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Editorial

Antibodies to the inward rectifying potassium channel 4.1 in multiple sclerosis: different methodologies—conflicting results?

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B-cells and antibodies are believed to play an important role in the pathogenesis of multiple sclerosis (MS). The occurrence of pathogenic antibodies in MS is most convincingly supported by histopathological findings, which demonstrate antibody deposits and activation of complement in subset of acute demyelinating lesions.[1] In addition, plasma exchange, which removes circulating antibodies from the blood, may be effective in treating severe MS relapses.[2] The effects observed in MS are similar to those seen in other diseases with known pathogenic antibodies such as myasthenia gravis and neuromyelitis optica. Despite the indirect evidence for the existence of disease relevant antibodies in MS, the search for the target(s) of these antibodies has turned out to be difficult.[3]

Recently, a serum antibody response against the inward rectifying potassium channel 4.1 (KIR4.1) was reported by our group. The KIR4.1 antibody (KIR4.1–IgG) was detected in 35–50% of MS patients but rarely in controls.[4,5] KIR4.1 is a membrane protein expressed in oligodendrocytes and a subset of astrocytes. The protein contains two extracellular domains and two transmembrane regions. KIR4.1 is expressed in vivo as a homotetramer (four units of KIR4.1) or a heterotetramer with KIR5.1 (two units of KIR4.1 and KIR5.1). KIR4.1 channels are essential for potassium homeostasis in the brain by maintaining the ionic and osmotic environment in the extracellular space. Previous studies and recent findings from our laboratory suggest that human KIR4.1 undergoes substantial posttranslational modification.[6,7] Indeed the molecular weight of KIR4.1 differs in various tissues such as kidney or brain tissue and even between white and grey matter.[6,7] At least part of these differences are explained by glycosylation, which occurs at the dome of the first extracellular domain of KIR4.1.[6]

Our initial study on KIR4.1–IgG was performed with an enzyme-linked immunosorbent assay (ELISA) based on KIR4.1 protein extracted from transiently transfected human embryonic kidney (HEK) cells. The isolated tetramers used for the assay best reflected the molecular weight of KIR4.1 expressed in human white matter. Covalent binding of protein to the surface of the ELISA plates allowed the fixation of the tetramers to expose the extracellular domain. Using this assay, significant differences in serum antibody reactivity were observed between MS patients and controls in four independent cohorts.[4,5] The MS autoantibody seems to target the first extracellular domain of KIR4.1, which is displayed in the tetramer formation by four loops, each composed of 25 amino acids. Tetrameric protein assembly, which allows cooperative binding of antibodies, seems to be important.[8] Also, a peptide comprising these 25 amino acids complemented by adjacent hydrophobic amino acids from the transmembrane region and biotinylated at the N-terminus was used to establish an ELISA. It was intended to bind the peptides to the plate by biotin–streptavidin interaction at the N-terminus (each streptavidin molecule would bind four biotinylated peptides) and hydrophobic interactions at the C-terminus aiming to achieve loop formation and tetrameric clustering that would mimic extracellular domain of a KIR4.1 tetramer. Although the assay had lower sensitivity and was difficult to control, a difference in antibody prevalence was observed between patients and controls.

Two follow up studies were not able to detect antibodies to KIR4.1 in MS patients and controls using

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a similar peptide-based assay.[9,10] Brickswana and colleagues also performed KIR4.1 antibody studies using HEK cells that were permanently transfected with fluorescence tagged KIR4.1 protein. Using immunohistochemistry and immunoprecipitation, they could not find any significant antibody reactivity against KIR4.1 in patients and controls.[10] In the current publication, Brill and colleagues used a modified peptide-based ELISA but ensured by competition assays that the ELISA was indeed picking up KIR4.1 specific antibody reactivities (Brill et al.). They found antibodies to KIR4.1 peptide in 26% of MS patients and 6% of controls. The difference reached a significant level as it did in the initial report. They also reported that antibody titers were higher during relapse than remission, suggesting that the titer could reflect disease activity. Moreover, they found antibodies also in 22% of neuromyelitis opitca (NMO) patients, which have not been investigated before.

How can these conflicting results emerging from different laboratories be explained? Complex folding of membrane proteins, tetramer formation and cell specific pre- and post-translation modifications make KIR4.1 a difficult target for antibody studies. Although peptides may cover the protein sequence of interest, they do not contain posttranslational modifications and do not easily form proper conformational structures. Even if one can ensure binding of the biotinylated peptide to the plate, which has not been done in most studies, proper tetrameric folding and clustering of the epitope, which mimics the extracellular domain of the KIR4.1 tetramer, has to be achieved. Therefore, a negative ELISA result might be due to lack of antibodies in the tested serum or due to the lack of proper epitope display in the ELISA. A positive result in peptide ELISA, however, may not necessarily ensure that the detected antibody binds the native KIR4.1 protein, which is folded, differentially glycosylated and has an oligomeric assembly.

Therefore, protein-based assays are more reasonable to investigate antibody responses to a complex membrane protein such as KIR4.1. However, given the differential posttranslational modifications of KIR4.1 that occur in vivo, it is essential that not only the formation of tetramers but also the posttranslational modifications need to be monitored while developing the assay. This is of particular importance, when cell lines are used for protein expression, which are not derived from the tissue that is targeted by the autoantibodies in MS (e.g. HEK cells, which are derived from the kidney). Indeed according to our own observations the composition and quantity of glycosylation in the extracellular domain of KIR4.1 is crucial for antibody binding.[7] In transfected HEK cells, the majority of KIR4.1 protein differs from KIR4.1 expressed in the brain by its molecular weight. Only a minor fraction reflects KIR4.1 expressed in white matter. Interestingly only this fraction is bound by KIR4.1–IgG from MS sera while other KIR4.1 protein variants are not recognized. Although cell-based assays are considered the gold standard for the detection of autoantibodies targeting membrane proteins, these assays can only work if the expressed protein reflects the properties of the protein expressed in the target tissue. The recombinantly expressed protein needs to be carefully analysed with respect to its molecular weight, posttranslational modification and tetramer formation to ensure that it precisely reflects the protein expressed in the target tissue. These comparative analyses are essential but rarely done in studies on autoantibodies in human autoimmune diseases.

To overcome the conflicting findings on KIR4.1 antibodies in MS, careful comparisons studies using the same samples in different assays are warranted. These studies will help to clarify the occurrence and biological relevance of autoantibodies to KIR4.1 in MS. These studies may also shed light on the role of posttranslational modifications for antibody binding that have largely been neglected in autoantibody research so far.

Conflict of interest

B. Hemmer has served on scientific advisory boards for Roche, Novartis, Bayer Schering, Merck Serono, Biogen Idec, GSK, Chugai Pharmaceuticals, Micromet and Genzyme Corporation; serves on the international advisory board of Archives of Neurology and Experimental Neurology; has received speaker honoraria from Bayer Schering, Novartis, Biogen Idec, Merck Serono, Roche, and Teva Pharmaceutical Industries Ltd; and has received research support from Biogen Idec, Bayer Schering, Merck Serono, Five prime, Metanomics, Chugai Pharmaceuticals and Novartis. He has been filed a patent for the detection of antibodies and T cells against KIR4.1 in a subpopulation of MS patients.

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