

## LETTER TO THE EDITOR

Comment on 'Interrelation of inflammation and APP in sIBM: IL-1 $\beta$  induces accumulation of  $\beta$ -amyloid in skeletal muscle'

Steven A. Greenberg

Brigham and Women's Hospital, Department of Neurology, 75 Francis Street, Boston, MA 02115, USA

Correspondence to: Steven A. Greenberg, Brigham and Women's Hospital, Department of Neurology, 75 Francis Street, Boston, MA 02115, USA

E-mail: [sagreenberg@partners.org](mailto:sagreenberg@partners.org)

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Sir,

Dr Schmidt and colleagues report that in muscle from patients with inclusion body myositis (IBM) there is a correlation between measures of inflammation and ' $\beta$ -amyloid-associated degeneration' (Schmidt *et al.*, 2008). They found that the number of immune system cells visible in hematoxylin/eosin stained muscle sections and the amount of certain transcripts produced by immune cells correlated with the abundance of  $\beta$ -amyloid precursor protein (APP) transcript. These and other data are interpreted as supporting an hypothesis suggested a decade ago in which in IBM muscle, interleukin-1-beta (IL-1 $\beta$ ) secreted by immune system cells results in APP production by myofibres, and APP production by myofibres results in IL-1 $\beta$  secretion by immune cells (Dalakas, 1998). This hypothesis is expanded here to one in which other inflammatory molecules, such as interferon-gamma (IFN- $\gamma$ ), also result in APP and  $\beta$ -amyloid production by myofibres.

What should be considered in the interpretation of these results is the previously published data that immune cells produce abundant APP transcript and produce and secrete APP protein (Monning *et al.*, 1990; Monning *et al.*, 1992; Schlossmacher *et al.*, 1992; Schubert *et al.*, 1993; Askanas *et al.*, 1995; Schubert *et al.*, 1997; Suh *et al.*, 1997). A correlation between the abundance of immune system cells and immune system transcripts in muscle with the amount of APP transcript would be expected simply because APP transcript is produced and carried into muscle by the very same immune cells producing the other immune transcripts.

The authors also report in the Discussion that not in PM or DM but 'only in sIBM, there was a significant and consistent correlation between the mRNA expression of  $\beta$ -amyloid-associated molecules and the major inflammatory markers'.

Actually, the PM data show a high correlation between APP transcript and inflammatory cell numbers and transcripts after a single outlier is removed. The Supplementary Figure shows removal of this outlier; for the grade of inflammation correlation with APP transcript abundance, the Pearson correlation coefficient for all PM samples graphed is  $-0.19$  ( $P=0.56$ ) in FIGURE 3A, but after removal of one outlier, it is  $0.69$  ( $P=0.02$ ) in the Supplementary Figure. Similarly, the correlation of IL-1 $\beta$  and APP transcript abundance is  $-0.08$  ( $P=0.81$ ) for all PM samples but after removal of one outlier, the correlation is  $0.75$  ( $P=0.007$ ). The IL-1 $\beta$  transcript correlation with APP is in fact significant only for PM, after removal of the outlier, and not for IBM ( $P=0.16$ ). The authors report in the Results that 'after removal of outliers, there was a significant correlation in the group of PM-patients between the mRNA-expression of APP and the grade of inflammation and the mRNA-expression of IL-1 $\beta$ '. The high correlation of APP transcript with inflammatory cells and transcripts present in PM weakens the hypothesis that the same relationship present in IBM relates to  $\beta$ -amyloid-mediated myofibre degeneration, given such degeneration is not hypothesized to occur in PM.

Lastly, the investigators state that a '*hallmark of sIBM is accumulation of aberrant molecules, most of all  $\beta$ -amyloid, within the myofibres*'. The claim that  $\beta$ -amyloid accumulates in IBM myofibres has been directly contradicted in published articles by three independent laboratories studying a combined 35 patients with IBM. These studies found no immunohistochemical evidence for the presence of either APP or  $\beta$ -amyloid protein in any myofibres in 28 of these patients and found five or less affected myofibres in each of the remaining seven patients (Leclerc *et al.*, 1993; Nalbantoglu *et al.*, 1994; Sherriff *et al.*, 1995). One of these laboratories found immunoreactivity that remained after

pre-absorption of antibody by synthetic  $\beta$ -amyloid, concluding that some anti- $\beta$ -amyloid antibodies that have been interpreted as showing  $\beta$ -amyloid presence immunostain diseased muscle non-specifically (Sherriff *et al.*, 1995). Dr Schmidt and colleagues found that APP transcript abundance was even higher in dermatomyositis muscle than in IBM muscle, in agreement with previous reports that APP transcript and protein are abnormally increased in myofibres (called 'regenerating' based on their staining for desmin) in a wide range of muscle diseases other than IBM (in 29 and 43 patients in six and seven disease categories) (Sarkozi *et al.*, 1994; Askanas *et al.*, 1995). In the current study by Dr Schmidt and colleagues,  $\beta$ -amyloid was reported present in 15.3% of IBM myofibres. However, the 6E10 antibody used and interpreted as reactive to  $\beta$ -amyloid reacts to APP as well (confirmed in western blot experiments shown in Figure 2 from Howland *et al.* (1998) and Figure 7b from Wojcik *et al.* (2007)), as the  $\beta$ -amyloid peptide sequence is a subsequence of APP. It therefore seems uncertain as to whether any  $\beta$ -amyloid protein is being seen in IBM myofibres in these experiments. More generally, given that no western blot study of IBM muscle demonstrating a 4kDa band (the approximate mass of  $\beta$ -amyloid) immunoreactive with any anti- $\beta$ -amyloid antibody has ever been published, together with multiple immunohistochemical studies that have failed to see  $\beta$ -amyloid in IBM muscle and the inability of any antibodies that have been used to discriminate  $\beta$ -amyloid from APP, it is important to consider the possibility that no  $\beta$ -amyloid protein has ever been demonstrated in any IBM patient muscle sample, let alone demonstration that its presence is disease specific. These issues should be kept in mind with regard to the view that the accumulation of  $\beta$ -amyloid is an IBM 'hallmark'.

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## 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>

<sup>1</sup>Neuromuscular Diseases Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA, <sup>2</sup>Department of Neurology and Department of Experimental and Clinical Neuroimmunology, University of Göttingen, Waldweg 33, 37073 Göttingen, Germany and <sup>3</sup>Neuromuscular Division, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA

Correspondence to: Dr Jens Schmidt, Department of Neurology and Department of Experimental and Clinical Neuroimmunology, University of Göttingen, Waldweg 33, 37073 Göttingen, Germany or Dr Marinos Dalakas, Neuromuscular Division, Thomas Jefferson University, 900 Walnut Street, Suite 200, Philadelphia, PA 19107, USA  
E-mail: j.schmidt@gmx.org or marinos.dalakas@jefferson.edu  
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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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