The role of molecular mimicry in the etiology of Guillain Barré Syndrome

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Abstract

Molecular mimicry between host tissue structures and microbial components has been proposed as the pathogenic mechanism for triggering of autoimmune diseases by preceding infection. Recent studies stated that molecular mimicry as the causative mechanism remains unproven for most of the human diseases. Still, in the case of the peripheral neuropathy Guillain-Barré syndrome (GBS) this hypothesis is supported by abundant experimental evidence. GBS is the most frequent cause of acute neuromuscular paralysis and in some cases occurs after infection with *Campylobacter jejuni* (*C. jejuni*). Epidemiological studies, showed that more than one third of GBS patients had antecedent *C. jejuni* infection and that only specific *C. jejuni* serotypes are associated with development of GBS. The molecular mimicry between the human gangliosides and the core oligosaccharides of bacterial lipopolysaccharides (LPSs) presumably results in production of antiganglioside cross-reactive antibodies which are likely to be a contributory factor in the induction and pathogenesis of GBS. Antiganglioside antibodies were found in the sera from patients with GBS and by sensitization of rabbits with gangliosides and *C. jejuni* LPSs animal disease models of GBS were established.

GBS as prototype of post-infection immune-mediated disease probably will provide the first verification that an autoimmune disease can be triggered by molecular mimicry.

Key words: Guillain Barré syndrome, molecular mimicry, *Campylobacter jejuni*, lipopolysaccharides, gangliosides

The molecular mimicry hypothesis

Molecular mimicry between host tissue structures and microbial antigens is postulated as mechanism for triggering of autoimmune disease by preceding infections. According to the mimicry hypothesis, due to the structural resemblance between microbial and some host antigens, antibodies and T cells that are induced by the antecedent infection and are initially directed against microbial antigens, not only destroy the invading pathogen but also attack host tissue (Albert and Inman, 1999). Molecular mimicry can be defined as presence of structurally similar epitopes in human and pathogen antigens, or in a wider sense it is sharing of antigens between hosts and microorganisms, leading

to immunological process by inducing cross-reactive antibodies or T cells that cause the symptoms of autoimmune disease (Damian, 1964).

The molecular mimicry hypothesis was first postulated by Koch and Witebsky as a mechanism by which infectious agents trigger an immune response against autoantigens, resulting in the development of autoimmune diseases (Falkow, 1988). The initial idea that molecular mimicry might play a role in autoimmune diseases was born in the 1980s, along with the development and use of monoclonal antibody technology. It was then first noted that an antibody might cross-react with both human and viral antigens. A number of similar cross-reactivity between microbial structures and host self determinants were subsequently recorded and molecular mimicry was postulated as part of pathogenesis in many diseases (Rose and Bona, 1993).

The claims whether molecular mimicry is a causative mechanism of autoimmune disease are numerous and are

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supported by abundant evidence of experiments. Still, to conclude that a disease is triggered by molecular mimicry, the following four criteria need to be satisfied:

- To establish an epidemiological association between the infectious agent and the immune-mediated disease
- To identify antibodies and/or T cells directed against the patient's target antigens
- To identify a microbial mimicry in the target antigen
- To reproduce the disease in an animal model

The concept of molecular mimicry as a possible mechanism by which infections may induce pathogenic immune responses has been linked to several diseases, such as acute rheumatic fever, multiple sclerosis and type 1 diabetes mellitus. Although there is some evidence that infectious agents play a part in the pathogenesis of a number of autoimmune diseases, it has not yet been proved that molecular mimicry is the initiating factor in any of these diseases (Rose and Mackay, 2000).

Mimicry between glutamate decarboxylase (GAD65) an enzyme present in pancreatic cells and coxsackie virus has been demonstrated (Kaufman et al., 1993). This mimicry might be responsible for cell destruction in patients with type I diabetes mellitus although convincing evidence for this in patients has yet to be found (Atkinson et al., 1992). A classic example of a disease in which molecular mimicry is postulated to be the pathogenic mechanism is rheumatic carditis with a cross-reactive immune response towards pneumococcal polysaccharides and cardiac tissue leading to rheumatic heart disease (Huber and Cunningham, 1996).

In the case of the peripheral neuropathy Guillain Barre syndrome (GBS) the hypothesis of the role of molecular mimicry in this immune-mediated disease is supported by abundant evidence of experiments. Therefore GBS is an excellent opportunity to study the relation between infections and development of neurological symptoms (Ang et al., 2004).

Guillain Barré syndrome

Guillain Barré syndrome (GBS) is the major cause of acute neuromuscular paralysis in the world since the eradication of polio, with an annual incidence of 1–2 per 100,000. It can affect all ages including children, but becomes increasingly common with age (Van Doorn et al., 2008). In the recent years much progress has been made in understanding its etiology and treatment. GBS is defined as an immune mediated disease of the peripheral nerves, involving both the myelin sheath and the axons. It is named after G. Guillain and J.A. Barre, two French neurologists who first described the syndrome in 1916 (Guillain et al., 1916). GBS as an autoimmune disorder of the peripheral nervous system is characterized by damage and dysfunction of the axon or myelin sheath of peripheral nerves which leads to

weakness, usually symmetrical, evolving over a period of several days or more. Components of the immune system such as antibodies, complement, T cells and macrophages are present in affected nerves and are likely to be involved in the pathogenesis of the disease (Hughes et al., 1999).

