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Research Article

Is aberrant N-glucosylation relevant to recognise anti-MOG antibodies in Rett syndrome?

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Abstract. Antibodies against myelin oligodendrocyte glycoprotein (MOG) are associated to several disorders, and their occurrence in patients presenting an acquired demyelinating disease affects a higher proportion of paediatric subjects, as compared to adults. Despite heterogeneity in clinical presentation, few connexions have been reported between the progressive neurodevelopmental disorder affecting child's brain development and cognitive ability, i.e. Rett syndrome (RTT), and a demyelination process. In order to identify the possible target of humoral autoimmune response in RTT patients, we set-up a home-made solid-phase ELISA, using the recombinant extracellular portion of human MOG(1-117) as an antigen. The screening to evaluate anti-MOG antibodies in RTT patient sera, compared to other relative non-RTT pervasive developmental disorders (non-RTT PDD), including mainly autism, and a healthy control group gave uncertain results. In fact, Student *t*-test and Mann-Whitney unpaired t test showed that differences in both IgG and IgM antibody titres between the different patient populations, were not statistically significant. We can conclude that the absence of anti-MOG antibody recognition in RTT has possibly to be ascribed to a different relevant protein folding and/or to the lack of a relevant aberrant post-translational modification, such as N-glucosylation, that we previously demonstrated, for the first time, fundamental to recognize antibodies in RTT.

Keywords. Myelin oligodendrocyte glycoprotein, Rett syndrome, antibody detection, ELISA.

INTRODUCTION

A precise myelination is crucial for optimal transmission of nerve impulses and in providing trophic support to axons. In the central nervous

system (CNS) oligodendrocytes shape the myelin sheath surrounding axons.[1] Intermittent uncovered short portions of the axon, called myelin-sheath gaps or the nodes of Ranvier, are fundamental for optimal myelin functioning.[2,3] Perturbations of the nodes of Ranvier and myelin can be due to several causes including autoimmune responses as in multiple sclerosis,[4] Guillain-Barré syndrome,[5] or in other immune-mediated neurological diseases.[6] Demyelination process can be unleashed either because of an attack directly on the myelin sheath and/or a disruption or death of oligodendrocytes. This clear difference in triggering the same end-stage of demyelination may not be obvious and sometimes damage to both may occur. The aetiology of myelin loss includes immune-mediated, viral, metabolic, toxic, and/or genetic causes. Moreover, brain damages that may occur during neonatal hypoxia or subsequent to traumatic injury may also result in successive demyelination.[3,7]

In this context, the involvement of CNS myelin proteins is fundamental for oligodendrocyte growth and myelination.[8-11] Myelin proteins include myelin proteolipid protein (PLP), the related DM20, myelin-associated oligodendrocyte basic protein (MOBP), myelin-associated glycoprotein (MAG), 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP), and particularly the myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG). Proteins as MBP and MOG, located in the external part of myelin, have been proposed as antigens in several immune-mediated disorders. MOG localization on the outermost surface of myelin sheath and the plasma membrane of oligodendrocytes[12] convert this protein into a partial exposed target (Figure 1). Despite the specific function of MOG has still to be clarified, its role as important surface marker of oligodendrocyte maturation, regulator of microtubule stability and mediator of interactions between myelin and the immune system have been described.[13,14] More controversial are the results obtained to identify and clarify the role of anti-MOG antibodies, which are still a matter of discussion,[15-17] particularly on their putative pathogenic involvement in autoimmune response in multiple sclerosis[15,18-20]. Interesting data about the diagnostic/prognostic role of anti-MOG antibodies in multiple sclerosis patient sera were published,[21] followed by contradictory studies that could not confirm these results. In fact, the same group of authors described other contrasting data in a conflicting array.[22-25] A recent review reports that methods to detect anti-MOG antibodies have improved substantially with cell-based assays.[26] However, a strong debate is still ongoing.[27] Anyway, from the molecular point of view definition of the peptide epitope (conformational and/or linear) involved in antibody recognition is a challenge. In fact, a maximum of 8-10 amino acids are involved in *in vivo* antibody binding.[28] MOG has a unique site of N-glycosylation at position 31 and the MOG(35-55) peptide has been the only MOG fragment able to induce neurological impairment in mice comparable with those observed in experimental autoimmune encephalomyelitis induced by MBP or PLP.[29] To assess the presence of a B-cell intramolecular epitope spreading mechanism, we tested synthetic peptides mapping MOG(1-117), including MOG(35-55). An intense IgG antibody response against both the recombinant protein and the immunizing peptide MOG(35-55) was observed, while no response was observed against the other synthetic fragments. Furthermore, as the properly refolded recombinant probe is able to bind antibodies with greater efficiency compared with MOG(35-55), we hypothesized the presence of both linear and conformational epitopes on MOG(35-55) sequence.[30]

