Chapter 14
Antibodies to Short Synthetic *Acinetobacter* and *Pseudomonas* Peptide Sequences Resembling Myelin and Neurofilaments in Multiple Sclerosis Patients

14.1 Introduction: The Use of Synthetic Peptide Sequences of Myelin and Neurofilaments to Study the Role of Antibodies in Multiple Sclerosis Patients

Peptide sequences of myelin basic protein and neurofilaments are relevant in the study of these epitopes for their role in the aetiology of multiple sclerosis.

Proteonomic investigations have identified an amino acid homology between a sequence present in 4-carboxy-muconolactone decarboxylase of *Acinetobacter calcoaceticus,* γ-carboxymuconolactone decarboxylase of *Pseudomonas aeruginosa* and a sequence of myelin basic protein (residues 110–124).

A peptide sequence of myelin basic protein (residues 110–124) was found to produce experimental allergic encephalomyelitis (EAE) in guinea pigs (Ben-Nun et al. 1981).

A similar epitope of myelin basic protein (residues 101–120) also induces experimental allergic encephalomyelitis in DA rats (Stepaniak et al. 1997).

Myelin basic protein (residues 82–100) have been demonstrated to be immunodominant in multiple sclerosis patients for both T cells and autoantibodies (Salvetti et al. 1993).

Generation of T cell clones from multiple sclerosis patients defined the specific peptide of myelin basic protein (residues 110–124) in their induction (Mazza et al. 2002).

Another sequence has been identified involving an epitope of myelin oligodendrocyte glycoprotein (MOG 43–57) which is known to induce experimental allergic encephalomyelitis in many mouse strains including Biozzi mice (Amor et al. 1994).

A closely related sequence to myelin oligodendrocyte glycoprotein (MOG) is found in 3-oxoadipate CoA transferase sub-unit in *Acinetobacter calcoaceticus* (residues 83–97) (Table 14.1). This sequence can also be found in the same enzyme of *Pseudomonas putida.*

A. Ebringer, *Multiple Sclerosis, Mad Cow Disease and Acinetobacter*,
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Table 14.1 15-mer peptides used in ELISA and EAE studies comparing similar sequences in mouse myelin basic protein and myelin oligodendrocyte glycoprotein to *Acinetobacter* sp. and *Pseudomonas aeruginosa* (identical amino acids in bold)

Peptide	Source	Amino acid sequence	Amino acid position	Location
1	*Acinetobacter sp.*	QNFIS**RF**A**WG**EVNSR	38–52	4-carboxy-muconolactone decarboxylase
2	*Pseudomonas aeruginosa*	QEMIT**R**H**AWG**D1WTR	38–52	γ-carboxy-muconolactone decarboxylase
3	MBP	GLSL**SRF**S**WG**AEGQR	110–124	
4	*Acinetobacter sp.*	DSYVFDE**LYR**A**GK**IB	83–97	3-oxoadipate Co A-transferase subunit A
5	MOG	PFSRVVH**LYR**N**GK**DQ	43–57	
6	Human papilloma virus (type 16)	TVIQDGDMVHTGFGA	219–233	Major capsid protein LI

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The human papilloma virus sequence was used as a control peptide
A alanine, *D* aspartic acid, *E* glutamic acid, *F* phenylalanine, *G* glycine, *H* histidine, *I* isoleucine, *K* lysine, *L* leucine, *M* methionine, *N* asparagine, *P* proline, *Q* glutamine, *R* arginine, *S* serine, *T* threonine, *V* valine, *W* tryptophan, *Y* tyrosine

Recombinant myelin oligodendrocyte glycoprotein (MOG) T cells from multiple sclerosis patients have been shown to respond to three main regions of myelin oligodendrocyte glycoprotein (MOG) including MOG (residues 34–56) (Kerlero de Rosbo et al. 1997).

Similar observations were made by other workers, linking the responses to the clinical course of the disease (Correla and de los Milagros 2003).

Anti-MOG antibodies in multiple sclerosis patients have a highly variable epitope specificity depending on the stage of the multiple sclerosis disease (Haase et al. 2001).

