Journal of Alzheimer's Disease 22 (2010) 1–4
IOS Press

Supplementary Data

Following Activation of the Amyloid Cascade, Apolipoprotein E4 Drives the *in vivo* Oligomerization of Amyloid-β Resulting in Neurodegeneration

Haim Belinson[a], Zehavit Kariv-Inbal[a], Rakez Kayed[b], Eliezer Masliah[c] and Daniel M. Michaelson[a,*]
[a]*Department of Neurobiology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel*
[b]*George P. and Cynthia Woods Mitchell Center for Neurodegenerative Diseases, University of Texas Medical Branch, Galveston, TX, USA*
[c]*Departments of Neurosciences and Pathology, University of California San Diego, LaJolla CA, USA*

Handling Associate Editor: Thomas Bayer

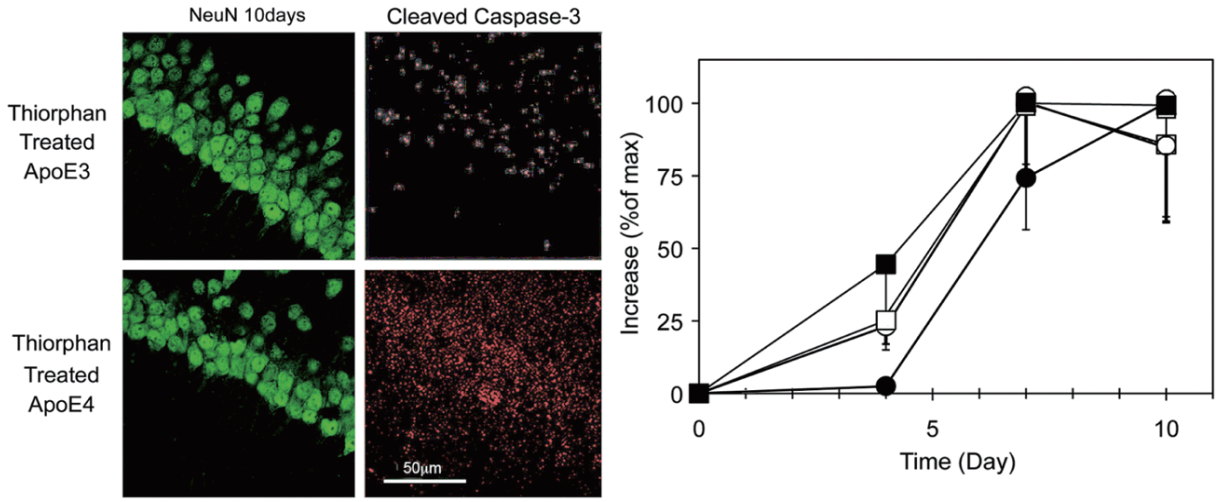
Accepted 1 August 2010

References

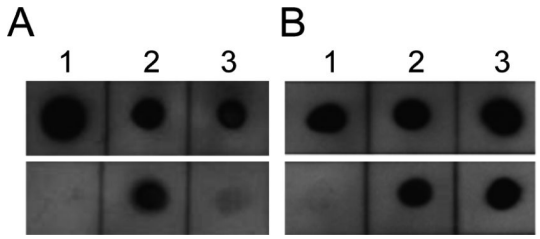
[1] Belinson H, Lev D, Masliah E, Michaelson DM (2008) Activation of the amyloid cascade in apolipoprotein E4 transgenic mice induces lysosomal activation and neurodegeneration resulting in marked cognitive deficits. *J Neurosci* **28**, 4690-4701.
[2] Kayed R, Head E, Sarsoza F, Saing T, Cotman CW, Necula M, Margol L, Wu J, Breydo L, Thompson JL, Rasool S, Gurlo T, Butler P, Glabe CG (2007) Fibril specific, conformation dependent antibodies recognize a generic epitope common to amyloid fibrils and fibrillar oligomers that is absent in prefibrillar oligomers. *Mol Neurodegener* **2**, 18.

*Correspondence to: Daniel M. Michaelson, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, 69978, Israel. Tel.: +972 3 6409624; Fax: +972 3 640764; E-mail: dmichael@post.tau.ac.il.

