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Lack of Cross-Reactivity between Casein and Gliadin in Sera from Coeliac Disease Patients

Key Words

Casein
Cross-reactivity
Gliadin
Coeliac disease

Abstract

Background: While searching for antigliadin and anticasein antibodies in sera from coeliac patients we investigated more than 20 persons suffering from this enteropathy. In some of sera, we registered antibodies against gliadin and in parallel the appearance of anticasein antibodies. A high percentage of glutamic acid has been proven by amino acid analysis in both these proteins. Therefore we try to demonstrate the existence of cross-reactivity between them in sera from coeliac patients. **Methods:** We analyzed sera from 5 adult patients by using Western blot (SDS-PAGE and immunoblot assay). All of them had previously proven serum antibodies against casein and gliadin. In the procedure, antigliadin-antibodies were removed from sera by adsorption with gliadin. Adsorbed antibodies were then eluted from the adsorbent. **Results:** Adsorbed samples retained anticasein activity. Eluates had a strong reactivity to gliadin but not to casein. **Conclusion:** No cross-reactivity restricted to any of the immunoglobulin isotypes (IgG, IgM, IgA) had been proven between these two proteins. Before the final conclusion, we believe the study has to be completed by using partly of totally disassembled proteins by intestinal enzymes. It might be that enzymatic digestion could open some epitopes shared by both these proteins.

Introduction

Circulating antigliadin antibodies and higher intestinal permeability are suggestive of coeliac disease [1, 2]. Antibodies reactive with cow milk proteins have been detected in serum from coeliac patients [3–5]. Having investigated patients suffering from this enteropathy, we found that some of them produce specific serum antibodies against casein and gliadin at the same time. On the other hand, amino acid analyses of casein and gliadin revealed a very high percentage of glutamic acid in both these proteins [6]. Taken together, these notions suggest the existence of common epitopes. Therefore we investigated the cross-reactivity between these two proteins.

Materials and Methods

Serum samples were collected from 5 (4 females, 1 male) adult coeliac patients before introduction of a gluten-free diet (table 1). Diagnosis was based (apart from strong clinical suspicion) on the histopathology of proximal small-bowel mucosa, serum antibodies against gliadin and clearcut clinical response to a gluten-free diet confirmed by jejunal rebiopsy (after 6 months) [1, 7–9]. All selected persons had destructive (type 3) lesions of the jejunal mucosa which failed to respond to a gluten-free diet until milk and dairy products were additionally withdrawn (6 months, second rebiopsy). None of them had severe malabsorption. We determined and proved specific antigliadin and anticasein antibodies by using Western blot in selected sera. All serum samples had been stored at –70°C in aliquots and then thawed only once. Antihuman IgG, IgA and IgM were purchased from Behringwerke (Germany) and horse radish peroxidase-labelled

Table 1. Patients and mode of selection for study of cross-reactivity

Age years	Patients, n		Abnormal jejunal biopsy type ^a				Positive sera, n						Mucosal response to diet, n		Figure 1 n
	M	F	1	2	3	4	antigliadin			anticasein			GF	GMF	
							G	M	A	G	M	A			
1–14 ^b	2	3					4	1	5	2	1	4	5		
15–24	2	1		1	2		3	1	2	3	2	2	2	1	2
25–34	1	1		1	1		2	1	1			1	2		
35–44	4	5	1	3	5		8	3	6	5	1	3	6	3	3, 4, 5
45–54	1	2		1	1	1	2		1	3	1	1	2	1	1
55–64	1	1			1	1	1		2	2		1	2		
Selected persons													5		

Figure 1 refers to patients 1–5 presented in figure 1. G = IgG; M = IgM; A = IgA, GF = gluten-free; GMF = gluten- and milk-free; n = patient number.

^a According to Marsh [8].

^b Criteria for diagnosis [9].

goat antirabbit IgG were purchased from BIO-RAD (USA). Gliadin adsorbent was prepared according to the procedure of Teruyner and Avramis [10].

Antigliadin antibodies were removed from the samples by adsorption with gliadin adsorbent for 2 h at room temperature. After adsorption supernatants were removed and stored until analyses. After three washings in phosphate-buffered solution adsorbed antibodies were eluted from the adsorbent by 0.2 M glycine/HCl buffer pH 2.8, and eluates were pH-neutralized with 1 M Tris solution. All samples (adsorbed serum samples and their eluates) were tested for anticasein and anti gliadin antibodies by Western blot.

SDS-PAGE was performed according to Laemmli [11] in a Mini Protean II apparatus and proteins were transferred to nitrocellulose sheets according to the Towbins electrophoretic transfer procedure [12].

