

Antigliadin Antibodies and Gluten-free Diet in Dermatitis herpetiformis

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Vainio E, Kalimo K, Viander M, Reunala T. Antigliadin antibodies and gluten-free diet in dermatitis herpetiformis. *Acta Derm Venereol (Stockh)* 1985; 65: 291–297.

IgA and IgG class antigliadin antibodies (AGA) were analysed with ELISA technique from serum samples of 30 dermatitis herpetiformis patients. Jejunal biopsies were performed to all patients before any treatment and high levels of IgA class AGA were found to be associated with subtotal villous atrophy. Fourteen patients started gluten-free diet (GFD) which caused a significant decrease in both IgA and IgG class AGA. The decrease of IgA AGA was faster than that of IgG AGA and IgA antibody levels fell to normal range during the GFD treatment in all but one patient. In contrast, 5 out of 8 patients followed on normal diet showed increasing IgA AGA levels and all of them had a rise in IgG AGA. IgA and IgG class antibodies to cow's milk were also measured in these patients but in contrast to AGA the diets had no clearcut effect on these antibodies. *Key words: Jejunal morphology.* (Received January 4, 1985.)

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Most patients with dermatitis herpetiformis (DH) have gluten-sensitive enteropathy although they rarely present with gastro-intestinal symptoms (1). Performing jejunal biopsies to every patient with DH is difficult but no other reliable methods have been available for examining how severely the small intestine is damaged. Several serological methods have been tried and various techniques have been developed to measure antigliadin antibodies (AGA) in coeliac disease (CD) and DH (2–7). We recently reported a sensitive ELISA assay to quantitate class specific AGA (8). With this method we showed that in children with CD IgA class AGA fell rapidly when gluten was withdrawn from the diet and rose on its reintroduction (9). Therefore, measurements of IgA class AGA seem to be useful when evaluating the progress of dietary treatment in CD. A strictly followed gluten-free diet (GFD) is effective also in DH and this treatment is now widely used in many centers (10–14).

The aim of the present study was to correlate the class specific AGA to jejunal morphology in DH and to follow up the antibody levels during the GFD treatment. As a control dietary antigen we examined cow's milk protein antibodies (CMPA) in our DH patients.

PATIENTS AND METHODS

Patients

Blood samples were collected from 30 DH patients. The mean age of the patients was 34 years (range 16 to 63 years). The diagnosis was based on clinical, histopathologic and immunofluorescent findings. Jejunal biopsy was performed to every patient. Villous atrophy was graded as subtotal (SVA) or partial (PVA) as previously described (11).

Fourteen patients started GFD and 8 continued on normal diet. Dapsone was given as a supportive therapy. These patients were then followed up from 3 to 26 months at a special out-patient clinic. Blood samples were taken at each visit to measure AGA and CMPA.

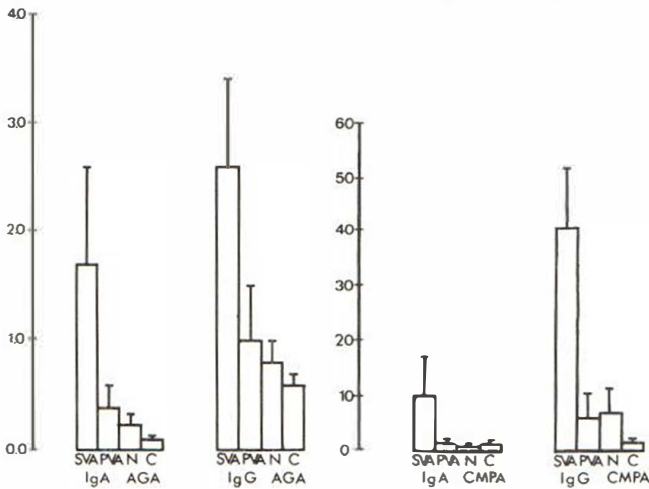


Fig. 1. Mean AGA (a) and CMPA (b) levels in DH patients divided according to jejunal morphology (SVA, PVA, N) and in healthy controls (C). The antibody levels are expressed as percentage of a positive reference serum. Standard error of means indicated by horizontal bars.

