Original article

Folia Neuropathologica

Recombinant forms of myelin antigens expressed on Chinese hamster ovary (CHO) cells as a tool for identification of autoantibodies in serum of multiple sclerosis patients

Ewa Jaśkiewicz[1,2], Grażyna Michałowska-Wender[3,4], Anna Pyszczek[2], Mieczysław Wender[4]

[1]Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland, [2]Institute of Biotechnology and Environmental Sciences, University of Zielona Góra, Poland, [3]Laboratory of Neurogenetics, University of Medical Sciences, Poznań, Poland, [4]Neuroimmunological Unit, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Poznań, Poland

Folia Neuropathol 2010; 48 (1): 45-48

Abstract

A contribution of B cells and autoantibodies has been demonstrated in MS leading to interest in the use of such autoantibodies as diagnostic or prognostic markers and as a basis for immunomodulatory therapy. ELISA and Western fail to detect reactivity against epitopes displayed by native antigens expressed on myelin sheats. We describe a cell-based assay that specifically identifies serum antibodies directed against three major myelin autoantigens: MBP, PLP and MOG. The method detects antibody binding to recombinant antigens in their native conformation on MBP, PLP and MOG transfected mammalian (hamster ovary) cells. 36 patients with relapsing-remitting MS diagnosed according to criteria of McDonald were recruited. Age 38.2 and duration of the disease 7.1. Serum anti-MBP, anti-PLP and anti-MOG IgG autoantibodies were detected in MS patients and 35 healthy donors by FACS analysis. Compared with healthy controls the titres of IgG autoantibodies directed against membrane-bound recombinant myelin antigens were most significantly increased for PLP, no quite significant for MBP and not significant for MOG. The titres of anti-MBP antibodies were low in contrast to high titre of anti-MOG antibodies in both groups suggesting a nonspecific binding. The cell-based assay detection of autoantibodies directed against recombinant myelin antigens could be a useful tool providing the serological markers in diagnosis and progression of MS. Indeed, it could allow obtaining molecular characteristics of disease in each patient in term of an antibody response against certain myelin and non-myelin antigens. We have shown that in RRMS patients elevated level of serum antibodies against PLP is significant, what should be considered in search for specific immunomodulatory therapy in MS.

Key words: *myelin antigens, multiple sclerosis, MBP, PLP, MOG.*

Communicating author:

Grażyna Michałowska-Wender, Laboratory of Neurogenetics, University of Medical Sciences, Poznań, Poland, phone +48 61 869 17 91, fax +48 61 869 16 97, e-mail: grazynawender@wp.pl

Introduction

The still actual hypothesis to pathogenesis of multiple sclerosis (MS) that the destruction of myelin in the plaques of MS within the central nervous system is due to antigen specific autoimmunity. Several myelin proteins may be encephalitogenic: myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendroglia glycoprotein (MOG), what is connected with contribution of B cells and autoantibodies in pathogenesis of MS.

That is why the interest in the studies of antibodies as basis for immunotherapy of MS as well as diagnostic or prognostic marker. One of the promising directions in therapy of MS is the search for the method of antigen – specific tolerance. The last developed trial is DNA vaccination using DNA plasmid vaccine encoding myelin basic protein, the vaccine named BHT-3009 [2]. The results indicate that treatment of relapsing-remitting MS patients with the low dose of BHT leads to the reduction of the rate of new enhancing lesions in magnetic resonanse imaging. The immunological data obtained in this study indicate that the above decribed treatment induced antigen specific immune tolerance to MBP.

The use of peripheral level of autoantibodies against MBP, PLP or MOG as marker of multiple sclerosis was proposed in several studies [9]. The diagnostic value of such an aproach is limited by fluctuation of autoantibodies titre with the phase of MS and the disease activity [1]. Nevertheless there are the opinions, that a combination of tests for autoantibodies, lymphocyte activation, cytokine production and MRI imaging may increase the sensitivity and specificity of MS diagnosis [10].

The significance of autoantibodies in the diagnosis and treatment of MS testify the nead of detailed study of the problem with the best available methods.

A common problem in studies of humoral immunity is that accurate detection of antibodies depends highly on the conformation of the antigen used for detection. Therefore widely used techniques, including ELISA and Western blotting, may fail to detect reactivity against pathogenic epitopes displayed by native antigens expressed on myelin sheath. We describe a cell-based assay that specifically identifies serum antibodies directed against three major myelin autoantibodies: MBP, PLP and MOG. The proposed method detecting antibody binding to recombinant antigens in their native conformation on MBP, PLP or MOG transfected mammalian (hamster ovary) cells.

