The neuroimmunology of gluten intolerance

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Abstract

The term Gluten Related Disorders (GRD) denotes a spectrum of diverse immune mediated diseases triggered by the ingestion of gluten (protein found in wheat, barley and rye). Coeliac disease (CD) or gluten sensitive enteropathy is the most recognised and studied entity within GRD. Extraintestinal manifestations, are gaining recognition and are increasingly the subject of further studies as they may hold the key to unravelling the pathophysiology of GRD. Such manifestations include skin involvement in the form of dermatitis herpetiformis (DH) and neurological dysfunction (eg gluten ataxia and gluten neuropathy). Furthermore the recent concept of extraintestinal manifestations without enteropathy (termed Non-Coeliac Gluten Sensitivity-NCGS) has become accepted as part of the same spectrum. In this chapter we review the neurological manifestations in GRD, discuss recent advances in diagnosis, and possible pathophysiological mechanisms.

Introduction

Coeliac Disease (CD) was first described by the Greek doctor Aretaeus the Cappadocian, in 100 AD. Long time after, Samuel Gee published his lecture (Gee, 1888) “on the coeliac affection” in which he described the classic presentation of the disease in children. The aetiological agent remained obscure until the observations of Willem Dicke, a Dutch paediatrician, in 1953 of “the presence in wheat, of a factor having a deleterious effect in cases of coeliac disease” (Dicke et al., 1953). The introduction of endoscopy and small

bowel biopsy in the 50's confirmed the bowel as the principal organ involved (Paulley, 1953). Such biopsies demonstrated for the first time the typical histological abnormalities that now define gluten sensitive enteropathy: villous atrophy, crypt hyperplasia and increased intraepithelial lymphocytes.

The first evidence of gluten sensitivity related extraintestinal manifestations became apparent in 1963 when a group of dermatologists published the interesting observation that dermatitis herpetiformis, an itchy vesicular rash, was in fact a form of gluten related dermatopathy sharing the same small bowel pathology, but less prominent or even no gastrointestinal symptoms (Marks et al., 1963). The only reason why small bowel biopsies were done in this group of patients was the observation of persistently low albumin suggestive of protein loss from the gut.

A small number of case reports of patients with presumed CD and neurological manifestations (Elders, 1925; Reed and Ash 1927; Woltman and Heck 1937) were published prior to the discovery of the aetiological agent and the introduction of small bowel biopsy. Such reports need to be treated with caution given that a diagnosis of CD in those patients was speculative.

The first comprehensive case series of neurological manifestations in the context of histologically confirmed CD was published in 1966 (Cooke and Thomas-Smith, 1966). This detailed clinical and pathology work described the range of neurological manifestations seen in 16 patients with established CD. Of interest was the fact that all patients had gait ataxia and some had peripheral neuropathy as well. The assumption was that such manifestations were nutritional. Indeed all of these patients were grossly malnourished and cachectic. Post-mortem data, however, demonstrated an inflammatory

process primarily affecting the cerebellum, but also involving other parts of the central and peripheral nervous systems, a finding that was in favour of an immune mediated pathogenesis.

Single and multiple case reports of patients with established CD who then developed neurological dysfunction continued to be published (Binder et al., 1967; Bundley, 1967; Morris et al., 1970; Coers et al., 1971; Kepes et al., 1975; Coers et al., 1971; Kepes et al., 1975; Finelli et al., 1980; Kinney et al., 1982; Ward and Murphy 1985; Lu et al., 1986; Kristoferitsch and Pointer, 1987; Kaplan et al., 1988; Tison et al., 1989; Hermaszewski et al., 1991; Colin et al., 1991; Dick et al., 1995; Bhatia et al., 1995; Muller et al., 1996)
Key findings from these reports were that ataxia and/or neuropathy were the commonest manifestations always seen in the context of established CD and almost always attributed to nutritional deficiencies. Some reports reported improvement of the neurological problems with adherence to a GFD whilst others did not. None of these reports however documented the strictness of adherence to the gluten free diet by regular serological testing.

Thirty years after the first comprehensive case series on neurological manifestations of CD saw the publication of a study (Hadjivassiliou et al., 1996) approaching the subject purely from a neurological perspective. This study investigated the prevalence of serological markers of gluten sensitivity (at the time, IgG and IgA antigliadin antibodies) in patients presenting with neurological dysfunction. The results demonstrated significantly higher prevalence of antigliadin antibodies (AGA) in the neurology group of patients with unclear diagnosis when compared to healthy blood donors and patients with a clear neurological diagnosis. Based on duodenal biopsies the study

showed that the prevalence of CD was 16 times higher than the prevalence of CD in the healthy population. This study sparked an interest for both neurologists and gastroenterologists in a possible link between sensitivity to gluten and neurological disease.

Epidemiology of neurological manifestations

There are now several epidemiological studies from Europe, America and a few from other continents demonstrating that the prevalence of CD in healthy individuals is on the increase (Catassi et al., 2014). Thus the prevalence of CD in the healthy population has been shown to be at least 1% (Sanders et al., 2003). There are no accurate figures of the prevalence of the neurological manifestations of gluten sensitivity in the general population. Some studies have concentrated on the prevalence of neurological dysfunction amongst patients with established CD. Figures of between 10% and 22.5% have been published (Holmes, 1997; Briani et al.,2008). Such figures are unlikely to be accurate because they are retrospective, derived solely from gastrointestinal clinics and concentrated exclusively on patients with the classic (ie gastrointestinal) CD presentation. Some of these studies also included neurological diseases that are highly unlikely to be gluten related (eg carpal tunnel syndrome, idiopathic Parkinson's disease).