The arguments discussed in the current literature regarding anti-MOG antibodies in multiple sclerosis can be extended to other inflammatory demyelinating diseases of the CNS. In particular, anti-MOG antibody-associated disorders account for a higher proportion of paediatric patients than adults who present an acquired demyelinating disease.[31]

Previously, we hypothesized the coexistence of a perturbation of the immune system in Rett syndrome

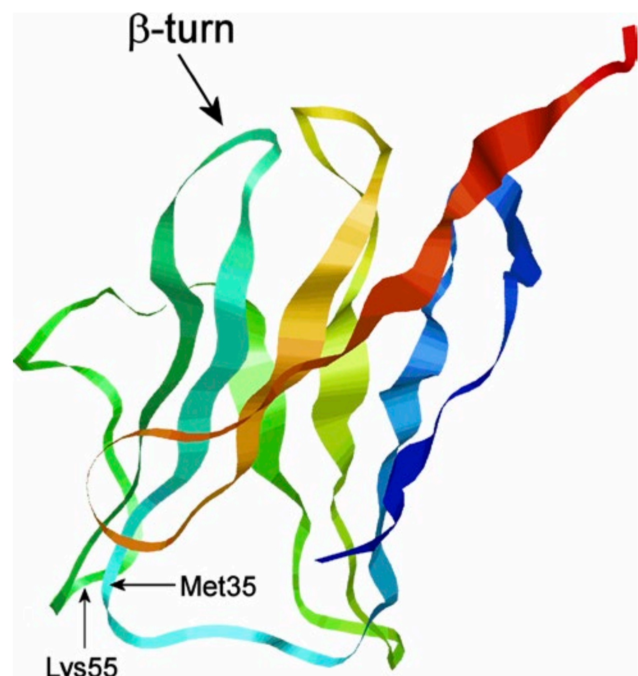

Figure 1. Homology model of the extracellular domain of human myelin oligodendrocyte glycoprotein (MOG), with the β-turn inside the fragment MOG(35-55) evidenced.

(RTT) patients.[32] RTT is a neurodevelopmental genetic disorder presenting neurological regression after development during infancy. A derangement of microglia immune responsiveness might be likely to occur in these paediatric patients, as neuroinflammation is a powerful modulator of the CNS immune system. We observed that RTT patients showed a consistent and highly significant increased titer of IgM antibodies relative to both healthy controls and non-RTT pervasive developmental disorders (non-RTT PDD) patient groups by using a diagnostic synthetic glycopeptide antigen of multiple sclerosis (Figure 2).[32-34]

Moreover, despite heterogeneity in clinical presentation, few connexions between RTT and demyelination process have been reported. In fact, Sharma *et al.* focused on the role of Methyl CpG binding protein 2 (MeCP2), one of the genes associated with RTT, and its involvement in regulation of myelin gene expression.[35] Additionally, a case report with similarities in RTT symptoms and anti-MOG antibody encephalitis has been described.[36] Convergence of these diseases could lead to a better understanding in demyelination process due to immune-mediated mechanisms.

