Chapter 11
Antibodies to *Acinetobacter* and *Pseudomonas* Bacteria in Multiple Sclerosis Patients

11.1 Introduction: Possible Immune Responses to *Acinetobacter* and *Pseudomonas* Bacteria in Multiple Sclerosis Patients

Multiple sclerosis is the most common demyelinating disease of the central nervous system, affecting, when formes frustes are included, almost 100,000 individuals in the U.K and over 500,000 in the USA with the condition having characteristic immunological features. Over two million individuals in the world are thought to suffer from multiple sclerosis.

It is generally considered that an autoimmune process is involved, triggered by an infectious agent and possibly through a process of molecular mimicry (Albert and Inman 1999). It involves production of anti-neuronal antibodies which lead to pathological and neurological complications.

A similar process was previously described for rheumatic fever (Kaplan and Meyeserian 1962) and more recently for rheumatoid arthritis (Wilson et al. 1995) and ankylosing spondylitis (Fielder et al. 1995).

Sydenham's chorea is a neurological disease associated with rheumatic fever and which is caused by anti-*streptococcal* antibodies that bind to basal ganglia. In this condition autoantibodies against brain tissues are evoked outside the nervous system but any IgG antibodies produced will cross the blood-brain barrier and if present in high enough concentrations, they will activate the complement cascade and cause tissue damage. Such tissue damage will manifest itself with a variety of neurological signs and symptoms.

It is possible that multiple sclerosis is produced by a similar mechanism and the demonstration that *Acinetobacter* bacteria are involved in BSE raises the question whether active multiple sclerosis patients also possess elevated levels of antibodies to these or related microorganisms (Wilson et al. 2003).

A. Ebringer, *Multiple Sclerosis, Mad Cow Disease and Acinetobacter*,
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It was decided to investigate multiple sclerosis patients for possible immune responses to bacterial pathogens that carry sequences crossreacting with brain sequences such as *Acinetobacter* species and *Pseudomonas aeruginosa.*

These two microbes had previously been identified to have sequences crossreacting with bovine myelin basic protein and myelin destruction is a characteristic feature of multiple sclerosis in both cows with BSE and in human patients affected by multiple sclerosis.

11.2 Materials and Methods: Serum Samples, Bacteria 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. Diagnosis was made according to the Poser criteria (Poser et al. 1983).

"Benign" multiple sclerosis patients are characterised by infrequent exacerbations but leading to full recovery.

"Relapsing remitting" multiple sclerosis patients have more frequent exacerbations. However they are followed by partial or complete remission in their clinical status. For this study, serum samples were obtained from "relapsing remitting" patients during exacerbations.

"Secondary progressive" multiple sclerosis patients are considered as those who continue to deteriorate following an initial relapsing remitting course of disease.

Primary progressive" multiple sclerosis patients are those who have a continuous deterioration without remission from the start of the disease

In addition serum samples were obtained from 20 patients in the Department of Geriatric Medicine at University College Hospital 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).

Furthermore, sera were also obtained from 10 patients with viral encephalitis (8 males and 2 females, mean age 38 years, range: 3–66 years), attending the National Hospital for Neurology and Neurosurgery.

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).

A further set of sera from 29 healthy control subjects attending the London Blood Donor services was used in the viral encephalitis study (15 males and 14 females, mean age 43 years; range 19–66 years).

Bacterial Cultures

Cultures *Acinetobacter sp. strain 11171*, *Acinetobacter sp. strain 19004*, *Acinetobacter junii 17908*, *Acinetobacter lwoffii 5866* and *Acinetobacter radioresistens (sp.12)* were provided by the Public Health Laboratory, Nottingham, United Kingdom.

Acinetobacter calcoaceticus (NCIMB 16904) was obtained from the National Collections of Industrial and Murine Bacteria Ltd. (Aberdeen, Scotland).

The Department of Microbiology at King's College provided *Pseudomonas aeruginosa* (NCTC 8203) and *Escherichia coli* (NCTC 9002) bacterial samples.