Clinical features of Guillain Barré Syndrome

GBS is clinically defined by progressive weakness of two or more limbs due to neuropathy, reduced or absent reflexes, less than 50 mononuclear leucocytes per liter of cerebrospinal fluid (CSF) and absence of other known causes of acute neuropathy. Symptoms typically begin with weakness, sensory disturbance or pain, and most patients develop rapidly progressive symmetrical weakness, worse in legs than arms along with partial or complete loss of reflexes (Asbury and McKhann, 1997). Most patients experience numbness, tingling and pain and weakness classically follows an ascending temporal pattern, starting in the lower limbs and then rapidly spreading to the upper limbs and the face. Frequently, when the lower cranial nerves are affected this results in bulbar weakness, bladder dysfunction, dysphagia and respiratory difficulties (Hughes et al., 1997). Sensory loss, which usually takes the form of loss of perception, is common, along with deep aching pain in the weakened muscles (Nachamkin et al., 1998). Autonomic disturbance is common, with fluctuations in blood pressure, heart rate and bowel function. The CSF protein concentration is elevated in 80% of patients. The weakness is most severe within two to four weeks from onset, when approximately 20% of patients require artificial ventilation because of respiratory muscle weakness, 40% are bedridden, 20% can walk only with assistance, 10% can walk but not run, and 10% have only mild symptoms. The disease can be self-limited, followed by partial or complete recovery over the period of several weeks to several months (Hughes et al., 1999).

Types of Guillain Barré Syndrome

GBS represents a spectrum of clinical and pathological entities with a number of subtypes being recognized. The disease subtype is determined by the clinical manifestations and by laboratory investigations. In general GBS can be classified into two major subtypes, demyelinating neuropathies where myelin sheaths are affected and axonal neuropathies affecting motor and sensory nerves (Asbury and Cornblath, 1990). Therefore the subtypes of GBS are acute inflammatory demyelinating neuropathy (AIDP) affecting the peripheral nerve myelin, acute motor axonal neuropathy (AMAN) affecting axons, acute motor and sensory axonal neuropathy (AMSAN) and Miller Fisher syndrome (MFS) (Table 1).

AIDP is typically characterized with muscle weakness which spreads proximally and can lead to respiratory paralysis. Numbness and paraesthesia are frequently pres-

Table 1. GBS subtypes and their relationships according to type of neuropathy

Guillain Barré syndrome
Demyelinating
Acute Inflammatory Demyelinating Polyneuropathy (AIDP)
Axonal
Acute Motor Axonal Neuropathy (AMAN)
Acute Motor Sensory Axonal Neuropathy (AMSAN)
Miller Fisher syndrome (MFS)

ent. AIDP is complicated by an autonomic neuropathy in 15% of cases, leading to life threatening dysfunction of the cardiovascular, alimentary and urinary systems. In severe cases, axonal degeneration may accompany the demyelination. The disease reaches its maximum at two weeks and recovery after a variable period of time. This can take weeks to months with up to 16% of patients suffering recurring episodes (Van der Meche and Van Doorn, 1995).

The axonal forms, AMAN and AMSAN, cause motor impairment or motor and sensory impairment respectively. In AMAN subtype motor nerves are unexcitable and axonal degeneration occurs at spinal roots and peripheral nerves with no demyelination, and little or no inflammation. In distal nerve there is a conduction block not found in AIDP (Hafer et al., 1996). The myelin sheath remains intact as the pathological process involves binding of antibodies to gangliosides located on the axolemma, resulting in infiltration of macrophages at nodes of Ranvier. AMSAN is subtype similar to AMAN, but sensory axons are also affected. It was first proposed by Griffin (Griffin et al., 1996) that AMAN and AMSAN could be part of a spectrum of a subtype where the target epitope is axonal. It would seem that they share a common immunological profile in regard to what anti-ganglioside antibodies are found in patient sera, which differs to those common to AIDP (Hafer et al., 2000). AMSAN is usually severe, involving both motor and sensory fibers. These axonal subtypes progress more rapidly, are more severe and more often require assisted ventilation.

MFS is characterized by an acute onset of ataxia, areflexia and ophthalmoplegia (Fisher, 1956) and if the condition is accompanied by disturbance of consciousness this syndrome is defined as Bickerstaff brainstem encephalitis (BBE) (Bickerstaff, 1957). An immunohistochemical study using a monoclonal anti-GQ1b antibody indicated the dense distribution of GQ1b at paranodal myelin of cranial nerves innervating extraocular muscles (Plomp et al., 1999; Hiraga et al., 2005). The distribution of the GQ1b antigen is critical for the symptomatology of MFS. It is now recognized that these disorders share many common features, in particular the antecedent infection, the cytological dissociation of albumins and the presence of anti-ganglioside antibodies in certain cases. The main clinical

method for distinguishing among subtypes is electrodiagnostic examination (Kuwabara et al., 2002).

The GBS subtypes show regional variations in incidence. AIDP is the most frequently subtype worldwide and constitutes 90% of cases in Europe and North America. The axonal forms (AMAN, AMSAN and MFS) constitute 5% of cases in North America and Europe but are common (30-47%) in China, Japan and South/Central America (McKhann et al., 1993). **The most severe and life threatening complication of GBS is paralysis of the respiratory muscles which, in 25% of cases, leads to a requirement for artificial ventilation. Ten to 20% of patients suffer permanent disability, one third needs to make substantial changes to their lifestyle, while the mortality rate is 4 to 15% of cases within year. GBS patients represent a clinical challenge due to the multitude of systems affected by the disease.**

