Apolipoprotein E-specific innate immune response in astrocytes from targeted replacement mice

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Abstract

Background: Inheritance of the three different alleles of the human apolipoprotein (apo) E gene (APOE) are associated with varying risk or clinical outcome from a variety of neurologic diseases. ApoE isoform-specific modulation of several pathogenic processes, in addition to amyloid β metabolism in Alzheimer’s disease, have been proposed: one of these is innate immune response by glia. Previously we have shown that primary microglia cultures from targeted replacement (TR) APOE mice have apoE isoform-dependent innate immune activation and paracrine damage to neurons that is greatest with TR by the ε4 allele (TR APOE4) and that derives from p38 mitogen-activated protein kinase (p38MAPK) activity.

Methods: Primary cultures of TR APOE2, TR APOE3 and TR APOE4 astrocytes were stimulated with lipopolysaccharide (LPS). ApoE secretion, cytokine production, and nuclear factor-kappa B (NF-κB) subunit activity were measured and compared.

Results: Here we showed that activation of primary astrocytes from TR APOE mice with LPS led to TR APOE-dependent differences in cytokine secretion that were greatest in TR APOE2 and that were associated with differences in NF-κB subunit activity.

Conclusion: Our results suggest that LPS activation of innate immune response in TR APOE glia results in opposing outcomes from microglia and astrocytes as a result of TR APOE-dependent activation of p38MAPK or NF-κB signaling in these two cell types.

Background

Humans are different from other mammals in that we have 3 common alleles of the apolipoprotein E gene (APOE): the ε2 (APOE2), ε3 (APOE3), and ε4 (APOE4) alleles [1]. Numerous genetic studies have associated inheritance of APOE4 with increased risk, earlier onset, or poorer clinical outcome for a number of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), traumatic brain injury, and HIV-encephalitis [2-10]. At least for AD, inheritance of APOE2 is associated with apparent neuroprotection, perhaps related to delayed onset of illness by many years [11]. While apoE isoforms play a role in the metabolism of beta amyloid (AB) pep-
tides and thereby may modulate the risk of developing AD [12], the influence of inheriting different APOE alleles extends well beyond diseases thought to involve Aβ peptide-mediated neurotoxicity, as noted above. For this reason, other apoE isoform specific mechanisms likely exist to explain the apparent influence of APOE alleles on such a broad spectrum of neurologic diseases; indeed, several have been proposed including synaptic stabilization, biologically active proteolytic fragments of apoE, anti-oxidant activity, and nitric oxide (NO) production [13-16]. ApoE also has an immune modulatory function, at least in the peripheral adaptive immune response to some bacteria and viruses [17]. We have recently shown that microglia from mice with targeted replacement (TR) of the mouse apoE gene with the coding sequences of human APOE alleles activated with LPS display an apoE isoform-specific innate immune response and result in apoE isoform-specific paracrine damage to neurons, both of which are dependent on p38 mitogen-activated protein kinase (p38MAPK) -mediated signaling.

One commonly used approach to investigate selectively innate immune response in neurodegeneration is to use a specific stimulus, lipopolysaccharide (LPS) [18-25]. LPS specifically activates CD14/Toll-like receptor (TLR) 4 co-receptors with subsequent increased gene transcription mediated through a bifurcated pathway that is dependent on both nuclear factor-kappa B (NF-κB) and p38MAPK signaling [26,27]. Indeed, LPS activation of CD14/TLR4 co-receptors on microglia leads to indirect damage to neurons and oligodendroglia in culture and in vivo [22,28-30]. Moreover, a role for CD14/TLR4 co-receptors is now understood to extend well beyond endotoxemia, as they are important in innate immune response to several endogenous ligands [31]. Indeed, CD14 binds Aβ fibrils and is responsible for most of Aβ-stimulated microglial-mediated neurotoxicity [32]. In addition, peptides and neoantigens expressed by apoptotic cells also activate this pathway [33]. Here we tested the hypothesis that innate immune response from CD14/TLR4 activation would show isoform-specific differences in primary cultures of astrocytes from TR APOE mice.