Some estimates of prevalence can be made from patient populations attending specialist clinics although caution must be exercised in extrapolating these as they are inevitably affected by referral bias. For example, data collected from the Sheffield dedicated CD clinic (the biggest in the UK) and from the dedicated gluten sensitivity/neurology clinic (the only one in the UK)

suggested that for every 7 patients presenting to the gastroenterologist who are then diagnosed with CD, 2 patients present to the neurologist with neurological dysfunction leading to the diagnosis of CD (Hadjivassiliou et al., 2010a). This ratio is likely to be an underestimate because it does not take into account those patients with neurological manifestations due to sensitivity to gluten that do not have enteropathy (NCGS). In fact, approximately two thirds of patients presenting with neurological dysfunction do not have enteropathy on duodenal biopsy. The authors believe that the prevalence of neurological dysfunction even in patients with CD presenting to gastroenterologists is likely to be much higher to what has been published if patients undergo rigorous neurological workup including MR spectroscopy of the cerebellum. Preliminary results from a prospective study using patients with newly diagnosed CD presenting to a gastroenterologist demonstrated that up to 50% of such patients have abnormal MR spectroscopy (low NAA/Cr ratios) of the cerebellum (Hadjivassiliou et al., 2013). One study in patients with established CD has shown such abnormalities in up to 80% of patients (Hadjivassiliou et al., 2011) whilst another study has shown that the prevalence of peripheral neuropathy in this group of patients was 23% (Luostarinen et al.,2003). The above figures are based on patients with CD. The frequency of neurological dysfunction in patients with NCGS is not known. Judging by the fact that 2/3 of the cohort of patients seen and assessed in a dedicated gluten/neurology clinic, Sheffield, UK have NCGS, it is likely that the prevalence of neurological cases with NCGS is even higher than those with CD.

Diagnosis of the spectrum of gluten related diseases

The diagnosis of CD in the hands of an experienced gastroenterologist and gastrointestinal histopathologist can be relatively straightforward. CD is after all defined by the presence of an enteropathy (triad of villus atrophy, crypt hyperplasia and increased intraepithelial lymphocytes), usually a reliable gold standard. It is now, however accepted, that the presence of enteropathy is not a prerequisite for the diagnosis of GRD particularly for those patients where neurological or other extraintestinal manifestations are the presenting feature. Furthermore, the triad of the small bowel mucosal changes mentioned above are merely one part of the small bowel histological spectrum (Marsh classification) that ranges from a normal mucosa to a pre-lymphomatous state (Marsh, 1992). Given that the bowel histology in some cases (as per Marsh classification) can be normal, trying to define GRD using solely the bowel biopsies becomes problematic. Whilst serological testing has enhanced the ability to identify patients with enteropathy, none of these tests are 100% specific or sensitive (ref). For example, endomysial antibody (EMA) and anti-transglutaminase-2 (TG2) IgA antibody detection are specific for the presence of an enteropathy. These markers are frequently negative in patients with neurological or other extraintestinal manifestations who do not have an enteropathy (Hadjivassiliou et al. 2008, 2013).

The majority of patients presenting with neurological manifestations have no gastrointestinal symptoms (ref). Even patients with CD may not have gastrointestinal symptoms. In patients without overt gastrointestinal involvement (enteropathy), serum antibodies to TG2 may be absent (Kaukinen et al., 2005). Patients with extraintestinal manifestations typically

have antibodies primarily reacting with different TG isozymes, TG3 in DH and TG6 in patients with gluten ataxia (Sardy et al., 2002; Hadjivassiliou et al, 2008). Reaction of such antibodies with TG2 that takes the form of IgA deposits against TG2 in the intestinal mucosa occurs prior to overt changes in small intestinal morphology and sometimes even before the antibodies are detectable in serum (Korponay-Szabo et al, 2003). Such antibody deposits seem to be present in patients with neurological and other extraintestinal manifestations as well, and may therefore be diagnostically useful (Hadjivassiliou et al, 2006). However, this test is not readily available and requires experience in its interpretation. In practise for suspected neurological manifestations, it is best to perform serological tests for both IgA and IgG antibodies to TG2 (and if available anti-TG6 and anti-TG3) as well as IgG and IgA antibodies to gliadin. Endomysium antibodies are very specific for the detection of enteropathy, but they detect the same antigen (transglutaminase 2) and have thus largely been replaced by TG2 antibody testing. Any differences between the 2 tests however are likely to be related to the different methodologies used (ELISA for TG2 vs immunofluorescence for the detection of EMA). The diagnosis of NCGS remains problematic by the absence of any biological markers. At the moment such diagnosis is based on symptomatic improvement after the introduction of a GFD and recurrence of symptoms on re-introduction of gluten in the diet. Antigliadin antibodies of the IgG type can be present in up to 25% of patients with NCGS attending gastroenterology clinics and such patients may also have increased intraepithelial lymphocytes (Volta et al.,2014).

CD has a strong genetic predisposition whereby ~40% of the genetic load comes from MHC class II association (Hunt, 2008). In Caucasian populations more than 90% of CD patients carry the HLA DQ2, with the remaining having the HLA DQ8. A small number of CD patients do not belong into either of these groups but these have been shown to carry just one chain of the DQ2 heterodimer (Jabri and Sollid, 2009). HLA genetic testing is therefore another useful tool, particularly as unlike other serological tests it is not dependent on an immunological trigger. However, the HLA DQ genotype can be used only as a test of exclusion of CD as the risk genotype DQ2 is common in Caucasian and Asian populations and many carriers will never develop GRD. Furthermore patients with NCGS may not have the HLA DQ2 or DQ8 (ref). Several genome wide association studies over the past decade have identified many non-HLA loci that each contribute a small amount of risk for celiac disease (Lundin and Sollid, 2014). Most of these additional genes are involved in immune functions and in fact, several risk loci are shared with other autoimmune conditions including ankylosing spondylitis, rheumatoid arthritis, type 1 diabetes and psoriasis. A recent study showed that including non-HLA variants in addition to HLA in the test for celiac disease risk improves the accuracy of disease prediction (Romanos et al., 2014). A bias for loci conferring risk for specific manifestations of GRD remains to be thoroughly investigated.

