Author's response to reviews

Title: Autoantibodies Against Retinal Proteins in Paraneoplastic and Autoimmune Retinopathy

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Enclosed is a revised version of our manuscript entitled "Autoantibodies against retinal proteins in paraneoplastic and autoimmune retinopathy" by Grazyna Adamus, Gaoying Ren, and Richard W. Weleber. We would like to thank the reviewers for their comments, which we considered when revising our manuscript.

Responses to Reviewer 1

1. As was stated in Results, "patients' autoantibodies" were called "patients' antibodies" for simplicity (page 9). We have also added "specificity" to the word "antibodies" where appropriate, e.g. "anti-recoverin antibodies". We have edited the text for consistency.

2. We divided the patients with visual symptoms into 2 groups (AR and PR), based on the diagnosis of cancer at the time of testing for anti-retinal antibodies. We agree with the reviewer that some AR patients with anti-retinal antibodies may develop cancer later in their lives, but the results from our studies show that more patients with visual symptoms developed anti-retinal antibodies after they have been diagnosed with cancer. We believe that if an AR patient still tests negative for cancer several months after positive antibody test, his/her retinal disease may not be related to the cancer. The results presented in Table 3 support this hypothesis. Table 3 shows that in some AR patients, the onset of visual symptoms and the presence of anti-retinal antibodies take place years after the diagnosis of cancer.

3. We corrected our statement regarding reactivity with "a single protein on the blot" to "a single band" (page 10). In the case of p35, the antigenic protein is still unknown although the candidate proteins are transducin-b and Muller cells protein (reference number #9).

4. Fig. 1 gives a summary of antibody testing for 193 patients over 9-year period. We believe that it is more important to present the overall reactivity of autoantibodies than individual 193 blots or immunocytochemistry pictures. Regarding anti-bipolar cell antibodies, they do not recognize any proteins on the blot, probably due to denaturation of the antigen by heat and SDS. The reactivity of anti-bipolar cell antibodies of MAR patients seems to require a native unchanged antigen in the tissue (page 9). It has been reported that even a slight fixation during tissue preparation for immunohistochemistry abolished their reactivity (see ref.#15). Some laboratories have attempted to identify this antigen without success.

5. Regarding the association of anti-retinal antibodies with retinal disease, ours is the first study of a larger group of patients with CAR and CAR-like symptoms to be evaluated for anti-retinal antibodies
in association with visual symptoms. Until now only case reports were available. We do not doubt that low levels of anti-retinal antibodies may be present in other patients and in normal subjects. In fact, we have previously shown that about 10% of patients have anti-enolase antibodies (ref #4) and these antibodies differed in epitopes and cytotoxicity. We now included a comment about the presence of anti-recoverin antibodies in non-CAR patients (page 4), and we have added 3 references as suggested.

6. Fig.3 and the legend were revised. Fig.3a - we added treatment (Tolpa) to the figure. To make this figure more consistent with other figures in this group, we removed the word "changes in visual fields" in Fig. 3b. The correlation of changes in specific visual symptoms with the antibody presence will be the subject of next paper.

7. Almost half of the patients tested had anti-retinal antibodies, which is quite high. However, to make the statement "we identified a much higher prevalence than expected" less ambiguous, we have changed the sentence to "we identified a high prevalence of anti-retinal antibodies" (Abstract and page 12).

Responses to Reviewer 2
1. We edited figures for missing labels.
2. As suggested, in the Abstract and the Methods section, we added the phenotype of patients in the study.
3. We defined and changed the term "reasonable observation period" to "within the next several months".
4. Normal controls were recruited from voluntary subjects. They have not reported problems regarding their vision. They had no physical exam at the time of collection.
5. Both Western blot results and immunocytchemistry data were evaluated by a masked observer and graded as positive and negative for the presence of antibodies. Antibody titers against recoverin and enolase were tested by ELISA (pages 7-8).
6. Serum antibodies were purified by ammonium sulphate precipitation and the purity was checked by SDS-gel electrophoresis (see the addition on page 8).
7. For controls, we used sera from 79 healthy volunteer subjects, including 47 men and 32 women who had no vision loss at the time of collection (now included on page 6). We used the same number of serum samples from each group of subjects. Fig. 2 shows the results obtained from 28 samples from each group (see figure legend and page 8). We also corrected our typing error in Fig. 3 and in the text (page 11) related to the p value for the AR group. It now reads p=0.0003.

Thank you for your kind considerstions.

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Professor