Bioinformatics Analyses Determined the CNS and Peripheral Lymphoid Surrogate Biomarker Candidates Between Two Distinct EAE Models for Progressive Multiple Sclerosis

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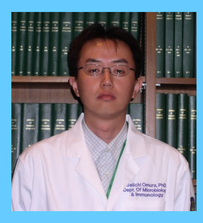

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Abstract

Background: Histologically, multiple sclerosis (MS) is heterogeneous with four patterns even among patients with the same (homogeneous) clinical course. We have established two distinct experimental autoimmune encephalomyelitis (EAE) models for primary progressive MS, sensitizing two *H-2*^{s} mouse strains with myelin oligodendrocyte glycoprotein (MOG) peptide$_{92\text{-}106}$. Although the two models have a progressive fatal clinical course without remission, A.SW mice develop ataxia with antibody deposition in the central nervous system (CNS), while SJL/J mice develop paralysis with CNS T cell infiltration.

Objective: To determine 1) common factors contributing to the progressive course seen in both models and 2) distinct factors associated with different clinical signs and pathology between the two models, the former and the latter may explain the homogeneity and heterogeneity of MS, respectively.

Materials and Methods: Using CNS and spleen microarray transcriptome and cytokine data, we conducted computational analyses [volcano plot, heat map, pathway, principal component analysis (PCA)].

Results: Temporal gene expression analysis indicated that T helper (Th) 1 and Th17 responses were associated with the disease progression in SJL/J mice, but not A.SW mice. PCA of microarray transcriptome data showed the contribution of a set of immune-related genes, including immunoglobulin, complements, lipocalin 2, and CXCL13, to separation of CNS samples between EAE and control groups. In the spleen, GATA1- and transporter-related genes were downregulated commonly in the EAE models, while cyclin-related genes were downregulated only in A.SW mice. Pattern matching analysis identified the peripheral lymphoid surrogate markers, including *Stfa2l1*, *Gypa*, and *Kel*, which reflected gene expression changes in the CNS.

Conclusions: Antibody-mediated immune responses could contribute to disease progression in common, while CNS cytokine and lymphoid cyclin differences may be associated with the heterogeneities between the two models.

Introduction

Multiple sclerosis is considered to be an immune-mediated disease, occurring in genetically susceptible individuals, precipitated by one or more environmental agents. The clinical courses of MS can differ among patients, including relapsing-remitting (RR) and progressive disease courses.

Myelin-specific Th1 cells produce interferon (IFN)-γ while Th17 cells produce interleukin (IL)-17. In RR-MS and RR-EAE, these proinflammatory cytokines have been proposed to contribute to demyelination and axonal degeneration. However, we do not know whether Th1 and Th17 cells play an effector role in progressive MS or progressive EAE.

We have previously demonstrated that MOG sensitization in different mouse strains results in different clinical and pathological outcomes. SJL/J mice sensitized with MOG developed RR-EAE with oligodendrocyte apoptosis and a high level of IFN-γ production from T cells. In contrast, A.SW mice sensitized with MOG developed primary progressive (PP) disease. In PP-EAE, we found that mice decreased IFN-γ production with sustained IL-4 production. Recently, we found that SJL/J mice sensitized with MOG and curdlan, which is a β-glucan derived from fungi and known as a Th17 inducer, also developed PP-EAE.

Materials & Methods

EAE induction: A.SW and SJL/J mice were injected with MOG$_{92\text{-}106}$ peptide subcutaneously and curdlan intraperitoneally to induce PP-EAE. RR-EAE was induced in SJL/J mice with PLP$_{139\text{-}151}$.

Real-time PCR: We reverse-transcribed 1 μg of total RNA into cDNA, using the ImProm-II™ Reverse Transcription System (Promega). Using 50 ng of cDNA, real-time PCR was conducted by the MyiQ™2 Real Time PCR Detection System (Bio-Rad). Fold changes were calculated by the ΔΔCt method.