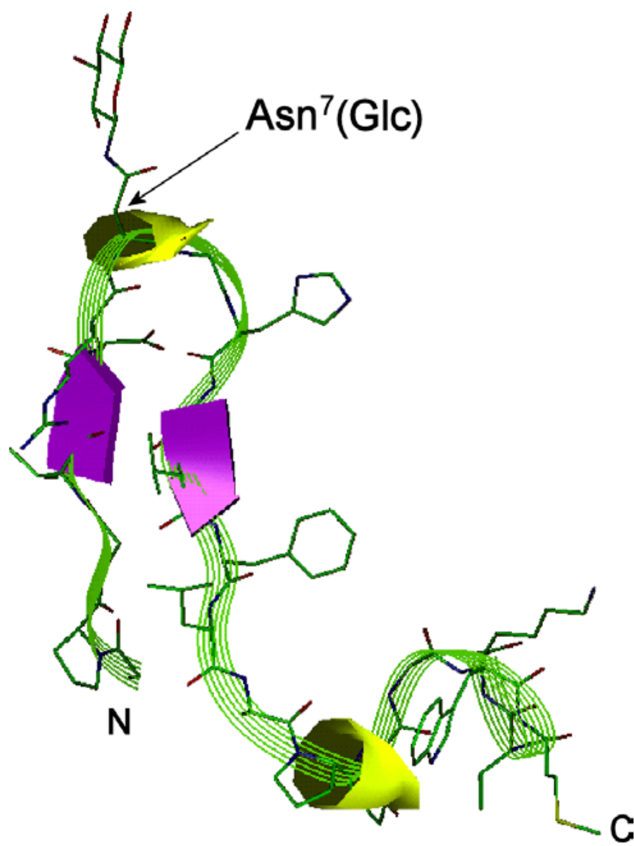

Figure 2. The β-turn peptide structure exposing at position 7 the N-glucosylation recognizing specific antibodies in Rett syndrome in a home-made ELISA.[32]

With all these considerations in mind, the main goal of our work was to identify the target of the humoral autoimmune response in RTT patients, recognised by the synthetic N-glucosylated β-turn peptide structure,[32] evaluating the possible cross-reaction with anti-MOG antibodies. Moreover, we focused on a better understanding of antibody response in Rett syndrome compared to other relative non-RTT PDD, including mainly autism, apparently connected (as they share some behavioural traits), but dramatically different for their severity, life-span expectancy, and immune system derangement. To this aim, a homemade SP-ELISA, based on the extracellular portion hMOG(1–117) expressed in *Escherichia coli* and properly refolded, was employed to test RTT patient population, other relative non-RTT PDD, and healthy control groups.

MATERIALS AND METHODS

Patients

In this study, a group of 110 children was enrolled. This population consisted of three clearly distinguishable groups: the RTT syndrome group (28) versus non-RTT pervasive developmental disorders (non-RTT PDD) group (48), classification based on the clinical features and the presence of mutated RTT-related genes and healthy, age-matched controls (34). These patients were hospitalized for 1 week every 6 months, in the Child Neuropsychiatric Unit, "Azienda Ospedaliera Universitaria Senese", Siena (Italy), during the course of the study. Criteria for inclusion in the study were clinical diagnosis of RTT syndrome coupled with positive identification for the presence/absence of mutated MeCP2, CDKL5, or FOXG1 genes. The age-matched non-RTT PDD group consisted of 48 patients, as diagnosed following well-established criteria. Blood samplings in the patient group were performed during the routine follow-up study at hospital admission, while the samples from the control group were carried out during routine health checks, sports, or blood donations, obtained during the periodic clinical checks. The healthy control subjects were age-matched. Patients were selected randomly and not previously tested for immune reactivity by ELISA. Parents, tutors, or guardians of all the participants provided their written informed consent for the minors to participate in this study. The study design, methods, and consent procedure were approved by the Institutional Review Board of Azienda Ospedaliera Universitaria Senese. All the data used in this study were anonymized.