Methods
Materials
Cell culture solutions and supplies were from GIBCO (Grand Island, NY). Poly-ornithine (0.01%) was from Sigma (St. Louis, MO). 4–15% SDS-polyacrylamide gels were from BioRad (Hercules, CA). LPS and the NO assay kit were from Calbiochem (La Jolla, CA). Primary antibodies used were polyclonal anti-human apoE antibody from Dako Corporation (Carpinteria, CA) and polyclonal anti-glial fibrillary astrocytic protein (GFAP) antibody from Novus Biologicals (Littleton, CO). The NF-κB transcription factor assay kit and purified human HDL were from Chemicon International (Temecula, CA)

Mice
Homozygous APOE2, APOE3 and APOE4 targeted replacement (TR) mice 'humanized' at apoE were developed by Dr. Maeda and colleagues [34,35]. Briefly, human APOE genomic fragments were used to replace mouse apoE via homologous recombination. All three lines of TR APOE mice contain chimeric genes consisting of mouse 5’ regulatory sequences continuous with mouse exon 1 (noncoding) followed by human exons (and introns) 2–4 [34]. These mice were backcrossed greater than six generations to C67BL/6 genetic background. Mice were housed in an ALAC-approved vivarium and methods approved by a University of Washington International Use and Care of Animals (IACUC) Committee.

Astrocyte cultures
Primary cultures of 1-day-old mouse cerebral cortical astrocytes were prepared according to the method of Gebicke-Haerter, et al. [36]. Confluent cultures were used on the 7th day in vitro (DIV). Our preparations were ≥93% pure for astrocytes, as demonstrated by glial fibrillary acidic protein (GFAP) antibody. Astrocytes were exposed to LPS in serum-free medium at a final concentration of 100 ng/ml (20 ng/10^5 cells). Vehicle control for LPS exposure was PBS.

Western blot analysis
Conditioned (serum-free) medium was removed from astrocyte cultures following LPS or vehicle exposure and centrifuged at 13,000 × g for 2 min at 4°C to remove cell debris. Equal volumes of conditioned media were diluted with 6X sample buffer (0.35 M Tris, 30% glycerol, 10% SDS, 0.93 g DTT, 1.2 mg bromophenol blue), heated to 95°C for 5 min, subjected to SDS-PAGE, transferred to PVDF membranes, and analyzed and quantified as previously described [37]. Anti-human apoE (Dako) was used at 1:2000 dilution. Secondary antibody was HRP-conjugated anti-rabbit (1:3000).

NO detection
NO levels in conditioned media following incubation with LPS or vehicle were measured using a colorimetric NO assay kit (Calbiochem) where nitrate is first converted to nitrite by the NADH-dependent nitrate reductase, followed by nitrite measurement using the Griess Reagent.

Cytokine measurements
Conditioned medium following incubation with LPS or vehicle was screened for cytokines with an array method, and selected cytokines further quantified individually by sandwich ELISAs. The bead-based Liquichip™ Mouse 10-Cytokine Kit (Qiagen Inc, Valencia CA) was used to simul-
and 24 hrs after exposure, spun briefly, mixed with 4 μl of
NF-κB transcription factor assay kit from Chemicon International. Briefly, cells were rinsed
with PBS, lysed in Buffer A (10 mM HEPES (pH 7.9), 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM DTT, 0.1% Triton X-100 and protease inhibitor cocktail), and a nuclear extract prepared in Buffer B (20 mM HEPES (pH 7.9), 1.5 mM MgCl₂, 0.42 M NaCl, 0.2 mM EDTA, 0.5 mM DTT, 1.0% Igepal CA-630, 25% (v/v) glycerol, and protease inhibitor cocktail). Double-stranded biotinylated oligonucleotide containing the flanked consensus sequence for NF-κB was mixed with the nuclear extract and the mixture immobi-

lized on a streptavidin-coated chemiluminescent plate, followed by immunologic detection of the bound NF-κB transcription factor subunits p50 and 065.

