Reviewer’s report

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

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Reviewer: Christopher Lock

Reviewer’s report:

General
The authors studied the effects of IFN-beta on gene expression in PBMCs from three normal subjects, at 3 hour and 24 hour time points, using microarray technology. Results were verified with quantitative RT-PCR for seven of the identified genes of interest. PBMCs from one of the subjects were also stimulated with IFN-gamma, TNF-alpha, or IL-1-beta for comparison in RT-PCR experiments.

There are a number of existing papers in the literature using microarrays to look at the effects of IFN-beta on gene expression in MS subjects and controls, as noted by the authors. This is a topic of considerable interest, in terms of identifying markers for predicting therapeutic response, or for predicting side-effects. The present paper differs mainly in identifying immediate early IFN-beta-response genes (IRGs) in PBMCs.

MS subjects often experience flu-like symptoms for the first 24 hours after an IFN-beta injection. These side-effects typically decline after about 3 months of treatment. Several proinflammatory CXCR3 ligand chemokines (SCYB11, SCYB10, SCYB9) and CCR2 ligand chemokines (SCYA8, SCYA2) were amongst the top 20 upregulated genes at 3 and 24 hours in the present study. These genes have not been reported in MS subjects on long-term IFN-beta treatment. The authors speculate therefore that CXCR3 ligand chemokines and CCR2 ligand chemokines may be significant in terms of IFN-beta related side effects. There is no direct proof of this idea; other proinflammatory mediators are upregulated, and other explanations for the side-effects of IFN-beta are possible.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

The methodology in the manuscript generally appears sound. No statistical analysis of the microarray data is provided however, perhaps because of the small sample size. There is the possibility of false positives and negatives in making a large number of comparisons, as is typical in a microarray experiment. This point should be addressed, as some measure of statistical significance is generally a standard requirement in microarray papers.

The authors point out that 11 of the upregulated IRGs seen at both time points in the present study were seen in a previous study of MS subjects treated for 3-6 months with IFN-beta. The top 20 upregulated genes in the present study were identified as known IRGs from databases. The induction of CXCR3 ligand and CCR2 ligand chemokine genes was verified in two other subjects by RT-PCR, but the data is not shown (why not?). These findings all tend to support the data presented. Error bars are given for the RT-PCR experiments, but as noted no p-values for the microarray data.

For the RT-PCR assays, PBMCs from three individuals (plus an additional two individuals not shown) were studied and samples were run in triplicate. In microarray experiments, were samples from all three individuals run, and are the listed fold-changes in the tables 2 through 5 the average of these? Were samples run more than once, or was this a single microarray experiment for each sample? It is noted that the arrays have duplicate spots for each gene represented. Cluster analysis is helpful for visualizing the data, and also tends to group genes functionally. I would also have liked to see the data for the different samples and time points displayed in this way.

Discretionary Revisions (which the author can choose to ignore)
What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.