Materials

Solid-phase ELISAs were performed using 96-well plates NUNC Maxisorp flat bottom (Sigma-Aldrich, Milan, Italy). Washing steps were performed using a microplate washer Hydroflex (Tecan, Männedorf, Switzerland). Fetal bovine serum (FBS) was purchased from Sigma-Aldrich (Milan, Italy). Secondary anti-human IgG and IgM antibodies conjugated with alkaline phosphatase were purchased by Sigma-Aldrich (Milan, Italy). p-Nitrophenyl phosphate was purchased from Fluka (Milan, Italy). Absorbance values were measured on a plate reader Tecan Sunrise purchased from Tecan (Tecan Italia, Milan, Italy). Electrocompetent ER2566 E. coli cells were purchased from New England Biolabs (Ipswich, MA, USA). Plasmid pET-22 was purchased from Novagen (Madison, WI, USA). Protein purification and refolding were performed using a Chelating Sepharose Fast Flow column on ÄktaBasic chromatography system (GE Healthcare, Milan, Italy). The far-UV circular dichroism (CD) spectra were recorded by using a J-810 Jasco spectropolarimeter (JASCO, Easton, MD).

Enzyme-Linked Immunosorbent Assay (ELISA)

The protein fragment hMOG(1–117) cDNA was subcloned into the His-tag expression vector pET-22. Recombinant hMOG(1-117) was produced according to the protocol published by Gori *et al.*[37] Recombinant hMOG(1-117) was dissolved in coating buffer (12mM Na_2CO_3, 35mM $NaHCO_3$, pH 9.6) to obtain a solution 10 µg/mL. Then 100 µl of solution were dispensed in each well of 96 well MaxiSorp flat bottom plate, pinch-bar design. Plates were incubated a +4°C overnight. Subsequently, plates were washed 3 times with Washing Buffer (0.9% NaCl, 0.01% Tween 20), and blocked 1 h at RT with 100 µl/well of FBS Buffer (10% FBS in Washing Buffer). After FBS buffer removal, 100 µl/well of diluted sera sample (1:100 in FBS Buffer) were dispensed in triplicates. Plates were incubated at +4°C overnight, and then washed 3 times with Washing Buffer, 100 µl/well of secondary Ab labeled with alkaline phosphatase diluted in FBS Buffer (anti-h IgG 1:8000 and anti-h IgM 1:200) were dispensed and incubated 3 h at room temperature. Plates were washed 3 times with Washing Buffer, then 100 µl/well of Substrate Solution (1mg/ml p-PNP in Carbonate Buffer containing 1mM $MgCl_2$, pH 9.8) were dispensed. Absorbance was read at 405 nm with a spectrophotometer. Sera values were calculated as (mean absorbance of triplicate) – (mean absorbance of blank triplicate).

Statistical analysis

Data are expressed as mean values and elaborated using the statistical software GraphPad Prism version 6.01. D'Agostino-Parson test was employed as normality test. Student *t*-test or Mann-Whitney unpaired t-test were used to compare continuous variables between groups. Spearman correlation analysis was used to test any relationship between pairs of variables. Differences were deemed statistically significant when $P < 0.05$ (two-tailored test).

RESULTS AND DISCUSSION

In order to study the antibody response against recombinant refolded h-MOG in RTT, we tested 28 RTT patients, 48 non-RTT PDD, and 30 healthy controls by using a home-made SP-ELISA. The recombinant hMOG(1-117) was tested as an antigen evaluating IgG and IgM type antibodies separately. Data distribution of IgG antibody titers detected to hMOG(1-117) in RTT, non-RTT PDD, and controls are plotted in Figure 3.

The overall data distribution were statistically analyzed using D'Agostino-Pearson test and results showed that none of the RTT, non-RTT PDD, or healthy controls group passed the normality test (alpha = 0.05). Then, antibody titer differences between groups were evaluated separately using the Mann-Whitney U-test. Results showed no discriminant differences between RTT and non-RTT PDD patients (P value = 0.6629, two-tailed), RTT and healthy controls (P value = 0.2583, two-tailed),

