Author's response to reviews

Title: Microarray analysis identifies a set of CXCR3 and CCR2 ligand chemokines as early IFNβ-responsive genes in peripheral blood lymphocytes: an implication for IFNβ-related adverse effects in multiple sclerosis

Authors:

Jun-ichi Satoh (satoj@ncnp.go.jp)
Yusuke Nanri (nanriyu@cc.saga-u.ac.jp)
Hiroko Tabunoki (tabunoki@my-pharm.ac.jp)
Takashi Yamamura (yamamura@ncnp.go.jp)

Version: 2 Date: 23 April 2006

Author’s response to reviews: see over
April 24, 2006

Melissa Norton, MD
Editor-in-Chief, BMC Neurology

Dear Prof Norton:

RE: MS: 5705286909804629 Revised “Microarray analysis identifies a set of CXCR3 and CCR2 ligand chemokines as early IFNβ-responsive genes in peripheral blood lymphocytes in vitro: an implication for IFNβ-related adverse effects in multiple sclerosis”

Thank you for your prompt review of our manuscript cited above. The comments of the three reviewers are well taken, and revisions have been made as follows:

To Editor-in-Chief

1. Following ethical regulations, written informed consents were obtained from the subjects involved in the present study according to the form approved by the Ethics Committee of National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan, as described in the text of the revised manuscript (p. 5, ls. 15-18).

2. New additional data, including microarray analysis performed on a MS patient requested by Referees #1 and #3 and validation of inter-experiment variability requested by Referee #3, are incorporated in the new version (Supplementary Table 6 and Supplementary Figure 1, respectively).

3. Two additional coauthors (Drs. Nanri and Tabunoki) have joined in the study to prepare the revised manuscript, and their contributions are described in the end of the text (p. 13, ls. 15-16).

To Referee #1

1. Following the reviewer’s suggestion, the title is changed to a new one “Microarray analysis identifies a set of CXCR3 and CCR2 ligand chemokines as early IFNβ-responsive genes in peripheral blood lymphocytes in vitro: an implication for IFNβ-related adverse effects in multiple sclerosis”. The conclusive remarks are also replaced by the sentence “IFNβ immediately induces a burst of gene expression of proinflammatory chemokines in vitro that have potential relevance to IFNβ-related early adverse effects in MS patients in vivo” (p. 12, ls. 25-27). The microarray results of two additional subjects, including a 28 year-old healthy man (the subject #2) and a 27 year-old woman with RRMS who was a dropout of IFNβ treatment due to induction of frequent severe relapses (the subject #4), validated the observations on the healthy subject #1, as described in the text of the revised manuscript (p. 8, ls. 7-13) and shown in Supplementary Table 6.
2. The details of standardization for real-time RT-PCR are described in the Methods section of the revised manuscript (p. 5, ls. 22-29; p. 6, ls. 1-4).

3. Enhanced expression of CXCL10 (SCYB10) in active MS lesions is emphasized in the Discussion section of the revised manuscript (p. 10, ls. 22-26). In addition, the role of IL-8 in MS is more discussed in the text of the revised manuscript (p. 11, ls. 14-20). The role of IRF family proteins is also described in the Discussion section of the revised manuscript (p. 9, ls. 19-29; p. 10, ls. 1-6).

**To Referee #2**

1. The microarray we utilized contains total 64 spots of G3PDH (Symbol GAPD), as shown in Supplementary Table 1. However, G3PDH was neither identified as a significantly upregulated gene nor a downregulated gene in the microarray analysis, suggesting that G3PDH represents a reliable housekeeping gene in gene expression analysis of PBMC following treatment with IFNβ. Therefore, quantitative real-time RT-PCR analysis was performed by evaluating the levels of expression of target genes standardized against those of G3PDH detected in the identical cDNA samples, as described in the text of the revised manuscript (p. 8, ls. 17-23).

2. The amounts of IFNβ used for in vitro stimulation are specified in the Methods section of the revised manuscript (p. 5, ls. 7-14).

3. The assays of real-time RT-PCR were performed in triplicate measurements of the same sample, and the results were expressed as the average (mean) with standard error (p. 5, ls. 22-29; p. 6, ls. 1-4).

4. The first paragraph on page 4 might provide the potential readers not only an introduction to the expediency of microarray analysis to characterize the immunopathogenesis of MS but also the clear background of the present study.

**To Referee #3**

1. The impact of inter-experiment variability in microarray analysis was verified by analyzing a scatter plot, as described in the Methods section of the revised manuscript (p. 6, ls. 24-25) and shown in Supplementary Figure 1. The average of FI of duplicate spots was calculated (p. 6, ls. 21-24). Although the analysis in the present study was a single microarray for each sample design, the results from two additional subjects, including a 28 year-old healthy man (the subject #2) and a 27 year-old woman with RRMS who was a dropout of IFNβ treatment due to induction of frequent severe relapses (the subject #4), validated the
observations on the healthy subject #1, as described in the text of the revised manuscript (p. 8, ls. 7-13) and shown in **Supplementary Table 6**.

In real-time RT-PCR, the levels of expression of target genes were **standardized** against those of the glyceraldehyde-3-phosphate dehydrogenase (**G3PDH**) gene detected in the **identical cDNA samples**. The assays were performed in triplicate measurements of the same sample and the results were expressed as the average (mean) with standard error (p. 5, ls. 22-29; p. 6, ls. 1-4). The \( p \) values in some real-time RT-PCR experiments are provided in the Results section of the revised manuscript (p. 9, ls. 3-8). Because the experiment number (\( n = 3 \) for microarray) is fairly small, the power of **statistical evaluation** becomes weak and hierarchical clustering analysis seems less valuable. We are currently enlarging the study population containing an increased number of the patients. The study of the large cohort will be reported separately in a forthcoming paper.

We thank you for the attention you have given to our manuscript.
We hope that this revised version meets with your approval.

Sincerely yours,

**Jun-ichi Satoh, M.D., Ph.D., the corresponding author**
Department of Bioinformatics
Meiji Pharmaceutical University
2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan.
E-mail: satoj@my-pharm.ac.jp or satoj@ncnp.go.jp

JS: