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Exacerbation of protracted-relapsing experimental allergic encephalomyelitis in DA rats by gluten-free diet

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The observation of neurological dysfunctions resembling multiple sclerosis (MS) seen clinically and/or by MRI in patients with celiac disease has focused attention on the possibility that cryptic gluten sensitivity may be involved in the pathogenesis of MS. Here we study the effects of a gluten-free diet on the course of protracted-relapsing EAE in DA rats, serving as a preclinical model of human MS. The data show not only that this nutritional approach failed to ameliorate development of the disease but rather that it exacerbated the course.

Key words: Gluten-free diet; experimental allergic encephalomyelitis; EAE; multiple sclerosis; MS; type 1 diabetes mellitus; celiac disease; DA rats; paresis.

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Increasing evidence suggests a link between dietary proteins and the immune system (1). Development of oral tolerance is the usual response of the immune system to dietary proteins. This may primarily occur through activation via transforming growth factor beta production from gut-associated lymphoid tissues of regulatory cells capable of downregulating potentially harmful immune-mediated pathways specifically directed against the dietary proteins (1).

Malfunctioning of regulatory mechanisms underlying oral tolerance induction may therefore play a role in the development of unwanted immunoinflammatory responses that may contribute to the development not only of food allergies and enteropathies but also of other organ-specific autoimmune diseases (2–4). Particular attention has been focused on the possibility that cryptic gluten sensitivity may play a part in neurological illness (5). This hypothesis arose from the observation that neurological dysfunctions resembling multiple sclerosis (MS) on clinical and/or MRI grounds can be observed in patients with celiac disease (5), an enteropathy resulting from immunological hypersensitivity to ingested gluten in individuals with a genetic predisposition (5). On this basis, and even though neither increased titres of antigliadin antibodies nor gross morphological and biochemical alterations of the jejunal mucosa could be found in patients with MS (6–8), withdrawal of gluten from the diet has speculatively been used with some anecdotal success in the management of MS (9, 10).

A pathological condition with clinical and histoimmunological characteristics similar to human MS can be induced in DA rats by immu-

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nization with either syngeneic or guinea pig spinal cord emulsified in incomplete (FIA) or complete Freund's adjuvant (FCA) (11). The thus induced experimental allergic encephalomyelitis (EAE) is characterized by a severe, protracted-relapsing and demyelinating course (protracted-relapsing [PR]-EAE), which makes this a useful *in vivo* model for studying immune-mediated mechanisms involved in the generation of chronicity and demyelination and for the preclinical screening of immunotherapeutic approaches worthy of being considered for the treatment of the human disease counterpart.

We have here studied the effects of a gluten-free diet on the course of PR-EAE in DA rats. The data show that not only did this nutritional approach fail to ameliorate the development of the disease but rather that it exacerbated its course.

MATERIALS AND METHODS

Animals

Male DA rats (B&K, Uppsala, Sweden), weighing 230–270 g, were used for the study. Animals were randomly distributed into cages, and then marked and maintained under standard laboratory conditions with free access to food and water.

Immunization

Guinea pig spinal cord (50 µg) (Sigma, St. Louis, MO), minced thoroughly, was emulsified with 100 µl of FIA (Sigma) and 2 mg *Mycobacterium tuberculosis*, strain H37RA (Difco, Detroit, MI) and injected subcutaneously (s.c.) at the base of the tail, as described elsewhere (12).

Treatment

A gluten-free diet (Altromin, Lage, Germany) was given under two different experimental conditions either from day of birth to day of immunization or from birth until 40 days after immunization. Control rats were treated under the same experimental conditions with the usual rat pellets containing standard amounts of gluten, as described previously (13). These treatment regimens were chosen to ascertain whether avoiding exposure of the immune system to gluten until immunization or throughout the course of the disease would impede the emergence or activation of encephalytogenic T cells.

Following this line of reasoning, another treatment regimen was also considered to see whether giving DA rats a gluten-free diet from day −7 until day +16 after immunization – in contrast to the control diet – influenced PR-EAE development.

