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APOE genotype alters immunoglobulin subtypes in knock-in mice

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Abstract

Apolipoprotein E (APOE) alleles are strongly related to the risk of Alzheimer's disease (AD). APOE genotype also affects inflammatory processes in response to damage. We tested whether APOE genotype affected the levels of specific immunoglobulins in healthy, uninfected APOE knock-in mice. We measured specific immunoglobulins in brain, spleen and plasma. Levels of total IgG in brain and spleen were highest in APOE-ε3 mice, significantly higher than in APOE-ε2 and APOE-ε4 mice; no differences were observed for levels of total IgG plasma. We also measured specific subtypes of IgG. IgG1 was only detectable in plasma, and did not differ by APOE genotype. IgG3 was detectable in plasma and spleen, and also did not differ by APOE genotype. IgG2b showed the same pattern as levels of total IgG by APOE genotype, with the highest levels of IgG2b in brain, spleen, and plasma of APOE-ε3 mice. IgG2a showed an entirely different pattern, with significantly higher levels in spleen and plasma of APOE-ε4 mice compared to APOE-ε2 and APOE-ε3 mice. We also measured IgM and IgA in spleens and plasma of these mice. In spleen, APOE-ε4 mice had the lowest IgA levels and the highest levels of IgM; both being significantly different from APOE-ε2 mice. In total, murine IgG2a and IgM were highest in APOE-ε4 mice, while total IgG and Ig2b were highest in APOE-ε3 mice. These dramatically different distributions of immunoglobulins could allow for human AD risk biomarkers based on specific immunoglobulin subtypes.

Keywords

Apolipoprotein E; immunoglobulin; inflammation; brain; spleen; plasma

Introduction

Polymorphisms in the gene for apolipoprotein E (APOE) greatly affect the risk of late-onset Alzheimer's Disease (AD) [1]. There are three common human APOE alleles (APOE- ϵ 2, APOE- ϵ 3 and APOE- ϵ 4), encoding apoE isoforms that differ from each other by single amino acids [2]. Individuals homozygous for APOE- ϵ 4 are 16 times more susceptible in AD than APOE- ϵ 3 homozygotes, while those who have inherited APOE- ϵ 2 have a significantly reduced risk of AD [3–5]. The strong association of APOE- ϵ 4 with risk of AD makes it possible to identify individuals at high risk for AD well before the onset of symptoms.

The mechanism of APOE increasing AD risk alters how APOE-directed therapies are developed. ApoE is a lipid transport molecule, associating with lipoproteins and promoting their endocytosis in various tissues [6]. ApoE affects metabolism of A β due to its partially hydrophobic nature [7], either through preventing A β clearance from the brain interstitial fluid [6] or promoting glial clearance [8,9]. However, the effects of APOE on AD pathogenesis may also be due in part to its role in regulation of inflammatory responses [10]. APOE knock-out [11] and APOE4 knock-in mice [12] have increased neuroinflammation in response to various agents [13–16], including the A β protein [17,18]. Microglia of APOE4 knock-in mice have a more active immune reaction in brain after infection compared to APOE3 mice, including increased proliferation and pro-inflammatory cytokine release [19,20]. In vitro studies also demonstrate the anti-inflammatory effects of apoE, with apoE4 being deficient compared to apoE2 or apoE3 [13,21]. The effects of APOE on inflammation are also supported by the observations that APOE knock-out mice have higher IgM levels, more T cell proliferation and more reactive nitric oxide production after antigen stimulation [22–24]. We hypothesize that APOE genotype may also affect neuroinflammation even in the absence of a proinflammatory stimulus.

In this study, we address the role of APOE as an immune modulator by examining the immunoglobulin levels in healthy APOE knock-in mice. We found that APOE4 mice had a higher level of IgG2a and IgM but lower levels of IgA; this being consistent with the role of apoE4 promoting a more inflammatory status in vivo. Furthermore, these findings identify several specific blood proteins that could be evaluated as biomarkers for APOE-associated risk of AD.

Materials and Methods

Mice

APOE2, APOE3 and APOE4 knock-in mice on a C57B16/J background each express human APOE isoforms regulated by the endogenous murine APOE promoter [25]. All mice were maintained in a constant room temperature and humidity under a 12h light/dark cycle at Georgetown University Medical Center, and freely provided food and water. Experiments were performed on age-matched nine month old female mice (APOE2 N=4; APOE3 N=6; APOE4 N=6). This age was chosen to allow analysis of immunoglobulins in adult animals but without alterations that may occur in aged mice; female mice were chosen for the sake of consistency. All experiments were performed in accordance with National Institutes of

Health Guide for the Care and Use of Laboratory Animals and approved by the Georgetown University Animal Care and Use Committee (protocol number 12-044).

