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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

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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/PGE_2) pathway has been implicated in preclinical AD development, both in human epidemiology studies and in transgenic rodent models of AD [2,3]. PGE_2 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).

Specifications	
Organism/cell line/tissue	Microglial cells from brains of 8- to 9-month-old Cd11b-Cre $EP2^{+/+}$ and Cd11b-Cre $EP2^{fl/fl}$ mice in C57BL/6 background
Sex	Female and male, see Table 1
Sequencer or array type	Affymetrix GeneChip Mouse Gene 2.0 ST
Data format	Raw
Experimental factors	Mice received intracerebroventricular injection of $Aβ_{42}$ fibrils or saline; 48 h later, the mice were sacrificed for brain dissection.
Experimental features	Primary microglia were isolated from whole brains using magnetic cell sorting and processed immediately to isolate RNA.
Consent	N/A
Sample source location	Stanford, CA

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57181.

2. Experimental design, materials and methods

2.1. Experimental design

In the context of $Aβ_{42}$ 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 $Aβ_{42}$ 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 $Aβ_{42}$ peptides. $Aβ_{42}$ 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.

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To examine cell-specific mechanisms of EP2-mediated innate immune responses in vivo, we generated an $EP2^{fl/-}$ C57BL/6 J 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 $EP2^{fl/fl}$ and control Cd11b-Cre $EP2^{+/+}$ C57BL/6 mice. To analyze the response to $A\beta_{42}$ 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 $EP2^{+/+}$ control and Cd11b-Cre $EP2^{fl/fl}$ mice were administered i.c.v. injection of $A\beta_{42}$ 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 to be sufficient for microarray analysis (RNA Integrity Number > 7.0 for all samples). cDNA synthesis, labeling, hybridization, and scanning were performed by the Stanford Protein and Nucleic Acid (PAN) Facility using GeneChip Mouse Gene 2.0 ST arrays (Affymetrix). Samples are described in Table 1.

2.3. Microarray and data analysis

Raw microarray data were statistically analyzed using Partek software (Partek, Inc.), using default RMA 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 treatment and genotype, with contrasts identifying the fold-change and significance of the Aβ-EP2 vs. Aβ-WT; Aβ-WT vs. Veh-WT; and Veh-EP2 vs. Veh-WT comparisons. We next identified differentially expressed genes in each contrast using an unadjusted P value of <0.05 to capture the widest possible number of differentially expressed genes. Genes that had a fold change of >1.5 between genotypes were used for unsupervised hierarchical clustering analysis and Gene Ontology (GO) expression analysis.

The first study [4] looked at changes in the innate inflammatory response to lipopolysaccharide (LPS) and the MPTP model of Parkinson's disease. Here we described the generation and initial analysis of the mice with Cd11b promotor-driven immune cell deletion of EP2 and the Cd11b-Cre $EP2^{fl/fl}$ versus Cd11b-Cre $EP2^{+/+}$ saline gene expression comparison. Quantification of EP2 mRNA in adult microglia used for microarray analysis revealed a decrease of 48% in Cd11b-Cre $Ep2^{fl/fl}$ microglia. A total of 136 genes were identified that were differentially regulated 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 PGH_2, the precursor of PGE_2 that activates the EP2 receptor, this suggests a feedforward cycle in which EP2-mediated increases in COX-2 expression results in further production of PGE_2. Thus, conditional knockout 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- 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 and i.c.v. vehicle treatment groups. IPA of upstream regulatory transcription factors demonstrated 2 major nodes of inflammatory gene regulation, Nfkb and Irf7. In this comparison, COX-2 was highly induced in vivo in microglia from i.c.v. $A\beta_{42}$-treated mice. Database for Annotation, Visualization and Integrated 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- 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	2.40	9.8
GSM1376788	Ab_EP2_2	Cd11b-Cre; EP2 f/f	Female	Aβ	2.40	9.9
GSM1376789	Ab_EP2_3	Cd11b-Cre; EP2 f/f	Female	Aβ	2.00	8.7
GSM1376790	Ab_WT_1	CD11b-Cre; +/+	Female	Aβ	2.30	9.7
GSM1376791	Ab_WT_2	CD11b-Cre; +/+	Female	Aβ	1.65	9.4
GSM1376792	Ab_WT_3	CD11b-Cre; +/+	Female	Aβ	2.30	7.0
GSM1376793	Ab_WT_4	CD11b-Cre; +/+	Female	Aβ	1.75	7.7
GSM1376794	Ab_WT_5	CD11b-Cre; +/+	Female	Aβ	1.65	8.5
GSM1376795	Ab_WT_6	CD11b-Cre; +/+	Female	Aβ	0.95	9.6
GSM1376796	Ab_WT_7	CD11b-Cre; +/+	Female	Aβ	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	1.90	9.1
GSM1376799	Veh_EP2_3	Cd11b-Cre; EP2 f/f	Female	Veh	1.65	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

