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pt point mutation in plp gene results in hyperexpression of MOG in hypomyelinated rabbit

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Abstract. Myelin/oligodendrocyte glycoprotein (MOG) is a minor myelin protein that belongs to the immunoglobulin gene superfamily and evokes demyelination based on immunological response. Localized preferentially at the external surfaces of myelin sheaths, it is one of the primarily target autoantigens in experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis. Elevated MOG content has been found in the myelin fraction of the rabbits affected by the mild form of paralytic tremor (*pt*) disease, evoked by natural, point mutation in exon 2 of plp gene. A single T→A transversion results in substitution of histidine^{36} by glutamine in PLP and it's splicing variant DM-20 molecules. The affected animals, although strictly controlled for *pt* trait, differ significantly in their phenotypes, distinguished by the severity of neurological symptoms. It was shown that the degree of CNS hypomyelination and deficiency of PLP/DM-20 correlates well with the severity of neurological symptoms and is highest in the most strongly affected animals. Variety of phenotypes generated from *pt* genotype together with previously observed MOG hyperexpression suggested possible contribution of immunological component to the *pt* disease. Present studies indicate that MOG expression depends both on the phenotype and the age of affected rabbits and most probably mirrors retardation in myelinogenesis process caused by *pt* mutation.

Key words: myelin/oligodendrocyte glycoprotein, MOG, hypomyelination, plp mutant, *pt* rabbit

INTRODUCTION

Term paralytic tremor (*pt*) is attributed to the hereditary, X-linked neurological disorder of Chinchilla rabbits and describes characteristic symptoms of the disease (Osetowska and Luszawski 1975). As it has been proved lately, the molecular base of the *pt* disease is a point mutation in plp gene resulting in a changed 36th aa (His→Glu) (Tosic et al. 1994). Typical feature of the mutation is significant hypomyelination of the rabbit central nervous system observed in many morphological (Zelman and Taraszewska 1984, Taraszewska and Zelman 1985) and biochemical studies (Domańska-Janik et al. 1988, Sypecka and Domańska-Janik 1995b), although glia cell number is even slightly elevated and they enter normal differentiation and maturation processes (Taraszewska and Zelman 1987, Sypecka et al. 1995). Amounts of myelin are reduced to different extent that mirrors spectrum of phenotypes, generated from *pt* genotype (Sypecka, submitted) and distinguished by the severity of neurological symptoms.

Myelin deficiency is accompanied by general depletion of other myelin constituents, as myelin specific proteins and glycolipids (Sypecka and Domańska-Janik 1994, 1995b, Sypecka et al.1995). Examination of myelin fraction isolated from *pt* brains revealed severe reduction of PLP and DM20, protein products of the mutated gene, whereas the expression of other myelin proteins is almost normal (MAG; 18,5 kD MBP isoform) or slightly reduced (CNP; 21,5 kD MBP isoform). Moreover, a scale of PLP/DM-20 deficiency depends on the expressed phenotype and is highest in the most strongly affected animals (Sypecka and Domańska-Janik 1994).

Hypomyelination and reduction of myelin-building proteins are the typical features of all plp gene mutations (Quarles 1990, Konat and Wiggins 1992). However, developmental studies on the myelinogenesis revealed hyperexpression of myelin/oligodendrocyte glycoprotein (MOG) that is phenomenon of pt mutation (Sypecka and Domańska-Janik 1995b). It seems to be interesting since this minor myelin protein belongs to the immunoglobulin gene superfamily (Gardinier et al. 1992, Pam-Dinh et al. 1993) and is highly immunogenic (Linington and Lassman 1987). Since MOG expresses also the L2/HNK-1 epitope (Burger et al. 1992, 1993), it is supposed to be a cell adhesion molecule.

Considering MOG properties and its hyperexpression in *pt* rabbits representing mild course of the disease, a question arises about possible contribution of immunological component to the *pt* disease. Adressing this question, we compared MOG contents in myelin fractions isolated from mutants that differ in their phenotypes.