Mouse tissue collection and preparation

Mice were fasted overnight before euthanasia by carbon dioxide asphyxiation. Blood was taken via heart puncture, treated to 10mM EDTA, and spun at 2,000 rpm for 20 minutes; plasma was stored at -20°C . Animal perfusion was performed using ice-cold saline (PBS, pH 7.4). Brains and spleens were rapidly removed (brains were then hemisected), frozen on dry ice, weighed and stored at -80°C . The spleens and left hemispheres of the brains were homogenized with a 7ml dounce with ice-cold Tris-buffered saline (TBS) buffer (50mM Tris-HCl, 150mM NaCl, 1x protease inhibitor and phosphatase inhibitor cocktails, pH 7.4). The homogenates were centrifuged in a Beckman Coulter Le-80 Ultracentrifuge (Brea, CA, USA, rotor#: sw55Ti) at 33,400 rpm for 45 minutes at 4°C , and the supernatants were removed and labeled as the TBS (soluble) fraction. The remaining pellet was sonicated in ice-cold TBS-X buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 1x protease inhibitor and phosphatase inhibitor cocktails, pH 7.4). The resuspended pellet was centrifuged at 33,400rpm for 45 min at 4°C , and the supernatants were removed and labeled as the TBS-X (membrane) fraction. Total protein concentration was determined by BCA Protein Assay Kit (Pierce, Rockford, IL, USA) and absorbance was analyzed by Origin 8.0 (OriginLab, Northampton, MA, USA). Total protein levels were adjusted to load equivalent amounts for Western blot analysis.

Western blots

All samples were reduced with 2-mercaptoethanol and heated at 95°C for 5 minutes before being subjected to SDS-PAGE: For brain and spleen samples, 20 μg of protein was analyzed by electrophoresis; for plasma, 3 μl was similarly analyzed. Separated proteins were transferred onto a nitrocellulose membrane. After blocking with 5% non-fat dried milk in TBS-T (TBS with 0.05% Tween 20) for 1 hour, the membranes were incubated with antibody diluted in TBS-T with 5% milk for at least 2 hours. The following antibodies were used: Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, (Sigma-Aldrich, St. Louis, MO, USA); HRP-conjugated goat anti-mouse IgM and HRP-conjugated goat anti-mouse IgA (SouthernBiotech, Birmingham, AL, USA); HRP-conjugated goat anti-mouse IgG1 (Abcam Inc, Cambridge, MA, USA); HRP-conjugated goat anti-mouse IgG2a (Santa Cruz Biotechnology, Santa Cruz, CA, USA); HRP-conjugated goat anti-mouse IgG2b (Life Technologies, Carlsbad, CA, USA) and HRP-conjugated goat anti-mouse IgG3 (Abcam Inc, Cambridge, MA, USA). Antibodies were diluted 1000 to 10,000 fold. After washing with TBS-T five times for 3 minutes, membranes were developed by SuperSignal West PICO (Pierce, Rockford, IL, USA) for 5 minutes and subjected to X-ray film development. The X-ray film was scanned and the density of bands was quantified using ImageJ software (National Institutes of Health). For loading control experiments in spleen and brain samples, rabbit anti-tubulin and HRP-conjugated goat anti-rabbit IgG (Sigma-Aldrich) were used. For plasma samples, nitrocellulose blots were stained with Ponceau to determine that there was no large variation in total protein levels.

Statistical analysis

Statistical analysis was performed by one-way ANOVA or T-test using GraphPad Prism (GraphPad, San Diego, CA, USA). Tukey's post hoc tests were applied to detect statistical differences among groups. Data are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to APOE3 mice; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared to APOE4 mice.

Results

APOE3 mice have higher levels of total IgG

In order to investigate the effect of APOE genotype on immunoglobulins throughout the body, we isolated brain, spleen and blood samples from nine month old female mice expressing APOE2, APOE3, or APOE4, using three to six samples from the various tissues of animals of each genotype. We first measured total IgG levels in these tissues, using immunoblots developed only with an anti-mouse IgG secondary antibody. We examined the distribution of IgG in extracts of brain and spleen, comparing levels in soluble (TBS) and membrane-associated (TBS-X) fractions. As expected, the assay demonstrated immunoglobulin bands of approximately 50 kDa. For both tissues, IgG was detected almost exclusively in the TBS-soluble tissue fractions compared to the membrane-associated fractions (Figure 1A, B), and APOE genotype did not affect whether immunoglobulins were associated with membrane fractions. In subsequent analyses, we used only tissue TBS fractions to test IgG levels.

In the various tissues, there was considerable variation in IgG levels between animals, as demonstrated by large standard error measures (Figure 1). In brain, APOE3 mice had the highest levels of IgG (fourteen fold higher than APOE2 mice ($p < 0.05$) and seven fold higher than APOE4 mice ($p = 0.06$) (Figure 1C, D). We observed a similar distribution in spleen: APOE3 mice had significantly higher levels of IgG (1.5 to 2 fold) compared to both APOE2 and APOE4 mice ($p < 0.05$) (Figure 1E, F). We found a similar distribution of IgG levels in brain tissue with another secondary anti-IgG antibody, with APOE3 mice having over four-fold higher levels of IgG than APOE2 mice ($p < 0.05$), and nearly two-fold higher levels than APOE4 mice (not significant) (data not shown). There were no significant differences in plasma levels of IgG among the APOE groups (Figure 1G, H). Together, these data suggested that there are APOE related differences in IgG subtypes, at least in some tissues.