Material

36 patients (9 males and 27 females) with relapsing-remitting multiple sclerosis diagnosed according to revised criteria of McDonald [8] were recruited. They were aged from 22 to 53 years (mean 38.2). The mean duration of MS was 7.1 years (ranging from 2 to 18 years). Mean EDSS was 3.0 (1.0 to 4.5). The patients were not treated previously by immunomodulatory treatment.The control group comprised 35 healthy adult blood donors (7 males and 28 females), aged from 26 to 48 years (mean 36.5 years). The study was approved by the Local Ethic Committee.

Methods

Expression vectors

cDNA encoding full length of human MOG (alpha1) in pcDNA3 eucariotic expression vector was kindly provided by Dr. D. Pham-Dinh (INSERM, Paris, France). Expression vectors containing cDNAs coding for 21.5 kDa isoform of human MBC/pcDNA3 or 26.6 kDa classic form of PLP/pcDNA3 were prepared as described previously [5].

Cell culture and transfection

Wild type Chinese Hamster Ovary Cells (CHO) were cultured in OPTI-Mem (Gibco BRL) containing 10% fetal calf serum (Gibco BRL) and 2mM glutamine (Sigma Chemical Co.). Stable transfection of CHO cells with MOG/pcDNA3 was performed in serum free medium using FuGENE 6 reagent (Roche Diagnostic) according to the manufacturer's protocol as described previously for expression vectors coding for human MBP and PLP [5]. Transfected CHO cells were selected in complete OPTI-Mem medium containing 0.4 mg/ml active geneticin (G418, Gibco BRL) and analysed for MOG expression by flow cytometry. Clonal cell lines expressing MOG recombinant proteins were isolated by repetitive cloning by limiting dilution.

Flow cytometry analysis

The transfected CHO cell clones expressing the recombinant forms of human MBP, PLP or MOG were detached with 0.2% EDTA in Hank's balanced salt solution pH 7.4, washed and collected in cold phos-

phate buffered saline pH 7.4 (PBS) containing 1% FCS. MBP expressing CHO cells were fixed and permeabilized with 0.1% saponin and 2% p-formaldehyde solution in PBS for 15 min at 4°C. All cells were blocked in PBS containing 10% FCS for 15 min at 4°C, washed and then incubated for 1 h at 4°C with human serum (1 : 10). Incubation with fluorescein (FITC) – conjugated goat anti-human Ig antibody (Jacson ImmunoResearch) was performed for 30 min at 4°C with intervening washing with PBS. Directly after labeling cells were analyzed for fluorescence intensity using a flow cytometer (FACSCalibur, Becton Dickinson).

Statistical evaluation

The t test assumed that the data are sampled from population that follow Gaussion distribution. This assumption was tested using the method Kolmogonow and Smirnow. The significance of differences was tested by means of Student test.

Results

Compared with healthy controls the titres of IgG autoantibodies directed against membrane-bound recombinant myelin antigens were most significantly increased for PLP ($p<0.0001$), no quite significant for MBP ($p= 0.00565$) and not significant for MOG ($p=0.7774$). There was not clear cut relation between myelin antigens and various clinical parameters. The detailed results are presented in Table I.

Discussion

Multiple sclerosis is an inflammatory demyelinating disease, of unknown etiology, but several lines of evidence – experimental as well as clinical support the hypothesis that autoimmune mechanism plays a dominating role in the development of the disease. The various clinical course, unpredictable therapeutic effects, heterogenous genetic background and diffe-

Table I. Recombinant forms of myelin antigens in serum of MS patients

		Controls	Multiple sclerosis	
Proteolipid protein (PLP)		**n = 35**	**n = 36**	
	Mean	0.605	2.957	*The titer was*
	SD	± 1.081	± 1.138	*increased*
	Medium	0.2700	2.830	*in all studied*
	Lower 95%	0.2336	2.572	*patients.*
	Upper 95%	0.9767	3.342	
Myelin basic protein (MBP)		**n = 35**	**n = 36**	
	Mean	1.011	1.602	*The titer was*
	SD	± 1.173	± 1.383	*increased*
	Medium	1.030	1.455	*in 12 patients.*
	Lower 95%	0.6076	1.134	
	Upper 95%	1.414	2.071	
Myelin oligodendrocyte glycoprotein (MOG)		**n = 20**	**n = 36**	
	Mean	3.168	3.340	*There were no*
	SD	1.923	2.304	*differences between*
	Medium	2.945	2.895	*particular MS*
	Lower 95%	2.268	2.560	*and control subjects.*
	Upper 95%	4.067	4.120	

rent immunopathological subtypes indicate for further studies of cellular and humoral aspects of MS. A contribution of B cells and autoantibodies lead to interest of sophisticated detection of myelin antigens in the peripheral blood, as easily possible in use in clinical praxis. The used methods detects antibody binding to recombinant antigens in their native conformation on MBP, PLP or MOG transferred mammalian (hamster ovary) cells. It should be stressed that only conformated antigens are pathogenic.