Treatment of Guillain Barré Syndrome

General supportive care as monitoring the respiratory capacity and autonomic functions of GBS patients is of great importance to prevent severe complications or even death by respiratory or autonomic dysfunction (Hughes et al., 2005). Therapeutic intervention is directed at suppressing the immune reactivity or blocking the effector mechanisms. The choice of therapy in any given patient or syndrome depends on the efficacy and safety of the treatment being considered (Van der Meche and Dutch Guillain Barré study group, 1992). The main therapy is corticosteroids or immunotherapy, either plasmapheresis or intravenous immunoglobulin (IVIg) (Hughes et al., 2007). Corticosteroids are useful for short term therapy, but prolonged use is associated with potentially severe side effects including osteoporosis, fractures, avascular necrosis, coronary artery disease, and stroke, as well as hypertension, obesity and diabetes. Plasmapheresis or IVIg both are equally effective at reducing disability and mortality and reducing the number of patients requiring artificial ventilation. Several randomized controlled trials reported a beneficial effect on outcome by plasma exchange and IVIg. A recent trial demonstrated that the combination of IVIg and methylprednisolone might be superior in those patients who are in the first two weeks of disease and unable to walk unaided.

ed and who have no contra-indication for the use of high dose steroids. However, even with immunotherapy intervention, 14% of patients still require assisted ventilation (Khan et al., 2009).

The current immunotherapy regimes are inadequate due their restricted efficacy, expense, inherent risks and lack of availability, particularly in developing countries. There is a need for a better understanding of the pathogenesis of GBS to allow development of novel targeted treatments and better prognostic indicators that will allow clinicians to predict who will require intensive treatment and who will benefit most from which intervention.

Guillain Barré Syndrome and Campylobacter jejuni infection

Approximately two-thirds of GBS patients develop this syndrome following various infection of the respiratory or gastrointestinal tract. The organisms most commonly associated with GBS are *Campylobacter jejuni* (4-66%), Cytomegalovirus (5-15%), Epstein-Barr virus (2-10%), *Mycoplasma pneumonia* (1-5%) (Jacobs et al., 1998). In rare cases of GBS, infective endocarditis, rubella virus infection, *Haemophilus influenzae* and *Legionella* infection may be associated (Hadden et al., 2001; Kaida et al., 2004). Vaccinations, including flu vaccine, immunization against group A streptococcus, rabies, and swine flu, may also trigger GBS (Yuki and Hirata 1998; Nachamkin et al., 2008). All of these pathogens have carbohydrate antigens in common with peripheral nerve tissue. Frequently most identified triggering agent of GBS is *Campylobacter jejuni* (*C. jejuni*), a spiral-shaped Gram-negative bacterium which is the main cause of acute gastroenteritis and bacterial diarrhea worldwide. Several reports described cases in which *C. jejuni* infection has been the preceding infection in GBS and showed strong epidemiological association between *C. jejuni* and GBS (Rees et al., 1995). Few of those reports have concluded that *C. jejuni* plays a major role in the onset of disease and that GBS associated with *C. jejuni* infection can be in more severe form and that it is more likely to be of the AMSAN variant (Kuwabara et al., 2004).

Recent data, present that 25-33% of GBS patients had conferred antecedent *C. jejuni* infection. There are more than 70 serotypes of *C. jejuni* but only few *C. jejuni* serotypes are associated with development of GBS (Kuroki et al., 1993). Biochemical and serological studies showed that GBS associated *C. jejuni* serotypes bear lipopolysaccharide (LPS) structures that mimic human gangliosides (Yuki et al., 1993). Gangliosides as components of the neuronal cell surface and myelin sheath are considered one of the possible target antigens for an autoimmune attack since serum anti-ganglioside antibodies are present in the acute phase of GBS subsequent to *C. jejuni* infection. Ganglioside-like structures are found more frequently in neuropathy associated *C. jejuni* strains than in strains isolated from patients with diarrhea (Ho et al., 1999; Sheikh et al., 1998).

Result of this molecular mimicry between the core oligosaccharides of bacterial LPS and carbohydrate moiety of the human gangliosides is induction of cross-reactive antibodies which are likely to be a contributory factor in the induction and pathogenesis of GBS (Fig. 1).

GM1 ganglioside

Lipopolysaccharide in *C. jejuni*

Fig. 1. Carbohydrate mimicry between GM1 ganglioside and *C. jejuni* lipopolysaccharide. Adapted from Ang C.W. et al., 2004

C. jejuni serotype commonly associated with GBS is serotype O:19, a serotype rarely isolated from uncomplicated gastroenteritis patients. Other *C. jejuni* serotypes identified in association with GBS include O:2, O:2/44, O:4/59, O:15, O:18, O:21, O:24, O:30, O:37 and O:53 (Aspinall et al., 1993).

In our previous work we have showed that some of the *C. jejuni* serotypes isolated from patient with enteritis and with no neurological symptoms also bear ganglioside like epitopes in their LPS molecules (Grozdanova et al., 2011). In our collection of 21 *C. jejuni* isolates from enteritis patient seven different serotypes of *C. jejuni* (O:27; O:6/O:7; O:38; O:3; O:1/O:44; O:19; O:37) were detected using Penner system. Unexpected one serotype from this group was detected as O:19, a serotype rarely isolated from enteritis patient and in close association with GBS. Binding studies with cholera toxin B subunit used as GM1 ligand and peanut agglutinin as marker for Gal β 1-3GalNAc structures showed presence of ganglioside-like epitopes in *C. jejuni* strains O:37, O:19 and O:27. Immune-reactivity with sera from patient with GBS with confirmed previous exposure to *C. jejuni* and with high titre of anti-ganglioside antibodies showed that the same three LPSs from *C. jejuni* serotypes O:37, O:19 and O:27 are bearing cross-reactive epitopes in their LPSs structures. These results are in correlation with studies from other research groups, that LPSs from certain *C. jejuni* serotypes are bearing cross-reactive ganglioside-like epitopes which might be involved in the induction of GBS after *C. jejuni* infection (Ang et al., 2004).