APOE genotype affects levels of IgG subtypes

We next examined the effects of APOE genotype on various mouse IgG subtypes: IgG1, IgG2a, IgG2b, and IgG3. For each, we focused on examining subtypes in the soluble brain and spleen fractions, as well as in plasma. We were unable to detect this IgG1 in brain and spleen samples (data not shown). IgG1 was detected in plasma as a 50–55 kDa band, as expected (Figure 2), but there were no significant differences in levels among the APOE2, APOE3, and APOE4 mice (Figure 2).

We were able to detect IgG2a in spleen and plasma samples, but not in brain extracts. Western blotting revealed significantly higher levels of IgG2a in spleen samples from

APOE4 mice compared to APOE2 and APOE3 mice (Figure 3A). Quantitation demonstrated that APOE4 mice had two fold higher levels of IgG2a than APOE3 mice and three-fold higher levels than APOE2 mice (Figure 3A). There was also a significantly higher level of IgG2a in plasma from APOE4 mice (three-fold higher than APOE3 mice and six-fold higher than APOE2 mice) (Figure 3B). Importantly, this distribution of IgG2a was different than we observed for total IgG levels, where the highest levels were observed in the APOE3 mice (Figure 1).

We next examined IgG2b. In contrast to IgG2a, IgG2b was detectable in brain, spleen and plasma samples. In all tissues, IgG2b levels were highest in the APOE3 mice (Figure 4), consistent with the findings for total IgG levels (Figure 1). In brain, APOE3 mice showed two-fold higher levels of IgG2b compared to APOE4 mice ($p<0.05$) (Figure 4A). In spleen, a similar trend was observed, with three fold higher levels in APOE3 mice compared to APOE4 (not significant, $p=0.06$) (Figure 4B). In plasma, IgG2b levels in APOE3 mice were nearly four-fold higher than in APOE4 mice ($p<0.01$) (Figure 4C). In all tissues, levels of IgG2b in APOE2 mice were intermediate to APOE3 and APOE4 (and statistically significantly lower than in plasma of APOE3 mice, $p<0.05$) (Figure 4C).

Finally, we analyzed IgG3. As with IgG2a, we were able to detect IgG3 in spleen and plasma samples, but not in brain samples, and again, as the expected 50–55 kDa protein. We found no significant differences in IgG3 levels in spleen or plasma among the three APOE genotypes (Figure 5).

APOE genotype affects other immunoglobulin subtypes

Since APOE genotype affected the IgG levels in various tissues, we examined other immunoglobulin subtypes. We measured levels of IgA, an important immunoglobulin in mucosal immunity that is partially regulated by the central nervous system [26]. As with IgG, we initially compared levels of IgA in TBS and TBS-X fractions of brain and spleen. Again, the TBS fraction showed a much higher level of IgA than the TBS-X fraction (Figure 6A). IgA could not be detected in brain samples, but was measurable in spleen and plasma. Quantifying spleen IgA levels across APOE mice, we found the APOE2 mice had the highest levels of IgA, significantly higher than APOE4 mice, but there were no significant differences with APOE3 mice (Figure 6B). There were no significant differences of plasma IgA among groups (Figure 6C).

Finally, we examined another type of immunoglobulin in these mice: IgM, which has a role in promoting neuronal survival and remyelination [27]. In these blots, IgM was visible as both 75kDa and 50 kDa proteins (Figure 7A), perhaps due to stable complexes between heavy and light chains. As with the other immunoglobulins, IgM was present predominantly in the soluble tissue fractions from both brain and spleen. In brain, there were no significant differences in IgM levels across APOE genotypes, although the average IgM levels were highest in the APOE4 mice and lowest in the APOE2 mice (Figure 7B). A similar but significant pattern was seen using samples from spleen: APOE4 mice had a significantly higher level of IgM in spleen than APOE2 and APOE3 mice (Figure 7C). Plasma samples revealed a similar pattern in IgM levels: APOE2 mice had a significantly lower level of IgM

compared to APOE3 and APOE4 mice (Figure 7D), although there were no statistically significant differences of IgM levels between APOE3 and APOE4 mice in plasma.

Discussion

We have hypothesized that APOE genotype affects basal levels of inflammation, which may lead to increased risk of disease. To explore whether APOE alleles affect immunoglobulins in the absence of infection or AD-related pathology, we used young (nine-month old) APOE2, APOE3 and APOE4 female knock-in mice and measured the levels of total IgG, IgG subtypes, IgA, and IgM in three different tissues: brain, spleen, and plasma. These mice allow analysis of the effect of APOE on immunoglobulins in a genetically controlled animal model. Although we were not able to measure each immunoglobulin in all tissues (in particular, several were not detectable in brain samples), and although there was variation within groups, we did observe significant differences by APOE genotype. Our most dramatic findings were that APOE3 mice had significantly higher levels of total IgG as well as the IgG2b subtype, and that APOE4 mice had higher levels of IgG2a. Several of these findings (for total IgG and for IgG2a) were demonstrated in several analyses with similar findings, although the magnitudes of the effect sizes were different. Together, these data demonstrate strong effects of APOE genotype on regulation of immunoglobulins in both the periphery and the CNS. APOE genotype effects on production, secretion, or clearance of immunoglobulins may account for these differences in the observed levels.