Cultures were grown as previously described.

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.

Pearson's correlation coefficient (r) was also calculated using the statistical package Prism 9.0 (GraphPad Software).

11.3 IgA Anti-*Acinetobacter* Antibodies in Multiple Sclerosis and CVA Patients

Elevated levels of IgA antibodies occur when the initiating antigen or microbe is crossing a mucosal surface such as the "gut associated lymphoid tissue" (GALT).

Levels of IgA antibodies to *Acinetobacter sp. strain 11171* ($p<0.0001$) (Fig. 11.1), *Acinetobacter sp. strain 19004* ($p<0.0001$) (Fig. 11.2), *Acinetobacter junii 17908* ($p<0.01$) (Fig. 11.3), *Acinetobacter lwoffii 5866* ($p<0.0001$) (Fig. 11.4) and *Acinetobacter radioresistens* ($p<0.0001$) (Fig. 11.5) in multiple sclerosis patients were significantly higher than those in the healthy control group.

Levels of IgA antibodies to *Acinetobacter sp. strain 11171* ($p<0.0001$) (Fig. 11.1), *Acinetobacter lwoffii 5866* ($p<0.001$) (Fig. 11.4) and *Acinetobacter radioresistens* ($p<0.0001$) (Fig. 11.5) were also shown to be significantly elevated in multiple sclerosis compared to CVA patients.

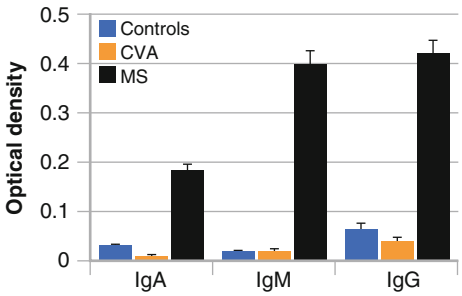

Fig. 11.1 Levels of IgA, IgM and IgG antibodies (mean ± SE with error bars) to *Acinetobacter sp. strain 11171* (Symbols: controls, CVA, MS) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors (Copyright © American Society for Microbiology, Hughes et al. (2001))

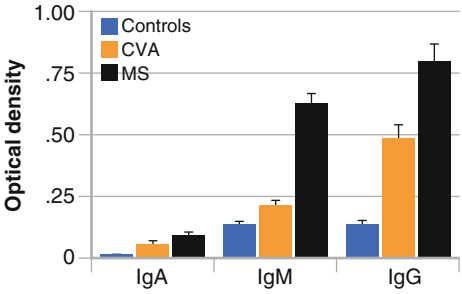

Fig. 11.2 Levels of IgA, IgM and IgG antibodies (mean ± SE with error bars) to *Acinetobacter sp. strain 19004* (Symbols: controls, CVA, MS) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors (Copyright © American Society for Microbiology, Hughes et al. (2001))

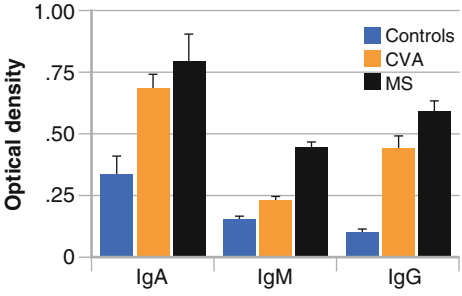

Fig. 11.3 Levels of IgA, IgM and IgG antibodies (mean ± SE with error bars) to *Acinetobacter junii sp. strain 17908* (Symbols: controls, CVA, MS) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors (Copyright © American Society for Microbiology, Hughes et al. (2001))

No significant difference was seen between multiple sclerosis patients and CVA patients for IgA antibodies to either *Acinetobacter sp. strain 19004* (Fig. 11.2) and *Acinetobacter sp. strain 17908* (Fig. 11.3).