METHODS

The animals were supplied by the Department of Comparative Neurology, Polish Academy of Sciences (Mińsk Maz., Poland). Mutants (homozygous or hemizygous) were strictly controlled for both their *pt* trait and the phenotype, those presenting either phenotype II or IV were selected for the study. Age matched control animals derived from the same Chinchilla rabbits lane. Sacrification was made by decapitation. Each brain, without cerebellum, was immediately frozen in a liquid nitrogen and stored in -70° C until use.

Myelin preparation

The hemispheres were homogenised in 0.32 M sucrose with protease inhibitors (0.1 mM PMSF; 10 μg/ml antipain; 5 μg/ml leupeptin; 5 μg/ml pepstatin; 2 mM EDTA; 2 mM EGTA). Myelin fractions were isolated according to Norton and Poduslo (1973).

Protein concentration was determined by the method of Lowry et al. (1951).

Immunoblotting

Samples containing 80 μg of proteins, prepared as described by Amiquet et al. (1992), were separated on 12% polyacrylamide gel (Leammli 1970) and subsequently either stained with Commassi

blue or transferred to nitrocellulose membranes (Amersham) using the electroblotting technique (Towbin et al. 1979). The immunoblots were incubated with anti-MOG antibody applied in 1:500 dilution (Linington et al. 1984) and subsequently with the horseradish peroxidase-conjugated sheep anti-mouse IgG (Amersham) as a secondary antibody, than detected by ECL technique (Amersham) and exposed to the Hyperfilm^{TM}-ECL (Amersham) for approximately 30 min.

RESULTS

Analysis of myelin fractions isolated from rabbits of various phenotypes revealed significant differences in MOG content that concerned not only particular phenotypical classes but also the age groups (Fig. 1). A tremendous hyperexpression of the protein is characteristic for both mutant phenotypes. Whereas MOG content slowly and gradually increases in control animals during development, no such tendency is observed in mutants. Contrary, in phenotype II it's highest content is observed in myelin of 4-weeks old mutants, i.e. at the beginning of the investigated developmental period, than decreases but still remains several-fold elevated when compared to controls. In phenotype IV, the MOG expression peaks at 6-weeks old animals than drops below the control level.

Studies on the 4 weeks old rabbits group, for which it was possible to collect four different phenotypes, revealed that the MOG content is lo-