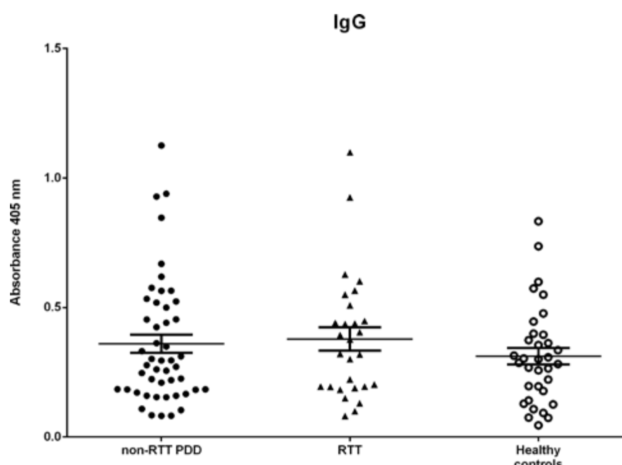

Figure 3. Comparison between IgG antibodies against the hMOG(1-117) identified by SP-ELISA in RTT (▲), non-RTT PDD patient sera (●), and healthy controls (○) respectively. Mean group values and standard error of mean (SEM) are represented.

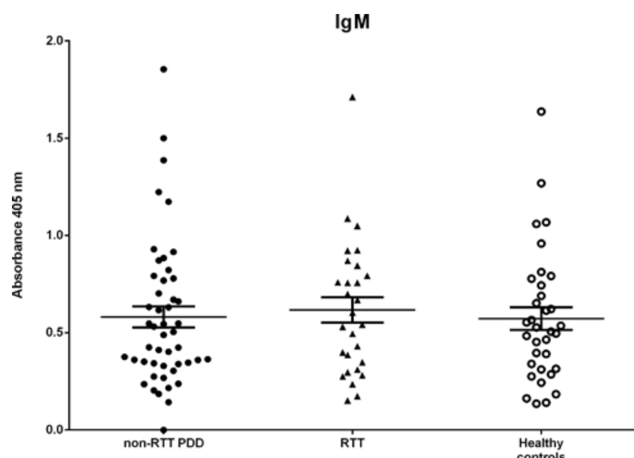

Figure 4. Data distribution of IgM antibodies against hMOG(1-117) identified by SP-ELISA in RTT (▲), non-RTT PDD patient sera (●), and healthy controls (○), respectively. Mean group values and standard error of mean (SEM) are represented.

or non-RTT PDD and healthy controls (P value = 0.6137, two-tailed).

Similar results were observed when IgM-type antibodies were evaluated. Data distribution of IgM antibody values are plotted in Figure 4. The overall data did not present a Gaussian distribution (D'Agostino-Pearson omnibus normality test, alpha = 0.05). Moreover, Mann-Withney test showed no significant statistic differences between groups (P value > 0.05, two-tailed) further evidencing no meaningful differences, thus allowing us to assume that MOG as a possible antigen in RTT and/or non-RTT PDD is irrelevant. Moreover, no relationship was found between IgG and IgM autoantibody levels (nonparametric Spearman correlation, P values > 0.05).

Evidences of anti-MOG antibody-associated diseases in children with acquired demyelinating syndromes, whose sera test were positive for anti-MOG antibodies, have been described.[38]. As discussed in the introduction, the genetic mechanism underlying the RTT syndrome appear directly linked to a demyelinating process. On the other hand, despite previous studies reporting a connection between multiple sclerosis and RTT humoral responses, the role of anti-MOG antibodies in these disorders cannot be clarified. The lack of a clear anti-MOG antibody identification in RTT, herein observed, reminds the open controversy around anti-MOG antibodies in the case of multiple sclerosis, as a kind of parallelism between these diseases.