Some groups have demonstrated increased autoantibodies to MOG (residues 35–55) especially in secondary progressive multiple sclerosis (Kennel de March et al. 2003).

Clearly elevated levels of autoantibodies to myelin basic protein and myelin oligodendrocyte glycoprotein have been reported by several groups to be present in patients with multiple sclerosis.

The aims of the present study were to determine whether multiple sclerosis patients have elevated levels of antibodies to the mimicry peptides found in *Acinetobacter* and *Pseudomonads aeruginosa* species, myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) when compared to stroke patients or healthy blood donors.

14.2 Materials and Methods: Serum Samples, Peptides and ELISA

Serum Samples

Sera from 26 multiple sclerosis patients (9 males and 17 females having a mean age 42 years, range: 29–55 years) were obtained from the Institute of Neurology at the Hospital for Nervous Diseases, Queen Square, London, as previously described.

Serum samples were also obtained from 20 patients in the Department of Geriatric Medicine at University College Hospital, London who had suffered from unilateral hemiplegia due to a "cerebro-vascular accident" (CVA) or stroke (10 males and 10 females having a mean age of 80.5 years, range 69–94 years).

Sera from 25 subjects attending the London Blood Donor services were used as healthy controls (12 males and 13 females, mean age 40.6 years, range 22–67 years).

Peptides

Myelin basic protein (MBP) (residues 110–124) with amino acid sequences according to mouse myelin basic protein was synthesized which resembles the sequence found in human myelin basic protein. The only difference is that MBP differs from the human sequence at position 122 (Lys to Arg).

Myelin oligodendrocyte glycoprotein (MOG) peptide (residues 43–57) was synthesized according to mouse MOG and closely resembles the amino acid sequence found in human MOG.

Bacterial mimicry peptides, *Acinetobacter* 4-carboxymuconolactone decarboxylase, *Pseudomonas* γ-carboxy-mucono-lactone decarboxylase and *Acinetobacter* 3-oxoadipate CoA-transferase subunit A were also synthesized (Table 14.1).

The 15-mer peptides incorporated a C-terminal amide and were purified via High-performance liquid chromatography (HPLC) (ABC Biotechnology, UK).

Preparation of MOG and MBP

Recombinant MOG (rmMOG) (amino acid residues 1–116) was synthesized as follows.

Escherichia coli strain JM109 was transfected with the cDNA encoding murine MOG N-terminal 1–116 amino acids sequence ligated to pRSET A (Invitrogen) expression vector.

The pRSET A vector encodes an N-terminal fusion peptide, upstream and in-frame of the MOG DNA insert.

The culture was grown in SOB media containing 100 mg/ml ampicillin and 17 mg/ml chloramphenicol (temperature 37 °C, 95 % O_2/5 % CO_2; 220 rpm).

RmMOG expression was initiated by adding 1 mM isopropyl-b-thiogalactopyranoside (IPTG) (Sigma).

Affinity purification of the soluble rmMOG was achieved by TALON metal affinity resin (Clontech), which binds the 6-histidine residues present in the fusion protein.

The protein-resin complex was added to the column and non-bound proteins released from the column by repeated washing.

The rmMOG protein was prepared by elution from columns and fractions containing the rmMOG collected, pooled and tested by UV spectrophotometer.

The eluted protein was dyalised for 16 h and concentrated via Centricon Biomax-5 K concentrating tubes at 4,000 g.

Myelin basic protein (MBP) from guinea pig brain was obtained from Sigma-Aldrich.

ELISA

ELISA studies were carried out as previously described.

Statistical Analysis

The mean OD units of control groups (CVA and healthy blood donors) were compared with the mean OD of the 26 multiple sclerosis patients, using a one-tail Student's t-test and 95 % confidence limits of control groups were calculated.

14.3 Molecular Mimicry Between MOG and *Acinetobacter*

The Swissprot database was used to identify any amino acid sequence that showed similarities or homologies between *Acinetobacter* and myelin oligodendrocyte glycoprotein (MOG).

A sequence similarity was found between MOG (residues 50–55), **LYRNGK** and *Acinetobacter* 3-oxoadipate CoA-transferase unit (residues 90–95) **LYRAGK.**

14.4 Multiple Sclerosis Patients Respond to Bacterial Peptide Sequences

The antibody responses to bacterial peptides was investigated by ELISA in sera collected from multiple sclerosis patients.