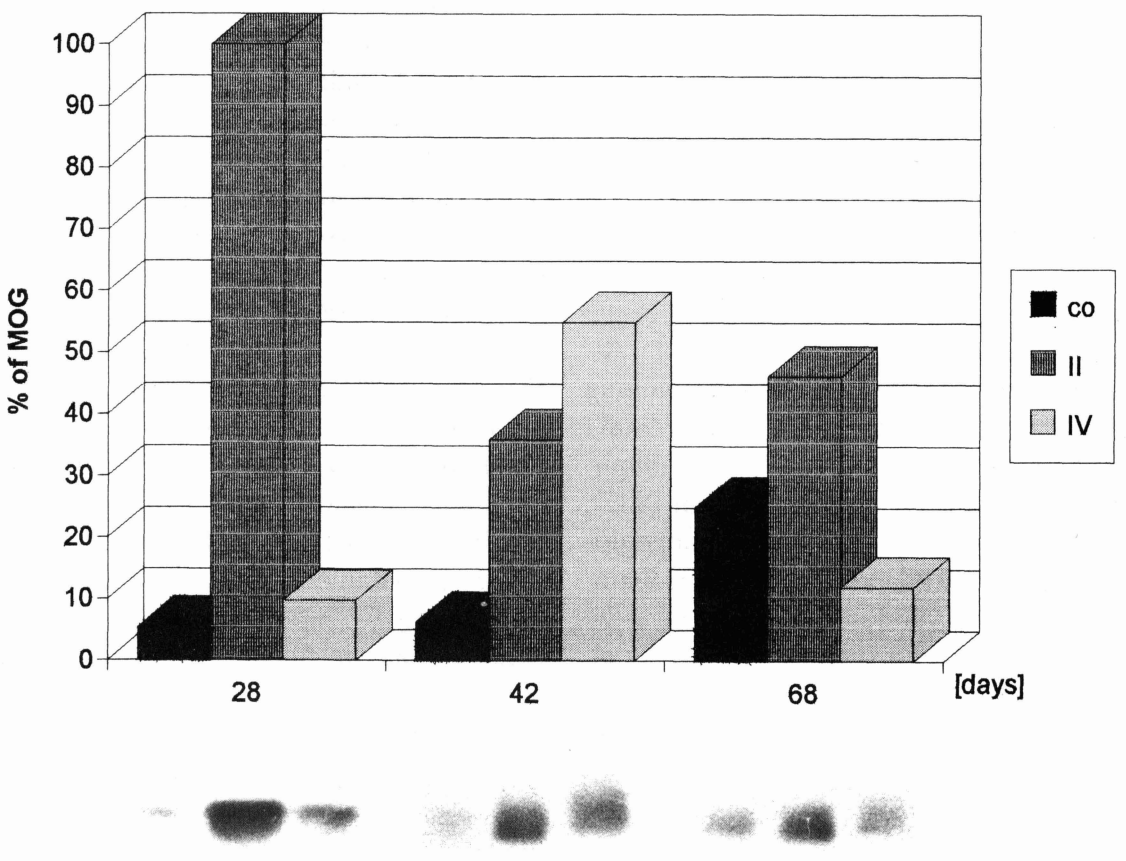

Fig. 1. Relative amounts of of MOG in controls and mutants of phenotype II and IV, respectively, as revealed by immunoblotting. Graph shows the densitometric analysis (GelScanXL) of the presented most representative immunoblot, indicating that MOG expression depends on the phenotype and the age of *pt* mutant.

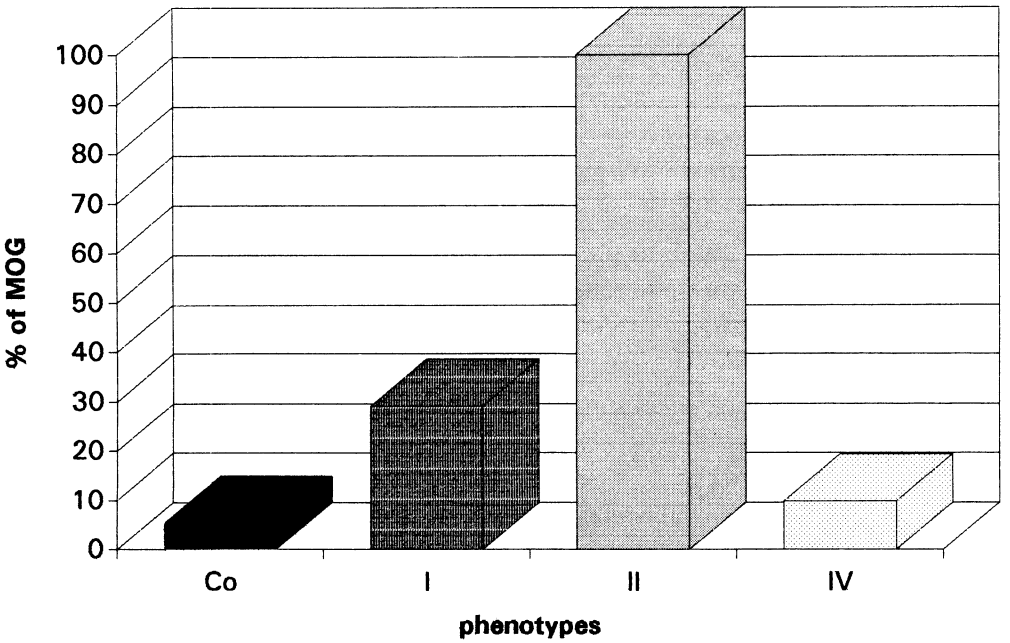

Fig. 2. Relative MOG contents in myelin fraction of 4-weeks old rabbits of different phenotypes, as revealed of densitometric analysis of immunoblots. Pattern of MOG expression in particular phenotypes seems to mirror retardation in myelinogenesis, caused by *pt* mutation.

west in control rabbits, nearly two-fold increased in most severely affected mutants and even more significantly elevated in asymptomatic course of the disease. The highest MOG amounts has been observed in moderately affected mutants (Fig.2).

