Data in Brief

Microarray analysis of the in vivo response of microglia to Aβ peptides in mice with conditional deletion of the prostaglandin EP2 receptor

Jenny U. Johansson 1, Nathaniel S. Woodling 1, Holden D. Brown, Qian Wang, Katrin I. Andreasson

Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305, United States

Abstract

Amyloid-β (Aβ) peptides accumulate in the brains of patients with Alzheimer’s disease (AD), where they generate a persistent inflammatory response from microglia, the innate immune cells of the brain. The immune modulatory cyclooxygenase/prostaglandin E2 (COX/PGE2) pathway has been implicated in preclinical AD development, both in human epidemiology studies and in transgenic rodent models of AD [2,3]. PGE2 signals through four G-protein-coupled receptors, including the EP2 receptor that has been investigated for its role in mediating the inflammatory and phagocytic responses to Aβ [4]. To identify transcriptional differences in microglia lacking the EP2 receptor, we examined mice with EP2 conditionally deleted in Cd11b-expressing immune cells. We injected Aβ peptides or saline vehicle into the brains of adult mice, isolated primary microglia, and analyzed RNA expression by microarray. The resulting datasets were analyzed in two studies [5,6], one describing the basal status of microglia with or without EP2 deletion, and the second study analyzing the microglial response to Aβ. Here we describe in detail the experimental design and data analyses. The raw data from these studies are deposited in GEO, accession GSE57181 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57181).

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2. Experimental design, materials and methods

2.1. Experimental design

In the context of Aβ42 peptides in AD, microglia generate a potent inflammatory response. Microglia are also intimately associated with neurons and synapses and perform essential nonimmune functions important to normal neural function. Microglial EP2 signaling broadly regulates inflammatory and anti-inflammatory pathways in vivo. Previous findings suggested a harmful function of microglial EP2 signaling both in vitro and in vivo in models of Aβ42 inflammation, with potentiation of proinflammatory responses, suppression of immune cell trafficking and Aβ peptide clearance [1,3]. We aimed to identify additional functions of microglial EP2 signaling and therefore examined microglial-specific gene expression in response to intracerebroventricular (i.c.v.) injection of Aβ42 peptides. Aβ42 peptide injection i.c.v. not only generates a robust, long-lasting innate immune response, but also disrupts memory consolidation, and thus represents a model in which to test effects of microglial EP2 on transcriptional responses. In a previous study [2], we analyzed the inflammatory response up to 7 days post i.c.v. injection. Forty-eight hours was chosen for this microarray study to avoid the immediate inflammatory response to the injection procedure while still capturing early changes in gene expression that may have subsided at later time points.

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57181.
To examine cell-specific mechanisms of EP2-mediated innate immune responses in vivo, we generated an EP2<sup>fl/fl</sup>−/− C57BL/6J mouse line to allow for conditional deletion of EP2. The Cd11b-Cre line, which drives expression of Cre recombinase in the monocyte lineage (macrophages and microglia), was used to generate Cd11b-Cre EP2<sup>fl/fl</sup>−/− and control Cd11b-Cre EP2<sup>+/+</sup> C57BL/6 mice. To analyze the response to Aβ<sub>42</sub> peptides in adult brain, we used 8–9 month-old mice, as isolation of primary microglia becomes progressively more difficult at older ages.

2.2. Sample preparation and quality control

Cd11b-Cre EP2<sup>+/+</sup> control and Cd11b-Cre EP2<sup>fl/fl</sup>−/− mice were administered i.c.v. injection of Aβ<sub>42</sub> fibrils (40 pmol) or saline vehicle as described previously [2]. At 48 h after surgery, mice were sacrificed by transcardiac perfusion with saline to ensure removal of blood cells from the brain. Brains were removed from the mice and pooled, 2 brains of the same genotype, sex, and treatment per sample. We found that pooling samples were required to ensure adequate cell and RNA yield. The brains were then enzymatically dissociated and microglia isolated using magnetic CD11b microbeads (Miltenyi Biotec) according to the manufacturer’s protocol.

RNA purification from primary microglia was performed using TRIzol (Life Technologies) followed by the RNeasy Mini Kit (Qiagen). RNA quality was assessed using a BioAnalyzer (Agilent) and determined using magnetic CD11b microbeads (Miltenyi Biotec) according to the manufacturer’s protocol.