The spectrum of gluten related neurological manifestations

Gluten ataxia

Gluten ataxia (GA) is defined as idiopathic sporadic ataxia with positive serological markers for sensitivity to gluten (Hadjivassiliou et al, 2003). The original definition was based on the serological tests available at the time (antigliadin IgG and IgA antibodies). In a series of 1000 patients with progressive ataxia evaluated over a period of 20 years in Sheffield, UK, GA had a prevalence of 15% amongst all ataxias but as high as 41% amongst idiopathic sporadic ataxias. Using the same AGA assay the prevalence of positive AGA in genetically confirmed ataxias was 14/110 (13%), and in healthy volunteers 149/1200 (12%). A number of studies looking at the prevalence of antigliadin antibodies in ataxias have been published (Hadjivassiliou et al., 1996; Pellecchia et al., 1999; Burk et al., 2001; Bushara et al., 2001; Luostarinen et al., 2001; Abele et al., 2002; Hadjivassiliou et al., 2003; Abele et al., 2003; Ihara et al., 2006; Anheim et al., 2006) The variations in prevalence may relate to geographical differences in the prevalence of CD, referral bias, variability in the AGA assays used, patient selection (some studies included as idiopathic sporadic ataxia patients with cerebellar variant of multi-system atrophy), small number of patients studied and no controls. The common theme in the majority of these studies is the consistently high prevalence of AGA antibodies in sporadic ataxias when compared to healthy controls.

GA usually presents with pure cerebellar ataxia or rarely ataxia in combination with myoclonus (see below), palatal tremor (Hadjivassiliou et al., 2008), opsoclonus (Deconinck et al., 2006) or rarely, chorea (Pereira et al., 2004). GA is usually of insidious onset with a mean age at onset of 53 years. Rarely the ataxia can be rapidly progressive mimicking paraneoplastic

cerebellar degeneration. Gaze-evoked nystagmus and other ocular signs of cerebellar dysfunction are common (80% of cases). All patients have gait ataxia and the majority have limb ataxia. Less than 10% of patients with GA will have any gastrointestinal symptoms but 40% will have evidence of enteropathy on biopsy.

Serological diagnosis still relies on the presence of IgG and/or IgA antigliadin antibodies but more specific biomarkers have been identified. TG6 antibodies have been found to be present in 73% of patients with idiopathic sporadic ataxia with positive AGA (Hadjivassiliou et al., 2008). Furthermore, 32% of patients with idiopathic sporadic ataxia negative for other serological markers of sensitivity to gluten were found to be positive for TG6 (Hadjivassiliou et al, 2013). This may suggest that the prevalence of gluten ataxia may even be higher than previously thought.

Patients with GA usually have evidence of cerebellar atrophy on MR imaging with particular predilection for the cerebellar vermis. MR spectroscopy of the vermis is abnormal in all patients with GA (low N-Acetyl Aspartate/Creatine ratio) with less prominent changes in the cerebellar hemispheres (ref). Even in patients with GA without cerebellar atrophy, MR spectroscopy is abnormal. MR spectroscopy is a useful monitoring tool. Patients who adhere to strict gluten free diet often have evidence of improvement of the NAA/Cr ratio within the vermis after a year on the diet.

The response to treatment with a gluten-free diet depends on the duration of the ataxia prior to the diagnosis of sensitivity to gluten. Loss of Purkinje cells in the cerebellum, the end result of prolonged gluten exposure in patients with GA, is irreversible, therefore prompt treatment is more likely to

result in improvement or stabilisation of the ataxia. Whilst the benefits of a gluten-free diet in the treatment of patients with CD and DH have long been established, there are very few studies, mainly case reports, of the effect of gluten-free diet on the ataxia. Most of these reports primarily concern patients with established CD who then develop ataxia (Pellecchia et al., 1999; Beversdorf et al., 1996; Hahn et al.,1998). These reports suggest overall favourable responsiveness to a gluten-free diet. A small, uncontrolled study and another case study looked at the use of intravenous immunoglobulins in the treatment of patients with GA with and without enteropathy (Bürk et al., 2001, Sander et al., 2003;). All patients improved. In all of these reports, strict adherence to the gluten-free diet was assumed and no serological evidence was provided. The best marker of strict adherence to a gluten-free diet is serological evidence of elimination of gluten related antibodies. Only one systematic study of the effect of gluten-free diet on a cohort of patients presenting with ataxia, with or without an enteropathy, has been published (Hadjivassiliou et al., 2003). This study also reported serological evidence of elimination of the antigliadin antibodies as a confirmation of strict adherence to the diet. Forty three patients with gluten ataxia were enrolled. Twenty six adhered strictly to the gluten-free diet, had serological evidence of elimination of antibodies and comprised the treatment group. Fourteen patients refused the diet and comprised the control group. Patient and control groups were matched at baseline for all variables (age, duration of ataxia etc). There was no significant difference in the baseline performance for each ataxia test between the two groups. There was significant improvement in performance in test scores and in the subjective global clinical impression scale in the

treatment group when compared to the control group. The improvement was apparent even after excluding patients with an enteropathy. The study concluded that gluten-free diet is an effective treatment for GA.

The current recommendation is that patients presenting with idiopathic progressive cerebellar ataxia should be screened for sensitivity to gluten using antigliadin IgG and IgA, anti-TG2, anti-TG6 and endomysium antibodies (Hadjivassiliou et al., 2013). Patients positive for any of these antibodies with no alternative cause for their ataxia should be offered a strict gluten free diet with regular follow up to ensure that the antibodies are eliminated (usually takes 6 to 12 months). Stabilisation or even improvement of the ataxia at 1 year would be a strong indicator that the patient suffers from gluten ataxia. The commonest reason for lack of response is compliance with the diet. If patients on strict gluten free diet continue to progress, with or without elimination of antibodies, the use of immunosuppressive medication (mycophenolate) should be considered. Such cases are rare.

Myoclonic ataxia and refractory coeliac disease

In 1986 Lu and colleagues published two cases with action myoclonus, ataxia and CD who in addition had epilepsy (Lu et al., 1986). The authors provided electrophysiological evidence for the cortical origin of the myoclonus. Similar findings of action, stimulus sensitive, cortical myoclonus were subsequently reported in another patient (Tison et al., 1989). This patient had cortical reflex and action myoclonus resembling epilepsia partialis continua, with constant arrhythmic myoclonic activity in the right hypothenar muscles. Electrophysiology confirmed the cortical origin of the myoclonus.