The excess of active sites were blocked for 2 h at room temperature by 3% gelatin in Tris buffer containing 0.5 M NaCl and 0.05% Tween 20 (TTBS). TTBS was used for dilution and all washings. After three washings, sheets were incubated in antihuman IgG, IgM or IgA antiserum (dilution was 1:100) for 30 min at room temperature. After another three washings sheets were incubated in goat antirabbit IgG antiserum (dilution 1:1,000) for 1 h at room temperature. Finally, after three additional washings reactions were visualized by 4-chloronaphthol in Tris/HCl/H₂O₂ buffer.

Results

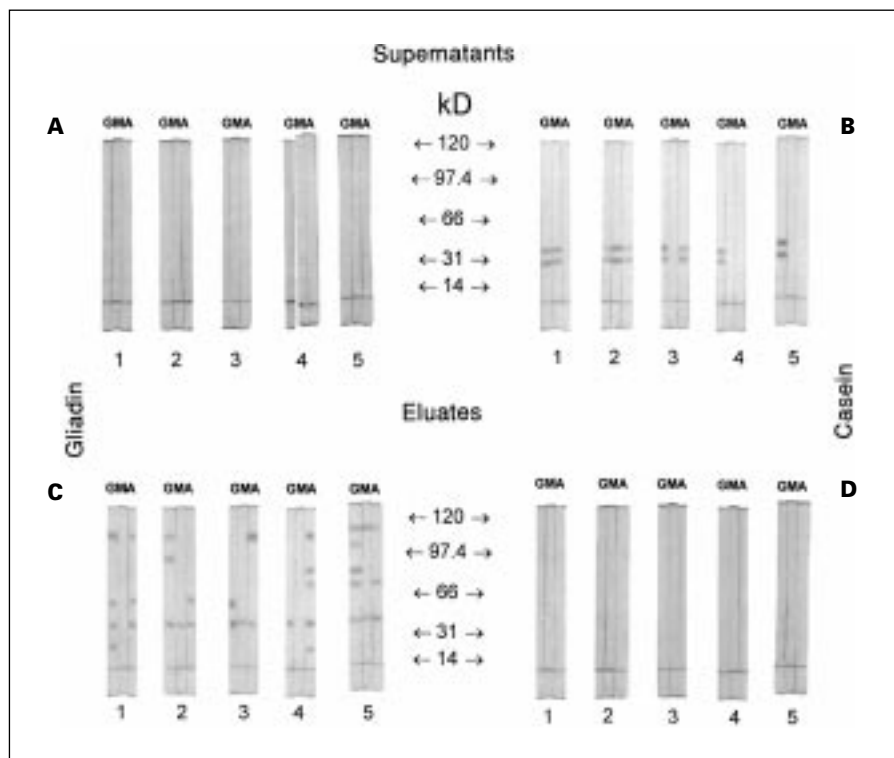
Western blot analysis confirmed successful removal of anti gliadin antibodies from all samples by adsorption (fig. 1A – negative reactions). The anticasein reactivity remained after that procedure giving a positive reaction (fig. 1B). We

proved the presence of anti gliadin antibodies in eluates (fig. 1C – positive reactions) while none of them expressed any reactivity to casein (fig. 1D – negative reactions).

Discussion

In the present study we assessed the cross-reactivity between cow milk casein and gliadin by using Western blot. We did not find any cross-reactivity restricted to either IgG, IgM or IgA isotype from sera of 5 coeliac patients. It has been shown that human serum IgG antibodies reactive with common dietary antigens (gliadin, casein and ovalbumin) are specific, non-cross-reactive and often of the IgG2 and/or IgG4 isotype [13]. In addition, no predominant IgG subclass restriction concerning gliadin was found in coeliac patients [13, 14]. In our patients we registered casein-specific antibodies of IgG, IgM and/or IgA isotype as well as gliadin-reactive antibodies distributed among all these immunoglobulin isotypes. The appearance of coeliac disease is closely related to the genetic profile of patients [1, 7]. It could be that quite different gliadin (and casein) epitopes are presented to immunocompetent cells in coeliac patients in comparison with cow milk-intolerant, asthmatic or healthy persons [13]. We believe that enzymatic digestion (by intestinal proteases) of these two proteins could open some sequestered epitopes with cross-reactive features that will be the subject of our future research.

Fig. 1. Western blot of cow milk casein (**B, D**) and of gliadin (**A, C**) with 5 individual sera (patients 1–5). Adsorbed serum samples (with remainder-specific antibodies to casein) were added in **A** (negative reactions) and **B** (positive reactions) while the eluates (with remainder-specific antigliadin antibodies) were added in **C** (positive reactions) and **D** (negative reactions). GMA = IgG, IgM, IgA.



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