Previously, our expertise in antibody detection using proteins[37,39,40] or peptides[41,42] prompted us to develop the so-called "chemical reverse approach" in which synthetic peptides were demonstrated to be more effective than native proteins.[43] In fact, their principal advantage is the complete control of the synthetic molecules. Mazzucco *et al.* showed that the N-glucosylation (N-Glc) of the hMOG peptide [Asn^{31}(N-Glc)]hMOG(30-50) allowed to detect antibodies in 40% of an unselected group of multiple sclerosis patients.[44] After almost 20 years, we discovered that anti-N-Glc antibodies from multiple sclerosis patients preferentially recognize adhesin of non-typeable *Haemophilus influenza* hyperglucosylated on asparagine residus exposed on β-turns.[39] Therefore, it is clear that the folding issue is relevant in antibody recognition, and synthetic peptides can be designed to adopt specific conformations, e.g. β-turns.[45,46] Moreover, synthetic conformational peptides can be efficient tools as antigenic probes for serum antibody detection, because they can also include unique chemical modifications, such as asparagine N-glucosylation, on strategic positions in selected sequences. This strategy has been, up to now, to the best of our knowledge, the only winner in detecting antibodies in RTT patient sera.[32,47] Our findings offer a new insight into the mechanism underlying the RTT as they unveil the possible participation of the immune system in this pathology.[48] Moreover, our previous work contributes to elucidate that two disorders such as RTT and autism, seemingly contiguous as they share some behavioral symptoms, but are in fact different for their ruthlessness, life-span expectation, and, as we previously demonstrated, for different immune system derangement. In this context and in light of the results herein presented, the connection of anti-MOG antibodies and RTT remains an uncertainty. In particular, the involvement of the correct folding, but also the lack of a mimicry effect reproducing N-glucosylation (and other molecules) as possible aberrant post-translational modifications on MOG amino acids (involved in triggering immune responses), require to be deeply investigated.

CONCLUSIONS

The screening of RTT patient sera, other relative non-RTT pervasive developmental disorders (non-RTT PDD) including mainly autism, and healthy controls group to evaluate anti-MOG antibodies was uncertain. Despite anti-MOG antibody detection in multiple sclerosis and generally speaking in MOG-IgG–related diseases have improved substantially with cell-based assays, in which the molecules involved in antibody recognition are not fully chemically characterised. On the other hand our preliminary results are in agreement with the idea that the reproduction of post-translational modifications possibly involved in the immune response

could be a must for antibody identification, as it occurs in other diseases connected with RTT, such as multiple sclerosis. In particular, investigating glycan-peptide mimicry in the context of immune response is an emerging topic, pointing toward the multiple roles that unique glycans of bacterial origin may play. These novel preliminary results pave the way to further studies, already ongoing in our laboratories, focused on understanding the responsible agents triggering the immune response in RTT, inducing aberrant conformation and/or N-glucosylation in native proteins, such as Myelin Oligodendrocyte Glycoprotein.