Antiganglioside antibodies in Guillain Barré Syndrome

Epidemiological studies showed that acute-phase sera of patients with *C. jejuni* associated GBS contain high titers of antibodies directed to various gangliosides, predominantly to GM1 in approximately 30%, and also antibody against asialoGM1, GD1b, GT1a, GM3, GD1a, and GT1b. Gangliosides are large and heterogeneous family of membrane glycolipids, highly expressed in nervous tissue, representing nearly 10% of the total lipid content of the peripheral and the central nervous systems (Ho et al., 1999). They are composed of a ceramide tail inserted in the lipid bilayer and a highly variable oligosaccharide moiety protruding externally, with one or more sialic acid(s). The number of sialic acid residues in a ganglioside is used to classify the different species, with one sialic acid designated by M (monosialo), two by D (disialo), giving rise to names such as GM1 (monosialo tetrahexosyl ganglioside), GM2 and GM3. Ganglioside localization studies have shown that GM1 and GM1-like epitopes are concentrated at the nodal regions and the paranodal myelin loops and in the motor nerve myelin of the human sensory and motor nerves (Sheikh et al., 1998). The suggestion that antiganglioside antibodies may play an important role in the pathogenesis of GBS came following the reports of high titers of antiganglioside antibodies in patients with a motor neuron disease. In 1988, Ilyas et al reported antiganglioside antibodies in 5 out of 26 GBS patients and the clinical improvement coincided with a reduction in the antibody titers (Ilyas et al., 1988). The presence of IgG anti-GM1 antibodies was later demonstrated in two patients who had AMAN associated with *C. jejuni* enteritis. Since then, there have been several other gangliosides detected that appear to be target pathogenic antigens in the development of AMAN. These include GD1a, GalNAc-GD1a and GM1b (Yuki et al., 1990). Several reports of patients developing GBS after ganglioside administration have also been reported, and IgG anti-GM1 and GD1a antibodies were identified in some of the patients (Illa et al., 1995). Autoantibodies are considered to be the pathogenic components which trigger GBS because plasma exchange is proven to be an effective treatment in GBS (Hirota et al., 1997).

Following the strong epidemiological data that linked GBS to previous *C. jejuni* infection, scientists were eager to elucidate the postulated association. Yuki and coworkers in 1993 reported that gas-liquid chromatography-mass spectrometric analysis of acute-phase sera from patients with GBS after *C. jejuni* infection has shown that the purified LPS contained Gal, GalNAc, and NeuAc, which are sugar components of GM1 ganglioside (Yuki et al., 1993). The following year they further isolated *C. jejuni* from 2 patients with Miller Fisher syndrome subsequent to enteritis and then extracted and separated crude LPS fractions from the bacteria by thin-layer chromatography. Using monoclonal antibodies to GQ1b ganglioside they showed that both LPS fractions reacted to the anti-GQ1b antiganglioside antibodies, indicating that the LPS bear the GQ1b

epitope (Yuki et al., 2004). These were the first evidence of molecular mimicry between neural tissue components and the often associated antecedent infectious agents of AIDP and MFS. A further work illustrated that anti-GM1 and anti-GD1a antibodies reacted with the LPSs of serotypes *C. jejuni* O:1, O:4 and O:19. In 1994, Aspinall and Penner elucidated the structure of the core oligosaccharide regions of *C. jejuni* strains O:4, and O:19 and concluded that they mimic human gangliosides in structure (Aspinall et al., 1994). Later on, other reports of the immunological mimicry of *C. jejuni* were published with focus into GBS associated *C. jejuni* serotypes (Wirguin et al., 1997).

In one of the study conducted by our research group we have determine the frequency of antecedent infection with *C. jejuni* in the population of our GBS patients, the association with anti-GM1 antibodies and the distribution of these antibodies within clinical forms of the disease. The diagnosis of GBS has been established in 17 patients according to clinical, electrophysiological and laboratory criteria. In relation to the clinical form and disability score two patients were ambulatory, five were ambulatory with support, seven were bedridden and two patients needed respirator. Five (29%) patients had pure motor, while 12 (71%) had sensor and motor GBS. Electromyoneurography showed primary axonal, predominantly motor neuropathy in 6 (35%) and demyelinating sensor and motor neuropathy in 11 (65%) patients. The CSF protein content ranged from 0.47 to 3.88 g/L. Anti-*C. jejuni* antibodies were found in 8 (73%) out of 11 patients with demyelinating GBS and in 4 (66.6%) out of 6 patients with axonal GBS. The antecedent infection with *C. jejuni* was shown by serum antibodies to *C. jejuni* flagellar protein in 12 (71%) patients. Fifteen (88%) patients had IgG anti-GM1 antibodies and twelve (71%) patients had both antibodies. The high titer of anti-GM1 antibodies was found in all patients (100%) with axonal and in 9 (82%) out of 11 patients with demyelinating GBS. The association of IgG anti-*C. jejuni* and IgG anti-GM1 antibodies was found in 4 (66.6%) out of 6 patients with axonal and in 8 (73%) out of 11 patients with demyelinating GBS. The main features of our patients with GBS were high frequency of antecedent infection with *C. jejuni*, unusually frequent association with anti-GM1 antibodies, and equally frequent association of anti-*C. jejuni* and anti-GM1 antibodies in both, axonal and demyelinating GBS (Basta et al., 2005).