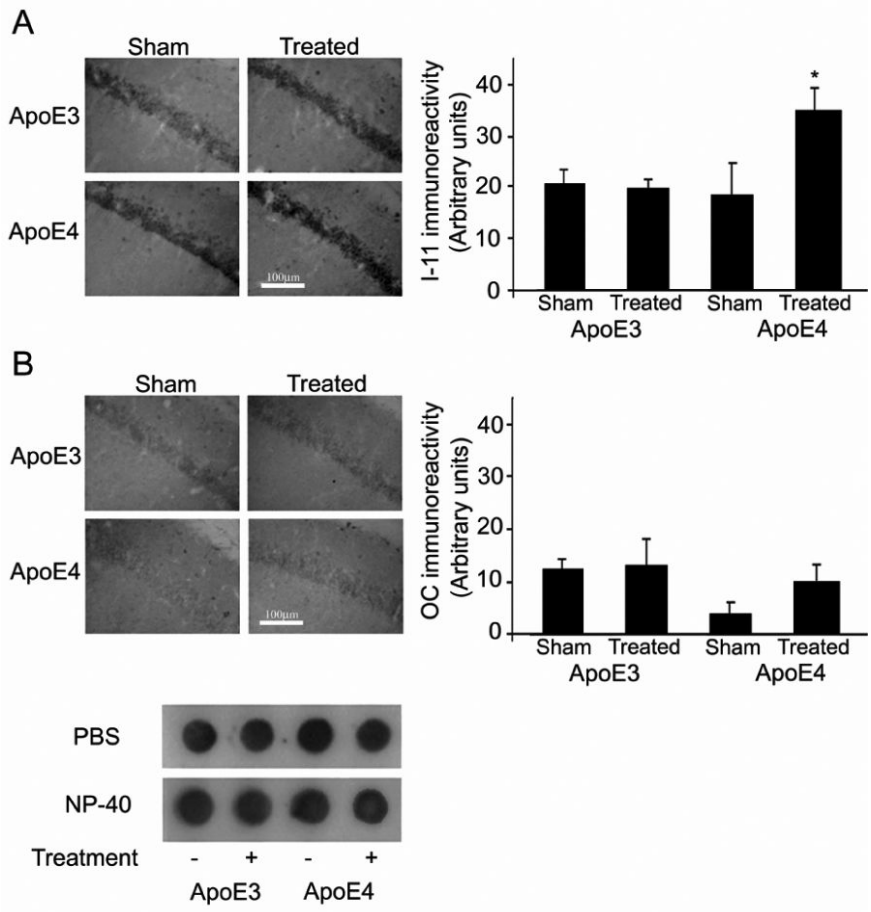
ISSN 1387-2877/10/$27.50



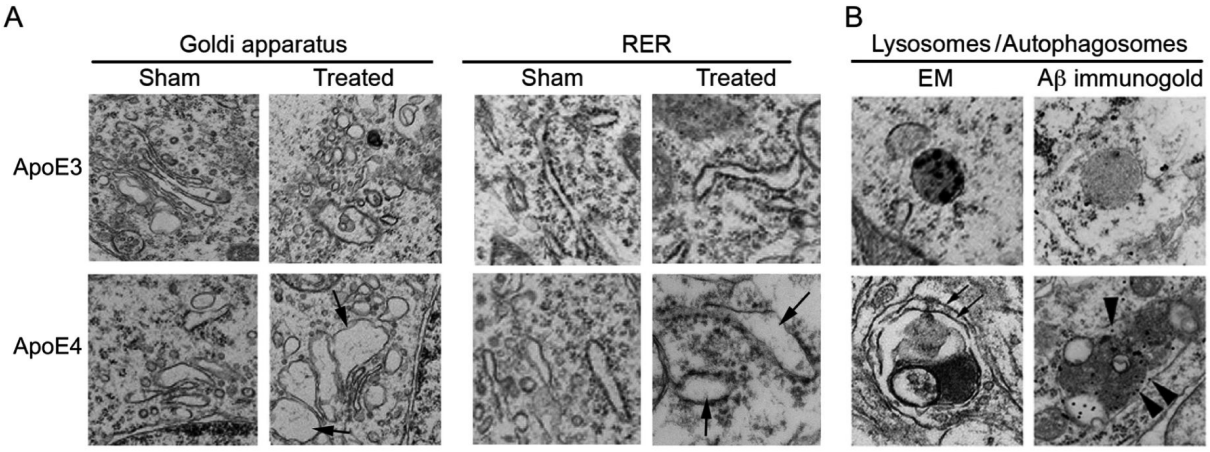
Supplementary Figure S1. Kinetics of the accumulation of Aβ_{42}, oligomerized Aβ (I-11), and cleaved caspase-3 levels, and of neuronal loss in CA1 hippocampal neurons of ApoE4 mice following inhibition of neprylisin. ApoE4 mice were processed for confocal immunohistochemistry at the indicated times following i.c.v. injection of thiorphan, as described in Materials and Methods. The sections were subjected to Aβ_{42}, I-11 and cleaved caspase-3 confocal microscopy, as described in Materials and Methods, and to NeuN immunohistochemistry as previously described [1]. Panels on the left depict representative confocal sections of ApoE3 and ApoE4 mice immunostained for NeuN and cleaved caspase-3. Quantitation of the results (mean $\pm$ SEM; $n = 4$–5 mice/group) are for Aβ_{42} (■), neuronal loss (●), cleaved caspase-3 (◯), I-11 (□), the percentage of the maximal change that was observed within 10 days after neprilysin inhibition was initiated. $^{*}p < 0.02$ for the Aβ_{42}, I-11, and cleaved caspase-3 and $p < 0.05$ for NeuN for a comparison of the results of the thiorphan-treated ApoE4 with those of the other mouse groups.