Microarray bioinformatics analyses: RNA samples from brains and spleens of PP-EAE and control mice at disease peak were processed to labeled cDNA fragments and hybridized to the GeneChip Mouse Gene 1.0ST Arrays (Affymetrix). Data were normalized using Robust Multi-array Average in the Expression Console (Affymetrix).

Volcano plots were drawn, using the OriginPro 8.1, to assess significance together with log ratio of transcriptome data.

Heat maps were drawn to determine the expression patterns of top 20 up- or downregulated genes in each organs and compare the expression levels with the other organs, using R version 2.15.1 and the packages 'gplots' and 'genefilter'.

***K*-means clustering** and **radar chart** were applied for microarray data to determine the differences of expression patterns among genes using R package 'cclust'.

Ingenuity Pathway Analysis® (IPA) was used to categorize the genes by the functions.

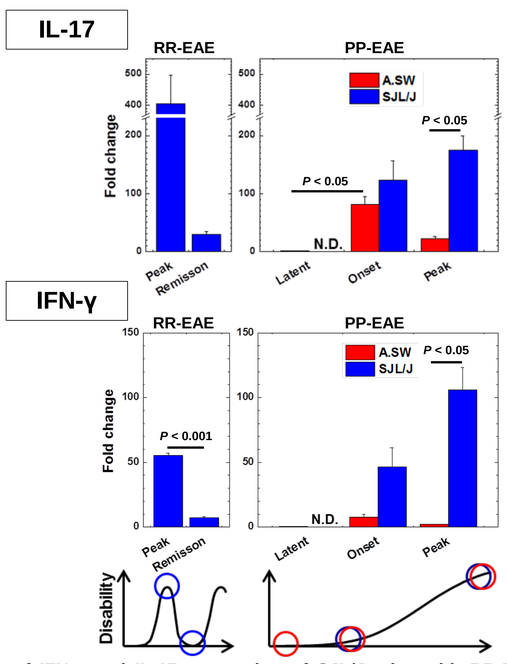
Principal component analysis (PCA) was conducted to determine the differences of gene expression patterns among samples, using R package 'prcomp'.

Pattern matching analysis was conducted to identify the peripheral surrogate markers that reflect the brain transcriptome changes using R.

Data mining was conducted to determine whether mouse peripheral surrogate markers were up- or downregulated in the blood from MS patients on Gene Expression Omnibus (GEO) database.

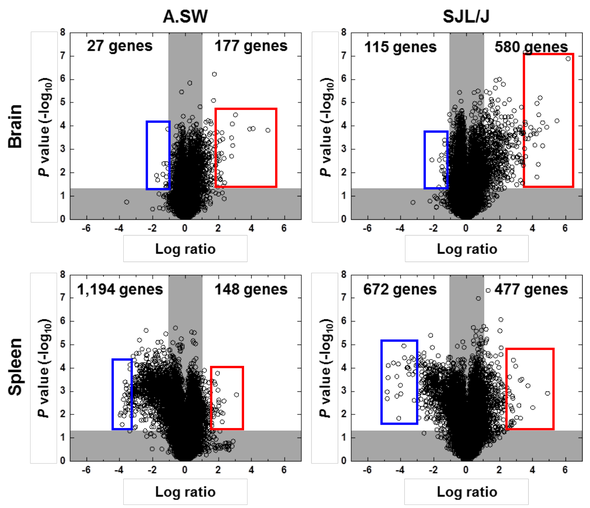
Results

Real-time PCR: the levels of IL-17 and IFN-γ are associated with EAE activity in SJL/J mice, but not A.SW mice