Significantly elevated levels of antibodies to the mimicry peptides were found to be present in multiple sclerosis patients when compared to CVA patients and healthy controls.

However no elevation was seen to an irrelevant peptide from Human Papilloma virus (HPV) in multiple sclerosis patients compared to the control groups.

Furthermore no correlations were seen between antibody responses to these peptides with either age or sex of the multiple sclerosis patients.

14.5 Results to Peptide 1: Antibodies to *Acinetobacter* 4-carboxy Muconolactone Decarboxylase (QNFISRFAWGEVNSR)

Levels of IgA antibody to **SRF**A**WG** peptide were shown to be significantly elevated in multiple sclerosis sera (0.044 ± 0.004) when tested against control sera (0.013 ± 0.001) ($t = 6.51$, $p < 0.0001$) and CVA patients (0.008 ± 0.002) ($t = 6.96$, $p < 0.0001$) (Fig. 14.1).

Increased levels of IgG antibody to anti-**SRF**A**WG** peptide (0.10 ± 0.007) were also seen in multiple sclerosis patients in comparison to the control group IgG (0.012 ± 0.003) ($t = 9.58$, $p < 0.0001$) and CVA patients IgG (0.02 ± 0.006) ($t = 7.26$, $p < 0.0001$).

Increased levels of IgM antibody to anti-**SRF**A**WG** peptide (0.059 ± 0.007) were also seen in multiple sclerosis in comparison to the control group IgM (0.011 ± 0.003) ($t = 5.66$, $p < 0.0001$) and CVA patients IgM (0.0005 ± 0.0004) ($t = 6.44$, $p < 0.0001$).

No significant differences were seen in the levels of either IgA or IgG anti-**SR**AWG peptide between control subjects and CVA patients.

Furthermore there was a significant difference between the CVA and the healthy blood donor groups when observing IgM antibody levels ($t = 3.26$, $p < 0.01$).

14.6 Results to Peptide 2: Antibodies to *Pseudomonas aeruginosa* γ-carboxy Muconolactone Decarboxylase (QEMITRHAWGDIWTR)

Levels of IgA antibody to **TR**H**AWG** peptide were shown to be significantly elevated in multiple sclerosis sera (0.047 ± 0.003) when tested against control sera (0.009 ± 0.001) ($t = 12.07$, $p < 0.0001$) and CVA patients (0.008 ± 0.001) ($t = 11.78$, $p < 0.0001$) (Fig. 14.2).

Increased levels of IgG antibody to anti-**TR**H**AWG** peptide (0.104 ± 0.009) were also seen in multiple sclerosis patients in comparison to the control group IgG (0.010 ± 0.002) ($t = 10.35$, $p < 0.0001$) and CVA patients IgG (0.012 ± 0.002) ($t = 1035$, $p < 0.0001$).

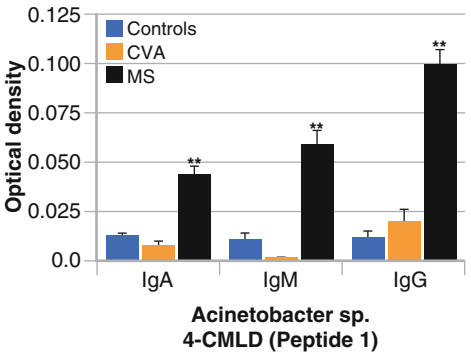

Fig. 14.1 Levels of IgA, IgM and IgG antibodies (mean ± SE with error bars) to *Acinetobacter* 4-carboxy muconolactone decarboxylase (Peptide 1) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors. (Symbols are indicated:) (Reprinted from Hughes et al. (2003), with permission from Elsevier)