A case series was published in 1995 reporting 4 patients with myoclonus and ataxia with electrophysiological evidence of stimulus sensitive myoclonus of cortical origin (Bhatia et al., 1995). Pathology showed atrophy of the cerebellar hemispheres with Purkinje cell loss. CD was diagnosed in all four, preceding the onset of the neurological manifestations by years. Such patients unlike those with gluten ataxia appear to be poorly responsive to gluten-free diet and follow a progressive course. The largest series published so far reported 9 patients (6 male, 3 female) with ataxia and asymmetrical irregular jerking (Sarrigiannis et al., 2014). The jerking affected one or more limbs and sometimes face and it was often stimulus sensitive. All patients later developed more widespread jerking. Six patients had a history of Jacksonian march and five had at least one secondarily generalised seizure. Electrophysiology showed evidence of cortical myoclonus. Four had a phenotype of epilepsia partialis continua. There was clinical, imaging and/or pathological evidence of cerebellar involvement in all cases. Eight patients adhered to a strict gluten-free diet with elimination of gluten-related antibodies, despite which there was still evidence of enteropathy in all thus suggestive of refractory celiac disease. One patient only just started the diet, 2 died from enteropathy-associated lymphoma. Five patients were treated with mycophenolate and one in addition with rituximab and IV immunoglobulins. Whilst their ataxia and enteropathy improved, the myoclonus remained the most disabling feature of their illness. This was the first report to highlight the strong association of this unusual phenotype with refractory CD and in 2 of the cases enteropathy associated lymphoma.

Gluten neuropathy

Up to 23% of patients with established CD on gluten-free diet have neurophysiological evidence of a peripheral neuropathy (Luostarinen et al., 2003). A large population based study of over 84,000 patients with CD in Sweden found that CD was associated with polyneuropathy with a hazard ratio of 3.4 (Ludvigsson et al., 2007). In a UK based study, 34% of patients with otherwise idiopathic sporadic sensorimotor axonal length dependent neuropathy were found to have circulating AGA (Hadjivassiliou et al., 2006). Using anti-TG2 antibody an Italian study also found 21% of patients with peripheral neuropathy to be positive (Mata et al.,2006). Finally, in a tertiary referral centre in the USA, retrospective evaluation of patients with neuropathy showed the prevalence of CD to be between 2.5 and 8% as compared to 1% in the healthy population (Chin et al., 2003).

Gluten neuropathy is defined as otherwise idiopathic sporadic neuropathy with serological evidence of sensitivity to gluten. The commonest types are symmetrical sensorimotor axonal length dependent peripheral neuropathy and sensory ganglionopathy (Hadjivassiliou et al., 2010b). Other types of neuropathies have also been reported including asymmetrical neuropathy (Kelkar et al., 1996; Hadjivassiliou et al., 1997; Chin et al., 2006) small fibre neuropathy (Brannagan et al., 2005) and rarely pure motor neuropathy (Hadjivassiliou et al., 1997) or autonomic neuropathy (Gibbons and Freeman, 2005). Gluten neuropathy is slowly progressive with a mean age at onset of the neuropathy being 55 years (range 24 to 77) and a mean duration of 9 years (range 1 to 33). A third of the patients will have evidence

of enteropathy on biopsy but the presence or absence of an enteropathy does not influence the effect of a gluten-free diet (Hadjivassiliou et al., 2006).

Limited pathological data available from post-mortem examinations and nerve biopsies are consistent with an inflammatory aetiology (perivascular lymphocytic infiltration). Gluten-free diet has been shown to be beneficial in single and multiple case reports. The only systematic, controlled study of the effect of a gluten-free diet on 35 patients with gluten neuropathy, with regular serological monitoring of the adherence to the gluten-free diet, found significant improvement in the treated compared with the control group after 1 year on gluten free diet (Hadjivassiliou et al., 2006). There was significant increase in the sural sensory action potential, the pre-defined primary endpoint, in the treatment group as well as subjective improvement of the neuropathic symptoms. Subgroup analysis showed that the capacity for recovery is less when the neuropathy is severe.

Sensory ganglionopathy (sometimes also called neuronopathy) is an asymmetric form of pure sensory neuropathy where the pathology is within the dorsal root ganglia. It can be a paraneoplastic syndrome or related to Sjogren's syndrome. It can also be seen in some inherited neurological illnesses such as Friedreich's ataxia and mitochondrial diseases (POLG-1). Sensitivity to gluten has proven to be the commonest cause of sensory ganglionopathy (ref). In such cases, there is evidence of inflammatory infiltrates within the dorsal root ganglia. The disease progresses slowly if untreated but strict adherence to a gluten free diet may result in stabilisation or even improvement of the neuropathy irrespective of the presence of enteropathy (Hadjivassiliou et al., 2010b).

Headache and gluten sensitivity (gluten encephalopathy)

Headache is a common feature in patients with GRD. The association was first reported in 2001 based in a series of 10 patients with GRD and headache who in addition had CNS white matter abnormalities on MRI scan (Hadjivassiliou et al., 2001). The term “gluten encephalopathy” was used to describe them. The headaches are usually episodic and intractable. They can mimic migraines but do not respond to the usual migraine medication. They characteristically resolve with the introduction of a gluten free diet. Some patients report a very strong association with ingestion of gluten. The white matter abnormalities are not always present but can be diffuse or focal. They do not always resolve following a gluten-free diet. The diet simply arrests progression of these changes but the white matter changes can be progressive if the patient does not adhere to a strict gluten free diet. Their distribution is more suggestive of a vascular rather than demyelinating aetiology. In a prospective study of patients newly diagnosed with CD frequency of intractable headaches was 44% (Hadjivassiliou et al., 2013)

In patients with migraine there is an overrepresentation of CD with a prevalence of 4.4% vs 0.4% in the control population (Gabrielli et al., 2003). Using PET brain imaging, a study on regional cerebral perfusion demonstrated that 73% of patients with CD not on a gluten-free diet, had at least one hypoperfused brain region as compared to 7% in healthy controls and in patients with CD on a gluten-free diet (Addolorato et al., 2004). Another study investigated the prevalence of white matter abnormalities in children

with CD and found that 20% of patients had such abnormalities (Kieslich et al., 2001).