We examined proteins from spleen as a major organ of production of immunoglobulins [28]. IgM is the first immunoglobulin produced by spleen. B cells that produce IgM are then differentiated into other types of B cells that produce the other classes of immunoglobulins (e.g., IgG and IgA), which reflects the process of immunoglobulin class switching [16]. In our study, the IgG and IgA levels of APOE4 mice were lower than APOE3 mice, but the IgG2a and IgM levels of APOE4 were quite high, suggesting that APOE4 mice might have a different immunoglobulin class switching from APOE3 or APOE2 mice. There are three major steps involved in class switching procedure: first, CD40 ligand on T helper cells binds to CD40 on B cells; second, the T helper cells secrete cytokines such as interleukin-4 (IL-4) and interferon- γ (IFN- γ); finally, B cells modulate their proliferation and immunoglobulin isotype switching [16]. B cell isotype switching can be altered by different cytokines. For instance, IL-4 and IL-5 can specifically increase the IgG1 and IgA levels, respectively [16]. APOE knock-out mice have immunodeficiency with unbalanced different types of cytokines [23], including IFN- γ . IFN- γ can stimulate IgM production, reduce IgM switching to IgG1, IgG2b, IgG3 and IgA, and increase the switching to IgG2a. In fact, the synthesis of IgG2 depends greatly on levels of IFN- γ and interleukins [19]. After septic insult in rats, APOE treatment in vivo decreased levels of IL-4 [24], which is an inhibitor of IFN- γ . Thus, modulation of the regulation of cytokines such as IFN- γ by APOE genotype may be responsible for the observed alteration in immunoglobulin expression.

Levels of immunoglobulins in APOE2 mice were similar to APOE4 mice for IgG, but different for the other immunoglobulins. While APOE4 is associated with an increased risk of Alzheimer's disease, APOE2 is protective [5]. The levels of IgG2a, IgM, and IgA correlate (positively or negatively) with the relative risk of each allele. APOE2 and APOE4

are both associated with increased amyloid deposition in an AD mouse model (14) and in human cerebral amyloid angiopathy (15). These effects of APOE genotype on amyloid may be through differences in A β clearance [29], either from the brain parenchyma (resulting in plaques) or through the brain vasculature (resulting in amyloid angiopathy). A β clearance is promoted by treatment against inflammation through increases in A β degrading enzymes [30]. Thus, some of the similar effects of APOE2 and APOE4 on total IgG levels may be related to similar effects on A β clearance altered by the brain inflammatory state.

Several studies have demonstrated effects of APOE genotype on the response of cells and animals to stimuli that induce inflammation in various brain and peripheral tissues [13,24,25,31]. ApoE can reduce inflammation in both microglia [25,32,33] and astrocytes [31] in response to endotoxins and A β . These effects are mediated at least in part by the apoE receptor LRP1 [32,34], with apoE4 less able to reduce inflammation than apoE3 [14]. APOE4 knock-in mice are more vulnerable to several types of brain damage that include inflammatory responses compared to APOE3 mice, such as stroke [13,14,35,36]. Injection of LPS into the brains of APOE knock-in mice produced greater responses in terms of cytokine production, invading T cells, and synaptic loss in APOE knock-out and APOE4 knock-in mice, compared to APOE2 and APOE3 mice [15].

The most significant findings here in young mice (the effects of APOE genotype on the levels of IgG, IgG2a, IgG2b) should be tested using other techniques, such as ELISAs, to test the reproducibility of the findings across assays. This work is relevant to identification of human biomarkers early in life for risk of AD. Trials for the prevention of AD will require identification of biomarkers that correlate with the later development of AD. Brain amyloid, as imaged by PET, is one biomarker that increases years before the onset of AD [37]. Our findings in mice should be followed with studies of human blood and CSF to determine if human immunoglobulin types could serve as biomarkers of APOE-associated risk of AD. Reduction in immunoglobulins (as seen in APOE4 mice) has a profound effect on plasma lipid and lipoprotein levels [20]. On a more basic science level, these data demonstrate an interaction between immunoglobulins and lipid metabolism.