Clinical scoring

The rats were weighed every day and clinical signs were scored by an observer who was unaware of the treatment regimen, as described elsewhere (11, 12). Clinical scoring was as follows: 0=no illness; 1=

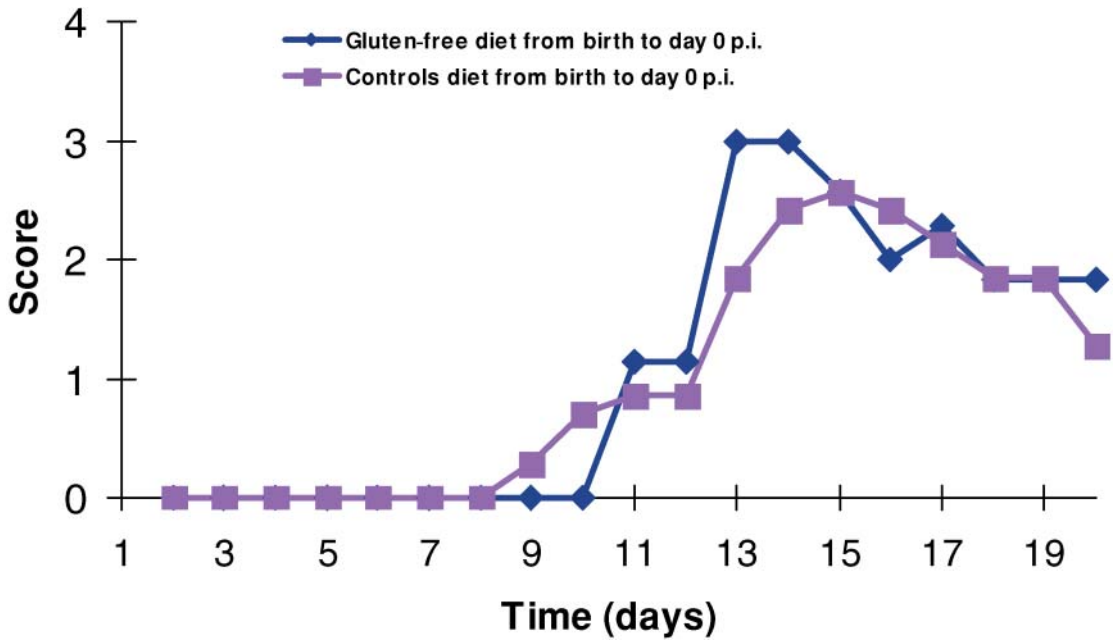

Fig. 1. DA rats fed *ad libitum* a gluten-free diet (diamond symbol, n=7) or with usual rat pellets containing standard amounts of gluten (square symbol, n=7) from day of birth until day of immunization (day 0). Clinical scores (mean values) are illustrated.

flaccid tail; 2=moderate paraparesis; 3=severe paraparesis; 4=tetraparesis; 5=death.

RESULTS

DA rats given a gluten-free diet (n=7) from day of birth until day of immunization exhibited a clinical course of EAE very similar to that of control rats (n=7) (Fig. 1), with a comparable number of relapses and mean cumulative score (data not shown). In addition, it was possible to observe that the rats treated with a gluten-free diet (n=7) from birth until day +40 exhibited a slight, though significant, more accelerated course of the disease than control rats (n=7) (Fig. 2). Accordingly, the maximum degree of paresis was reached on average 14.8±0.7 (SEM) days after immunization in the gluten-free treated group compared to 19.3±0.9 days in the control group ($p<0.002$, Student's *t*-test). Also, the maximum mean paresis score within the first 25 days after immunization was higher in the gluten-free treated group [2.9] than in the control group [2.4], although this was not significant. Number of relapses and mean EAE cumulative score were, however, comparable between the two groups of rats and no significant differences between the two groups could be obtained by nonparametric Mann-Whitney.

No effects on the course of the PR-EAE were observed when a gluten-free diet was started on day −7 and continued until day +16 (data not shown).

DISCUSSION

Organ-specific autoimmune diseases such as type 1 diabetes mellitus, MS and thyroiditis share many common features. The diseases are localized and involve mononuclear cells and cytokines (14, 15). Various immunomodulatory treatments have beneficial effects but are often impossible to implement in the long run because of the side effects (14, 15). Most of the diseases are linked to certain MHC alleles, but not necessarily the same alleles (16). Partly due to this, hereditary factors are involved, but environmental factors also play an important role.