Over the last 20 years we have encountered 100 patients with gluten encephalopathy, a figure that includes the initial 10 patients reported in the 2001 series. Gluten encephalopathy does not always occur in isolation and such patients will often have additional neurological features such as ataxia. A study from the Mayo clinic emphasised the significant cognitive deficits encountered in 13 such patients (Hu et al., 2006). By comparison to gluten ataxia and gluten neuropathy there is a higher prevalence of enteropathy in patients with gluten encephalopathy (59/100), but the age at onset is the same. The observed improvement of the headaches and arrest of progression in the MRI brain abnormalities, suggest a causal link with gluten ingestion (Serratrice et al., 2003). Gluten encephalopathy represents a spectrum of clinical presentations with episodic headaches responsive to a gluten-free diet at one end, to severe debilitating headaches sometimes associated with focal neurological deficits. MRI findings range from normal to extensive white matter abnormalities.

Epilepsy

A link between epilepsy and CD was proposed as far back as 1978 (Chapman et al., 1978; Fois et al., 1994; Cronin et al.,1998). Whilst studies examining the prevalence of CD amongst patients with epilepsy have suggested a prevalence of 1.2-2.3%, others failed to demonstrate an increased prevalence (Ranua et al., 2005). A more recent large (28,885 subjects with CD) population-based cohort study showed that patients with

CD were at an increased risk of future epilepsy (HR=1.42). The absolute risk of future epilepsy in patients with CD was 92/100,000 person-years which equates to an excess risk of 27/100,000 person-years (Ludvigsson et al., 2012). Most studies on the subject suffer from the same methodological problem of treating epilepsy as a homogeneous disorder. The only study that attempted to look at the prevalence of GRD in well characterised subgroups of patients with epilepsy found a significant association between AGA and temporal lobe epilepsy with hippocampal sclerosis (Paltola et al., 2009). Of interest are some case reports on patients with CD and epilepsy whose epilepsy improves following the introduction of gluten-free diet (Mavroudi et al., 2005; Harper et al., 2007).

There is a particular form of focal epilepsy associated with occipital calcifications that appears to have a strong link with CD (Gobbi et al., 1992). This entity is common in Italy but rare in other countries. It tends to affect young patients (mean age 16 years) and in the majority the seizures are resistant to antiepileptic drugs. The pathogenesis of the cerebral calcifications remains unclear. An autopsy study showed that these depositions consisted of both calcium and silica, and microscopically were found in three main types: psammoma-like bodies without any identifiable relationship to cells, vessels or other structures; small granular deposition along small vessels; and focal scanty areas of calcium within neurons (Toti et al, 1996). As most of the reported cases are from Italy, Spain, and Argentina, it has been hypothesized that the syndrome of coeliac disease, epilepsy, and cerebral calcifications is “a genetic, non-inherited, ethnically and geographically restricted syndrome associated with environmental factors” (Gobbi, 2005). A case study of a 4

year old boy with refractory epilepsy, occipital calcifications and coeliac disease reported positive antibody binding to neurones and glia using indirect immunofluorescence. High levels of TG6 antibodies were found in the patients serum. After the introduction of gluten free diet the child became seizure free (Johnson et al., 2012).

Myopathy

This is a relatively rare neurological manifestation of GRD, first described by Henriksson et al. (1982). This study from Sweden reported that out of 76 patients with suspected polymyositis investigated at a neuromuscular unit, 17 had a history of gastrointestinal symptoms with evidence of malabsorption. Fourteen of these fulfilled the diagnostic criteria for polymyositis and of those 5 were diagnosed with CD. A more recent study from Spain (Selva-O'Callaghan et al, 2007) demonstrated the prevalence of AGA antibodies amongst patients with inflammatory myopathies to be 31%. This was accompanied by a higher prevalence of CD within the same population when compared to healthy controls.

A case series of 19 patients are based on what we have encountered in the gluten neurology clinic, Sheffield, UK over the last 20 years. Thirteen of these patients have been reported previously (Hadjivassiliou et al., 2007). Enteropathy was identified following duodenal biopsy in 11 of these patients. The mean age at onset of the myopathic symptoms was 54 years. Ten patients had predominantly proximal weakness, 6 patients had both proximal and distal weakness, and 4 patients had primarily distal weakness. Two patients had ataxia and neuropathy, and one patient had just neuropathy in

addition to the myopathy. Serum creatine kinase (CK) level ranged between normal to 4380 IU/L at presentation (normal, 25-190 IU/L). Inflammatory myopathy was the most common finding on neuropathological examination. Six patients received immunosuppressive treatment in addition to starting a gluten-free diet whereas the others went on a gluten-free diet only. In the majority of those patients who did not receive immunosuppressive treatment, there was clinical improvement of the myopathy with gluten-free diet, suggesting that the myopathy was aetiologically linked to the GRD. One patient developed a profound myopathy after inadvertently eating rye flour. He made a full recovery by re-establishing a strict gluten-free diet. Two patients had histological evidence of inclusion body myositis. It is interesting to note that inclusion body myositis shares the same HLA genetic predisposition with CD (ref). One patient was known to have CD already when he developed the myopathy. He was on gluten free diet already with negative serology for CD. Muscle biopsy showed an inflammatory myopathy and repeat duodenal biopsy showed a flat mucosa. Further immunohistological examination of the biopsy did not suggest refractory CD. The patient admitted the occasional dietary indiscretion. He underwent further dietary review and has been started on steroids with some clinical improvement.