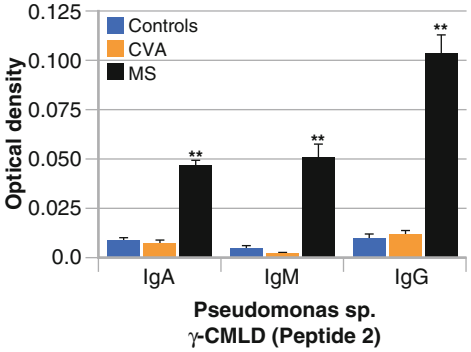

Fig. 14.2 Levels of IgA, IgM and IgG antibodies (mean ± SE with error bars) to *Pseudomonas aeruginosa* γ-carboxy-muconolactone decarboxylase (Peptide 2) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors. (Symbols are indicated:) (Reprinted from Hughes et al. (2003), with permission from Elsevier)

Increased levels of IgM antibody to anti-**TR**HAWG peptide (0.051 ± 0.007) were also seen in multiple sclerosis patients in comparison to the control group IgM (0.005 ± 0.001) ($t=6.03$, $p<0.0001$) and CVA patients IgM (0.001 ± 0.001) ($t=5.88$, $p<0.0001$).

No significant differences were seen in the levels of either IgA or IgG anti-**TR**HAWG peptide between control subjects and CVA patients.

However there was a significant difference between the CVA and the healthy blood donor groups when observing IgM antibody levels ($t=2.58$, $p<0.01$).

14.7 Results to Peptide 3: Antibodies to Myelin Basic Protein (MBP Residues 110–124) (GLSLSRFSWGAGQR)

Levels of IgA antibody to **SRFSWG** peptide were shown to be significantly elevated in multiple sclerosis sera (0.037 ± 0.005) when tested against control sera (0.010 ± 0.004) ($t=4.08$, $p<0.0001$) and CVA patients (0.003 ± 0.002) ($t=5.33$, $p<0.0001$) (Fig. 14.3).

Increased levels of IgG antibody to anti-**SRFSWG** peptide (0.080 ± 0.010) were also seen in multiple sclerosis patients in comparison to the control group IgG (0.004 ± 0.002) ($t=5.79$, $p<0.0001$) and CVA patients IgG (0.001 ± 0.001) ($t=5.41$, $p<0.0001$).

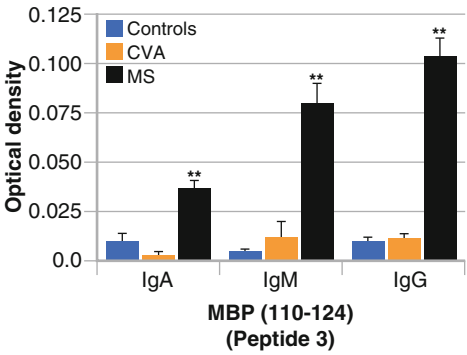

Fig. 14.3 Levels of IgA, IgM and IgG antibodies (mean ± SE with error bars) to myelin basic protein (residues 110–124) (Peptide 3) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors. (Symbols are indicated:) (Reprinted from Hughes et al. (2003), with permission from Elsevier)

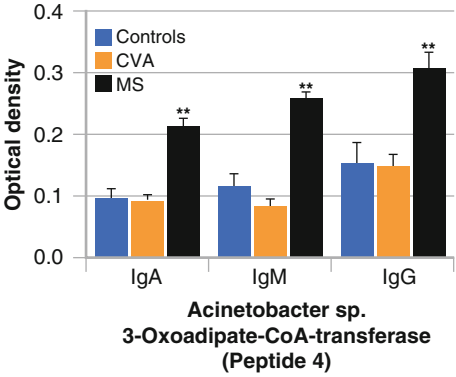

Fig. 14.4 Levels of IgA, IgM and IgG antibody levels to *Acinetobacter* 3-oxoadipate-CoA-transferase (Peptide 4) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors (Symbols are indicated:) (Reprinted from Hughes et al. (2003), with permission from Elsevier)

Increased levels of IgM antibody to anti-**SRFSWG** peptide (0.080 ± 0.010) were also seen in multiple sclerosis patients in comparison to the control group IgM (0.004 ± 0.002) ($t = 6.15$, $p < 0.0001$) and CVA patients IgM (0.012 ± 0.008) ($t = 4.53$, $p < 0.0001$).

No significant differences were seen in the levels of either IgA, IgG or IgM anti-**SRF**SWG peptide between control subjects and CVA patients.