Results
We have recently reported that LPS activation of TR APOE glial-wt neuron mixed cultures for 24 hours results in apoE isoform-specific paracrine damage to neurons [30]. For activated microglia, TR APOE4 is more neurotoxic than TR APOE2 or APOE3. For activated astrocytes, which produce much less neurotoxicity than microglia, both TR APOE4 and TR APOE3 are mildly damaging to neurons, while TR APO2 shows no neurotoxic effect. In this previous work, we pursued apoE-isoform specific mechanisms in LPS-activated microglia and showed that these were p38MAPK-dependent. Here, we pursued the basis of apoE isoforms-specific differences in LPS activation of astrocytes from these TR mice.

We first showed that there was no difference among the three TR APOE astrocytes in the amount of secreted apoE following LPS exposure for up to 24 hours (Figure 1), in agreement with our findings for microglia [30]. We also determined that similar to microglia, there was no difference in medium nitrate plus nitrite levels (a measure of NO secretion) compared to wild type (wt) at 12 or 24 hours after LPS exposure (P > 0.05), although we did observe increased medium nitrate plus nitrite levels in TR APOE4 (205 ± 41 % of wt) but not TR APOE2 (116 + 15% of wt) astrocytes 72 hours after LPS incubation. As with microglia, this temporal mismatch suggests increased NO secretion by TR APOE4 lies distal to the processes underlying the TR-APOE isoform-specific differences in astrocyte-mediated neurotoxicity seen within 24 hours of LPS incubation [30].

Previously, we observed TR APOE-dependent differences in cytokine secretion by microglia in response to LPS exposure [30]. Here we measured cytokine secretion in the three TR APOE astrocyte cultures. We screened for changes in medium cytokine concentrations using the LiquiChip™ Mouse 10-Cytokine assay and a Luminex 100 X-Map reader that simultaneously determines 10 mouse cytokines in medium from TR APOE astrocytes. The cytokines quantified were GM-CSF, INF-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, and TNF-α. Only IL-6 and TNF-α changed significantly following LPS exposure for 12 hr; IL-1β was near the limit of detection for this assay. The magnitude of induction for these cytokines was TR APOE-dependent with IL-6 and TNF-α concentrations following the gradient of TR APOE2 > TR APOE3 > TR APOE4. We confirmed our IL-6 and TNF-α findings with individual ELISAs and extended our analysis to IL-1β, since many others have shown it to be overexpressed and secreted from LPS-stimulated glia; TR APOE-dependence
of IL-1β secretion followed the same pattern as the other two cytokines (Figure 2).

LPS activation of CD14/TLR4 co-receptors leads to subsequent increased gene transcription mediated through a bifurcated pathway that is dependent on NF-κB and p38MAPK signaling. We have previously demonstrated apoE isoform-specific p38MAPK activation following LPS exposure of microglia but not astrocytes [30]. We therefore determined the activity of two NF-κB subunits, p50 and p65, in astrocytes from TR APOE mice (Figure 3). Following LPS exposure, both p50 and p65 activity significantly increased in all 3 genotypes. p50 activity showed an apoE isoform-specific increase, with a larger increase in TR APOE2 than the other two (P < 0.01 for both) and no difference between TR APOE3 and TR APOE4. p65 showed a similar trend in apoE isoform-specific effect; however, this was not significantly different in corrected multiple comparison tests.

Discussion

Inheritance of APOE alleles is associated with varying clinical outcomes in several neurodegenerative diseases, including AD, PD, ALS, head trauma, multiple sclerosis, and HIV-encephalitis [2-10]. Although apoE isoforms likely modulate AD pathogenesis by influencing metabolism of Aβ [12,38], the pathophysiologic significance of apoE isoforms appears to go beyond interacting with Aβ since these other diseases of brain are not thought to involve Aβ peptides in their pathogenesis. Indeed, others have suggested more general mechanisms of neurotrophism or neurotoxicity from inheritance of different APOE alleles that potentially could contribute to multiple neurologic diseases [13-16]. Since activation of innate immunity also is associated with these same diseases, we tested the hypothesis that apoE isoforms may act by modulating glial innate immune response and thereby altering neurotoxicity. Previously, we showed that microglia from TR APOE mice show apoE isoform-specific innate immune activation and paracrine damage to neurons that was greatest with TR APOE4 and dependent on p38MAPK signaling [30]. Here, we showed that identical activation of astrocytes from these same TR APOE mice had apoE isoforms-specific innate immune response that was greatest with TR APOE2 astrocytes and associated with NF-kB-mediated signaling.

