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IgA antibodies against gliadin and gluten in multiple sclerosis

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Background – Multiple changes in antibodies against various antigens are found in multiple sclerosis (MS). *Objective* – We wanted to measure immunoglobulin A (IgA) antibodies to some common food antigens in MS and also IgG against gliadin and gluten. *Methods* – The IgA antibodies were measured in serum against gluten, gliadin, lactoglobulin, lactalbumin, casein and ovalbumin in patients with MS and controls using ELISA technique. IgG was likewise measured for gluten and gliadin. *Results* –Highly significant increases compared with controls were found for IgA and IgG antibodies against gliadin and gluten. IgA antibodies against casein were significantly increased. Anti-endomycium and anti-transglutaminase antibodies were negative. *Conclusions* – The data presented indicates that there may be a possible moderately increased uptake of some specific proteins from the gut in MS compared with controls.

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Key words : immunoglobulin A; antibodies; multiple sclerosis; gliadin; gluten; casein

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Multiple sclerosis (MS) is a debilitating neurological disease without known cause. However, immunological changes found in this disease are thought to reflect an autoimmune reaction (1, 2) in genetically susceptible individuals (3). Antibodies that bind to brain basic myelin protein have been detected, and peptides from myelin basic protein can induce experimental demyelinating encephalitis in animal models.

As immunoglobulin A (IgA) antibodies in serum against gliadin and gluten could be demonstrated in gluten-induced cerebellar ataxia with peripheral neuritis and ataxia (4), and as these antibodies have a high affinity for the brain barrier vasculature (5), we decided to measure these antibodies in serum also in MS.

Patients and methods

The patients were obtained through the Multiple Sclerosis Society of Norway, which is thanked for their outstanding efforts. All the patients volunteered after having received an informed letter describing why and how the examination was going to be performed.

The patients all had their diagnosis determined by the neurological departments of the University of Oslo at either Ullevål City Hospital or The National Hospital (Rikshospitalet) [Poser criteria]. None were first episode patients and had experienced from two or more worsening episodes with at least two separate lesions (intermittent relapsing type). Those included in the study had typical oligoclonal immunoglobulin band in the CSF and half had been examined by NMR. Two had an unremitting course but of slow worsening (progressive subtype). The total number of patients were 36 with an age range of 27–67 years (median 44 years). Of them, 21 were females and 15 males. We do not have the HLA classification on these patients collected systematically. Normal controls ($n = 26$) were obtained through Fürsts laboratory in Oslo (Clinical Chemistry Laboratory of high standard) were of the age range 21–50 years (median 38 years). Of them, 16 were females and 8 males. Excluding the five oldest persons in the patient group older than the oldest control did not have any impact on the results.

Venous blood was obtained by venous tap and the IgA antibodies measured by ELISA technique as described (6, 7). Costar ERIA microplates 2 (no. 3590) were coated with antigens at concentrations found optimal for different antigens (0.001–0.01 g/l), and the patients' sera were tested at a ratio

of 1:400. The measurements were taken at Fürsts laboratory on a commercial basis but blinded.

These tests are part of the celiac screening battery run routinely. Known standards were run with each analytical series. Whenever values were above the cut-off for celiac disease, endomycium and lately anti-trans-glutaminase antibodies were run.

Statistics

As the distribution of the IgA antibodies is extremely skewed (not parametrically distributed) we chose to use nonparametric statistics: the Mann–Whitney *U*-test, two-tailed.

Results

The results of the measurements are illustrated in Table 1. We thus obtained the following statistics: gluten against control showed serum IgA antibodies that were higher than controls with $P < 0.001$ (two-tailed) and statistical characteristic with $U = 138.5$ and $U' = 773.5$. IgA antibodies against gliadin (Table 2) also had $P < 0.001$ ($U = 88$ and $U' = 824$). For casein *P*-value (two-tailed) was 0.036 ($U = 322$ and $U' = 614$).

immunoglobulin A antibodies against lactoglobulin, lactalbumin and ovalbumin did not show statistical increases. However, one patient had IgA antibodies against ovalbumin six times higher than the upper cut-off value. Total IgA levels were in the normal range (not shown), although two of the 28 patients had no demonstrable IgA antibodies against the specific proteins tested.

The trans-glutaminase test was negative also for those with IgA levels in serum above the cutoff for celiac disease, however, celiac disease is unlikely.