Supplementary Figure S2. Determination of the specificity of I-11 to oligomerized Aβ. Panel A) Dot blots of 0.36 μg soluble Aβ_{42} (1), oligomerized Aβ_{42} (2) and fibrillar Aβ_{42} (3) were probed either with the anti Aβ_{42} mAb 6E10 (upper row) or with Ab I-11 (lower row). Determination of the specificity of I-11 to oligomerized Aβ by blocking experiments is depicted in Panel B. Each dot has 0.36 μg of Aβ_{42} oligomer. The I-11 reactivity with the unblocked Aβ_{42} oligomers is shown in the upper row whereas the blots in the lower row were blocked with Aβ_{42} oligomers (1), Aβ_{42} fibril (2) and soluble Aβ_{42} (3). The dot blots experiments and oligomerized and fibrillar Aβ were prepared as described in [2].



Supplementary Figure S3. Comparison of I-11 and OC levels of oligomerized amyloid in CA1 hippocampal neurons of ApoE4 and ApoE3 mice following inhibition of neprilysin. ApoE3 and ApoE4 male mice were injected i.c.v. with thiorphan or sham-treated for 7 days. The mice were then killed and their brains subjected to anti-I-11 and OC immunohistochemistry and dot-blot experiments, as described in Materials and Methods. A) Representative coronal sections of thiorphan-treated and sham-treated ApoE3 and ApoE4 mice immunostained with I-11 are shown on the left (bar $= 100$ μm). Quantification of the density of I-11 staining (mean $\pm$ SEM; $n = 4$–5 mice/group) in the CA1 neurons is shown on the right. $^*p < 0.007$ for the effect of group $\times$ treatment and $p < 0.001$ for a comparison of the results of the thiorphan-treated ApoE4 with those of the other mouse groups. B) Representative coronal sections of thiorphan-treated and sham-treated ApoE3 and ApoE4 mice immunostained with OC are shown on the left (bar $= 100$ μm). Quantification of the density of OC staining (mean $\pm$ SEM; $n = 4$–5 mice/group) in the CA1 neurons is shown on the right. The lower panel depicts representative OC dot blots of the PBS and NP-40 hippocampal extracts from the mouse groups.



Supplementary Figure S4. Golgi, rough endoplasmic reticulum (RER), and secondary lysosomal pathology in CA1 hippocampal neurons of ApoE4 and ApoE3 mice following inhibition of neprilysin. ApoE3 and ApoE4 mice were injected i.c.v. with thiorphan or sham-treated for 7 days. The mice were then killed and their brains subjected to EM, as described in Materials and Methods. A) Representative Golgi apparatus and RER EM images of thiorphan-treated and sham-treated ApoE3 and ApoE4 mice. B) Representative EM images (left) and EM Aβ immunogold images (right) of secondary lysosomes/autophagosomes of thiorphan-treated ApoE3 and ApoE4 mice.

Supplementary Video. Schematic presentation of the intracellular processes underlying the synergistic pathological cross-talk between ApoE4 and oligomerized Aβ. http://www.j-alz.com/issues/22/belinson_video.mov.