2.3. Microarray and data analysis

Raw microarray data were statistically analyzed using Partek software (Partek Inc.,) using default RNA normalization and log2 transformation of data. These raw data were deposited in GEO (accession no. GSE57181). We used Partek to perform 2-way ANOVA analysis on the factors of Aβ<sub>42</sub> treatment and genotype, with contrasts identifying the most highly enriched (enrichment score, 94.42). There was a 1.3 fold downregulated and used to create the node map. Ingenuity Pathway Analysis (IPA, Ingenuity Systems) was used for pathway analysis. Unsupervised hierarchical clustering revealed a striking downregulation of a majority of microglial genes with EP2 deletion, with 116 genes significantly downregulated 1.5-fold and 20 genes upregulated by 1.5-fold. The principal biological functions represented by the differentially regulated genes included the immune response, cytoskeletal function, and cell cycle/mitosis. Immune molecules functioning in cytokine and chemokine signaling, chemotaxis and cell adhesion, and immune cell activation were significantly downregulated. Interestingly, cell cycle and mitosis as well as signaling molecules involved in cell cycle progression, cytoskeletal function, and chromatin assembly were also significantly downregulated. Together, the suppression of gene expression in these biological pathways suggests a decreased inflammatory and proliferative state of EP2 conditional knock-out microglia. To further define connections between immune molecules that were regulated by microglial EP2 signaling, we performed Ingenuity Pathway Analysis to define networks of differentially regulated immune genes (1.5-fold and greater) that were connected to each other either through regulation of expression or protein–protein binding. Interestingly, COX-2 was highly downregulated with EP2 deletion, and as COX-2 catalyzes the formation of PGE2, the precursor of PGE2 that activates the EP2 receptor, this suggests a feedback cycle in which EP2-mediated increases in COX-2 expression results in further production of PGE2. Thus, conditional knock-out of microglial EP2 resulted in the downregulation of most inflammatory genes represented in the pathway, in large part through downregulation of COX-2, which drives expression of multiple immune genes.

In the second study [5], the comparison between Aβ<sub>42</sub>- versus vehicle-injected Cd11b-Cre mice showed the Immune System Process as the most highly enriched (enrichment score, 94.42). There was a 1.3 fold induction of microglial EP2 in the Cd11b-Cre control genotype. Unsupervised hierarchical clustering of differentially expressed genes revealed a striking distinction between the i.c.v. Aβ<sub>42</sub> and i.c.v. vehicle treatment groups. IPA of upstream regulatory transcription factors demonstrated 2 major nodes of inflammatory innate gene regulation, Nfkβ and Irf7. In this comparison, COX-2 was highly induced in vivo in microglia from i.c.v. Aβ<sub>42</sub>-treated mice. Database for Annotation, Visualization and Integrating Discovery (DAVID) functional annotation software (version 6.7; NIAID, NIH) was used to identify KEGG molecular pathways significantly overrepresented among lists of the 416 transcripts differentially expressed in Aβ<sub>42</sub>- versus vehicle-treated Cd11b-Cre mice. This analysis revealed 20 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways

### Table 1
Samples used for microarray analysis.

| GEO ID ref | Sample ID | Genotype | Sex | Treatment | Cells (×10^6) | RIN score |
|-----------|-----------|----------|-----|-----------|--------------|-----------|
| GSM1376787 | Ab_EP2_1  | Cd11b-Cre; EP2 f/f | Female | Aβ<sub>42</sub> | 2.40 | 9.8 |
| GSM1376788 | Ab_EP2_2  | Cd11b-Cre; EP2 f/f | Female | Aβ<sub>42</sub> | 2.40 | 9.9 |
| GSM1376789 | Ab_EP2_3  | Cd11b-Cre; EP2 f/f | Female | Aβ<sub>42</sub> | 2.00 | 8.7 |
| GSM1376790 | Ab_WT_1   | Cd11b-Cre; +/-   | Female | Aβ<sub>42</sub> | 2.40 | 9.7 |
| GSM1376791 | Ab_WT_2   | Cd11b-Cre; +/-   | Female | Aβ<sub>42</sub> | 2.30 | 9.4 |
| GSM1376792 | Ab_WT_3   | Cd11b-Cre; +/-   | Female | Aβ<sub>42</sub> | 1.65 | 7.0 |
| GSM1376793 | Ab_WT_4   | Cd11b-Cre; +/-   | Female | Aβ<sub>42</sub> | 1.75 | 7.7 |
| GSM1376794 | Ab_WT_5   | Cd11b-Cre; +/-   | Female | Aβ<sub>42</sub> | 1.65 | 8.5 |
| GSM1376795 | Ab_WT_6   | Cd11b-Cre; +/-   | Female | Aβ<sub>42</sub> | 0.95 | 9.6 |
| GSM1376796 | Ab_WT_7   | Cd11b-Cre; +/-   | Female | Aβ<sub>42</sub> | 2.75 | 9.0 |
| GSM1376797 | Veh_EP2_1 | Cd11b-Cre; EP2 f/f | Male | Veh   | 1.50 | 9.4 |
| GSM1376798 | Veh_EP2_2 | Cd11b-Cre; EP2 f/f | Female | Veh   | 1.50 | 9.4 |
| GSM1376799 | Veh_EP2_3 | Cd11b-Cre; EP2 f/f | Female | Veh   | 1.50 | 9.9 |
| GSM1376800 | Veh_WT_1  | Cd11b-Cre; +/-   | Male | Veh   | 1.12 | 9.8 |
| GSM1376801 | Veh_WT_2  | Cd11b-Cre; +/-   | Female | Veh   | 1.25 | 9.6 |
| GSM1376802 | Veh_WT_3  | Cd11b-Cre; +/-   | Female | Veh   | 1.50 | 8.6 |
| GSM1376803 | Veh_WT_4  | Cd11b-Cre; +/-   | Female | Veh   | 1.70 | 10.0 |
that were significantly enriched, almost all of which corresponded to inflammatory signaling networks. Comparison of Aβ-treated Cd11b-Cre Ep2β/β versus Cd11b-Cre mice revealed 55 regulated genes (Tables 2 and 3), and hierarchical clustering of these genes across conditions demonstrated a clear segregation of Aβ-regulated genes in Cd11b-Cre Ep2β/β mice. Comparison of KEGG pathways revealed shared pathways between the vehicle-treated Cd11b-Cre Ep2β/β and vehicle-treated Cd11b-Cre groups. The complete set of enriched KEGG pathways in the Cd11b-Cre Ep2β/β versus Cd11b-Cre comparison included cell cycle, proteolysis, and immune pathways [5]. Interestingly, the majority of differentially regulated genes in the Aβ-treated Cd11b-Cre Ep2β/β versus Aβ-treated Cd11b-Cre comparison were not regulated by Aβ, but were specifically changed with microglial EP2 deletion (39 genes, Table 3). This

| Genes regulated > 1.5-fold by both conditional EP2 deletion and Aβ treatment. |
|---------------------------------------------------------------|
| Gene symbol | Gene name | Fold change Aβ-EP2 vs. Aβ-WT | Fold change Aβ-WT vs. Veh-WT |
|----------------|-----------------|--------------------------|--------------------------|
| Mir3094 | MicroRNA 3094 | 1.825 | −1.582 |
| Ly6c1 | Lymphocyte antigen 6 complex, locus C1 | 1.716 | −1.714 |
| Rjabggb | Rab geranylgeranyl transferase, b subunit | 1.638 | −1.844 |
| Serpinb1b | Serine (or cysteine) peptidase inhibitor, clade B, member 1b | 1.514 | −1.687 |
| Pin | Phospholamban | 1.511 | −1.980 |
| Syn1 | Syn1//synaptic nuclear envelope 1 | 1.511 | −1.660 |
| Osm | Oncostatin M | −1.524 | 1.749 |
| Dusp1 | Dual specificity phosphatase 1 | −1.542 | 1.824 |
| Dusp2 | Dual specificity phosphatase 2 | −1.570 | 2.139 |
| Thap6 | THAP domain containing 6 | −1.602 | 1.839 |
| Pmaip1 | Phorbol-12-myristate-13-acetate-induced protein 1 | −1.660 | 2.036 |
| Bco49715 | cDNA sequence BCO49715 | −1.715 | 1.594 |
| Gm20269 | Predicted gene, 20269 | −1.873 | 1.848 |
| Il12 | Interleukin 1 receptor, type II | −1.892 | 2.451 |
| Gm129 | Predicted gene 129 | −1.901 | 1.880 |
| Fam71a | Family with sequence similarity 71, member A | −2.585 | 2.403 |