We used a model of selective activation of CD14/TLR4 co-receptors that is now appreciated to initiate innate immune response to endogenous ligands relevant to neurodegenerative diseases such as Aβ fibrils as well as peptides and neoantigens expressed by apoptotic cells [32,33]. LPS activation of CD14/TLR4 co-receptors leads to increased gene transcription through a bifurcated pathway; one arm is NF-κB-dependent and the other is p38MAPK-dependent [26,27]. Our data indicated that the intracellular signaling that mediates altered gene transcription in response to LPS is different between astrocytes and microglia expressing TR APOE. Specifically, NF-κB-mediated signaling, which is associated with immune modulation and protection of cells from undergoing apoptosis [39], was greatest in TR APOE2 astrocytes, the only cell line that did not yield paracrine damage to neurons following activation with LPS [30]. In contrast, apoE isoforms-specific effects in microglia, including much p50 and p65, in astrocytes from TR APOE mice (Figure 3). Following LPS exposure, both p50 and p65 activity significantly increased in all 3 genotypes. p50 activity showed an apoE isoform-specific increase, with a larger increase in TR APOE2 than the other two (P < 0.01 for both) and no difference between TR APOE3 and TR APOE4. p65 showed a similar trend in apoE isoform-specific effect; however, this was not significantly different in corrected multiple comparison tests.
κ-dependent activation of p38MAPK or NF-κB from microglia and astrocytes as a result of TR APOE-response in TR APOE glia results in opposing outcomes. Our results suggest that LPS activation of innate immune response in TR APOE glia associates with LPS-activated astrocytes that we reported previously [30] and innate immune activation may be related to diminished NF-κB signaling [30]. We speculate that the inverse relationship between low-level neurotoxicity associated with p38MAPK signaling [30] and innate immune activation may be related to diminished NF-κB-dependent trophic factors in TR APOE3 and TR APOE4 astrocytes.

Conclusion
Our results suggest that LPS activation of innate immune response in TR APOE glia results in opposing outcomes from microglia and astrocytes as a result of TR APOE-dependent activation of p38MAPK or NF-κB signaling in these two cell types.

Abbreviations
AD (Alzheimer's disease); ALS (amyotrophic lateral sclerosis); apo (apolipoprotein); APOE (human apoE gene); Aβ (beta amyloid); DIV (days in vitro); GFAP (glial fibrillary astrocytic protein); hHDL (human high density lipoprotein); IL (interleukin); INF (interferon); LPS (lipopolysaccharide); NF-κB (nuclear factor kappa B); NO (nitric oxide); p38MAPK (p38 mitogen-activated protein kinase); PD (Parkinson’s disease); RLU (relative light units); TLR (Toll-like receptor); TNF (tumor necrosis factor); TR (targeted replacement); wt (wild type).

Competing interests
The author(s) declare that they have no competing interests.

Authors’ contributions
IM carried out the experiments described. NM developed the mouse line that was used in all experiments. TJM conceived the study and its design and helped to draft the manuscript. KSM assisted in experimental design, analyzed the data, and drafted the manuscript.

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References
1. Mahley RW: Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 1988, 240(4852):622-630. Apr 29
2. Alberts MJ, Graffagnino C, McClenney C, DeLong D, Strittmatter WJ, Saunders AM, Roses AD: APOE genotype and survival from intracerebral hemorrhage. Lancet 1995, 346:675.
3. Newman MF, Croughwell ND, Blumenthal JA, Lowry E, White WD, Spillane W, Davis RD, Glower DD, Smith LR, Mahanna EP: Predictors of cognitive decline after cardiac operation. Ann Thorac Surg 1995, 59:1326-1330.
4. Strittmatter WJ, Roses AD: Apolipoprotein E and Alzheimer disease. Proc Natl Acad Sci U S A 1995, 92:4725-4727.
5. Jordan BD, Relkin NR, Ravdin LD, Jacobs AR, Bennett A, Gandy S: Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. JAMA 1997, 278:136-140.
6. Corder EH, Robertson K, Lannfelt L, Bogdanovic N, Eggertsen G, Wilkins J, Hall C: HIV-infected subjects with the E4 allele for APOE have excess dementia and peripheral neuropathy. Nat Med 1998, 4:1182-1184.
7. Nathoo N, Chetty R, van Dellen JR, Barnett GH: Genetic vulnerability following traumatic brain injury: the role of apolipoprotein E. Mol Pathol 2003, 56:132-136.
8. Enzinger C, Ropele S, Smith S, Strasser-Fuchs S, Poltrum B, Schmidt H, Matthews PM, Fazekas F: Accelerated evolution of brain atrophy and "black holes" in MS patients with APOE-epsilon 4. Ann Neurol 2004, 55:563-569.
9. Li YJ, Hauser MA, Scott WK, Martin ER, Booze MW, Qin XJ, Walter JW, Nance MA, Haines JL, Siddique T: Apolipoprotein E is associated with age at onset of amyotrophic lateral sclerosis. Neurogenetics 2004, 5:209-213.
11. Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PCJ, Rimmler JB, Locke PA, Conneally PM, Schmader KE, et al.: Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet 1994, 7:180-184.