One potential treatment for Alzheimer's disease is intravenous injection of pooled human immunoglobulins (IVIG) [38]. A mechanism of this treatment is unclear, although it may be through introduction of naturally-occurring antibodies against A β isoforms [39,40]. IVIG also reduces inflammation [41], and is used in treatments of autoimmune and inflammatory diseases. IVIG treatment of a mouse model of AD altered the inflammatory response to amyloid [42]. Thus, IVIG may act at least partially through an inhibition of AD-related inflammation. Anti-inflammatory effects of IgG depend on glycosylation of IgG [39], which was evident in some of our analyses of IgG, particularly for brain IgG. Interestingly, in human trials, the subgroup of APOE- ϵ 4-positive individuals responded more positively to IVIG treatment [43]. Since the APOE4 mice had lower levels of total IgG, it may be that introduction of IgG into APOE- ϵ 4 humans may be particularly beneficial in delaying the accumulation or downstream effects of amyloid.

APOE- ϵ 4-positive humans also had an altered response to immunotherapy approaches to treatment of AD. In clinical trials of one A β antibody, bapineuzumab, APOE- ϵ 4 individuals

had significantly higher levels of side effects related to edema [44]. These effects may be related to the preferential effects of apoE4 on the accumulation of A β in amyloid angiopathy [23], and the enhanced movement of A β from plaques to vascular amyloid by apoE [45]. There may also be a stronger IgG response in APOE- ϵ 4 individuals if the starting levels of IgG are lower than APOE- ϵ 3 individuals.

The specific effects of APOE genotype on AD therapies related to immunoglobulins will depend on preclinical studies. Recent work has established a tractable mouse model of amyloid deposition in the presence of the three different apoE isoforms, the EFAD mice [22]. In these mice, APOE genotype affects the level and morphology of A β deposits [18,22]. It will be interesting to test whether APOE genotype affects the response of these mice to active or passive immunotherapies to reduce A β levels. We speculate that the effects of APOE genotype on A β in the brains of EFAD mice is due to altered levels of chronic inflammation, evidenced in part by differences in specific IgG levels. ApoE4 reduces the recycling of its receptors [46] and may similarly affect the levels of Fc γ receptors, thus altering levels of circulating IgG subtypes, a suggested contributor to neurodegeneration [47].

Our findings that APOE genotype has a marked effect on levels of specific subtypes of immunoglobulins in mice demonstrate that APOE alters the resting immune system. These observations suggest potential immunoglobulin biomarkers in blood or CSF that may correlate with APOE-associated risk of AD in humans. This area of research also could elucidate ways that APOE genotype affects the risk of AD.

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References

1. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A*. 1993; 90:1977–1981. [PubMed: 8446617]
2. Mahley RW, Rall SC. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet*. 2000; 1:507–537. [PubMed: 11701639]
3. Schipper HM. Apolipoprotein E: implications for AD neurobiology, epidemiology and risk assessment. *Neurobiol Aging*. 2011; 32:778–790. [PubMed: 19482376]
4. Jordan BD, Relkin NR, Ravdin LD, Jacobs AR, Bennett A, Gandy S. Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. *JAMA J Am Med Assoc*. 1997; 278:136–140.
5. Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC Jr, Rimmler JB, Locke PA, Conneally PM, Schmechel KE. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet*. 1994; 7:180–184. [PubMed: 7920638]
6. Verghese PB, Castellano JM, Garai K, Wang Y, Jiang H, Shah A, Bu G, Frieden C, Holtzman DM. ApoE influences amyloid- β (A β) clearance despite minimal apoE/A β association in physiological conditions. *Proc Natl Acad Sci U S A*. 2013; 110:E1807–1816. [PubMed: 23620513]

