synaptic scaling (Stellwagen et al., 2006). The activation of TNFR1 can induce the activation of pro-survival pathways, but under certain circumstances it can also trigger the activation of the extrinsic apoptotic cascade (Micheau and Tschoop, 2003). One of these situations is when the expression of FAIM-L is down-regulated (Segura et al., 2007). Recently, we have established for the first time that FAIM-L could be relevant in neurodegenerative diseases. In the context of AD, we demonstrated that amyloid beta ($A\beta$) down-regulates the expression of FAIM-L promoting that the protection conferred by TNF α against $A\beta$ toxicity is terminated, thus accelerating neuronal loss (Carriba et al., 2015).

The TNF system is clearly implicated in AD, therefore the study of the role of FAIM-L could give clues about how to prevent or decelerate neurodegeneration. In human hippocampus, we detected a significant decrease in the mRNA and protein levels of FAIM-L with the advance of the disease. In the transgenic mouse model of AD PS1xAPP (PS1M146LxAPP751sl), which reproduces neuroinflammation and neurodegeneration, the expression of FAIM-L was also reduced with ageing compared with age matched wild type animals. Such reduction was observed in areas that the disease most affects, the hippocampus and the entorhinal cortex. It is noteworthy that the levels of FAIM-L are reduced before the onset of neuronal loss. Collectively, these observations suggest that the advance of neurodegeneration probably is linked to reduced levels of FAIM-L, and therefore FAIM-L expression could be a good biomarker of disease progression. We discovered that the oligomers of $A\beta$ were responsible for the FAIM-L reduction. The expression of endogenous FAIM-L was reduced when we treated primary mouse cortical cultures with the cortical soluble fractions of older transgenic animals. It has been described that in these animals the content of soluble oligomers increases with the age. Accordingly with this, the levels of FAIM-L were also reduced when neuronal cultures were treated with soluble oligomers of $A\beta$ (also termed ADDL, *i.e.*, $A\beta$ derived diffusible ligands). As happened with human and transgenic animals, the reduction of FAIM-L in cortical neurons in culture was also detected at mRNA levels, suggesting that $A\beta$ modulates its expression rather than its degradation. In all of those experiments, the levels of FAIM-S did not change, indicating that the expression of the two isoforms of FAIM in neurons is controlled by different mechanisms (Carriba et al., 2015). The expression of FAIM-S in B lymphocytes is regulated by the interferon (IFN) regulatory factor 4 (IRF4) (Kaku and Rothstein, 2009). Nonetheless, nothing is known about the regulation of FAIM-L expression in neurons. We could speculate that $A\beta$ would be able to alter the expression of FAIM-L by directly interfering with proteins that physiologically regulate its expression. Alternatively, we could presume that the reduction might be due to signals or factors



released by neighboring neurons in response to the presence of A β and/or damaged cells. If the latter was the case, FAIM-L levels would be an indicator of pathological conditions, and its potential applicability as a pharmacological target would be expanded to other neurodegenerative diseases besides AD. In this regard, Yu et al. (2008) proposed that in PD the expression of FAIM-L in midbrain dopaminergic neurons after trophic factor deprivation could be reduced making this type of neurons more vulnerable to Fas-induced death. Epigenetic and alternative splicing studies could provide evidence for the differential regulation of the two isoforms of FAIM in pathological conditions. As extracted from EPITRANS, a database that integrates epigenome and transcriptome data, the FAIM gene could be subjected to epigenetic regulation by DNA methylation. However, this information is obtained from non-neuronal studies, and does not differentiate between the two isoforms.

The quantity of FAIM-L determines the response of neurons to TNF α . The initial protection conferred by TNF α against A β toxicity (Barger et al., 1995; Carriba et al., 2015) vanished when we knocked down in cortical neurons the endogenous FAIM-L with a short hairpin RNA (shRNA-FAIM-L), or by the treatment with ADDLs. In the first case, we were able to recover the protection of TNF α when we co-expressed shRNA-FAIM-L with a mutant form of FAIM-L that is not down-regulated by the shRNA-FAIM-L; and in the treatment with ADDLs when we overexpressed FAIM-L. These results indicate that FAIM-L is necessary for TNF α to protect against A β toxicity. More importantly, the restoration of FAIM-L protein quantities allows to recover the protective function of TNF α . These results also explain the controversy about the function of TNF α during the progression of AD (Carriba et al., 2015). **Figure 2** exemplifies the conclusions obtained.

To conclude, the described properties of FAIM-L suggest that this neuronal specific protein has a huge potential as a pharmacological target and as a diagnostic tool in neurodegenerative diseases. However, some important points have to still be resolved. From how FAIM-L expression is regulated to fully understanding how it functions. The quantity of FAIM-L seems to be a key determinant in the outcome of neurodegeneration, since it regulates the survival or apoptotic neuronal response to inflammatory molecules such as FasL and TNF α . Consequently, the evolution of its expression may be indicative of susceptibility or disease progression. In this regard, it is unknown whether the *faim*-null mice will be prone to neurodegeneration or be more susceptible to neurodegenerative diseases. Remarkably, the restoration of FAIM-L levels could be a promising therapeutic approach to decelerate the progression of neuronal loss meanwhile there is not developed therapies to prevent these diseases.

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Paulina Carriba*, Joan X. Comella*

Institut de Recerca de l'Hospital Universitari de la Vall d'Hebron (VHIR), Passeig Vall d'Hebron 119-129, Barcelona 08035, Spain
Institut de Neurociències, Departament de Bioquímica i Biologia Molecular, Facultat de Medicina, Universitat Autònoma de Barcelona, Campus de Bellaterra (Edifici M), Bellaterra 08193, Spain; Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Barcelona 08035, Spain

*Correspondence to: Paulina Carriba, Ph.D. or Joan X. Comella, Ph.D., paulina.carriba@gmail.com or joan.comella@vhir.org.

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orcid: 0000-0002-6980-2277 (Paulina Carriba)

0000-0002-6218-0786 (Joan X. Comella)

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