Myelopathy

Clinical evidence of a myelopathy in the absence of vitamin B12 and other deficiencies (particularly copper) can be a rare manifestation of CD. It is usually associated with normal imaging of the spinal cord although cases of transverse myelitis like MR appearances have been encountered in our cohort

of patients. The neurological presentation often coincides with the diagnosis of CD. There have been some case reports of patients with neuromyelitis optica and sensitivity to gluten who have antibodies to aquaporin-4 (Jacob et al., 2005; Jarius et al., 2008). Such patients clearly have abnormal MRI of the spinal cord but the diagnosis of CD was only made at the time of their neurological presentation. Neuromyelitis optica and CD share the same HLA genetic susceptibility (HLA DQ2). There is very limited data on the effect of the diet on the likelihood of relapse of the disease particularly given the fact that most patients with neuromyelitis optica end up on long term immunosuppressive medication.

Stiff-Man Syndrome

Stiff-Man syndrome (SMS) is a rare autoimmune disease characterised by axial stiffness, painful spasms and positivity for anti-GAD. It has a strong association with other autoimmune diseases (eg IDDM, hypothyroidism). We have found a high prevalence of gluten related antibodies in patients with this condition over and above that expected from an association of 2 autoimmune diseases. The relapsing remitting nature of the condition makes a study of any responsiveness to gluten free diet difficult. There is however evidence of reduction of the anti-GAD antibody titre following the introduction of a gluten free diet suggesting that the diet may be beneficial in treating the condition (Hadjivassiliou et al., 2010c). This finding also supports the concept of prevention of autoimmunity in patients with GRD if the gluten free diet is introduced early enough (Ventura et al., 2002).

The concept of hyperexcitability of the central nervous system in the context of CD is of interest. We have already discussed above the entity of cortical myoclonus and refractory CD and the association with SPS. We have encountered patients with other hyperexcitable CNS disorders such as progressive encephalomyelitis with rigidity and spasms and patients with startle myoclonus who also have CD. A recent study from Italy has demonstrated that a group of 20 patients with newly diagnosed CD (no neurological complaints) had significantly shorter cortical silent period, reduced intracortical inhibition and enhanced intracortical facilitation by comparison to 20 age matched healthy controls. The authors concluded that a pattern of cortical excitability was found in patients with CD and that immune system dysregulation may be responsible for this (Pennisi et al., 2014).

Pathogenesis

Post mortem data from patients with gluten ataxia demonstrate patchy loss of Purkinje cells throughout the cerebellar cortex, a rather end stage non-specific finding in many cerebellar disorders. However, findings supporting an immune mediated pathogenesis include diffuse infiltration mainly of T-lymphocytes within the cerebellar white matter as well as marked perivascular cuffing with inflammatory cells (Hadjivassiliou et al., 1998). The peripheral nervous system also shows sparse lymphocytic infiltrates with perivascular cuffing being observed in sural nerve biopsy of patients with gluten neuropathy (Hadjivassiliou et al., 2006), in dorsal root ganglia in patients with sensory neuronopathy (Hadjivassiliou et al., 2010) and in patients with gluten myopathy (Hadjivassiliou et al., 2007). GRD patients produce an immune

response to gluten involving both the innate and adaptive arm of the immune system (Sollid and Jabri, 2009; Junker et al., 2012). Antibodies to gliadin are part of this response, and their systemic levels appear to mirror the immune reaction triggered by gluten in the intestine including their reduction in response to a clinical improvement of the intestinal mucosa. There is cross-reactivity of these antibodies with antigenic epitopes on Purkinje cells. Serum from patients with GA and from patients with CD but no neurological symptoms recognize Purkinje cells of both human and rat origin (Hadjivassiliou et al., 2002). This reactivity can also be seen using polyclonal AGA and the reactivity eliminated by absorption with crude gliadin. When using sera from patients with GA there is evidence of additional antibodies targeting Purkinje cell epitopes since elimination of AGA alone is not sufficient to eliminate such reactivity. There is evidence that the additional antibodies that may be causing such reactivity, are antibodies against one or more transglutaminase isoenzymes (TG2, TG3, TG6) (Boscolo et al., 2010).

TG2 belongs to a family of enzymes that covalently crosslink or modify proteins through transamidation, deamidation or esterification of a peptide-bound glutamine residue (Aeschlimann and Thomazy, 2000). Notably, the deamidation reaction may occur in preference over the transamidation reaction, even under conditions that should favour amine incorporation, and this appears to be substrate sequence context-dependent (Stamnaes et al., 2008). Gluten proteins (from wheat, barley and rye), the immunological trigger of GRD, are glutamine rich donor substrates amenable to deamidation. Deamidation of gluten peptides enhances binding with disease-relevant HLAs and thereby enhances presentation, leading to the development of gluten-specific Th1-like

$CD4^+$ T cells (Jabri and Sollid, 2009; Tye-Din et al., 2010). The resulting inflammatory cytokine environment drives TG2 expression through direct transcriptional regulation (Aeschlimann and Thomazy, 2000; Nurmiskaya and Belkin, 2012), thereby further increasing the production of the immunological trigger. Therefore, Activation of TG2 and deamidation of gluten peptides appears to be central to disease development and is now well understood at a molecular level (Jabri and Sollid, 2009). In genetically predisposed individuals, this is at the centre of a destructive chronic inflammatory reaction manifesting as aphthous stomatitis in the oral cavity or villous atrophy in the upper small intestine at sites where the gluten load through food ingestion is high.