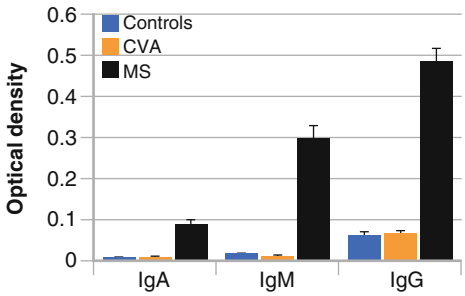

Fig. 11.4 Levels of IgA, IgM and IgG antibodies (mean ± SE with error bars) to *Acinetobacter lwoffii strain 5866* (Symbols: controls, CVA, MS) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors (Copyright © American Society for Microbiology, Hughes et al. (2001))

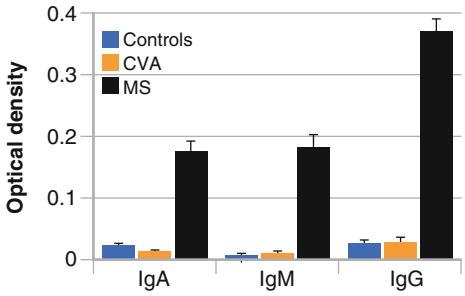

Fig. 11.5 Levels of IgA, IgM and IgG antibodies (mean ± SE with error bars) to *Acinetobacter radioresistens* (Symbols: controls, CVA, MS) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors (Copyright © American Society for Microbiology, Hughes et al. (2001))

Significantly elevated levels of IgA antibodies to *Acinetobacter sp. strain 19004* ($p < 0.0001$) and *Acinetobacter junii 17908* ($p < 0.001$) were observed in CVA patients compared to controls.

Levels of IgA antibodies to *Acinetobacter sp. strain 11171* were shown to be significantly higher in controls ($p < 0.001$) than in CVA patients.

No other significant difference were seen in the levels of IgA antibodies to *Acinetobacter lwoffii* 5866 (Fig. 11.4) or *Acinetobacter radioresistens* (Fig. 11.5) between the control and CVA groups.

11.4 IgG Anti-*Acinetobacter* Antibodies in Multiple Sclerosis and CVA Patients

Circulating monomeric IgG antibodies, because of their relatively low molecular weight compared to dimeric IgA or pentameric IgM antibodies can readily cross the blood-brain barrier.

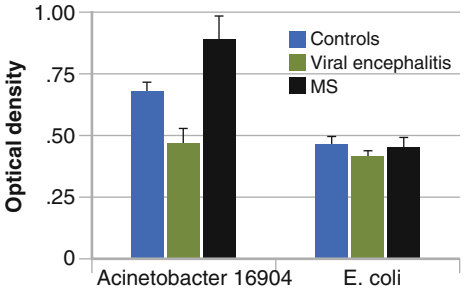

Fig. 11.6 IgG antibody levels (mean ± SE with error bars) to *Acinetobacter calcoaceticus 16904* and *Escherichia coli* in sera from 26 multiple sclerosis patients, 10 viral encephalitis patients and 29 healthy blood donors. Copyright © American Society for Microbiology, Hughes et al. (2001))

Monomeric IgG autoantibodies thus can penetrate to brain tissues such as myelin of nervous tissues and if present in sufficiently high concentrations produce pathological damage, as is observed in Sydenham's chorea patients.

Levels of IgG antibodies to *Acinetobacter sp. strain 11171* ($p < 0.0001$), (Fig. 11.1), *Acinetobacter sp. strain 19004* ($p < 0.0001$) (Fig. 11.2), *Acinetobacter junii 17908* ($p < 0.0001$) (Fig. 11.3), *Acinetobacter lwoffii 5866* ($p < 0.0001$) (Fig. 11.4) and *Acinetobacter radioresistens* ($p < 0.0001$) (Fig. 11.5) were significantly elevated in multiple sclerosis patients compared to healthy controls.