7. Kim J, Basak JM, Holtzman DM. The role of apolipoprotein E in Alzheimer's disease. *Neuron*. 2009; 63:287–303. [PubMed: 19679070]
8. Lee CYD, Landreth GE. The role of microglia in amyloid clearance from the AD brain. *J Neural Transm Vienna Austria* 1996. 2010; 117:949–960.
9. Zhao W, Zhang J, Davis EG, Rebeck GW. Aging reduces glial uptake and promotes extracellular accumulation of A β from a lentiviral vector. *Front Aging Neurosci*. 2014; 6:210. [PubMed: 25177293]
10. Liu C-C, Liu C-C, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol*. 2013; 9:106–118. [PubMed: 23296339]
11. Van Ree JH, van den Broek WJ, Dahlmans VE, Groot PH, Vidgeon-Hart M, Frants RR, Wieringa B, Havekes LM, Hofker MH. Diet-induced hypercholesterolemia and atherosclerosis in heterozygous apolipoprotein E-deficient mice. *Atherosclerosis*. 1994; 111:25–37. [PubMed: 7840811]
12. Sullivan PM, Mezdoor H, Aratani Y, Knouff C, Najib J, Reddick RL, Quarfordt 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. [PubMed: 9218423]
13. Vitek MP, Brown CM, Colton CA. APOE genotype-specific differences in the innate immune response. *Neurobiol Aging*. 2009; 30:1350–1360. [PubMed: 18155324]
14. Lynch JR, Tang W, Wang H, Vitek MP, Bennett ER, Sullivan PM, Warner DS, Laskowitz DT. APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *J Biol Chem*. 2003; 278:48529–48533. [PubMed: 14507923]
15. Zhu Y, Nwabuisi-Heath E, Dumanis SB, Tai LM, Yu C, Rebeck GW, LaDu MJ. APOE genotype alters glial activation and loss of synaptic markers in mice. *Glia*. 2012; 60:559–569. [PubMed: 22228589]
16. Stavnezer J. Immunoglobulin class switching. *Curr Opin Immunol*. 1996; 8:199–205. [PubMed: 8725943]
17. Zhao W, Dumanis SB, Tamboli IY, Rodriguez GA, LaDu MJ, Moussa CEH, Rebeck GW. Human APOE genotype affects intraneuronal A β 1–42 accumulation in a lentiviral gene transfer model. *Hum Mol Genet*. 2013:ddt525.
18. Rodriguez GA, Tai LM, LaDu MJ, Rebeck GW. Human APOE4 increases microglia reactivity at A β plaques in a mouse model of A β deposition. *J Neuroinflammation*. 2014; 11:111. [PubMed: 24948358]
19. Kawano Y, Noma T, Yata J. Regulation of human IgG subclass production by cytokines. IFN- γ and IL-6 act antagonistically in the induction of human IgG1 but additively in the induction of IgG2. *J Immunol Baltim Md 1950*. 1994; 153:4948–4958.
20. Reardon CA, Blachowicz L, White T, Cabana V, Wang Y, Lukens J, Bluestone J, Getz GS. Effect of immune deficiency on lipoproteins and atherosclerosis in male apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2001; 21:1011–1016. [PubMed: 11397712]
21. Barger SW, Harmon AD. Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. *Nature*. 1997; 388:878–881. [PubMed: 9278049]
22. Youmans KL, Tai LM, Nwabuisi-Heath E, Jungbauer L, Kanekiyo T, Gan M, Kim J, Eimer WA, Estus S, Rebeck GW, Weeber EJ, Bu G, Yu C, Ladu MJ. APOE4-specific changes in A β accumulation in a new transgenic mouse model of Alzheimer disease. *J Biol Chem*. 2012; 287:41774–41786. [PubMed: 23060451]
23. Nelson PT, Pious NM, Jicha GA, Wilcock DM, Fardo DW, Estus S, Rebeck GW. APOE- ϵ 2 and APOE- ϵ 4 correlate with increased amyloid accumulation in cerebral vasculature. *J Neuropathol Exp Neurol*. 2013; 72:708–715. [PubMed: 23771217]
24. Kelly ME, Clay MA, Mistry MJ, Hsieh-Li HM, Harmony JA. Apolipoprotein E inhibition of proliferation of mitogen-activated T lymphocytes: production of interleukin 2 with reduced biological activity. *Cell Immunol*. 1994; 159:124–139. [PubMed: 7994749]
25. Pocivavsek A, Burns MP, Rebeck GW. Low-density lipoprotein receptors regulate microglial inflammation through c-Jun N-terminal kinase. *Glia*. 2009; 57:444–453. [PubMed: 18803301]