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REFERENCES

1. C.S. Raine, In Myelin, 2nd ed. (Ed: P. Morell) New York: Plenum, **1984**, pp. 1-41.
2. M.H. Grider, C.Q. Belcea, S. Sharma, In: Neuroanatomy, Nodes of Ranvier. Treasure Island (FL): StatPearls Publishing, **2019**, Available from: https://www.ncbi.nlm.nih.gov/books/NBK537273/
3. I.L. Arancibia-Carcamo, D. Attwell, *Acta Neuropathol.*, **2014**, 128(2), 161-175.
4. O.W. Howell, A. Palser, A. Polito, S. Melrose, B. Zonta, C. Scheiermann, A.J. Vora, P.J. Brophy, R. Reynolds, *Brain*, **2006**, 129(12), 3173-3185.
5. K. Susuki, *Clin. Exp. Neuroimmunol.*, **2016**, 7(4), 324-329.
6. K. Susuki, *ASN Neuro.*, **2013**, 5(3), e00118.
7. Y.P. Zhang, Q.L. Huang, C.M. Zhao, J.L. Tang, Y.L. Wang, *Braz. J. Med. Biol. Res.*, **2011**, 44(6), 553-561.
8. B. Emery, *Science*, **2010**, 330(6005), 779-782.
9. B. Emery, *Curr. Opin. Neurobiol.*, **2010**, 20(5), 601-607.
10. L. He, Q.R. Lu, Neurosci. Bull. 2013, 29(2), 129–143.
11. H. Huang, X.F. Zhao, K. Zheng, M. Qiu, *Neurosci Bull.*, **2013**, 29(2), 155-164.
12. C. Brunner, H. Lassmann, T. V. Waehneldt, J. M. Matthieu, C. Linington, *J. Neurochem.*, **1989**, 52, 296-304.
13. T.G. Johns, C.C.A. Bernard, *J. Neurochem.*, **1999**, 72, 1-9.
14. P. Patrick, B. Monika, H. Romana, B. Thomas, R. Markus, *Frontiers in Immunology*, **2017**, 8, 529.
15. P.H. Lalive, *Swiss Med. Wkly.*, **2008**, 138, 692-707.
16. H. C. von Büdingen, A. Bar-Or, S.S. Zamvil, *Curr. Opin. Immunol.*, **2011**, 23, 713-720.
17. A. Ben-Nun, N. Kaushansky, N. Kawakami, G. Krishnamoorthy, K. Berer, R. Liblau, R. Hohlfeld, H. Wekerle, *J. Autoimmun.*, **2014**, 54, 33-50.
18. M.C. Mayer, E. Meinl, *Ther. Adv. Neurol. Disord.*, **2012**, 5, 147-159.
19. M. Reindl, F. Di Pauli, K. Rostásy, T. Berger, *Nat. Rev. Neurol.*, **2013**, 9, 455-461.
20. R. Aharoni, *Expert. Rev. Clin. Immunol.*, **2013**, 9(5), 423-440.
21. T. Berger, P. Rubner, F. Schautzer, R. Egg, H. Ulmer, I. Mayringer, E. Dilitz, F. Deisenhammer, M. Reindl. *N. Engl. J. Med.*, **2003**, 349, 139-145.
22. F. Angelucci, M. Mirabella, G. Frisullo, M. Caggiula, P.A. Tonali, A.P. Batocchi, *Dis. Markers*, **2005**, 21, 49-55.
23. S. Gaertner, K. de Graaf, B. Greve, R. Weissert, *Neurology*, **2004**, 63, 2381-2383.
24. J. Kuhle, R.L.P. Lindberg, A. Regeniter, M. Mehling, F. Hoffmann, M. Reindl, T. Berger, E.W. Radue, D. Leppert, L. Kappos, *J. Neurol.*, **2007**, 254, 160-168.
25. T. Menge, H.C. von Budingen, P.H. Lalive, C.P. Genain, *Eur. J. Immunol.*, **2007**, 37, 3229-3239.
26. M. Reindl, S. Jarius, K. Rostasy, T. Berger, Curr. *Opin. Neurol.*, **2017**, 30(3), 295-301.
27. P.J. Waters, L. Komorowski, M. Woodhall, S. Lederer, M. Majed, J. Fryer, J. Mills, E.P. Flanagan, S.R. Irani, A.C. Kunchok, A. McKeon, S.J. Pittock, *Neurology*, **2019**, 92(11), e1250-e1255.
28. J.T. Ballew, J.A. Murray, P. Collin, M. Mäki, M.F. Kagnoff, K. Kaukinen, P.S. Daugherty, *Proc. Natl. Acad. Sci. U.S.A.*, **2013**, 110(48), 19330-19335.
29. I. Mendel, N. Kerlero de Rosbo, A. Ben-Nun, *Eur. J. Immunol.*, **1995**, 25(7), 1951-1959.
30. G. Pacini, M. Ieronymaki, F. Nuti, S. Sabatino, M. Larregola, R. Aharoni, A.M. Papini, P. Rovero, *J. Pept. Sci.*, **2016**, 22(1), 52-58.
31. M. Reindl, P. Waters, *Nat. Rev. Neurol.*, **2019**, 15(2), 89-102.
32. A.M. Papini, F. Nuti, F. Real-Fernandez, G. Rossi, C. Tiberi, G. Sabatino, S. Leoncini, C. Signorini, A. Pecorelli, R. Guerranti, S. Lavielle, L. Ciccoli, P. Rovero, C. De Felice, J. Hayek, *J. Immunol. Res.*, **2014**, 260973.
33. F. Lolli, B. Mulinacci, A. Carotenuto, B. Bonetti, G. Sabatino, B. Mazzanti, A.M. D'Ursi, E. Novellino, M. Pazzagli, L. Lovato, M.C. Alcaro, E. Peroni, M.C. Pozo-Carrero, F. Nuti, L. Battistini, G. Borsellino, M. Chelli, P. Rovero, A.M. Papini, *Proc. Natl. Acad. Sci. U.S.A.*, **2005**, 102(29), 10273-10278.