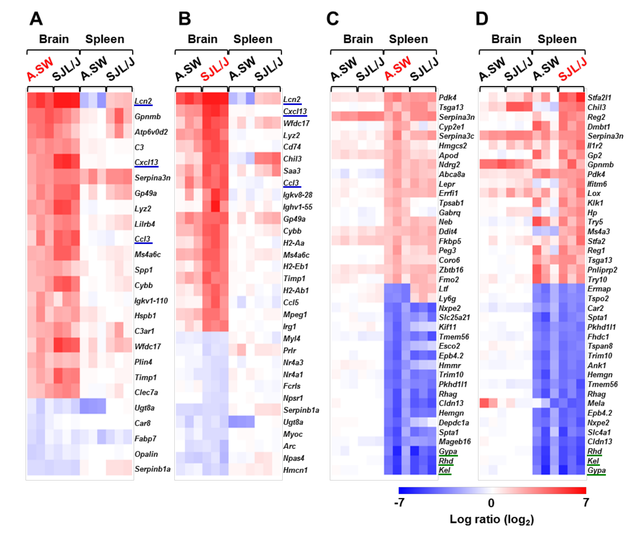
Kinetics of IFN-γ and IL-17 expression of SJL/J mice with RR-EAE (left) or PP-EAE (right) and A.SW with PP-EAE, determined by real-time PCR. Expression levels were showed as fold changes compared with control mice. Levels of both IFN-γ and IL-17 were correlated with disease activity in SJL/J mice with EAE, while they were high at the onset but decreased at the disease peak in A.SW mice with EAE.

Volcano plot: many genes are upregulated in the brains and downregulated in the spleens from both mouse strains



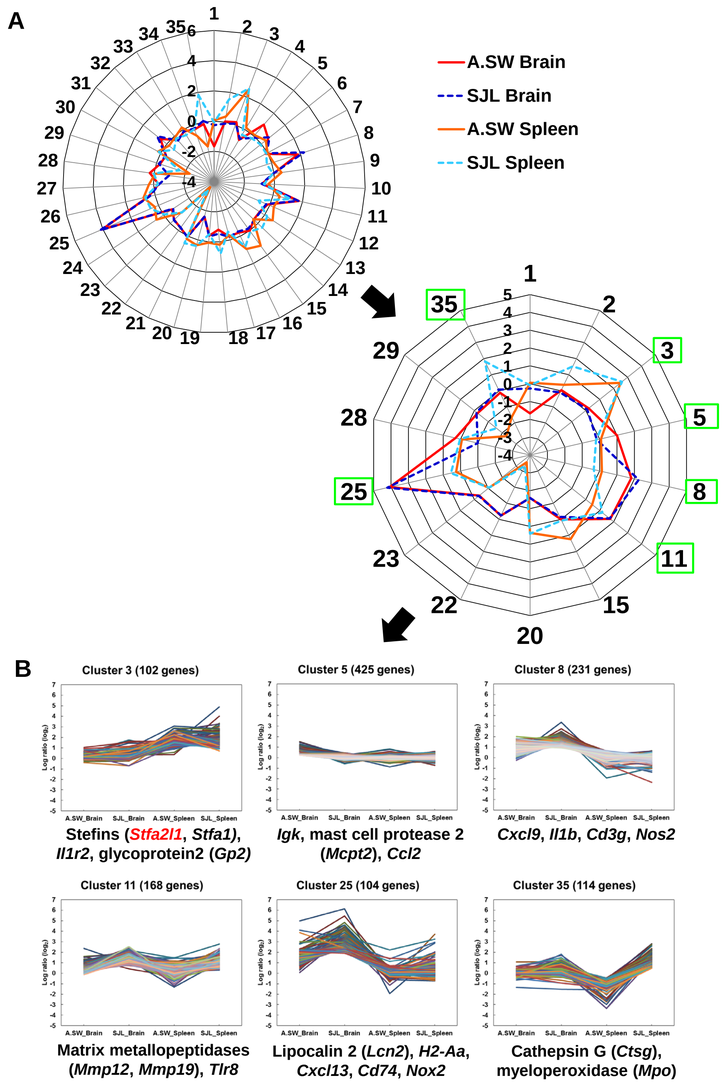
Volcano plots of up- or down-regulated genes in the brains and spleens of PP-EAE mice, compared with control mice, at disease peak. White areas indicate that the expression ratios were less than 0.5-fold or more than 2-fold and *P* values were less than 0.05. The number of upregulated genes was more than that of downregulated genes in the brains of both A.SW and SJL/J mice with EAE, while upregulated genes were less than downregulated genes in the spleens. Red and blue boxes indicate positive and negative top 20 genes, shown in heat maps, respectively.