Antibody measurements

AGA were measured with an ELISA in which gliadin (British Drug Houses Ltd, Laboratory Chemicals Division, Poole, England) was the solid phase antigen (8). IgA and IgG class AGA was quantitated from serum samples diluted in 1:100 and always tested in duplicates. Serial samples from each patient were analysed concurrently.

IgA class CMPA were measured with a modified ELISA. Paper discs, coupled with cow's milk protein antigen (f2 Phadebas RAST, Pharmacia Diagnostics, Uppsala, Sweden) were used as the solid phase. Fifty microliters of patient serum, diluted in 1:500 with phosphate buffered saline supplemented with 1% bovine serum albumin (BSA-PBS), was incubated with the discs in polystyrene tubes for 2 h at 37°C. Then the discs were washed with physiological saline containing 0.05% Tween 20, alkaline phosphatase labelled swine antihuman IgA (Orion Diagnostica, Espoo, Finland) in a dilution of 1:250 was added, and the tubes were incubated at room temperature overnight. After washing the discs were transferred to microtiter plates. P-nitrophenyl phosphate substrate was added and after 20 min at 37°C the reaction was stopped with 1 N NaOH and the discs were removed. The absorbance was measured at 405 nm with a Titertek Multiscan analyser (Eflab, Helsinki, Finland). All samples were analysed in duplicates.

A radioimmunoassay was developed to measure IgG class CMPA. The paper discs coupled with cow's milk protein were incubated with 50 µl of patient serum diluted in 1:100 with 1% BSA-PBS for 2 h at 37°C. After washing the specific IgG bound to the discs was detected with ¹²⁵iodine labelled staphylococcal protein A as recently described (15). The radioactivity was measured with a gamma counter (Wallac, Turku, Finland). The samples were analysed in triplicates.

The AGA and CMPA results were expressed as percentage of a positive reference serum which was included in every assay. Sera from healthy blood donors and laboratory personnel were included as controls for the assays. Standard deviations were calculated from these measurements and the antibody levels higher than the mean + 2SD were regarded as positive.

Statistics

Mann Whitney U test was used to analyse the differences in AGA and CMPA levels between the patient groups with different jejunal morphology. The effect of the diet treatment on the antibody levels was analysed with Wilcoxon matched-pairs signed-ranks test.

RESULTS

Antibodies and jejunal morphology (Fig. 1)

Thirteen patients had SVA, seven PVA and ten had normal intestinal mucosa. In patients with SVA serum IgA AGA levels were significantly higher than those found in patients with PVA or normal mucosa ($p < 0.05$ and $p < 0.02$). Patients with SVA had also significant-