Levels of IgG antibodies to *Acinetobacter sp. strain 11171* ($p < 0.0001$) (Fig. 11.1), *Acinetobacter sp. strain 19004* ($p < 0.01$) (Fig. 11.2), *Acinetobacter junii 17908* ($p < 0.05$) (Fig. 11.3), *Acinetobacter lwoffii 58666* ($p < 0.0001$) (Fig. 11.4) and *Acinetobacter radioresistens* ($p < 0.0001$) (Fig. 11.5) were significantly higher in multiple sclerosis patients compared to CVA patients.

Significantly elevated levels of IgG antibodies to *Acinetobacter sp. strain 19004* ($p < 0.0001$) (Fig. 11.2) and *Acinetobacter junii 17908* ($p < 0.0001$) (Fig. 11.3) were found in CVA patients compared to healthy controls.

However there was no significant difference in the levels of IgG antibodies to *Acinetobacter sp. strain 11171*, *Acinetobacter lwoffii 5866* and *Acinetobacter radioresistens* between the CVA patients and healthy controls.

A further study, measuring IgG antibody levels to *Acinetobacter calcoaceticus 16904* demonstrated a slight elevation in sera from multiple sclerosis patients compared to levels in sera from viral encephalitis patients ($p < 0.02$) and healthy blood donor controls ($p < 0.05$) (Fig. 11.6).

11.5 IgM Anti-*Acinetobacter* Antibodies in Multiple Sclerosis and CVA Patients

Circulating IgM antibodies cannot cross the blood-brain barrier.

Levels of IgM antibodies to *Acinetobacter sp. strain 11171* ($p < 0.0001$) (Fig. 11.1), *Acinetobacter sp. strain 19004* ($p < 0.0001$) (Fig. 11.2), *Acinetobacter junii 17908* ($p < 0.0001$) (Fig. 11.3), *Acinetobacter lwoffii 5866* ($p < 0.0001$)

(Fig. 11.4) and *Acinetobacter radioresistens* ($p<0.0001$) (Fig. 11.5) were significantly higher in multiple sclerosis patients than in the healthy control group.

Levels of IgM antibody to *Acinetobacter sp. strain 11171* ($p<0.0001$) (Fig. 11.1), *Acinetobacter sp.strain 19004* ($p<0.0001$) (Fig. 11.2), *Acinetobacter junii 17908* ($p<0.0001$) (Fig. 11.3), *Acinetobacter lwoffii 5866* ($p<0.0001$) (Fig. 11.4) and *Acinetobacter radioresistens* ($p<0.0001$) (Fig. 11.5) were also shown to be significantly elevated in multiple sclerosis patients compared to CVA patients.

Significantly elevated levels of IgM antibodies to *Acinetobacter sp strain 19004* ($p<0.001$) and *Acinetobacter sp. strain 17908* ($p<0.001$) were found in CVA patients compared to healthy controls.

No significant differences were seen in the level of IgM antibodies to *Acinetobacter sp. strain 11171*, *Acinetobacter lwoffii 5866* and *Acinetobacter radioresistens* between the CVA patients and the control groups.

11.6 Antibodies to *Pseudomonas aeruginosa*

Levels of IgA antibodies to *Pseudomonas aeruginosa* were shown to be significantly higher in multiple sclerosis patients than in the control group ($p<0.001$) (Fig. 11.6) and CVA patients ($p<0.05$) (Fig. 11.7).

There was also a slight elevation in the level of IgA anti-*Pseudomonas* antibodies in the CVA patients ($p<0.05$) when compared to healthy controls.

Elevated levels of IgG antibodies to *Pseudomonas* were also observed in multiple sclerosis sera compared to controls ($p<0.0001$) and also to CVA patients ($p<0.05$).

Furthermore, there was also a slight elevation in the level of IgG antibodies to *Pseudomonas* ($p<0.05$) in the CVA group when compared to controls.