26. Campos-Rodríguez R, Godínez-Victoria M, Abarca-Rojano E, Pacheco-Yépez J, Reyna-Garfias H, Barbosa-Cabrera RE, Drago-Serrano ME. Stress modulates intestinal secretory immunoglobulin A. *Front Integr Neurosci*. 2013; 7:86. [PubMed: 24348350]
27. Correale J, Villa A. The neuroprotective role of inflammation in nervous system injuries. *J Neurol*. 2004; 251:1304–1316. [PubMed: 15592725]
28. Weinstein JS, Hernandez SG, Craft J. T cells that promote B-Cell maturation in systemic autoimmunity. *Immunol Rev*. 2012; 247:160–171. [PubMed: 22500839]
29. Tai LM, Mehra S, Shete V, Estus S, Rebeck GW, Bu G, LaDu MJ. Soluble apoE/A β complex: mechanism and therapeutic target for APOE4-induced AD risk. *Mol Neurodegener*. 2014; 9:2. [PubMed: 24386905]
30. Kalinin S, Richardson JC, Feinstein DL. A PPARdelta agonist reduces amyloid burden and brain inflammation in a transgenic mouse model of Alzheimer's disease. *Curr Alzheimer Res*. 2009; 6:431–437. [PubMed: 19874267]
31. Guo L, LaDu MJ, Van Eldik LJ. A dual role for apolipoprotein e in neuroinflammation: anti- and pro-inflammatory activity. *J Mol Neurosci*. 2004; 23:205–212. [PubMed: 15181248]
32. Pocivavsek A, Mikhailenko I, Strickland DK, Rebeck GW. Microglial low-density lipoprotein receptor-related protein 1 modulates c-Jun N-terminal kinase activation. *J Neuroimmunol*. 2009; 214:25–32. [PubMed: 19586665]
33. Pocivavsek A, Rebeck GW. Inhibition of c-Jun N-terminal kinase increases apoE expression in vitro and in vivo. *Biochem Biophys Res Commun*. 2009; 387:516–520. [PubMed: 19615334]
34. May P, Bock HH, Nofer J-R. Low density receptor-related protein 1 (LRP1) promotes anti-inflammatory phenotype in murine macrophages. *Cell Tissue Res*. 2013; 354:887–889. [PubMed: 23963646]
35. Lei B, Mace B, Bellows ST, Sullivan PM, Vitek MP, Laskowitz DT, James ML. Interaction between sex and apolipoprotein e genetic background in a murine model of intracerebral hemorrhage. *Transl Stroke Res*. 2012; 3:94–101. [PubMed: 23935764]
36. Laskowitz DT, Horsburgh K, Roses AD. Apolipoprotein E and the CNS response to injury. *J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab*. 1998; 18:465–471.
37. Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, Holtzman DM, Santacruz A, Buckles V, Oliver A, Moulder K, Aisen PS, Ghetti B, Klunk WE, McDade E, Martins RN, Masters CL, Mayeux R, Ringman JM, Rossor MN, Schofield PR, Sperling RA, Salloway S, Morris JC. Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. *N Engl J Med*. 2012; 367:795–804. [PubMed: 22784036]
38. Fillit H, Hess G, Hill J, Bonnet P, Toso C. IV immunoglobulin is associated with a reduced risk of Alzheimer disease and related disorders. *Neurology*. 2009; 73:180–185. [PubMed: 19620605]
39. Schwab I, Nimmerjahn F. Intravenous immunoglobulin therapy: how does IgG modulate the immune system? *Nat Rev Immunol*. 2013; 13:176–189. [PubMed: 23411799]
40. Britschgi M, Olin CE, Johns HT, Takeda-Uchimura Y, LeMieux MC, Rufibach K, Rajadas J, Zhang H, Tomooka B, Robinson WH, Clark CM, Fagan AM, Galasko DR, Holtzman DM, Jutel M, Kaye JA, Lemere CA, Leszek J, Li G, Peskind ER, Quinn JF, Yesavage JA, Ghiso JA, Wyss-Coray T. Neuroprotective natural antibodies to assemblies of amyloidogenic peptides decrease with normal aging and advancing Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2009; 106:12145–12150. [PubMed: 19581601]
41. Nimmerjahn F, Ravetch JV. Anti-inflammatory actions of intravenous immunoglobulin. *Annu Rev Immunol*. 2008; 26:513–533. [PubMed: 18370923]
42. Sudduth TL, Greenstein A, Wilcock DM. Intracranial Injection of Gammagard, a Human IVIg, Modulates the Inflammatory Response of the Brain and Lowers A β in APP/PS1 Mice Along a Different Time Course than Anti-A β Antibodies. *J Neurosci*. 2013; 33:9684–9692. [PubMed: 23739965]
43. Relkin N. Clinical trials of intravenous immunoglobulin for Alzheimer's disease. *J Clin Immunol*. 2014; 34(Suppl 1):S74–79. [PubMed: 24760112]
44. Salloway S, Sperling R, Fox NC, Blennow K, Klunk W, Raskind M, Sabbagh M, Honig LS, Porsteinsson AP, Ferris S, Reichert M, Ketter N, Nejadnik B, Guenzler V, Miloslavsky M, Wang D, Lu Y, Lull J, Tudor IC, Liu E, Grundman M, Yuen E, Black R, Brashear HR. Two Phase 3

- Trials of Bapineuzumab in Mild-to-Moderate Alzheimer's Disease. *N Engl J Med*. 2014; 370:322–333. [PubMed: 24450891]
45. Sakai K, Boche D, Carare R, Johnston D, Holmes C, Love S, Nicoll JAR. A β immunotherapy for Alzheimer's disease: effects on apoE and cerebral vasculopathy. *Acta Neuropathol (Berl)*. 2014; 128:777–789. [PubMed: 25195061]
46. Chen Y, Durakoglugil MS, Xian X, Herz J. ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. *Proc Natl Acad Sci U S A*. 2010; 107:12011–12016. [PubMed: 20547867]
47. Fuller JP, Stavenhagen JB, Teeling JL. New roles for Fc receptors in neurodegeneration-the impact on Immunotherapy for Alzheimer's Disease. *Front Neurosci*. 2014; 8:235. [PubMed: 25191216]

Figure 1. APOE3 mice have higher levels of IgG in spleen and brain

Nine-month old female APOE2 (n=4), APOE3 (n=6) and APOE4 (n=6) mice were euthanized and IgG levels measured by Western blot. Brain (A) and spleen (B) tissues were homogenized sequentially in TBS (for soluble proteins) and TBS-X (for membrane-associated proteins); IgG was detected primarily in the soluble tissue fractions as a 50 kDa protein. (C, D) APOE3 mice demonstrated the highest level of IgG in soluble brain fractions (3.0 ± 0.9 in APOE2 mice, 42.0 ± 17.9 in APOE3 mice, 6.0 ± 0.9 in APOE4 mice; E2 vs E3, $*p < 0.05$; E3 vs E4, $p = 0.056$). (E, F) APOE3 mice also showed a significantly higher level of IgG in soluble spleen fractions (4.4 ± 1.3 in APOE2 mice, 9.0 ± 0.9 in APOE3 mice, 5.8 ± 0.9 in APOE4 mice; E2 vs E3, $*p < 0.05$; E3 vs E4, $*p < 0.05$). (G, H) No significant differences of IgG levels were detected in plasma among the APOE groups (1.4 ± 0.1 in APOE2 mice, 1.6 ± 0.2 in APOE3 mice, 1.5 ± 0.1 in APOE4 mice). Data are presented as mean $\pm$ SEM.