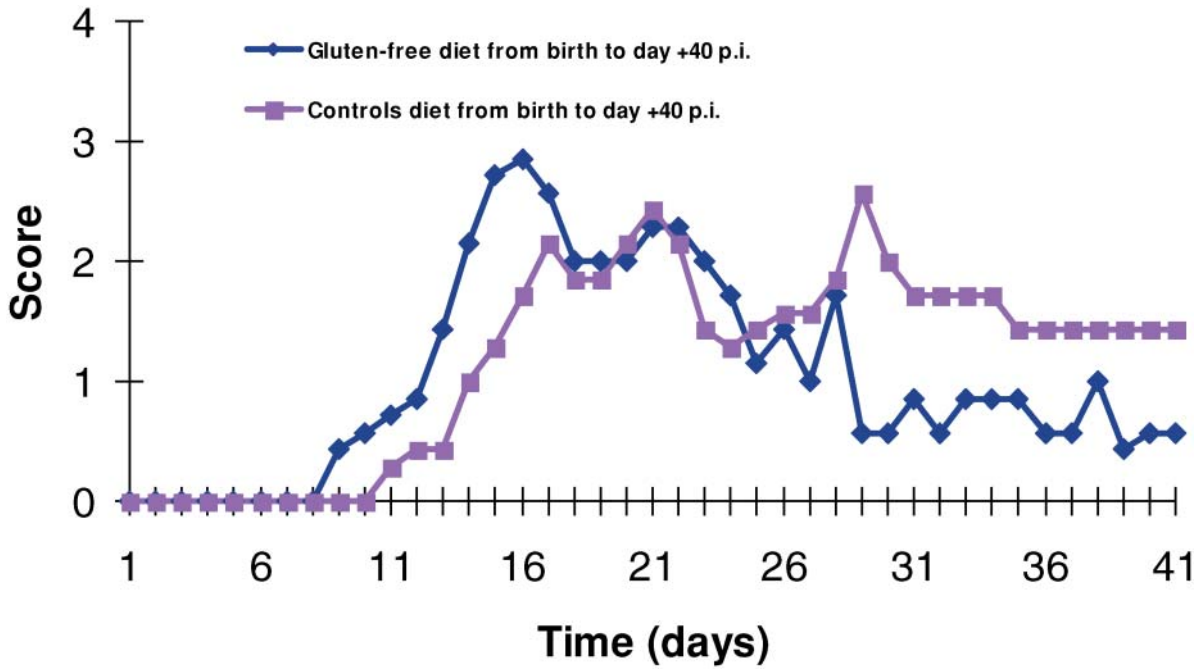

Fig. 2. DA rats fed *ad libitum* a gluten-free diet (diamond symbol, n=7) or with usual rat pellets containing standard amounts of gluten (square symbol, n=7) from day of birth until day 40 post immunization (+40). Clinical scores (mean values) are illustrated. Maximum degree of paresis was reached on average 14.8±0.7 (SEM) days after immunization in the gluten-free treated group compared to 19.3±0.9 days in the control group ($p<0.002$, Student's *t*-test).

The nature of these environmental factors is largely unknown, but influences from virus and bacteria are often mentioned (14–16). Much attention has, furthermore, been focused on the role played by diet as a possible triggering or protective factor in the development of these diseases (2, 3, 17).

In particular, studies conducted in rodent models of type 1 diabetes have clearly shown that disease development may be efficiently prevented by feeding the animals a gluten-free diet (13). Though the mechanism of this beneficial effect is unknown, the experimental findings have recently been strongly supported by the observation that a gluten-free diet exerts beneficial effects in individuals at high risk of developing type 1 diabetes (18). In addition, the question is raised whether this treatment can also be extended to other organ autoimmune diseases.

MS shares some pathogenic characteristics with human type 1 diabetes, including the infiltration of the target organs by mononuclear cells and the apparent contribution to the development of the diseases of type 1 proinflammatory cytokines such as Interleukin (IL)-1, IL-12, IL-18 and Interferon (IFN)-γ as opposed to the beneficial action of type 2/type 3 anti-inflammatory cytokines such as IL-4 and IL-10 and TGF-β1 (14, 15). For both diseases, environmental factors seem to be important, though they are largely unknown. Similarities can also be observed in animal models of type 1 diabetes and MS, including the favourable response to immunomodulatory treatments with specific inhibitors (receptor antagonist, soluble receptor, monoclonal antibodies) of IL-1, IL-12 and TNF-α (19). The clear-cut beneficial effects observed with a gluten-free diet in the NOD mouse led us to anticipate that similar treatment might have also have a favourable influence on EAE in DA rats.