Molecular Mimicry in Guillain Barré Syndrome

The last 20 years there have been the emergence of abundant clinical data clearly showing a disease-specific correlation between peripheral neuropathies and anti-ganglioside antibodies. Many clinical and serological studies support the concept that anti-ganglioside antibodies play a key active role in pathogenesis of GBS. Experimental evidence obtained from human and animal studies continues to support the model of post infectious neuropathy as a dis-

ease involving molecular mimicry between bacterial and neural oligosaccharides. As previously stated, in order to conclude that a disease is triggered by molecular mimicry four criteria need to be satisfied.

1. Establishment of an epidemiological association between an infectious agent and the immune-mediated disease

In the case of GBS there is strong epidemiological evidence for the association between acute infection with *C. jejuni* and GBS. Majority of GBS patients showed occurrence of neurological symptoms in no more than few weeks after the preceding infectious disease (Goodyear et al., 1999). Because of the short period between the acute infection and the development of symptoms, identification of the triggering infectious agents thru case control studies and using *in vitro* culture and serological techniques is possible. Therefore there is a strong and documented epidemiological evidence for the association between acute infection with *C. jejuni* and development of GBS. Those findings fulfill the first criteria for determining the infectious agent associated with the autoimmune disease (Ang et al., 2001).

2. Identification of antibodies or T cells directed against host target antigens in patients

In GBS patients, the association of *C. jejuni* infections is conferred with presence of autoantibodies against host ganglioside. The pathogenic effect of the induced antibodies or T cells in patients must be demonstrated *in vivo* or *in vitro*. It is very hard to satisfy this criterion, since there are a lot of experimental limitations and not always the observed symptoms directly reflect the clinical pathway of the human disease. In the acute phase serum from GBS patients, antibodies against GM1, GM1b, GD1a and GalNAc-GD1a gangliosides, are present and the specificity of these autoantibodies is closely related to the nature of the preceding infections and the pattern of clinical features of GBS (Ang et al., 2002). Therefore in GBS subtypes affecting only motor nerves (AMAN) the detected antibodies are specific for GM1, GM1b GD1a and GalNAc-GD1a gangliosides, while in Miller Fisher syndrome more present are anti-GQ1b antibodies. This means that a clinical feature of the illness is in part mediated by the ganglioside specificity which is determined by the infectious agent. The isotype of the antiganglioside antibodies in GBS patients beside the expected IgM class is presented in IgA and IgG antibodies. The IgG1 and IgG3 subclass are less expected having in mind that this is not "classical" antibody response against carbohydrate antigens, and pointing to an isotype switch involving T-cell which has been identified in the affected nerves and acute phase blood samples from GBS patients. The pathogenic effect of anti-ganglioside antibodies is mediated by binding to the Schwann cell surface,

nodes of Ranvier and axons in peripheral nerves, disturbing the function and integrity of the motor nerve terminal, in a complement-dependent manner (Ariga and Yu, 2005). Therefore, the presence of ganglioside autoantibodies with neuropathogenic potential in GBS patients satisfies the criterion of identification of T cells or antibodies against host target antigens.

3. Identification of microbial mimic of target antigen

Association between GBS, previous infections and the presence of antiganglioside antibodies has been investigated most extensively in *C. jejuni* induced GBS. Biochemical and serological studies showed that the LPS fraction of the outer cell wall of *C. jejuni* contains structures that mimic gangliosides (Yuki et al., 1993). The terminal tetrasaccharide of LPS extracted from a *C. jejuni* isolate of an AMAN patient was completely identical to that of the GM1 ganglioside and IgG, IgM and IgA anti-ganglioside antibodies from GBS patients recognized the *C. jejuni* LPS. This is strong evidence that these antiganglioside antibodies have been induced by an infection with *C. jejuni*. The complete genome of *C. jejuni* has recently become available, enabling the identification of neuropathogenic virulence factors (Parkhill et al., 2000). Serological and biochemical studies have demonstrated that the LPS fraction of *C. jejuni* contains structures that mimic gangliosides (Yuki et al., 1993). Some strains appear to have a higher potential for inducing neurological complications and this is related to the presence of specific genes in the LPS biosynthesis locus (Van Belkum et al., 2001). This comprises demonstration of cross-reactivity of autoreactive T cells or antibodies with a microbial antigen, derived from an organism that has been epidemiologically linked to the disease (Kaida et al., 2009). The identification and biochemical characterization of *C. jejuni* LPS as the microbial mimic for gangliosides satisfies the third criterion for molecular mimicry.

4. Reproduction of the disease in an animal model

Animal models used to reproduce the disease can be achieved either by infection or by immunization with the infection agent or purified antigens in order to induce cross-reactive immune response, with similar specificity, clinical symptoms and pathological features as seen in patients. Animal models are used also to investigate other aspects of proposed molecular mimicry. The first animal studies in the case of GBS, showed that immunization with *C. jejuni* or with purified LPS result in a cross-reactive antiganglioside response (Ang et al., 2001). The animals did not develop neuropathy but the anti-ganglioside antibodies generated in animals share pathogenic properties with human GBS sera. Yuki and colleagues immunized Japanese white rabbits with purified gangliosides as self-antigen and with purified *C. jejuni* LPS and induces neurological disorders,

resulting in a neuropathy with clinical, electrophysiological and histopathological features closely resembling GBS. An animal model of AMAN was established by the sensitization of rabbits with a bovine brain ganglioside mixture. The rabbits developed high titres of IgG anti-GM1 antibodies, followed by an acute onset of flaccid limb weakness with a monophasic course which corresponds well with pathological findings for human AMAN (Yuki et al., 2004). These findings forms strong evidence that the ganglioside autoantibodies in humans can be induced by molecular mimicry between *Campylobacter* LPS and gangliosides, and therefore the last criterion for molecular mimicry, reproduction of the disease in an animal model is fulfilled.