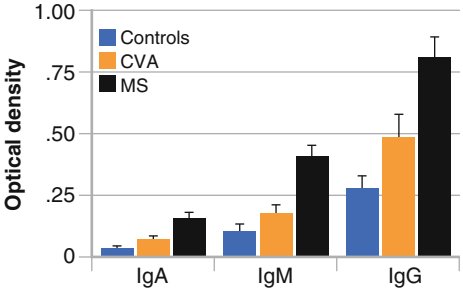

Fig. 11.7 Levels of IgA, IgM and IgG antibodies (mean ± SE with error bars) to *Pseudomonas aeruginosa* (Symbols: controls, CVA, MS) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors (Copyright © American Society for Microbiology, Hughes et al. (2001))

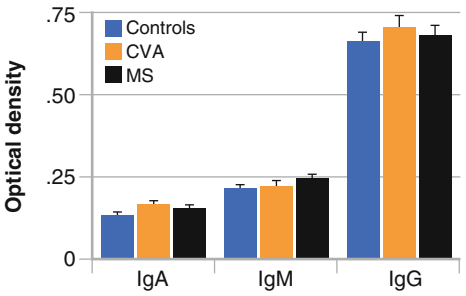

Fig. 11.8 Levels of IgA, IgM and IgG antibodies (mean ± SE with error bars) to *Escherichia coli* (Symbols: controls, CVA, MS) in sera from 26 multiple sclerosis patients, 20 CVA patients and 25 healthy blood donors (Copyright © American Society for Microbiology, Hughes et al. (2001))

IgM antibody levels to *Pseudomonas* were shown to be significantly elevated in multiple sclerosis patients compared to controls ($p < 0.0001$) and also to CVA patients ($p < 0.001$).

There was no significant difference in the level of IgM antibodies to *Pseudomonas* between the CVA patients and the healthy control group.

11.7 Antibodies to *Escherichia coli* in Multiple Sclerosis Patients

No significant differences in the IgA, IgG and IgM antibody levels to *Escherichia coli* were observed between any of the groups tested (Figs. 11.6 and 11.8).

11.8 Correlation Coefficient Analysis

The correlation coefficient (r) was calculated between all strains of *Acinetobacter* tested and *Pseudomonas aeruginosa.*

There was a significant positive correlation between the IgM levels of *Acinetobacter junii 17908* and *Pseudomonas aeruginosa* ($r = +0.831$; $p < 0.0001$).

A significant positive correlation was also seen between the IgM levels of *Acinetobacter sp. strain 19004* and *Pseudomonas aeruginosa* ($r = +0.819$; $p < 0001$).

There was also a significant correlation between IgA antibodies to *Acinetobacter sp. strain 19004* and *Pseudomonas aeruginosa* ($r = +0.407$, $p < 0.01$).

11.9 Discussion and Pathological Implications

Elevated levels of antibodies directed against several strains of *Acinetobacter* bacteria have been found in multiple sclerosis patients when compared to CVA patients or healthy control subjects.

This would appear to be the first report that multiple sclerosis patients have antibodies against microbial species, such as *Acinetobacter* which are readily found in the environment, for instance on skin, in soil samples and in nasal cavities. The pathological implications would appear to be unclear but it is relevant to note that *Acinetobacter* bacteria possess molecular sequences which resemble those found in myelin and nerve filaments.

Previous studies have suggested that respiratory infections may be involved in the onset of multiple sclerosis. In this study we have looked at potential respiratory pathogens such as *Acinetobacter* bacteria and *Pseudomonas aeruginosa.*

A sequence homology has been found between a known encephalitogenic myelin peptide and the enzyme 4-carboxymucono lactone decarboxylase in both *Acinetobacter* species and *Pseudomonas aeruginosa.*

Another approach has been to suggest that molecular mimicry may operate through variable T-cell recognition (Gran et al. 1999). Several viral and bacterial peptides have been found to activate three of seven T-cell clones isolated from multiple sclerosis patients, specific against myelin basic protein especially involving amino acid positions 85-99.

The bacterial peptide identified was phospho-mannomutase protein in *Pseudomonas aeruginosa* (Wucherpfennig and Strominger 1995).

These results would appear to suggest that antibodies to *Acinetobacter* bacteria are present in multiple sclerosis patients but whether such antibodies have activity against human myelin or human neurofilaments and therefore behave as autoantibodies awaits further studies.

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