34. F. Lolli, B. Mazzanti, M. Pazzagli, E. Peroni, M.C. Alcaro, G. Sabatino, R. Lanzillo, V. Brescia Morra, L. Santoro, C. Gasperini, S. Galgani, M.M. D'Elios, V. Zipoli, S. Sotgiu, M. Pugliatti, P. Rovero, M. Chelli, A.M. Papini, *J. Neuroimmunol.*, **2005**, 167(1-2), 131-137.
35. K. Sharma, J. Singh, P.P. Pillai, E.E. Frost, *J. Mol. Neurosci.*, **2015**, 57(2), 176-84.
36. H. Tani, N. Ishikawa, Y. Kobayashi, S. Yamaoka, Y. Fujii, K. Kaneko, T. Takahashi, M. Kobayashi, *Brain Dev.*, **2018**, 40(10), 943-946.
37. F. Gori, B. Mulinacci, L. Massai, C. Avolio, M. Caragnano, E. Peroni, S. Lori, M. Chelli, A.M. Papini, P. Rovero, F. Lolli, *J. Neuroimmunol.*, **2011**, 233(1-2), 216-220.
38. Y. Hacohen, T. Rossor, K. Mankad, W. Chong, A. Lux, E. Wassmer, M. Lim, F. Barkhof, O. Ciccarelli, C. Hemingway, *Developmental medicine & child development*, **2018**, 60(4), 417-423.
39. M.T.C. Walvoort, C. Testa, R. Eilam, R. Aharoni, F. Nuti, G. Rossi, F. Real-Fernandez, R. Lanzillo, V. Brescia Morra, F. Lolli, P. Rovero, B. Imperiali, A.M. Papini, *Scientific Reports*, **2016**, 6, 39430.
40. F. Real-Fernández, R. Cimaz, G. Rossi, G. Simonini, T. Giani, I. Pagnini, A.M. Papini, P. Rovero, *Anal. Bioanal. Chem.*, **2015**, 407(24), 7477-7485.
41. F. Nuti, A. Gallo, F. Real-Fernandez, M. Crulli, C. Rentier, F. Piarulli, E. Peroni, G. Rossi, P. Traldi, P. Rovero, A. Lapolla, A.M. Papini, *Archives of Biochemistry and Biophysics*, **2018**, 659, 66-74.
42. M. Di Pisa, S. Pascarella, M. Scrima, G. Sabatino, F. Real Fernández, M. Chelli, D. Renzi, A. Calabrò, A.M. D'Ursi, A.M. Papini, P. Rovero, *J. Med. Chem.*, **2015**, 58(3), 1390-1399.
43. A.M. Papini, *J. Pept. Sci.*, **2009**, 15(10), 621-628.
44. S. Mazzucco, S. Matà, M. Vergelli, R. Fioresi, E. Nardi, B. Mazzanti, M. Chelli, F. Lolli, M. Ginanneschi, F. Pinto, L. Massacesi, A.M. Papini, *Bioorg. Med. Chem. Lett.*, **1999**, 9(2), 167-172.
45. A. Carotenuto, A.M. D'Ursi, B. Mulinacci, I. Paolini, F. Lolli, A.M. Papini, E. Novellino, P. Rovero, *J. Med. Chem.*, **2006**, 49(17), 5072-5079.
46. A. Carotenuto, M.C. Alcaro, M.R. Saviello, E. Peroni, F. Nuti, A.M. Papini, E. Novellino, P. Rovero, *J. Med. Chem.*, **2008**, 51(17), 5304-5309.
47. F. Real Fernández, M. Di Pisa, G. Rossi, N. Auberger, O. Lequin, M. Larregola, A. Benchohra, C. Mansuy, G. Chassaing, F. Lolli, J. Hayek, S. Lavielle, P. Rovero, J.M. Mallet, A.M. Papini, *Biopolymers (Pept Sci).*, **2015**, 104(5), 560-576.
48. C. De Felice, S. Leoncini, C. Signorini, A. Cortelazzo, P. Rovero, T. Durand, L. Ciccoli, A.M. Papini, *J. Hayek, Autoimmunity Reviews*, **2016**, 15, 411-416.