Heat map: the expression patterns of highly up- or down-regulated genes are similar between the two strains and different between the brains and spleens



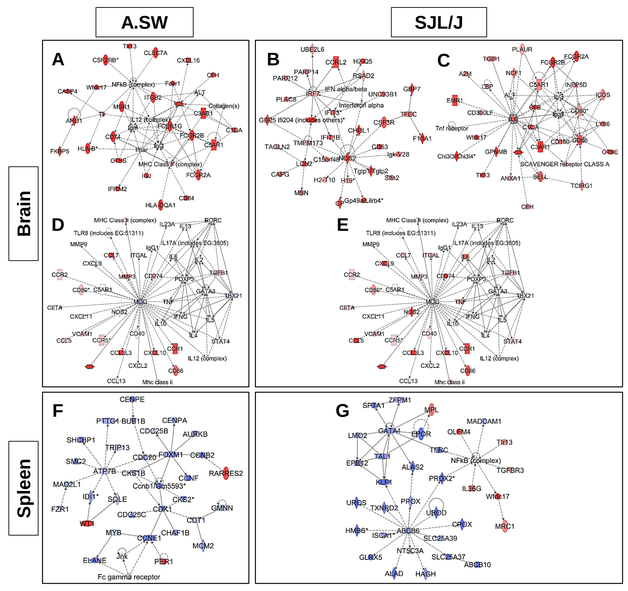
Heat maps of most highly up- or down-regulated genes in the brains and spleens of mice during the disease peak of PP-EAE. Heat maps were drawn based on brain microarray data of A.SW mice (A) or SJL/J mice (B), or spleen microarray data of A.SW mice (C) or SJL/J mice (D). Overall, heat maps between A.SW and SJL/J mice were similar in the brains and spleens. Inflammation-related genes, such as ***Lcn2***, ***Cxcl13***, and ***Ccl3***, were upregulated in the brains of both mouse strains, while erythrocyte-related genes, such as ***Gypa***, ***Kel***, and ***Rhd***, were downregulated in the spleens.

***K*-means clustering** and **radar chart**: the different gene expression patterns between the strains or organs



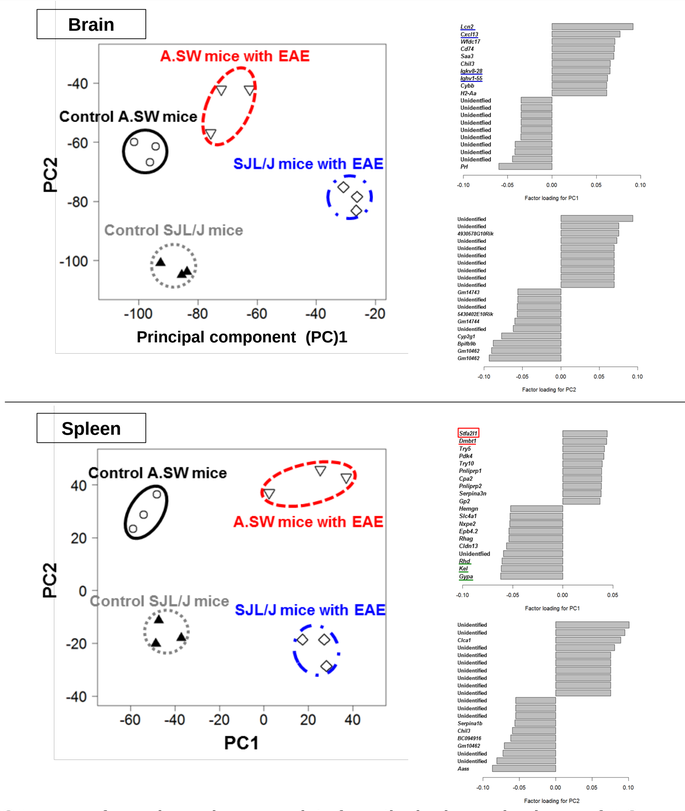
To find the differences of gene expression profiles between the strains or organs, we conducted *k*-means clustering. A) Radar chart visualized the different expression patterns of 35 clusters (left); among which 13 clusters showed differentially expressed patterns (right). The number at each vertex is the cluster number (1, 2, 3, 5, 8, 11, 15, …), while the numbers along the axis (-4 to 5) are log ratios, compared with controls. B) Typical genes included in 6 clusters were mainly related to immune response or inflammation.