In the central nervous system myelin basic protein (MBP) and proteolipid protein (PLP) are the major part of the myelin protein. Several techniques have been employed to search the presence of anti-MBP antigens in MS patients [7]. Despite of some controversial results there exist the overhelming opinion that the antibodies against MBP may be detected in sera of MS patients. In our studies performed with recombinant form of myelin antigen we have established only mild increase in MS patients. It should be added that the titres of anti-MBP antibodies was low not only in MS patients but also in healthy blood donors.

The myelin proteolipid protein (PLP), reffered also as lipophilin, is an intristic protein associated with myelin lipids. In our studies we have established a significantly higher titres of antibodies directed against PLP, than in control material.

Conformational epitopes of MOG (myelin oligodendrocyte glycoprotein) provide a target for demyelinating autoantibodies in experimental autoimmune encephalomyelitis (EAE) and probable also in multiple sclerosis. The survey of published studies concerning serum antibodies against MOG in patients with multiple sclerosis was presented in review paper of Reindl *et al*. [9]. The results and used method are very different. Percentage of anti-MOG antibodies varied from 0 to 59%. Our results should be considered as negative. The titres of antibodies against MOG were high in both MS and control studies, what suggest a nonspecific binding.

Antigen specific approaches are effective in EAE [4]. In MS the situation is more complex, due to probable existence of immunological subtypes. Very impressive in this respect are the findings of Greer *et al.* [3], who found a correlation of blood T cells and antibody reacting to various myelin proteins with HLA-type and lesion distribution in MS. The strongest immunoreactivity was connected with presence of brain and cerebellar lesions.

The heterogenity of immunoreactions in particular MS cases determines the necessity of individualised immunological treatment of MS patients. Already in 1994 Warren *et al*. [11] expressed opinion, that there are two distinct forms of multiple sclerosis: anti-myelin basic protein asssociated MS versus anti-proteolipid associated MS. In view of our studies the greater interest should be concentrated on antigen-specific therapy against myelin proteolipid protein.

The problem to the answered is, if the treatment of MS with methylprednisolone, what was noticed concerning the peripheral blood immunomarkers [6] influences the level of circulating anti-myelin antibodies.

References

1. Angelluci F, Mirabella M, Frisullo G, Caggiula M, Tonali PA, Batocchi AP. Serum levels of anti-myelin antibodies in relapsing-remitting multiple sclerosis patients during different phases of disease activity and immunomodulatory therapy. Dis Markers 2005; 21: 49-55.
2. Garren H, Robinson WH, Krasulová E, Havrdová E, Nadj C, Selmaj K, Losy J, Nadj I, Radue EW, Kidd BA, Gianettoni J, Tersini K, Utz PJ, Valone F, Steinman L; BHT-3009 Study Group. Phase 2 trial of a DNA vaccine encoding myelin basic protein for multiple sclerosis. Ann Neurol 2008; 63: 611-620.
3. Greer JM, Csurhes PA, Muller DM, Pender MP. Correlation of blood T cell and antibody reactivity to myelin proteins with HLA type and lesion localization in multiple sclerosis. J Immunol 2008; 180: 6402-6410.
4. Holmøy T, Vartdal F. The immunological basis for treatment of multiple sclerosis. Scand J Immunol 2007; 66: 374-382.
5. Jaśkiewicz E, Jedynak A, Zioło E. Recombinant forms of myelin basic protein and proteolipid protein expressed in CHO cells. Acta Biochimica Polonica 2005; 52: 863-866.
6. Michałowska-Wender G, Wender M. Peripheral blood cell immunomarkers in the course of methylprednisolone treatment of multiple sclerosis relapses. Folia Neuropathol 2008; 46: 134-138.
7. O'Connor KC, Chitnis T, Griffin DE, Piyasirisilp S, Bar-Or A, Khoury S, Wucherpfennig KW, Hafler DA. Myelin basic protein-reactive autoantibodies in the serum and cerebrospinal fluid of multiple sclerosis patients are characterized by low-affinity interactions. J Neuroimmunol 2003; 136: 140-148.
8. Polman Ch, Reingold S, Edan G et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 2005; 58: 840-846.
9. Reindl M, Khalil M, Berger T. Antibodies as biological markers for pathophysiological processes in MS. J Neuroimmunol 2006; 180: 50-62.
10. Vojdani A, Vojdani E, Cooper E. Antibodies to myelin basic protein, myelin oligodendrocytes peptides, alpha-beta-crystallin, lymphocyte activation and cytokine production in patients with multiple sclerosis. J Intern Med 2003; 254: 363-374.
11. Warren KG, Catz I, Johnson E, Mielke B. Anti-myelin basic protein and anti-proteolipid protein specific forms of multiple sclerosis. Ann Neurol 1994; 35: 280-289.