

## LETTER TO THE EDITOR

Inflammation interrelates to APP in sIBM: IL-1 $\beta$  induces accumulation of  $\beta$ -amyloidJens Schmidt<sup>1,2</sup> and Marinos C. Dalakas<sup>1,3</sup>

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Sir, Dr Greenberg has unfortunately misinterpreted some of our data or looked at them rather narrowly. He raises the following four points with respect to our conclusions drawn from our results:

- (1) Dr Greenberg argues that immune cells produce amyloid precursor protein (APP) and suggests that a correlation between APP and inflammatory mediators would hence be expected. This is true and in line with our immunohistochemical analysis as shown in Fig. 2B (Schmidt *et al.*, 2008). Probably Dr Greenberg did not appreciate that Fig 2B clearly demonstrates that the majority of the staining signal of APP in sIBM derives from the muscle fibres while the contribution by immune cells is minimal. The production of APP by the muscle fibres themselves was further substantiated by our *in vitro* experiments with exposure of muscle cells to IL-1 $\beta$  and IFN- $\gamma$  (Fig. 5A and B).
- (2) Dr Greenberg notes that after removing the outlier, there is a significant correlation between IL-1 $\beta$  and APP in PM. This is true, but we had already pointed this out. We have underscored however, that, regardless of whether calculated with or without the outliers, the majority of the inflammatory mediators including CXCL-9, CCL-3, CCL-4 and IFN- $\gamma$  significantly correlate with APP only in sIBM. By immunohistochemical analysis, there is a striking difference between the distribution of both APP as well as all inflammatory mediators. As we emphasized, these molecules are mostly confined to immune cells and connective tissue
- in PM and DM, whereas in sIBM they are predominantly produced by the muscle fibres themselves. Based on the combination of both, the mRNA-data and the immunohistochemical analysis, our conclusion is absolutely correct that only in sIBM, but not in PM or DM, there is a consistent correlation between inflammation and  $\beta$ -amyloid-associated molecules.
- (3) Dr Greenberg comments that the antibody clone 6E10 may detect  $\beta$ -amyloid as well as APP. This is correct and particularly true for western blot, where the size of protein allows discrimination of APP from  $\beta$ -amyloid. However, the immunohistochemical staining patterns of APP and  $\beta$ -amyloid are fundamentally different: APP is a transmembrane-protein, which is frequently observed on the surface of cells. In contrast, the signal for  $\beta$ -amyloid is predominantly present in accumulations within muscle fibres. Preferential binding of the 6E10 antibody to  $\beta$ -amyloid as opposed to APP is facilitated by fixation with paraformaldehyde as used in our article. Staining for APP in this article has been performed by a polyclonal antibody, which — unlike 6E10 — has a low affinity to accumulations of  $\beta$ -amyloid. Taken together, the immuno-labelling of APP as well as  $\beta$ -amyloid in the respective figures of the paper are in line with what is expected.
- (4) Dr Greenberg's last point is very surprising. In various state-of-the-art publications (Tawil and Griggs, 2002; Askanas and Engel, 2006) international workshops on sIBM, and a classic Myology textbook (Engel, 2004), the presence of accumulation of  $\beta$ -amyloid in the

muscle pathology of sIBM appears out of question. A crucial role of APP overexpression or accumulation of  $\beta$ -amyloid has been also seen *in vitro* (Askanas *et al.*, 1996), in biopsies of human skeletal muscle (Askanas and Engel, 2006) as well as in mouse models of IBM (Kitazawa *et al.*, 2006; Sugarman *et al.*, 2006).

In line with this, staining for  $\beta$ -amyloid or APP was present in all of our sIBM patients. It should be also stressed that accumulations of  $\beta$ -amyloid may require advanced competence of immunostaining, particularly regarding an appropriate processing of the tissue or observation by special filters (Askanas and Engel, 2006). Specifically, detection of  $\beta$ -amyloid by antibodies as well as histochemical methods such as Congo-staining may be too insensitive. As part of the widely accepted disease pathomechanism, an overexpression of APP may initially lead to a generation of oligomers of  $\beta$ -amyloid, which are known to be cell toxic (Querfurth *et al.*, 2001; Askanas and Engel, 2007) and even APP itself has been shown to be cell toxic (Lu *et al.*, 2003).

Lack of vacuoles and Congo-positive material in the old publications cited by Dr Greenberg can be seen early in sIBM muscle, where chronic inflammation and COX-negative fibres are the overwhelming finding. Accumulation of  $\beta$ -amyloid is clearly more evident in late stages of the muscle pathology, which has been repeatedly emphasized (Dalakas, 2006a, b; Askanas and Engel, 2006) and is in line with a recent publication (Chahin and Engel, 2008). Dr Greenberg's contention 'that no  $\beta$ -amyloid protein has ever been demonstrated in an IBM muscle patient tissue', is incorrect and inconsistent with the present knowledge of IBM. We agree that  $\beta$ -amyloid is not only seen in sIBM but also in other vacuolar myopathies and appreciate the possibility that its accumulation may be a secondary event (Dalakas, 2008) or, as we have previously proposed and shown here, produced in response to inflammatory mediators (Dalakas, 1998, 2006b; Schmidt *et al.*, 2008). The universal presence of  $\beta$ -amyloid in sIBM and its value in the diagnosis

remains one of the major advances in sIBM pathology during the last decade.

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