IPA: gene networks in the brains and spleens from two mouse strains with PP-EAE



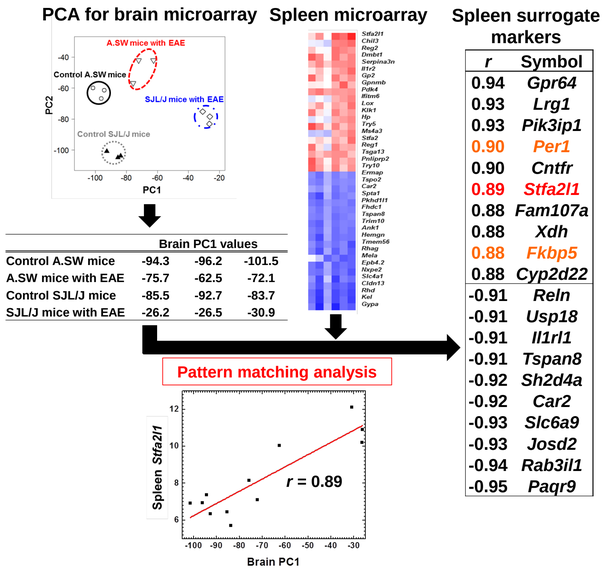
IPA ranked IgG/complement-related pathway in the brain from A.SW mice as a top pathway (A), while both IFN-α- (B) and IL6/IgG-related pathways (C) were ranked in SJL/J mouse brain. Proinflammatory molecules, such as IFN-γ, IL-23, MMP9 and NOS were downregulated in A.SW mice (D), while proinflammatory molecules were upregulated and Th2-associated molecules, IL-4, 5, and GATA3 were downregulated in SJL/J mice (E). Downregulation of cell cycle-related genes were ranked as a top pathway in A.SW mouse spleen (F), while downregulation of ion transporter-related pathway was ranked in SJL/J mouse spleen (G).

PCA: samples are separated between the two mouse strains with or without EAE



PCA was performed on microarray data from the brains and spleens of A.SW and SJL/J mice with PP-EAE and control mice. PCA separated the samples of both brains and spleens clearly. PC1 distinguished between EAE and control mice, while PC2 distinguished between the two mouse strains. Factor loadings suggested that ***Lcn2***, ***Cxcl13***, and **immunoglobulins** might be useful as the biomarkers for progressive EAE in the brain, while ***Stfa2l1***, ***Dmbt1***, and **erythrocyte-related genes** might be useful in the spleen.

Pattern matching analysis: identification of spleen surrogate markers that reflect the transcriptome changes in the brain



To identify the peripheral surrogate markers, which reflect the transcriptome changes in brain, we conducted pattern matching analysis, using brain PC1 values and spleen microarray data. ***Gpr64*** and ***Paqr9*** were correlated significantly with brain PC1. Among them, ***Stfa2l1*** was listed as a biomarker candidate in the spleen PCA of EAE mice. In a data mining on GEO database, ***Per1*** and ***Fkbp5*** were upregulated in blood samples from MS patients.

Conclusions

- Lipocalin 2, CXCL13, and immunoglobulins might be useful as the biomarkers for progressive EAE in the brain.
- Stefin A2L1 and erythrocyte-related genes might be useful as peripheral surrogate markers which reflect the changes in the brain.
- Period circadian clock 1 (*Per1*) and FK506 binding protein (*Fkbp5*) might be useful as blood surrogate markers in MS patients.