DISCUSSION

Present study reveals that one of the pleiotropic effects of *pt* mutation is MOG hyperexpression. Although MOG is minor myelin protein, accounting for about 0.05% of total myelin protein content (Amiguet et al. 1992), it is known to posses immunogenic properties (Linington and Lassman 1987). Since this glycoprotein belongs to the immunoglobulin gene superfamily (Gardinier et al. 1992, Pam-Dinh et al. 1993) and is preferentially located at the external surface of myelin sheaths (Brunner et al. 1987), it might be an easy target for antibodies leading to immunological reactions (Linington and Lassman 1987, Kerlero de Rosbo et al. 1990). It's contribution to the diseases based on autoimmunological responses has been postulated, e.g. for multiple sclerosis (Xiao et al. 1991). Another MOG property is expression of the L2/HNK-1 epitope (Burger et al. 1992, 1993) characteristic for the cell adhesion molecules (Schacher 1989).

Concerning the known MOG properties, it's hyperexpression in *pt* mutants is an interesting phenomenon. As indicated by developmental study on myelinogenesis in rabbits (Sypecka and Domańska-Janik 1995a), MOG appears at the end of active myelination period, after expression of major protein constituents of myelin (PLP, MBP, CNP, MAG). On the other hand, it has been proven that myelination is not only deficient but also delayed in *pt* mutants (Sypecka and Domańska-Janik 1995a). Due to occurring retardation, active myelination period is shifted in time and this might possibly explain why MOG expression is highest in mutant phenotype II aged 4 weeks. Active myelinogenesis is followed by the period of steady-state level of the process, what in normal rabbits corresponds to approximately 6-7 weeks of life. At this age, MOG content peaks in mutant phenotype IV (most affected animals) but is also high in phenotype II and such pattern of MOG hyperexpression probably mirrors the retardation in myelinogenesis in *pt* rabbits, since it's recognized as a marker of oligodendrocyte maturation (Scolding et al. 1989). Decreased amounts of this glycoprotein in phenotype IV in older animals might indicate an active elimination of the myelin fractions containing the large portions of the antigens. This hypothesis

should be however further evaluated by examination of MOG mRNA levels, since it's still possible that aberration in subsequent myelin lamellae appositing, resulting in uncompaction of myelin sheaths characteristic for *pt* disease leads to different exposure of the antigen in mutants. It should be mentioned here, that no evident signs of inflammatory processes or demyelination are observed in pt mutant brain under light or electron microscopy (Sypecka et al. 1995).

On the other hand, studies on mutants with defects in different myelin genes, as PLP in jimpy, MBP in myelin deficient and MAG in quacking mice indicated a significant deficit in MOG amounts independently on the type of the mutation (Matthieu and Amiguet 1990, Amiguet et al. 1992). However, in shiverer mutation characterized by a huge deficit of most of the myelin specific proteins (e.g. MBP content represents about 5% of control values), MOG expression is not so drastically reduced (70% of control values in the period of active myelination.

It's well known that program of myelinogenesis starts at the strictly determined period of ontogenesis and is regulated or influenced by a spectrum of factors as transcription and signal transduction factors. A common cis-regulatory elements have been found in promotor regions of some myelin proteins genes (Berndt et al. 1992). Expression of certain genes seems however to be induced/regulated in the different way, as probably MAG (Konat et al. 1988) and MOG what could explain that in the particular cases they are not affected by the mutation to the same extent as other myelin genes. It has been observed, that MOG mRNA has no canonical polyadenylation signal (AAUAAA) and polyA tail is limited to only 13 A residues (Gardinier et al. 1992) what suggest that its half life might be much shorter than mRNAs of other myelin specific proteins.

As it was shown, *pt* mutation affects expression of various myelin proteins to different extent (Sypecka and Domańska-Janik 1995b) and most probably accelerates their turn-over (Domaska et al. 1987). The defect in plp gene causes disturbances in trafficking in oligodendrocyte RER leading to alteration in protein transport towards forming myelin (Sypecka et al. 1995). Short half life of MOG mRNA together with hypothetically increased turn over might be responsible for protection from influence of different factors, resulting in elevated MOG biosynthesis.

Significant differences in MOG hyperexpression between mutant phenotypes II and IV indicated by present study seems to be interesting for further investigations. A possible contribution of immunological component will be further evaluated.

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