| Table 3 | Genes regulated > 1.5-fold by conditional EP2 deletion but not by Aβ treatment. |
|---------------------------------------------------------------|
| Gene symbol | Gene name | Fold change Aβ-EP2 vs. Aβ-WT | Fold change Aβ-WT vs. Veh-WT |
|----------------|-----------------|--------------------------|--------------------------|
| Gpr114 | G protein-coupled receptor 114 | 2.413 | 1.453 |
| Lux | Lysyl oxidase | 2.232 | 1.227 |
| Atp6v0d2 | ATPase, H+ transporting, lysosomal V0 subunit D2 | 1.924 | 1.166 |
| Stbisa6 | STB alpha-N-acetylated-lysine alpha-2,8-sialyltransferease | 1.814 | −1.075 |
| Rdh13 | Retinol dehydrogenase 13 (all-trans and 9-cis) | 1.781 | −1.058 |
| Mamdc2 | MAM domain containing 2 | 1.752 | −1.335 |
| Igf1 | Insulin-like growth factor 1 | 1.727 | 1.148 |
| Slc26a7 | Solute carrier family 26, member 7 | 1.701 | 1.344 |
| Gsdmc4 | Gasdermin C4 | 1.689 | 1.055 |
| 2210404009Rik | RIKEN cDNA 2210404009 gene | 1.684 | −1.087 |
| Tgtp2 | T cell specific GTPase 2 | 1.681 | 1.103 |
| Pdc1 | Programmed cell death 1 | 1.652 | 1.015 |
| Ctsf | Cystatin F (leukocystatin) | 1.643 | 1.132 |
| Cd3g | CD3 antigen, gamma polypeptide | 1.617 | 1.025 |
| Rxrg | Retinoid X receptor gamma | 1.605 | 1.195 |
| Olfr1212 | Olfactory receptor 1212 | 1.599 | 1.095 |
| Vmn11184 | Vomeronasal 1 receptor, 184 | 1.570 | −1.114 |
| Afs29169 | cDNA sequence AFS29169 | 1.553 | 1.088 |
| Olfr1052 | Olfactory receptor 1052 | 1.549 | 1.011 |
| Olfr110 | Olfactory receptor 110 | 1.549 | −1.291 |
| Olfr1313 | Olfactory receptor 1313 | 1.540 | 1.009 |
| Axel | AXL receptor tyrosine kinase | 1.537 | 1.464 |
| Mir7-2 | MicroRNA 7-2 | 1.524 | −1.187 |
| Gm4787 | Predicted gene 4787 | 1.519 | −1.483 |
| Lpl | Lipoprotein lipase | 1.516 | 1.055 |
| Gm4934 | Predicted gene 4934 | 1.502 | 1.441 |
| Olfr221 | Olfactory receptor 221 | −1.505 | 1.137 |
| Bco31361 | cDNA sequence BCO31361 | −1.513 | 1.225 |
| Mir423 | MicroRNA 423 | −1.524 | 1.080 |
| Gm10584 | Predicted gene 10584 | −1.527 | 1.264 |
| A430078002Rik | RIKEN cDNA A430078002 gene | −1.532 | 1.380 |
| Hist3h2a | Histone cluster 3, H2a | −1.585 | 1.291 |
| 4933433G15Rik | RIKEN cDNA 4933433G15 gene | −1.623 | 1.343 |
| Mir3096b | MicroRNA 3096b | −1.649 | 1.381 |
| G530011006Rik | RIKEN cDNA G530011006 gene | −1.671 | −1.081 |
| Hist4h4 | Histone cluster 4, H4 | −1.698 | 1.513 |
| 4633427F10Rik | RIKEN cDNA 4633427F10 gene | −1.780 | 1.492 |
| Ifnb1 | Interferon beta 1, fibroblast | −1.876 | 1.443 |
| G530011006Rik | RIKEN cDNA G530011006 gene | −5.550 | −1.492 |
suggested that rather than simply reversing Aβ42-induced inflammatory changes, Cd11b-Cre Ep2\textsuperscript{fl/fl} microglia engaged alternative response pathways. Functional annotation of these genes using DAVID revealed an enrichment of PPAR signaling pathway genes. There were 16 genes regulated by >1.5-fold in both the Aβ\textsuperscript{42}-treated Cd11b-Cre versus vehicle-treated Cd11b-Cre comparison and the Aβ\textsuperscript{42}-treated Cd11b-Cre EP2\textsuperscript{fl/fl} versus Aβ\textsuperscript{42}-treated Cd11b-Cre comparison (Table 2). Notably, all 16 of these genes are regulated in opposite directions by Aβ and EP2 deletion, suggesting that EP2 deletion reverses the transcriptional effects of Aβ on microglia cells. Included among these genes are the immune genes dual specificity phosphatase 1 and 2 and interleukin 1 receptor, type II.

3. Summary

We analyzed in vivo Cd11b-Cre control and Cd11b-Cre EP2\textsuperscript{fl/fl} mouse brain microglia gene expression by microarray and following GO expression and pathway analyses. The results are described in two publications [4,5], and here we provide a detailed description of the experiment and analysis, in addition to two new tables with gene changes. Of the three comparisons, vehicle- and Aβ\textsuperscript{42}-treated Cd11b-Cre showed the largest number of genes altered. Our results identify new candidates for further study in the inflammatory response of microglia in AD.

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