12. Holtzman DM: In vivo effects of ApoE and clustering on amyloid-beta metabolism and neuropathology. J Mol Neurosci 2004, 23:247-254.

13. Hyvysa M, Smith JD: Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. Nature Genetics 1996, 14:55-61.

14. Brown CM, Wright E, Colton CA, Sullivan PM, Laskowitz DT, Vitek WA: Pharmacologic suppression of neuronal oxidative damage and dendritic degeneration following direct activation of the antioxidant ferulic acid. J Neurochem 2004, 87:463-470.

15. Bock HH, Jossin Y, May P, Bergner O, Herz J: Apolipoprotein E receptors are required for reelin-induced proteasomal degradation of the neuronal adaptor protein Disabled-1. J Biol Chem 2004, 279:33471-33479.

16. Montine TJ, Milatovic D, Gupta RC, Valyi-Nagy T, Morrow JD, Breyer RM: Neuronal oxidative damage from activated innate immunity is EP2 receptor-dependent. J Neurochem 2002, 83:463-470.

17. Lehnardt S, Massillon L, Follett P, Jensen FE, Ratan R, Rosenberg PA, Brecht WJ, Harris FM, Chang S, Tessier L, Yu GQ, Xu Q, Dee Fish J, Akira S: In vivo effects of ApoE and clustering on amyloid-beta metabolism and neuropathology. J Mol Neurosci 2004, 23:247-254.

18. Johnson GB, Devitt A, Bell ED, Simmons DL, Gregory CD: Macrophage recognition of ICAM-3 on apoptotic leukocytes. J Immunol 1999, 162:6800-6810.

19. Sullivan PM, Mezdour H, Aratani Y, Knouff C, Najib J, Redick RL, Quarforids SH, Maeda N: Targeted replacement of the mouse apolipoprotein E gene with the common human APOE3 allele enhances diet-induced hypercholesterolemia and atherosclerosis. J Biol Chem 1997, 272:17972-17980.

20. Sullivan PM, Mezdour H, Quarforids SH, Maeda N: Type III hyperlipoproteinemia and spontaneous atherosclerosis in mice resulting from gene replacement of mouse Apoe with human Apoe*3. J Clin Invest 1998, 102:130-135.

21. Gebicke-Haerter PJ, Bauer J, Schobert A, Nordhoff H: Lipopolysaccharide-free conditions in primary astrocyte cultures allow growth and isolation of microglial cells. J Neurosci 1989, 9:183-194.

22. Maezawa I, Jin LW, Wolger RL, Maeda N, Martin GM, Montine TJ, Montine KS: Apolipoprotein E isoforms and apolipoprotein A1 protect from amyloid precursor protein carboxy terminal fragment-associated cytotoxicity. J Neurochem 2004, 91:1312-1321.

23. Bredenda RP, Bales KR, Paul SM, Holtzman DM: Role of apoE/Abeta interactions in Alzheimer's disease: insights from transgenic mouse models. Mol Psychiatry 2002, 7:132-135.

