

Article Addendum

Stimulation of autophagy by autoantibody-mediated activation of death receptor cascades

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Activation of membrane death receptors has been connected to apoptosis and, recently, other non-apoptotic events. For example, we reported recently that sera from either a subset of patients with type 2 diabetes with neuropathy or a subpopulation of patients with neurogenic chronic intestinal pseudo-obstruction (CIP) stimulate autophagy in SH-SY5Y human neuroblastoma cells via complement-independent, autoantibody-mediated activation of Fas (CD95). Activation of the Fas pathway causes minimal activation of apoptosis in these cells since procaspase-8 shows low constitutive levels of expression in neuroblastoma cells. The observation that anti-Fas autoantibodies induce autophagy is novel and provocative. This finding has implications regarding the pathophysiology of diabetic neuropathy, CIP and, perhaps, other autoimmune disorders. For example, recent reports suggest that expression or activity of proapoptotic caspases can be enhanced by activation of more than one membrane death receptor, as could happen by combinations of cytokines and autoantibodies. The observation that autophagy, a putative cytoprotective pathway that has also been implicated in non-apoptotic cell death, is activated by autoantibodies against Fas, may represent an early cellular protective response. An increase in cytotoxic cytokine levels or the ratio of agonist:antagonist autoantibodies may “tip” the balance of the cellular response to activation of programmed cell death pathways.

We recently reported that complement-inactivated sera from a subset of patients with type 2 diabetes and neuropathy and a subset

of patients with neurogenic chronic intestinal pseudo-obstruction (CIP) stimulate autophagy in cultures of the human neuroblastoma SH-SY5Y cell line.^{1,2} This effect appears to involve autoantibodies that bind to the Fas (CD95) receptor complex in both diseases. This observation may have important pathophysiological implications. The development of diabetic neuropathy in patients with Type 2 diabetes typically occurs over a time frame of years.³ The loss of nerve function is attributed to nerve fiber loss.^{4,5} However, apoptotic cell death is evident only in a small proportion of nerve fibers and the rate of cell drop-out is slow.⁶⁻⁸ As the etiology of diabetic neuropathy is multifactorial,⁵ the protracted development of nerve fiber loss is consistent with a process in which cytoprotective mechanisms in nerve cells are eventually overcome by the inimical endocrine and metabolic milieu of diabetes.

Among the different factors involved in the pathophysiology of diabetic neuropathy, a potential role for autoimmune immunoglobulins remains controversial. Reports exist in the literature suggesting that anti-Fas autoantibodies may play a role in the demise of the nerve cell.⁹ Additionally, the presence of both agonistic and antagonistic anti-Fas autoantibodies present in Ig preparations from sera of healthy individuals has been reported,¹⁰ and anti Fas autoantibodies have been implicated in the pathophysiology of other diseases such as silicosis.¹¹ Whereas stimulation of the Fas cascade has been traditionally considered to lead to stimulation of the extrinsic pathway of apoptosis through the CD95 death receptor,^{12,13} recent research has shown that engagement of the Fas receptor can activate other cell responses, including cell proliferation.¹²⁻¹⁴ These non-apoptotic effects underscore the need to better understand the cellular events triggered by Fas activation.

Our recent reports on the stimulation of autophagy by anti-Fas autoantibodies present in diabetic neuropathic sera and sera from patients with CIP, suggest a potentially novel insight involving stimulation of the putative cytoprotective autophagy pathway through autoantibodies. The stimulatory effect of autoantibodies is in line with a recent report showing that sialic acid binding immunoglobulin-like lectin-9 (Siglec-9) autoantibodies stimulate autophagy and autophagic cell death depending on the cytokine and oxidative stress environment.¹⁵ In our studies, the autoantibody nature of this effect on the neuroblastoma cells is substantiated by our finding that enriched IgG and IgM fractions from the diabetic neuropathic sera increase the level of autophagy. Additionally, in

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and

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our experimental design, sera are complement-inactivated in order to remove the potential compounding, cytotoxic effects of complement. Nevertheless, it is not possible to discount the potential effects of death receptor-binding cytokines that co-migrate with the auto antibodies in the preparations and that, if present, might act additively or synergistically to stimulate autophagy.^{16,17} The serum-mediated stimulation of autophagy is eliminated by preincubation of the sera with a soluble Fas/Ig chimera (extracellular domain), suggesting that autoantibodies present in the sera are responsible for this effect. It is noteworthy that immunohistochemical analysis of the SH-SY5Y cells demonstrates that there is increased colocalization of microtubule-associated light chain (LC3) (functional homologue of Atg 8, and whose conversion to LC3-II is a marker for mature autophagosomes),¹⁸ with Fas-Associated Death Domain (FADD), a key component of the first steps of the Fas transducing cascade.^{12,13} Specifically, the interaction of FADD with the autophagic molecule Atg5 has been reported and is considered to be a pivotal step for the development of the autophagosome.¹⁶ In addition, our data suggest that the interaction of FADD with the autophagic assembly machinery may be more complex, because LC3 is a component of the mature autophagosome. The immunohistochemical data in our manuscripts do not address the important issues of whether FADD is sequestered in the mature autophagosome, thereby reducing activation of the extrinsic apoptotic cascade or whether FADD has additional interactions with other modulators of autophagy. Nevertheless, our results suggest that this is a potentially important point of interaction between the autophagy and apoptosis cascades. The data in our studies are also in line with our earlier observations that exposure of diabetic neuropathic sera to protein L and protein G beads abolishes the ability of the sera to increase the pixel density of the LC3-II band in immunoblots from extracts of SH-SY5Y cultures.¹⁹ Similar results are observed when a subset of sera obtained from patients with neurogenic CIP are pretreated with either protein L beads or the Fas/Ig chimera before adding the sera to the cultures.

The induction of autophagy by conventional stimulation of cell death receptors has been reported, as has the lack of stimulation of extrinsic apoptotic pathways in cells that demonstrate low constitutive levels of expression of caspase 8, such as neuroblastoma cells.²⁰ It has also been reported in this and other similar types of cells, resistant to extrinsic stimulation of apoptosis, that stimulation of more than one cell death receptor may increase the expression or activity of caspases and shift the post receptor transducing pathway towards activation of executioner caspases and an increase in apoptosis.²¹⁻²³ It is therefore possible that an increase in agonist vs. antagonist anti-Fas autoantibodies may tip the initial cytoprotective reaction involving autophagy to a cytotoxic response involving apoptotic or non-apoptotic programmed cell death in the presence of prolonged cell stress or stimulation of multiple cell death receptors. Relevant to this discussion, a recent report supports the presence of non-apoptotic (necrotic) cell death triggered by TNF α , although this study did not indicate if autophagy is increased.²⁴ Furthermore, cell death associated with activation of autophagy has also been described following binding of autoantibody.¹⁵

In summary, our recent reports provide potentially useful insights regarding the interaction between the humoral immune system and the stimulation of autophagy through engagement of cell death receptors. Our results add to a growing body of research

examining interactions between putative cytoprotective, apoptotic and non-apoptotic cellular pathways triggered by activation of surface membrane death receptors.

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