Reviewer's report

Title: COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglia of multiple sclerosis and amyotrophic lateral sclerosis spinal cord

Version: 1 Date: 15 December 2005

Reviewer: Noel G Carlson

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General
Review of manuscript by Yiangou et al entitled COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglia of multiple sclerosis and amyotrophic lateral sclerosis spinal cord.

Summary: In this study the authors use immunohistochemical and immuno-blot methodology to describe an increase of three antigens (COX-2, CB2 and P2X7) in MS and ALS post-mortem tissues relative to controls. While elements of this paper such as COX-2 increase in ALS and MS are not novel, the relationship between increased COX-2 expression in macrophage/microglia with increased CB2 and P2X7 is potentially important and interesting. However, there a several aspects that should be addressed prior to publication. No clear question is posed, as such the parts of the paper come across as a collection of several findings that may or may not be related. Also, relevant literature is not cited with respect to increased expression of COX-2 in MS plaques (see Rose et al, 2004 J. Neuroimmunology 149:40).

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
The methods used in this study are relatively straight forward by are not always adequately described. Several issues should be addressed.
1) Controls for expression of these antigens are described in graphs but are never shown in IHC. This is important to illustrate qualitative aspects of the expression as well.
2) No western blots are shown. A representative western blot should be shown for each protein.
3) In the western blots it is not clear how the samples are normalized for differences in loading. It is noted on page 8 in the methods that the inter gel variation was corrected by comparison of the OD of the positive control in each blot and adjusted to the OD reading accordingly. What was the positive control? Was actin or some other standard used? This should be spelled out in the methods and an example western blot shown with the standard along with the protein being assessed.
4) Image analysis in not adequately described. What magnification(s) was used? Also, the authors only present the % of the image area of a particular antigen relative to the area of the field scanned. However, in images such as Figs 10 and 12 where portions of the field don not contain tissue, this is misleading. The % reactive area should be assessed relative to the tissue area in the field.
5) There is essentially no information describing the tissues used in the study. For instance, what types of MS plaques were examined? (Were the plaques classified according to Lassmann et al. 1998 J Neuroimmunology?). In several instances in the paper the authors refer to expression near active plaques. How do they identify whether the plaques were active with respect to demyelination? Were the plaques stained with LFB or some other myelin stain to assess demyelination? This should be described for the MS tissues. Also, more information describing the ALS tissues needs to be included, particularly since the findings in this study are different from those of Almer et al regarding COX-2 expression in neurons.

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

Many of the figures are confusing. Perhaps labeling the panels with the antigens examined could help (eg. Fig 1, A—PK1195, B- CD68, E-COX-2, etc)

Figure 3 is confusing. What is stained red and what is stained black? What tissue is presented in E and F?

Labeling “MND” in Figs 4,11,13 is not defined in the figure legends.

The results presented in Fig 9A are different than described in the text and Fig legend. Not all the COX-2 co-localizes with Ferritin (as seen left side of Figure).

The writing in the paper could also be improved. The paper needs a stronger rationale for why each experiment was done.

In many cases the data are presented both in the figures and in the text (eg page 14, CD68 section and Fig 11). In other examples such as on pages 12-13 (CB2 data) should be summarized in a graph or table.

Discretionary Revisions (which the author can choose to ignore)

A brief description of what PK11196 is should be included in the initial presentation in the results.

The entire results section is almost in outline form with no transitions or introductory statements for each section.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

Quality of written English: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests’ below.