24. Clevers H: At the crossroads of inflammation and cancer. Cell 2004, 118:671-674.

25. Imler JL, Hoffmann JA: Toll receptors in innate immunity. Trends Cell Biol 2001, 11:304-311.

26. Akira S: Toll-like receptor signaling. J Biol Chem 2003, 278:38105-38108.

27. Milatovic D, Zaja-Milatovic S, Montine KS, Shie FS, Montine TJ: Neuronal oxidative damage and dendritic degeneration following activation of CD14-dependent innate immune response in vivo. J Neuroinflammation 2004, 1:20.

28. Shie FS, Montine KS, Breyer RM, Montine TJ: Microglial EP2 is critical to neurotoxicity from activated cerebral innate immunity. Glia 2005, 52:70-77.

29. Maetzawa I, Nivison M, Montine KS, Maeda N, Montine TJ: Neurotoxicity from innate immune response is greatest with targeted replacement of E4 allele of apolipoprotein E gene and is mediated by microglial p38MAPK. FASEB J 2006, 20:797-799.

30. Johnson GB, Brunn GJ, Platt JL: Activation of mammalian Toll-like receptors by endogenous agonists. Crit Rev Immunol 2003, 23:15-44.

31. Fassbender K, Walter S, Kuhl S, Landmann R, Ishii K, Bertsch T, Stalder AK, Muehlihauser F, Liu Y, Ulmer AJ, Rivest S, Lentschat A, Gulbins E, Jucker M, Staufenbriel M, Brechel K, Walter J, Multhaup G, Penke B, Adachi Y, Hartmann T, Beyreuther K: The LPS receptor (CD14) links innate immunity with Alzheimer's disease. FASEB J 2004, 18:203-205.

32. Moffatt OD, Devitt A, Bell ED, Simmons DL, Gregory CD: Macrophage recognition of ICAM-3 on apoptotic leukocytes. J Immunol 1999, 162:6800-6810.

33. Sullivan PM, Mezdour H, Aratani Y, Knouff C, Najib J, Redick RL, Quarforids SH, Maeda N: Targeted replacement of the mouse apolipoprotein E gene with the common human APOE3 allele enhances diet-induced hypercholesterolemia and atherosclerosis. J Biol Chem 1997, 272:17972-17980.

34. Sullivan PM, Mezdour H, Quarforids SH, Maeda N: Type III hyperlipoproteinemia and spontaneous atherosclerosis in mice resulting from gene replacement of mouse Apoe with human Apoe*3. J Clin Invest 1998, 102:130-135.

35. Gebicke-Haerter PJ, Bauer J, Schobert A, Nordhoff H: Lipopolysaccharide-free conditions in primary astrocyte cultures allow growth and isolation of microglial cells. J Neurosci 1989, 9:183-194.

36. Maezawa I, Jin LW, Wolger RL, Maeda N, Martin GM, Montine TJ, Montine KS: Apolipoprotein E isoforms and apolipoprotein A1 protect from amyloid precursor protein carboxy terminal fragment-associated cytotoxicity. J Neurochem 2004, 91:1312-1321.

37. Bredenda RP, Bales KR, Paul SM, Holtzman DM: Role of apoE/Abeta interactions in Alzheimer's disease: insights from transgenic mouse models. Mol Psychiatry 2002, 7:132-135.

38. Clevers H: At the crossroads of inflammation and cancer. Cell 2004, 118:671-674.

39. Imler JL, Hoffmann JA: Toll receptors in innate immunity. Trends Cell Biol 2001, 11:304-311.

40. Akira S: Toll-like receptor signaling. J Biol Chem 2003, 278:38105-38108.

41. Milatovic D, Zaja-Milatovic S, Montine KS, Shie FS, Montine TJ: Neuronal oxidative damage and dendritic degeneration following activation of CD14-dependent innate immune response in vivo. J Neuroinflammation 2004, 1:20.

42. Shie FS, Montine KS, Breyer RM, Montine TJ: Microglial EP2 is critical to neurotoxicity from activated cerebral innate immunity. Glia 2005, 52:70-77.

43. Maetzawa I, Nivison M, Montine KS, Maeda N, Montine TJ: Neurotoxicity from innate immune response is greatest with targeted replacement of E4 allele of apolipoprotein E gene and is mediated by microglial p38MAPK. FASEB J 2006, 20:797-799.