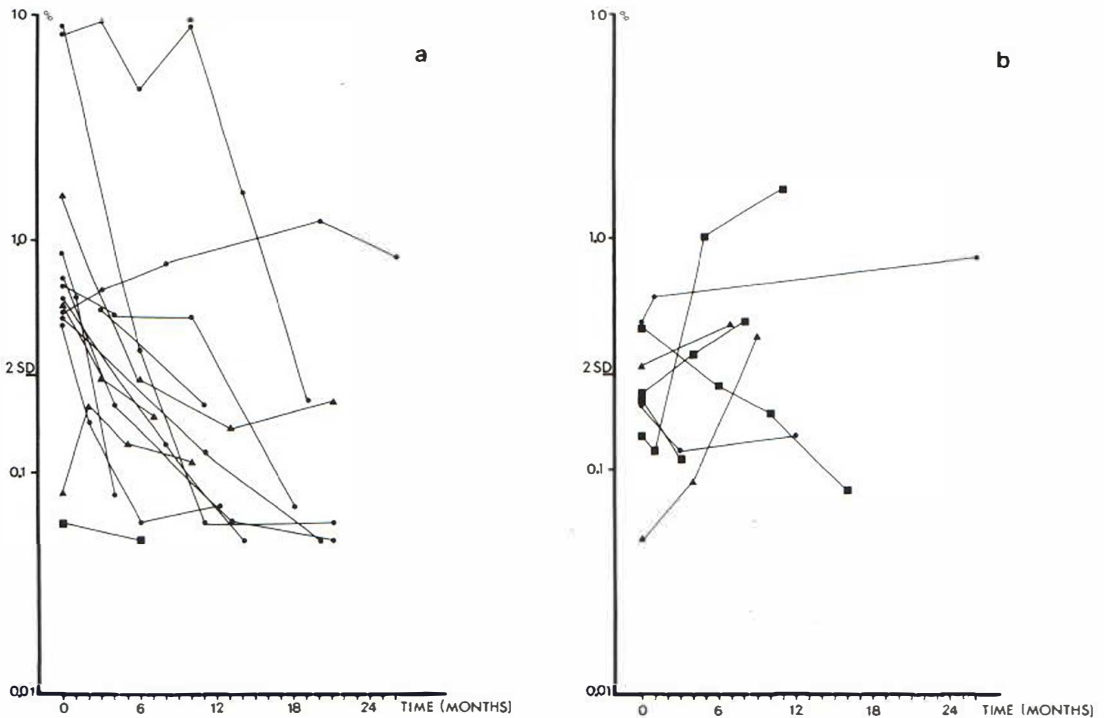


Fig. 2. Individual IgA class AGA levels during (a) GFD and (b) normal diet expressed as percentage of a positive reference serum (patients with SVA ●—●, PVA ▲—▲, normal mucosa ■—■). GFD was started on point 0 except in one case, starting point marked with an asterisk. The 2 SD limit indicated in the scale.

ly higher serum IgG AGA levels than patients with normal jejunal mucosa ($p < 0.02$) but the difference in IgG antibody levels was not statistically significant between patients with SVA and PVA ($p < 0.1$).

Patients with SVA had significantly higher levels of IgA class CMPA than the patients with normal jejunal mucosa ($p < 0.05$) whereas no statistical difference could be observed in IgA CMPA levels between patients with SVA and PVA, nor in IgG class CMPA between any groups.

Antibodies and gluten-free diet treatment

Fourteen patients (10 with SVA, 3 with PVA and one with normal mucosa) started GFD. Twelve had increased levels of IgA class AGA and in eleven of them the antibodies decreased to normal levels during the GFD treatment (Fig. 2a). One patient with SVA showed continuously high levels of IgA AGA during the follow-up on GFD. Her jejunal morphology was controlled with a biopsy at the end of the study and SVA was still found. Then she admitted some deviations from GFD. However she had had only mild skin symptoms and had been able to stop dapsone treatment earlier. All the other patients on GFD still needed dapsone to control the skin symptoms although 10 of these 13 patients had been able to reduce the daily dapsone dose indicating a diminished disease activity in the skin.

Eight of the 14 patients had increased levels of IgG class AGA before GFD, in half of them the levels normalized during diet (Fig. 3a). The decrease in both IgA and IgG AGA levels during GFD was significant ($p < 0.01$ and $p < 0.02$, respectively).