The IgG antibody levels against gluten were different from controls with $P < 0.001$ ($U = 138$ and $U' = 773.5$). For gliadin a $P < 0.001$ was obtained ($U = 88$ and $U' = 824$). There was no increase in IgA antibodies against beta-lactoglobulin, lact-albumin and ovalbumin (not shown).

Table 1 Serum IgA antibody levels in multiple sclerosis (MS) and controls

	Mean	Median	Number	95% CI	*P*-value
MS					
IgA gliadin	0.327	0.441	38	0.156–0.498	<0.001
IgA gluten	0.249	0.263	38	0.147–0.351	<0.001
IgA casein	0.103	0.159	38	0.041–0.165	0.035
Control					
IgA gliadin	0.061	0.067	27	0.035–0.088	
IgA gluten	0.056	0.051	27	0.035–0.076	
IgA casein	0.039	0.078	27	0.007–0.072	

P-values using Mann–Whitney *U*-test for patient data against controls. Six of 38 patients had IgA values against gliadin and gluten above the cutoff for celiac disease, while none of the controls showed such increases. Counting numbers with IgA antibody levels above the cutoff for celiac disease, we find chi-square values with $P < 0.01$.

Table 2. IgG antibodies against gluten and gliadin in MS

	Mean	Median	Number	95% CI	*P*-value
MS					
Gliadin	0.466	0.400	38	0.377–0.556	<0.001
Gluten	0.451	0.400	38	0.358–0.543	<0.001
Control					
Gliadin	0.154	0.100	24	0.073–0.236	
Gluten	0.158	0.100	24	0.066–0.251	

ELISA measured IgG antibodies are as described (6, 7). Although not very high, the level was very significant compared with controls run simultaneously.

Discussion

As MS is found mainly in the temperate zone, which is also the dairy farming sector of the world, we expected that the possible specific IgA increase to be against casein. However, the highly significant differences from the controls were found in serum IgA antibodies against gliadin and gluten which was extremely significant ($P < 0.001$; Table 2). Increase in specific serum IgA antibodies is thought to reflect increased uptake of protein from the gut. In addition, normal persons take up proteins from the gut (8–10) as well as fragments of proteins such as peptides (11). Very many epitopes that induce antibody formation are indeed peptides. The fact that dietary proteins are found in mothers milk (12–14) further reflect this uptake.

For MS patients in remission, no increase in antibodies against milk proteins were found (15), while in our case most of the patients if not all were in an active phase of their disease based on their subjective evaluation of worsening. Using a sheep erythrocytes agglutination assay, another group did not find antibody increase towards gluten and gliadin (16). This may be the result of methodical differences, but again might be caused by differences in disease process (not stated). Furthermore, a general antibody test instead of measuring specific subclasses, may, because of increase in one and decrease in another species of antibodies, mask specific changes.

Gliadin is especially noxious as shown for cerebellar atrophy and ataxia (4), celiac disease and gluten/gliadin related epilepsy (17, 18) .The longer one is exposed to gluten in celiac disease the greater is the risk of developing an autoimmune disorder (19). In celiac disease, IgA antibodies against gliadin and gluten are usually increased and these antibodies do have a high affinity for the blood–brain barrier vasculature (5), probably altering the permeability of this barrier.

Quite intriguing and possibly of relevance to MS, is the observation that chronic inflammatory bowel disease causes lesions in the white-matter of the brain (20, 21). Lesions of the gut would increase the permeability of the gut, and also the uptake of gluten and gliadin and thus increase the IgA antibodies to these proteins. Increased intestinal permeability has been reported in MS(22) as well as possible inflammatory bowel disease (23).

On a purely speculative note, is it possible that the reported connection between MS and measles antibodies (24, 25) could be the result of a chronic gut inflammation by measles as in regressive autism (26), where genetic material from measles viruses has been found in active lymph nodes of the gut (27, 28).

The IgA antibody increase may be secondary to an increased gut uptake or an epi-phenomenon. It could, however, also be a primary cause. Possibly relevant to this is that butyrophilin from milk, can induce anti-myelin oligo-dendrocyte glycoprotein antibodies and a CD4+ T-cell response in animals with Experimental autoimmune encephalitis (29).

Conclusion

Increased levels of IgA and IgG antibodies in serum against gluten and gliadin and possibly also for casein in MS patients may indicate a possible increased gut permeability to certain proteins.

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