Figure 2. IgG1 was detected only in plasma

Plasma from APOE2 (n=4), APOE3 (n=4) and APOE4 (n=4) were analyzed for IgG1 by immunoblot. Quantification of films demonstrated that no significant differences in levels of IgG1 were detected across APOE genotypes. Data are presented as mean $\pm$ SEM.

Figure 3. APOE4 mice have significantly higher levels of IgG2a in plasma and spleen
(A) Spleen samples were analyzed for IgG2a. APOE4 mice showed a significantly higher level of Ig2a in the soluble spleen fractions (1.03 ± 0.39 in APOE2 mice, 1.87 ± 0.93 in APOE3 mice, 3.56 ± 0.77 in APOE4 mice; E2 vs E4, $##p < 0.01$; E3 vs E4, $\#p < 0.05$). **(B)** In plasma samples, IgG2a was significantly higher in APOE4 mice (0.98 ± 0.50 in APOE2 mice, 1.78 ± 1.49 in APOE3 mice, 5.75 ± 1.15 in APOE4 mice; E2 vs E4, $###p < 0.001$; E3 vs E4, $###p < 0.001$). Data are presented as mean $\pm$ SEM.

Figure 4. IgG2b levels are highest in APOE4 tissues

IgG2b was measured in brain (A), spleen (B), and plasma (C) by western blotting (n=3–4). (A) APOE3 mice showed significantly higher levels of brain IgG2b compared to APOE4 mice (1.1 ± 0.4 in APOE2 mice, 1.6 ± 0.3 in APOE3 mice, 0.69 ± 0.12 in APOE4 mice; E3 vs E4, $*p < 0.05$). (B) APOE3 mice also showed highest levels of spleen IgG2b, although not significantly different from APOE2 or APOE4 mice (1.5 ± 0.9 in APOE2 mice, 2.3 ± 1.1 in APOE3 mice, 0.72 ± 0.2 in APOE4 mice; ns). (C) APOE3 mice showed significantly higher levels of plasma IgG2b compared to APOE2 or APOE4 mice (1.6 ± 0.5 in APOE2 mice, 3.4 ± 0.9 in APOE3 mice, 0.87 ± 0.42 in APOE4 mice; E3 vs E2, $*p < 0.05$; E3 vs E4, $**p < 0.01$). Data are presented as mean $\pm$ SEM.

Figure 5. APOE genotype had no effects on IgG3 levels

IgG3 was measured in TBS-soluble spleen samples (A), and plasma (B) by western blotting. There were no significant differences in IgG3 levels by APOE genotype.

Figure 6. APOE4 mice have lower levels of IgA in spleen

IgA levels were examined by Western blot. **(A)** IgA bands were detected in the TBS fraction of immunoblots of spleen samples (inset). APOE4 mice showed a significantly lower level of IgA in the soluble spleen fractions compared to APOE2 mice (94.8 ± 17.7 in APOE2 mice, 53.3 ± 21.0 in APOE3 mice, 15.8 ± 12.5 in APOE4 mice; E2 vs E4, # $p < 0.05$). **(B)** No significant differences of IgA in plasma were observed among groups (8.4 ± 1.9 in APOE2 mice, 6.8 ± 2.6 in APOE3 mice, 8.9 ± 2.0 in APOE4 mice). Error bars expressed as SEM.

Figure 7. APOE4 mice have higher levels of IgM in spleen and plasma

Western blots of IgM in soluble fractions of spleen revealed proteins of 50 and 75 kDa (A). (B) Quantification of soluble brain fractions revealed that APOE4 mice had the highest levels of IgM, but differences with APOE2 and APOE3 mice were statistically not significant. (C) APOE4 mice showed a significantly higher level of IgM in spleen (44 ± 33 in APOE2 mice, 136 ± 38 in APOE3 mice, 275 ± 33 in APOE4 mice; E2vsE3, $p < 0.05$, E3vsE4, $\#p < 0.05$, E2vsE4, $##p < 0.01$). (D) APOE2 mice showed a significantly lower level of IgM in plasma (1.4 ± 0.3 in APOE2 mice, 2.2 ± 0.2 in APOE3 mice, 2.5 ± 0.1 in APOE4 mice; E2vsE3, $*p < 0.05$, E2vsE4, $##p < 0.01$). Error bars expressed as SEM.