14.8 Results to Peptide 4: Antibodies to *Acinetobacter* sp 3-oxoadipate CoA-transferase Subunit A. (DSYVFDE LYRAGKIE)

Levels of IgA antibody to **LYRAGK** peptide were shown to be significantly elevated in multiple sclerosis sera (0.212 ± 0.014) when tested against control sera (0.098 ± 0.015) ($t = 4.66$, $p < 0.0001$) and CVA patients (0.094 ± 0.010) ($t = 5.46$, $p < 0.0001$) (Fig. 14.4).

Increased levels of IgG antibody to anti-**LYRAGK** peptide (0.306 ± 0.027) were also seen in multiple sclerosis patients in comparison to the control group IgG (0.155 ± 0.032) ($t = 3.25$, $p < 0.01$) and CVA patients IgG (0.149 ± 0.019) ($t = 3.91$, $p < 0.001$).

Increased levels of IgM antibody to anti-**LYRAGK** peptide (0.257 ± 0.012) were also seen in multiple sclerosis patients in comparison to the control group IgM (0.117 ± 0.020) ($t = 6.26$, $p < 0.0001$) and CVA patients IgM (0.085 ± 0.012) ($t = 9.31$, $p < 0.0001$).

No significant differences were seen in the levels of either IgA, IgG or IgM anti-**LYR**AGK peptide between control subjects and CVA patients.

14.9 Results to Peptide 5: Antibodies to Myelin Oligodendrocyte Glycoprotein (MOG) (Residues 43–57) (PFSRVVH LYRNGKDQ)

Levels of IgA antibody to **LYRNGK** peptide were shown to be significantly elevated in multiple sclerosis sera (0.245 ± 0.016) when tested against control sera (0.083 ± 0.019) ($t = 5.65$, $p < 0.0001$) and CVA patients (0.123 ± 0.018) ($t = 4.54$, $p < 0.0001$) (Fig. 14.5).

Increased levels of IgG antibody to anti-**LYRNGK** peptide (0.272 ± 0.019) were also seen in multiple sclerosis patients in comparison to the control group IgG (0.170 ± 0.020) ($t = 3.29$, $p < 0.001$) and CVA patients IgG (0.145 ± 0.019) ($t = 4.34$, $p < 0.0001$).

Increased levels of IgM antibody to anti-**LYRNGK** peptide (0.285 ± 0.024) were also seen in multiple sclerosis in comparison to the control group IgM (0.078 ± 0.015) ($t = 5.54$, $p < 0.0001$) and CVA patients IgM (0.150 ± 0.018) ($t = 3.83$, $p < 0.001$).

No significant differences were seen in the levels of either IgA or IgG anti-**LYR**NGK peptide between control subjects and CVA patients.

However there was a significant difference between the CVA and the healthy blood donor groups when observing IgM antibody levels ($t = 2.97$, $p < 0.01$).

14.10 Multiple Sclerosis Patients have Antibodies to Bacterial *Acinetobacter/Pseudomonas* Peptides

Elevated levels of IgA, IgM and IgG antibodies directed against peptide sequences from *Acinetobacter* 4-carboxy-muconolactone decarboxylase, *Acinetobacter* 3-oxo-adipate-CoA-transferase subunit A and *Pseudomonas aeruginosa*

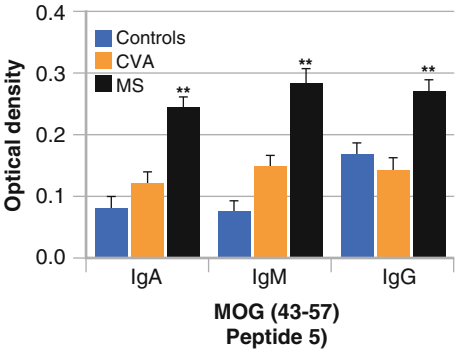

Fig. 14.5 Levels of IgA, IgM and IgG antibody levels to myelin oligodendrocyte glycoprotein (MOG) (residues 43–57) (Peptide 5) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors (Symbols are indicated:) (Reprinted from Hughes et al. (2003), with permission from Elsevier)

γ-carboxy-muconolactone decarboxylase were found in the sera from multiple sclerosis patients compared to CVA patients or healthy controls (Hughes et al. 2003). Dilution studies showed that antibody activity against the peptides could be detected up to a dilution of 1/6,400.