However, we have here shown that – in spite of these similarities – feeding DA rats with a gluten-free diet under different experimental conditions not only failed to prevent disease development but rather caused a slight, though significant, acceleration in disease onset. Furthermore, the amount of paresis tended to be increased. Though the mechanistic mode of action by which the gluten-free diet led to a worsened EAE development in DA rats has not been ascertained, the present findings do not support -on the contrary they rather warn against – the use of a gluten-free diet in the management of MS patients.

REFERENCES

1. Parish NM, Hutchings PR, O'Reilly L, Quartey-Papafio L, Healey D, Ozegbe P, Cooke A. Tolerance induction as a therapeutic strategy for the control of autoimmune endocrine disease in mouse models. Immunol Rev 1995;144:269–300.
2. Hunt BS. Diet and multiple sclerosis. Lancet 1974;2:1512.
3. Scott FW, Marliss EB. Conference summary: diet as an environmental factor in development of insulin-dependent diabetes mellitus. Can J Physiol Pharmacol 1990;69:311–9.
4. Barbeau WE. Interactions between dietary proteins and the human system: implications for oral tolerance and food-related diseases. Adv Exp Med Biol 1997;415:183–93.
5. Ghezzi A, Zafferoni M. Neurological manifestations of gastrointestinal disorders with particular reference to the differential diagnosis of multiple sclerosis. Neurol Sci (Suppl) 2001;2: S117–22.
6. Hunter AL, Rees BW, Jones LT. Gluten antibodies in patients with multiple sclerosis. Hum Nutr Appl Nutr 1984;38:142–3.
7. Hadjivassiliou M, Gibson A, Davies-Jones GA, Lobo AJ, Stephenson TJ, Milford-Ward A. Does cryptic gluten sensitivity play a part in neurological illness? Lancet 1996;347:369–71.
8. Bateson MC, Hopwood D, MacGillivray JB. Jejunal morphology in multiple sclerosis. Lancet 1979;26:1108–10.
9. Hafner FH. Gluten-free diet as treatment for multiple sclerosis. Postgrad Med 1976;59:20.
10. Hawson DC. Is there a role for gluten-free diets in multiple sclerosis? Hum Nutr Appl Nutr 1984; 38:417–20.
11. Lorentzen JC, Issazadeh S, Storch M, Mustafa MI, Lassman H, Linington C, Klareskog L, Olsson T. Protracted, relapsing and demyelinating experimental autoimmune encephalomyelitis in DA rats immunized with syngenic spinal cord and incomplete Freund's adjuvant. J Neuroimmunol 1995;63:193–205.
12. Di Marco R, Khademi M, Wallstrom E, Iacobeus E, Salvaggio A, Caracappa S, Papoian R, Nicoletti F, Olsson T. Curative effects of recombinant human interleukin-6 in DA rats with protracted relapsing experimental allergic encephalomyelitis. J Neuroimmunol 2001;116:168–77.
13. Funda DP, Kaas A, Bock T, Tlaskalová-Hogenová, Buschard K. Gluten-free diet prevents dia-

betes in NOD mice. Diabetes Metab Res Rev 1999;15:323–7.
14. Bach JF. Insulin-dependent diabetes mellitus as an autoimmune disease. Endocr Rev 1994;15: 516–42.
15. Hunter SF, Rodriguez M. Multiple sclerosis: a unique immunopathological syndrome of the central nervous system. Springer Semin Immunopathol 1995;17:89–105.
16. Wucherpfenning KW. Insights into autoimmunity gained from structural analysis of MHC-peptide complexes. Curr Opin Immunol 2001;13: 650–6.
17. Hoorfar J, Buschard K, Dagnaes-Hansen F. Prophylactic nutritional modification of the incidence of diabetes in autoimmune non-obese diabetic (NOD) mice. Br J Nutr 1993;69:597–607.
18. Pastore MR, Bazzigaluppi E, Belloni C, Arcovio C, Bonifacio E, Bosi E. Six months of gluten-free diet do not influence autoantibody titers, but improve insulin secretion in subjects at high risk for type 1 diabetes. J Clin Endocrinol Metab 2003; 88:162–5.
19. Prud'homme GJ. Gene therapy of autoimmune diseases with vectors encoding regulatory cytokines or inflammatory cytokine inhibitors. J Gene Med 2000;2:222–32.