Besides the strong gluten-specific T cell response, one of the hallmarks of GRD is a robust IgA autoantibody response to TG2 or TG2 and further TG isozymes (Dietrich et al., 1997; Sardy et al., 2002; Hadjivassilou et al., 2008). Assessment of serum anti-TG2 antibodies has become an important tool in CD diagnosis, and new ESPGHAN guidelines enable their use as a surrogate marker of disease (Husby et al., 2012). However, events leading to the formation of autoantibodies against TG2 or other TG isozymes are less clear. The recent characterisation of an unusual and overwhelming immature plasma cell response in the small intestine goes some way to explain the strict association of gluten related disorders with autoantibodies to TGs (Di Niro et al., 2012). Notably, intestinal deposits of IgA antibodies targeting TG2 are present at all stages of CD, including early developing CD (Kaukinen et al., 2005) as well as late stage refractory CD (Salmi et al.,2006) where patients may be seronegative. With regards to B cell activation and differentiation, the

hapten carrier model proposed by Sollid et al. (1997), although not formally demonstrated *in vivo*, appears to hold true, whereby gluten-specific T cells can provide help to TG-specific B cells. This unusual scenario is enabled by the ability of TGs to form stable complexes with gliadin peptides (Stamnaes et al., 2010) leading to uptake and ultimately presentation of MHC-gliadin complexes by B cells expressing TG-specific IgD. Recent *in vitro* studies confirmed that this is indeed possible (Di Niro et al., 2012). Given the relative lack of somatic hypermutation of the antibody repertoire present in adult patients that should have undergone extensive affinity maturation (Di Niro et al, 2012; Iversen et al., 2013), questions remain as to the mechanism by which B cell maturation takes place, and this could involve an extrafollicular pathway (Mesin et al., 2012). Plasmablasts re-enter the lamina propria via the circulation and form the IgA secreting plasma cell niche. It is important to keep in mind that B cells have roles beyond antibody production, most notably as highly effective antigen presenting cells for T cell responses. Therefore, B cells may drive clonal expansion of gluten–specific T cells which in turn may support development of B cells specific for TGs as well as deamidated gluten peptides and thereby create an amplification loop. This potentially puts B cells center stage of GRD pathogenesis.

Questions also remain as to the contribution of these autoantibodies to organ-specific deficits. Anti-TG2 antibodies have been shown to be deposited in the small bowel mucosa of patients with GRD and may contribute to the formation of the lesion. Furthermore such deposits have been found at extraintestinal sites, such as muscle and liver (Korponay-Szabo et al., 2004).

Widespread deposition of IgA antibodies has also been found around brain vessels in GA (Hadjivassiliou et al., 2006). The deposition was most pronounced in the cerebellum, pons and medulla. This finding suggests that such autoantibodies could play a role in the pathogenesis of the whole spectrum of manifestations seen in GRD and that effector functions of antibodies could contribute to tissue damage. IgM antibodies are present in GRD patients and may activate the complement cascade and promote inflammation.

Variations in the specificity of antibodies produced in individual patients could explain the wide spectrum of manifestations. Whilst TG2 has been shown to be the autoantigen in CD (Dietrich et al., 1997), the epidermal transglutaminase TG3 has been shown to be the autoantigen in DH (Sardy et al., 2002). Antibodies against TG6, a primarily brain expressed transglutaminase (Thomas et al., 2013), have been shown to be present in patients with GA (Hadjivassiliou et al., 2008). Similar to anti-TG2 and anti-deamidated gluten peptide antibodies, the production of these anti-TG3 and anti-TG6 antibodies in DH and GA patients, respectively, is gluten-dependent which substantiates the link to a gluten-specific T cell population (Sardy et al., 2002; Hadjivassilou et al., 2013). In GA and DH, IgA deposits of TG6 and TG3 respectively seem to accumulate in the periphery of blood vessels at sites where in health the respective proteins are absent. Recent data on DH suggests that the deposits originate from immune complexes forming locally as a consequence of enhanced vascular leaking, and that TG3 although potentially present in health may normally be rapidely cleared (Zone et al., 2011). Furthermore, TG3 within immune complexes retains enzymatic activity

and through crosslinking to fibrinogen and cell surface receptors drives innate immune cell activity (Taylor et al., 2015). Importantly, the demonstration that circulation-derived anti-TG3 antibodies can induce a dermatitis herpetiformis-like pathology in human skin-grafted SCID mice emphasis the central role antibodies play in disease establishment in different organ systems (Zone et al., 2011). By inference, this suggests that adaptive immune cell development likely occurs in the gut and is not driven by trafficking of gut derived T cells to other organ systems. It is likely that vasculature centered inflammation is also at the heart of GA. Indeed perivascular cuffing with lymphocytes is a common finding in brain tissue from patients with GA but is also seen in peripheral nerve and muscle in patients with gluten neuropathy or myopathy (ref). However, it is unclear at present how immune complexes develop and to what extent a compromised blood-brain barrier is a pre-requisite to disease development. In most sera reactive with more than one TG isoenzyme, distinct antibody populations are responsible for such reactivity rather than this being a result of cross-reactivity with different TG isozymes (Hadjivassiliou et al, 2008). The absence of crossreactivity was recently substantiated in an analysis of clonal antibodies constituting the antibody repertoire in CD (Iversen et al., 2013). This makes shared epitopes less likely to be the cause for B cell development to other TGs and points to the possibility that TG isozymes other than TG2 can be the primary antigen in GRD. All 3 TG isozymes (TG2, TG3, TG6) for which autoantibodies have been described, can form thioester-linked complexes with gluten peptides which are thought to be responsible for the B cell response to TG isozymes (Stamnaes et al, 2010). This implicates the shared activity of these enzymes rather than their sequence

similarity in stimulation of antibody production, and explains the exquisite specificity of the antibody response to TG family members. While antibodies targeting other autoantigens have been reported, the development of such antibodies is much more sporadic among the GRD patient population (Dieterich et al., 2006).