Both genetic and environmental factors would appear to be implicated in the aetiology of multiple sclerosis and any infectious agent must be ubiquitous within the risk groups (Granieri et al. 2001).

Acinetobacter and *Pseudomonas* bacteria are found frequently in the environment of multiple sclerosis patients and it would not be inconceivable to consider these bacteria as potential pathological agents.

Acinetobacter is a ubiquitous, common microbe found in soil, water and on the skin of many animals. It is also frequently encountered in the mucous membranes of animals and man, especially the nasal sinuses.

Acinetobacter is an opportunistic pathogen and frequently described as being associated with nosocomial infections, especially in hospital environments, resulting in bacteraemia and pneumonia (Villers et al. 1998) and sometimes leading to chest and sinus infections.

Pseudomonas microbes are also opportunistic pathogens of man and causing wound, urinary tract and even upper respiratory tract infections. They are widely distributed throughout the environment of man and can cause transient colonization of skin and intestinal tract.

The search for an aetiological agent in multiple sclerosis has been focussed on viruses but no microorganisms have so far been implicated.

It has been shown that 52 % of multiple sclerosis patients have a history of repeated upper respiratory tract infections. Increased risk of clinical relapses in multiple sclerosis have suggested links with upper respiratory tract viral infections (Edwards et al. 1998) although 41 % of multiple sclerosis patients also have a concomitant bacterial infection (Rapp et al. 1995).

Several mechanisms have been suggested as to how an infection could initiate autoimmune disease, including molecular mimicry, determinant spreading and bystander activation (Talbot et al. 2001).

Molecular mimicry has been suggested as a potential pathogenic mechanism leading to the development of multiple sclerosis (Steinman 2001) probably through a sequence or structural homology (Kohm et al. 2003).

Some microorganisms have been identified to possess amino acid sequences with homology to myelin antigens including Herpes virus 6 (HHV-6) (Tejada-Simon et al. 2003), Epstein-Barr virus, Herpes simplex virus, influenza and *Pseudomonas aeruginosa* however it is unclear whether these agents are involved in the pathogenesis of multiple sclerosdis.

Our studies have previously identified potential molecular mimicry sequences between *Acinetobacter* and *Pseudomonas* and brain components.

Elevated levels of antibodies to these microorganisms as well as against these bacterial peptides were found in multiple sclerosis patients.

Class specific immune responses to peptide sequences from *Acinetobacter* and *Pseudomonas* bacteria, which mimic the brain components of myelin basic protein and myelin oligodendrocyte glycoprotein (MOG) were found in multiple sclerosis patients.

Autoantibodies to myelin antigens, including MOG, myelin basic protein (Warren and Catz 1997), myelin associated glycoproteins (Wajgt and Gorny 1983) and gray matter neurofilaments are well recognised in multiple sclerosis patients.

Our studies indicate that amino acid sequences can also function as a B cell epitope as increased levels of IgA, IgM and IgG antibodies were observed to myelin basic protein (MBP) (residues 110–224) in multiple sclerosis patients when compared to healthy controls or CVA patients.

Both T cell and antibody responses to MOG (residues 35–55) have been shown in multiple sclerosis patients (Kennel de March et al. 2003).

We show that IgA, IgM and IgG antibodies to a similar epitope, namely MOG (residues 43–57) are elevated in multiple sclerosis patients compared to CVA patients or healthy controls.

The results suggest that the mimicry sequences identified between *Acinetobacter, Pseudomonas,* myelin basic protein and myelin oligodendrocyte glycoprotein (MOG) could be potentially cross-reactive.

It is therefore suggested that the antibody responses to the specific bacterial peptide antigens seen in multiple sclerosis patients could cross-react and bind to myelin constituents within the brain, thereby enhancing the pathological lesions seen in multiple sclerosis.

Further studies are required to evaluate the role of *Acinetobacter* and *Pseudomonas* bacteria in multiple sclerosis.

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