Conclusion

GBS is probably the first true model of molecular mimicry having fulfilled all four criteria as follows: (i) establishment of an epidemiological association between GBS and *C. jejuni* infection by a prospective case-control study, (ii) identification of autoantibodies against GM1 and GD1a gangliosides in patients with AMAN subsequent to *C. jejuni* enteritis, (iii) identification of molecular mimicry between GM1 or GD1a and LPS of *C. jejuni* isolated from AMAN, and (iv) reproduction of the AMAN models by active immunization of rabbits with GM1 or with *C. jejuni* LPS as well as by passive transfer of anti-GD1a antibodies in mice. This makes GBS probably the first disease in humans to verify that an autoimmune disease is triggered by molecular mimicry.

Still, many key issues in the pathogenesis of GBS remain unresolved, like genetic and/or environmental host factors that confer disease susceptibility to a small number of infected individuals, specific nature of pathology according to antibody specificity, the involvement of T cell providing help to antibody production, and the nature of the immunological injuries. There is a need for a better understanding of the pathogenesis of GBS that will allow development targeted immunotherapy in order to prevent or limit the devastating injury that can result from this autoimmune-mediated neuropathy.

References

- Albert, L.J., Inman, R.D., 1999. Molecular mimicry and autoimmunity. *N. Engl. J. Med.* 341, 2068–2074.
- Ang, C.W., De Klerk, M.A., Endtz, H.P., Jacobs, B.C., Laman, J.D., Van der Meche, F.G.A., Van Doorn, P.A., 2001. Guillain-Barre syndrome and Miller-Fisher syndrome-associated *Campylobacter jejuni* lipopolysaccharides induce anti-GM1 and anti-GQ1b antibodies in rabbits. *Infect. Immun.* 69, 2462–2469.
- Ang, C.W., Laman, J.D., Willison, H.J., Wagner, E.R., Endtz, H.P., De Klerk, M.A., Tio-Gillen, A.P., Van den Braak, N., Jacobs, B.C., Van Doorn, P.A., 2002. Structure of *Campylobacter jejuni* lipopolysaccharides determines antiganglioside specificity and clinical features of Guillain-Barre and Miller Fisher patients. *Infect. Immun.* 70, 1202–1208.
- Ang, C.W., Jacobs, B.C., Laman, J.D., 2004. The Guillain-Barre syndrome: a true case of molecular mimicry. *Trends Immunol.* 25, 61–66.
- Ariga, T., Yu, R.K., 2005. Anti-glycolipid antibodies in Guillain-Barre syndrome and related diseases: review of clinical features and antibody specificities. *J. Neurosci. Res.* 80, 1–17.
- Asbury, A.K., Cornblath, D.R., 1990. Assessment of current diagnostic criteria for Guillain-Barre syndrome. *Ann. Neurol.* 27, 21–24.
- Asbury, A.K., McKhann, G.M., 1997. Changing views of Guillain Barré syndrome. *Ann. Neurol.* 41, 287–288.
- Aspinall, G.O., McDonald, A.G., Raju, T.S., Pang, H., Moran, A.P., Penner, J.L., 1993. Chemical structures of the core regions of *Campylobacter jejuni* serotypes O:1, O:4, O:23, and O:36 lipopolysaccharides. *Eur. J. Biochem.* 213, 1017–1027.
- Aspinall, G.O., McDonald, A.G., Pang, H., Kurjanczyk, L.A., Penner, J.L., 1994. Lipopolysaccharides of *Campylobacter jejuni* serotype O:19: Structures of core oligosaccharide regions from the serostrain and two bacterial isolates from patients with Guillain-Barre syndrome. *Biochemistry* 33, 241–249.
- Atkinson, M.A., 1992. Islet Cell Autoantibodies Reactive to Glutamate Decarboxylase in Insulin Dependent Diabetes. *J. Clin. Invest.* 91, 350–356.
- Basta, I., Suturkova, Lj., Vujic, A., Aleksic, S., Poceva, A., Paskoska, A., Milenikova, K., Trikić, R., Apostolski, S., 2005. Antibodies to ganglioside GM1 and *Campylobacter jejuni* in patients with Guillain-Barre syndrome. *Srp. Arh. Celok. Lek.* 133, 123–128.
- Bickerstaff, E.R., 1957. Brain-stem encephalitis: further observations on a grave syndrome with benign prognosis. *BMJ.* 1, 1384–1387.
- Damian, R.T., 1964. Molecular mimicry: antigen sharing by parasite and host and its consequences. *Am. Nat.* 98, 129–149.
- Falkow, S., 1988. Molecular Koch's postulates applied to microbial pathogenicity. *Rev. Infect. Dis.* 10, 274–276.
- Fisher, M., 1956. An unusual variant of acute idiopathic polyneuritis (syndrome of ophthalmoplegia, ataxia and areflexia). *N. Engl. J. Med.* 255, 57–65.
- Goodyear, C.S., 1999. Monoclonal antibodies raised against Guillain-Barre syndrome-associated *Campylobacter jejuni* lipopolysaccharides react with neuronal gangliosides and paralyze musculenerve preparations. *J. Clin. Invest.* 104, 697–708.
- Griffin, J. W., Li, C. Y., Macko, C., 1996. Early nodal changes in the acute motor axonal neuropathy pattern of the Guillain Barré syndrome. *J. Neurocytol.* 25, 33–51.
- Grozdanova, A., Poceva, A., Brezovska, K., Trajkovska-Dokic, E., Apostolski, S. and Suturkova, Lj., 2011. Cross-reactive epitopes in *Campylobacter jejuni* strains isolated from enteritis patients. *Prilozi MASA.* June 2011 (in press).
- Guillain, G. Barré, J.A., Ströhl, A., 1916. Sur un syndrome de radiculonévrite avec hyperalbuminose du liquid céphalo-rachidien sans réaction cellulaire: remarques sur les caractères cliniques et graphiques des réflexes tendineux. *Bulletins et mémoires de la Société des Médecins des Hôpitaux de Paris* 40, 1462–1470.