Table 2
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
Rabggtb	RAB geranylgeranyl transferase, b subunit	1.638	−1.844
Serpinb1b	Serine (or cysteine) peptidase inhibitor, clade B, member 1b	1.514	−1.687
Pln	Phospholamban	1.511	−1.980
Syne1	Syne1//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
BC049715	cDNA sequence BC049715	−1.715	1.594
Gm20269	Predicted gene, 20269	−1.873	1.848
Il1r2	Interleukin 1 receptor, type II	−1.892	2.451
Gm129	Predicted gene 129	−1.899	1.880
Fam71a	Family with sequence similarity 71, member A	−2.585	2.403

that were significantly enriched, almost all of which corresponded to inflammatory signaling networks. Comparison of Aβ-treated Cd11b-Cre $Ep2^{fl/fl}$ 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 $Ep2^{fl/fl}$ mice. Comparison of KEGG pathways revealed shared pathways between the Aβ-treated Cd11b-Cre and vehicle-treated Cd11b-Cre groups and between the vehicle-treated Cd11b-Cre $Ep2^{fl/fl}$ and vehicle-treated Cd11b-Cre groups. The complete set of enriched KEGG pathways in the Cd11b-Cre $Ep2^{fl/fl}$ 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 $Ep2^{fl/fl}$ versus Aβ-treated Cd11b-Cre comparison were not regulated by Aβ, but were specifically changed with microglial EP2 deletion (39 genes, Table 3). This

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
Lox	Lysyl oxidase	2.232	1.227
Atp6v0d2	ATPase, H+ transporting, lysosomal V0 subunit D2	1.924	1.166
St8sia6	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransfe	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
2210404O09Rik	RIKEN cDNA 2210404O09 gene	1.684	−1.087
Tgtp2	T cell specific GTPase 2	1.681	1.103
Pdcd1	Programmed cell death 1	1.652	1.015
Cst7	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
Vmn1r184	Vomeronasal 1 receptor, 184	1.570	−1.114
AF529169	cDNA sequence AF529169	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
Axl	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
BC031361	cDNA sequence BC031361	−1.513	1.225
Mir423	MicroRNA 423	−1.524	1.080
Gm10584	Predicted gene 10584	−1.527	1.264
A430078I02Rik	RIKEN cDNA A430078I02 gene	−1.532	1.380
Hist3h2a	Histone cluster 3, H2a	−1.584	1.291
4933433G15Rik	RIKEN cDNA 4933433G15 gene	−1.623	1.343
Mir3096b	MicroRNA 3096b	−1.649	1.381
G530011O06Rik	RIKEN cDNA G530011O06 gene	−1.671	−1.081
Hist4h4	Histone cluster 4, H4	−1.698	1.513
4833427F10Rik	RIKEN cDNA 4833427F10 gene	−1.780	1.492
Ifnb1	Interferon beta 1, fibroblast	−1.876	1.443
G530011O06Rik	RIKEN cDNA G530011O06 gene	−5.550	−1.492

suggested that rather than simply reversing $A\beta_{42}$-induced inflammatory changes, Cd11b-Cre $Ep2^{fl/fl}$ 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-treated Cd11b-Cre versus vehicle-treated Cd11b-Cre comparison and the Aβ-treated Cd11b-Cre $EP2^{fl/fl}$ versus Aβ-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 $EP2^{fl/fl}$ 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-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.

Acknowledgments

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