IgA deposition in brain vessels and the pathological finding of perivascular cuffing with inflammatory cells, may indicate that vasculature-centred inflammation may compromise the blood-brain barrier, allowing exposure of the CNS to pathogenic antibodies, and therefore be the trigger of nervous system involvement. Indeed, TG2 is expressed by smooth muscle and endothelial cells in non-inflamed brain, is an abundant component of the choroid plexus extracellular matrix (Aeschlimann et al., 2001) and autoantibody binding could initiate an inflammatory response. However, expression of anti-TG2 antibodies in mice by themselves did not precipitate CD-like lesions in the small intestine or overt systemic manifestations akin of GRD (Di Niro et al., 2008), and no antibody deposition in brain vessels was reported. This may relate to the fact that it involves a specific subset of anti-TG2 antibodies that was not represented by the analysed clonal antibodies. It could also suggest that development of antibodies targeting antigens other than TG2 may be a critical step in the precipitation of specific extraintestinal manifestations as illustrated by anti-TG3 antibodies in DH (Zone et al., 2011). It is also possible that additional factors other than the autoantibodies themselves play a role. These may either affect vascular permeability, blood brain barrier integrity or antigen availability. With regards to the latter, TG2 and other TGs adopt a number of vastly different conformations dependent on biological context (Pinkas et al., 2007), and the recognition of TG2 by antibodies is conformation dependent (Simon-Vecsei et al., 2012; Iversen et al.,

2013), or binding sites of TG2 may be masked *in situ* by other interaction partners as recently documented (Iversen et al., 2013). One might speculate that an unrelated infection or other insult that causes local inflammation may in the presence of circulation-derived autoantibodies bring about pathogenic immune complexes at the blood brain barrier. This hypothesis is consistent with experimental evidence showing that antibody-mediated neuronal damage in mice harbouring pathogenic antibodies does only occur upon compromise of the blood-brain barrier (Kowal et al., 2006). Furthermore, brain areas affected in experimental animals, and therefore induced functional deficits, differed dependent on the mechanism underlying the breach of the blood brain barrier (Kowal et al., 2006). It appears therefore that regionally-specific vascular permeability leads to localized neuronal damage. Similarly, localized exposure to pathogenic antibodies may explain why patients with cerebellar ataxia or stiff person syndrome present with similar dysfunctions affecting preferentially the cerebellum or spinal cord, respectively.

It could be argued that development and deposition of antibodies is an epiphenomenon rather than being pathogenic. One method to demonstrate the pathological effect of an antibody is the passive transfer of the disease through antibody injection into a naïve animal. While for only very few antibody-mediated diseases such experimental evidence exists, IgG fractions of patients with anti-GAD ataxia and stiff-man syndrome have been shown to compromise motor function and impair learning in rodents, an effect possibly ascribed to antibodies against GAD (Manto et al., 2007). A common problem in such studies is to be able to demonstrate whether it is these specific antibodies or other autoantibodies in the IgG-fraction of patient sera that

cause neuronal damage. Using a mouse model we have recently shown that serum from GA patients as well as clonal monovalent anti-TG immunoglobulins derived using phage display, cause ataxia when injected intraventricularly in mice (Boscolo et al., 2010). The fact that not only Ig fractions but also monospecific scFv's mediate functional deficits shows that there is no requirement for complement activation or for the engagement of Fc receptors on Fc-receptor bearing cells in the brain. These data therefore provide evidence that anti-TG immunoglobulins (derived from patients) compromise neuronal function in selected areas of the brain once exposed to the CNS and suggest that this involves an immune system independent mode of action.

A bias of the immune response towards TG6 in GRD patients presenting with neurological deficits (Hadjivassiliou et al., 2008, 2013) implicates neuronal TG6 in pathogenesis, at least of GA but possibly also other neurological problems. Further support for this notion comes from the identification of mutations in the gene encoding TG6 in families with autosomal dominant ataxia (Wang et al., 2010; Li et al., 2013). This form of spinocerebellar ataxia is now referred to as SCA35. Clinical features associated with TGM6 mutations are those of late onset cerebellar ataxia, slow progression of gait and limb ataxia, hyperreflexia, and cerebellar degeneration but with no cognitive impairment, autonomic and peripheral nerve involvement, or epilepsy (Wang et al., 2010; Li et al., 2013). This is in keeping with the presentation in patients with immune-mediated cerebellar ataxia (GA), and provides strong evidence for an essential function of TG6 in the CNS. TG6 is,

however, expressed by other cells including various epithelia (Thomas et al., 2013), and one of the TGM6 mutations also associated with acute myeloid leukemia (Pan et al., 2014). Functional data on the physiological role of TG6 protein are sparse at present. We have begun to characterise the enzyme biochemically and analyse the gene expression pattern during development, which identified a complex system with splice variants that are differentially expressed and presumably functionally distinct (Thomas et al., 2013). Using molecular modeling and biochemical assays, we have shown that TG6 is regulated by GTP and Ca^{2+} similar to TG2, and adopts compact or extended conformations with transamidation activity, respectively (Thomas et al. 2013). The expression of TG6 during CNS development demonstrated an association with neurogenesis, and this was further confirmed by *in vitro* differentiation of neuronal precursor cells (Thomas et al., 2013). All single nucleotide exchanges reported to date lead to alteration of amino acid residues that are strictly conserved in TG6 among different species. Based on structural modeling and biochemical analysis (Thomas et al., 2013), we hypothesise that the biological significance of TGM6 mutations lies in the impairment of regulation of transamidation activity. This implicates TG6 in an extracellular function that is critical to neuronal differentiation and survival. However, how crosslinking or modification of extracellular proteins contributes to neuronal survival remains to be identified. Autoantibody binding may sequester TG6 or block its activity and thereby act as a competitive inhibitor of enzyme function.

Conclusions

GRD include immune mediated diseases triggered by ingestion of gluten proteins. While coeliac disease has been the most comprehensively studied of all GRD, to fully understand the immunological aftermath from gluten ingestion, there is a need to further study extraintestinal manifestations. In addition there is a need for the early identification of those patients that are specifically at risk of irreversible complications (eg gluten ataxia). To that effect, new diagnostic tools are now becoming available (e.g. antibodies against TG6) which may make a more reliable identification of those patients with neurological manifestations a reality. Up to 40% of patients presenting to the gastroenterologist who are ultimately diagnosed with CD also have antibodies against TG6 in addition to antibodies against TG2. This subgroup of patients with classic CD presentation may well be the ones susceptible to the development of neurological dysfunction if they continue to consume gluten, although this remains to be shown in longitudinal studies. The presence of gastrointestinal symptoms, however, offers a major potential advantage to this group, as it substantially increases their chances of being diagnosed and treated early, whereas the diagnosis of those patients presenting purely with extraintestinal manifestations may be more difficult.

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