- Hadden, R.D., Cornblath, D.R., Hughes, R.A.C., 1998. Electrophysiological classification of Guillain-Barre syndrome: clinical associations and outcome. *Ann. Neurol.* 44, 780-788.
- Hadden, R.D., Cornblath, D.R., Hughes, R.A.C., 2001. Preceding infection, immune factors and outcome in Guillain-Barre syndrome. *Neurology* 56, 758-765.
- Hafer-Macko, C., Hsieh, S.T., Li, C.Y., 1996. Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. *Ann. Neurol.* 40, 635-644.
- Hafer-Macko, C., Hsieh, S.T., Li, C.Y., 2000. Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. *Ann. Neurol.* 39, 625-635.
- Hiraga, A., Kuwabara, S., Ogawara, K., 2005. Patterns and serial changes in electrodiagnostic abnormalities of axonal Guillain-Barre syndrome. *Neurology* 64, 856-860.
- Ho, T.W., Willison, H.J., Nachamkin, I., 1999. Anti-GD1a antibody is associated with axonal but not demyelinating forms of Guillain-Barre syndrome. *Ann Neurol.* 45, 168-173.
- Huber, S.A., Cunningham M.W., 1996. Streptococcal M protein peptide with similarity to myosin induces CD4+ T cell dependent myocarditis in MRL++ mice and induces partial tolerance against coxsackie viral myocarditis. *J. Immunol.* 156, 3528-3534.
- Hughes, R.A., Rees J.H., 1997. Clinical and epidemiologic features of Guillain-Barre syndrome. *J. Infect. Dis.* 176, 92-98.
- Hughes, R.A., 1999. Pathogenesis of Guillain-Barre' syndrome. *J. Neuroimmunol.* 100, 74-97.
- Hughes, R.A., 2005. Multidisciplinary Consensus Group. Supportive care for patients with Guillain Barré syndrome. *Arch. Neurol.* 62, 1194-1198.
- Hughes, R.A., Swan, A. V., Raphael, J., Annane, D., Van Doorn, P.A. 2007. Immunotherapy for Guillain-Barre syndrome: a systematic review. *Brain* 130, 2245-2257.
- Illa, I., Ortiz, N., Gallard, E., Juarez, C., Grau, J.M., Dalakas, M.C., 1995. Acute axonal Guillain-Barresyndrome with IgG antibodies against motor axons following parenteral gangliosides. *Ann. Neurol.* 38, 218-24.
- Ilyas, A.A., Willison, H.J., Quarles, R.H., Jungalwala, F.B., Cornblath, D.R., Trapp, B.D., Griffin, D.E., Griffin, J.W., McKhann, G.M., 1988. Serum antibodies to gangliosides in Guillain-Barre syndrome. *Ann. Neurol.* 23, 440-447.
- Jacobs, B.C., 1998. The spectrum of antecedent infections in Guillain-Barre syndrome: a case-control study. *Neurology* 51, 1110-1115.
- Kaida, K., Ariga, T., Yu, R.K., 2009. Antiganglioside antibodies and their pathophysiological effects on Guillain-Barre syndrome and related disorders. *Glycobiology* 19, 676-692.
- Kaufman, D.L., 1993. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature* 366, 69-72.
- Khan, F., Nag, L., 2009. Rehabilitation for Guillain Barre Syndrome. *IJRT.* 16, 1-8.
- Kuroki, S., 1993. *Campylobacter jejuni* strains from patients with Guillain-Barre syndrome belong mostly to Penner serogroup 19 and contain b-N-acetylglucosamine residues. *Ann. Neurol.* 33, 243-247.
- Kuwabara, S., Ogawara, K., Sung, J.Y., 2002. Differences in membrane properties of axonal and demyelinating Guillain-Barre syndromes. *Ann. Neurol.* 52, 180-187.
- Kuwabara, S., Ogawara, K., Misawa, S., 2004. Does *Campylobacter jejuni* infection elicit "demyelinating" Guillain-Barre syndrome. *Neurology* 63, 529-533.
- McKhann, G. M., Cornblath, D. R., Griffin, J. W., 1993. Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China. *Ann. Neurol.* 33, 333-342.
- Nachamkin, I., Allos, B. M., Ho, T. 1998. *Campylobacter* species and Guillain-Barre syndrome. *Clin Microbiol. Rev.* 11, 555-567.
- Nachamkin, I., 2001. *Campylobacter* Enteritis and the Guillain-Barre Syndrome. *Curr. Infect. Dis. Rep.* 3, 116-122.
- Nachamkin, I., Shadomy, S.V., Moran, A.P., Cox, N., Fitzgerald, C., Ung, H., Corcoran, A.T., Iskander, J.K., Schonberger, L.B., Chen, R.T., 2008. Antiganglioside antibody induction by swine (A/NJ/1976/H1N1) and other influenza vaccines: Insights into vaccine-associated Guillain-Barre syndrome. *J. Infect. Dis.* 198, 226-233.
- Parkhill, J., Wren, B.W., Mungall, K., Ketley, J.M., Churcher, C., Basham, D., Chillingworth, T., Davies, R.M., Feltwell, T., Holroyd, S., 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* 403, 665-668.
- Plomp, J.J., Molenaar, P.C., O'Hanlon, G.M., Jacobs, B.C., Veitch, J., Daha, M.R., Van Doorn, P.A., Van Der Meche, F.G., Vincent, A., Morgan, B.P., 1999. Miller Fisher anti-GQ1b antibodies: a-latrotoxin-like effects on motor endplates. *Ann. Neurol.* 45, 189-199.
- Rees, J.H. Soudain, S.E., Gregson, N.A., Hughes, R.A.C., 1995. *Campylobacter jejuni* infection and Guillain-Barre syndrome. *N. Engl. J. Med.* 333(21), 1374-1379.
- Rose, N.R., Bona C., 1993. Defining criteria for autoimmune diseases. *Immunol. Today* 14, 426-430.
- Rose, N.R., Mackay I.R., 2000. Molecular mimicry: a critical look at exemplary instances in human diseases. *Cell. Mol. Life Sci.* 57, 542-551.
- Sheikh, K.A., Nachamkin, I., Ho, T. W., Willison, H. J., Veitch, J., Ung, H., Nicholson, M., Li, C. Y., Wu, H. S., Shen, B. O., Cornblath, D. R., Asbury, A. K., McKhann, G. M., Griffin, J. W., 1998. *Campylobacter jejuni* lipopolysaccharides in Guillain-Barre syndrome: molecular mimicry and host susceptibility. *Neurology* 51, 371-378.
- Van Belkum, A., 2001. A *Campylobacter jejuni* gene associated with immune-mediated neuropathy. *Nat. Med.* 7, 752-753.
- Van der Meche, S., PIM, Dutch Guillain Barré Study Group. 1992. A randomized trial comparing intravenous immune globulin and plasma exchange in Guillain Barré syndrome. *N. Engl. J. Med.* 326, 1123-29.
- Van der Meche, S., Van Doorn, P.A., 1995. Guillain-Barre'syndrome and chronic inflammatory demyelinating polyneuropathy: immune mechanisms and update on current therapies. *Ann. Neurol.* 37, 14-31.
- Van Doorn, P.A., 2008. Clinical features, pathogenesis, and treatment of Guillain-Barre syndrome, *The Lancet Neurology* 7(10), 939-950.
- Wirguin, I., Briani, C., Suturkova-Milosevic, L., Fisher, T., Della-Latta, P., Chalif, P., Latov, N., 1997. Induction of anti-GM1 ganglioside antibodies by *Campylobacter jejuni* lipopolysaccharides. *J. Neuroimmunol.* 78, 138-142.
- Yuki, N., Yoshino, H., Sato, S., Miyatake, T., 1990. Acute axonal polyneuropathy associated with anti-GM1 antibodies following *Campylobacter* enteritis, *Neurology* 40(12), 1900-1902.
- Yuki, N., Taki, T., Inagaki F., 1993. A bacterium lipopolysaccharide that elicits Guillain-Barre syndrome has a GM1 ganglioside-like structure, *Journal of Experimental Medicine* 178(5), 1771-1775.

