Author's response to reviews

Title: Passively transferred human NMO-IgG exacerbates demyelination in mouse experimental autoimmune encephalomyelitis

Authors:

Harleen Saini (harleen.sn@gmail.com)
Robert A Rifkin (rrifkin7@gmail.com)
Michael Gorelik (mg007m@gmail.com)
Hwa Huang (hhuang35@jhu.edu)
Zachary Ferguson (zfergus1@jhu.edu)
Melina V Jones (mjones96@jhmi.edu)
Michael Levy (mlevy@jhmi.edu)

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Author's response to reviews: see over
Response to Reviewers.

Reviewer #1:
1. The final volume of demyelinated lesions were actually similar in all the 3 groups. Not clear therefore the underlying pathology that was responsible for the persistent worse clinical status in the NMO transferred group. A better explanation as considering a more axonal damage, ischemic events while affecting more the subpial area? Not clear why the more deeper lesions (as seen in the control group) would be less clinical dysfunctional than the subpial lesions. The more affected ventral cord areas vs. the lateral maybe actually more relevant.

Axonal damage may certainly accompany the increased EAE scores and disability observed in the mice with passively transferred NMO-IgG. Our group could not demonstrate a reliable correlation between axonal count and disability in rat EAE models in the past (Brain. 2007 Aug;130(Pt 8):2199-210) so we did not attempt to count axons in this model. Ischemia seems less likely as the demyelinated areas do not appear necrotic, but we acknowledge spinal cord ischemia likely plays a role in the disability in patients with NMO.

We propose that although all groups had equal total demyelination, perhaps the simplest explanation is that confluent involving the dorsolateral corticospinal tracts may be the cause of the higher EAE scores in this model. We added this hypothesis to the manuscript.

2. A better explanation/relation between the more subpial location found in the NMO group and the temporary increase in granulocyte accumulation is desirable.

Our hypothesis is that NMO-IgG targets AQP4, which is preferentially expressed on the astrocytic foot processes of the pia-glia limitans. As a result of the antibody binding, a humoral-mediated inflammatory process ensues that includes granulocyte infiltration. It is temporary because the human NMO-IgG is cleared shortly after passive transfer.

We added our hypothesis above to the discussion section of the manuscript.

3. Although limited data on the clinical relation between NMO levels is available, the authors did not mentioned the reference “Mult Scler. 2008 Sep;14(8):1061-7” showing a relation between disease activity and presence of NMO IgG and the possibility of complete reversal (negative status) in patients with stabilized clinical status on immunosuppressive therapy.

Our results are consistent with this publication pointed out by the reviewer. We added it to our references in the discussion about the mechanism of a pathogenic NMO antibody.
Reviewer #2:

1. Please define “behavioral worsening” in relation to EAE in the mice. The term is used several times but not explained.

   “Behavioral worsening” in relation to EAE in the mice is defined as higher EAE disability scores. This has been clarified in two sections of the manuscript to be clearer.

2. The legend for Figure 2 needs to be clarified. Although one could guess, it is not stated that micrographs A and B were stained with anti-human antibody, as is said for micrograph C. If the tissue section in D is stained with anti-AQP4, what is section E stained with? The relationship between the sections and the staining needs explanation, i.e. which colors are associated with which antibody? This is not even in their Supplementary Table 1, which gives the details of the antibodies used. Is E a merger of C and D?

   Figure legend 2 was clarified to identify the colors and stains used for all of the sections. We also marked section E as a merged section in the legend.

3. In the legend for Figure 3, the staining or lack thereof (since black and white) in micrographs G, H and I is not addressed, nor what the arrows are pointing to: supposedly AQP4 staining, but that was red in Figure 2 and only one anti-AQP4 antibody was used, according to Table 1. This is not explained in the text either (i.e. fluorescence versus light microscopy). It is not acceptable to show sections that were wrinkled during processing. Of all the mice included in the experiment shown and the mentioned repeat, there should be examples that show the tissues better for publication.

   Micrographs G, H, and I were pseudo-colored red to be consistent with the others anti-AQP4 stained figures in this manuscript.

   The wrinkled sections were included only to show a low field view of the spinal cord section. These particular sections highlight the pial pathology of the NMO-IgG group and the dorsal column pathology of the control groups. The insets and magnified sections are not wrinkled. We can replace the low power sections with sketches to make the same point, if preferred by the reviewer.

4. The text in the legend of Figure 4 is incorrect in stating that 80% of all the demyelinated lesions in the NMO-IgG group were greater than 10,000um2. Both the data in Figure 4B and the text in Results section, paragraph 4 show close to 40%. Figure 4A also shows the same section of wrinkled tissue as Figure 3A.

   Thank you for pointing out the error in the figure legend. We corrected the figure legend. The wrinkled tissue is provided here again as a low power image just to show the relevant areas that were measured. This particular
section showed three EAE lesions, including a pial lesion, all in the same cut. Again, we could use a sketch to make the same point, if the reviewer prefers.

5. The micrographs in Figure 6 are not of a high enough resolution to see the cell types being referred to. A higher magnification is needed. As is the issue in all of the figures with tissue staining, reference needs to be made to which sections were stained with which antibody, rather than glossed over as if it was text in the body of the text. Why are there pictures labeled E and F without a D?

The resolution of Figure 6 has been corrected so the identified cells can be clearly visualized. The antibodies and colors have been added to clarify the figure legend. And the figure was re-labeled so as not to skip D.