Yuki, N., Yamada M., Koga, M., 2001. Animal model of axonal Guillain-Barre syndrome induced by sensitization with GM1 ganglioside, *Ann. Neurol.* 49(6), 712–720.

Yuki, N., Susuki, K., Koga, M., Nishimoto, Y., Odaka, M., Hirata, K., Taguchi, K., Miyatake, T., Furukawa, K.,

Kobata, T., Yamada, M., 2004. Carbohydrate mimicry between human ganglioside GM1 and *Campylobacter jejuni* lipooligosaccharide causes Guillain-Barre syndrome. *Proc. Natl. Acad. Sci.* 101, 11404–11409.

Резиме

Улогата на молекуларната мимикрија во етиопатогенезата на Guillain Barré синдромот

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Клучни зборови: Guillain Barré syndrome, молекуларна мимикрија, *Campylobacter jejuni*, липолисахариди, ганглиозиди

Молекуларната мимикрија помеѓу сопствени ткивни структури и микробни структури е предложена како патоген механизам за иницирање на автоимуни заболувања кај кои претходи инфекција. Неодамнешните испитувања посочија дека молекуларната мимикрија како можен механизам сеуште не е докажана кај најголем број на хумани заболувања. Сепак, во случајот на периферната невропатија Guillain Barré синдром (GBS) оваа хипотеза е поткрепена со огромен број на експериментални докази. GBS е најчестата причина за акутна невромускулна парализа и често се јавува по инфекција со бактеријата *Campylobacter jejuni* (*C. jejuni*). Епидемиолошките студии, покажаа дека повеќе од една третина од пациентите со GBS имале претходна инфекција со *C. jejuni* и дека само одредени серотипови на *C. jejuni* се асоцирани со развој на GBS. Молекуларната мимикрија помеѓу хуманите ганглиозиди и олигосахаридите на бактерискиот липополисахарид (LPS) најверојатно резултира со создавање на антиганглиозидни вкрстено реактивни антитела кои најверојатно се фактор кој влијае на патогенезата на GBS. Антиганглиозидните антитела се присутни во серумот на пациентите со GBS, а со сензибилизација на зајци со ганглиозиди и *C. jejuni* LPS воспоставени се експериментални животински модели на GBS.

GBS е класичен пример на пост инфективно имуно-посредувано заболување кое најверојатно ќе биде дефинирано како прв вистински модел на автоимуно заболување кое е резултат на молекуларна мимикрија.