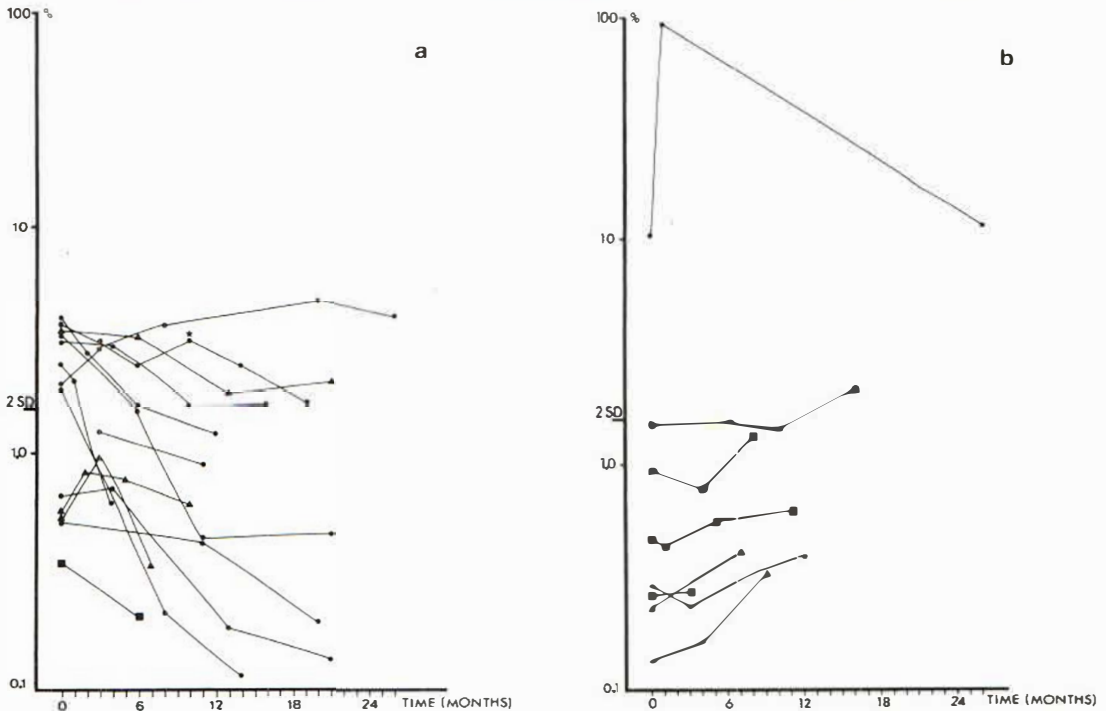


Fig. 3. Individual IgG class AGA levels during (a) GFD and (b) normal diet expressed as percentage of a positive reference serum (patients with SVA ●—●, PVA ▲—▲, normal mucosa ■—■). GFD was started on point 0 except in one case, starting point marked with an asterisk. The 2 SD limit indicated in the scale.

Eight patients (2 with SVA, 2 with PVA and 4 with normal mucosa) continued on normal diet (Figs. 2b and 3b). Three were initially IgA AGA positive and another three became positive during the study whereas two patients remained IgA AGA negative. One became IgA AGA negative and she was the only one on normal diet who was able to reduce the dapsons dose. All eight patients on normal diet had an increase in IgG AGA. This increase was statistically significant ($p < 0.02$) although in six patients the levels were still below normal range.

The results from CMPA measurements during GFD and normal diet are summarized in Table I. The changes in individual CMPA levels were not statistically significant.

Table I. CMPA in DH patients before and after the diet treatments

	IgA		IgG	
	Positive	Negative	Positive	Negative
Gluten-free diet (n=14)				
Before	2	12	6	8
After	3	11	4	10
Normal diet (n=8)				
Before	0	8	1	7
After	1	7	3	5

DISCUSSION

There are several reports describing the prevalence of antigluten or antigliadin antibodies in patients with DH. Unsworth et al. (16) used an immunofluorescent method and found IgG class AGA in 47% and IgA class AGA in 22% of their DH patients on normal diet and an assessment with dissecting microscope revealed an association between jejunal damage and the occurrence of AGA. Ljunghall et al. (17) measured gluten and gliadin antibodies with fluorometric methods using wheat grain and gliadin as antigens. They found antibodies in 45% of their patients and both IgG and IgA class antibodies seemed to be equally effective indicators of mucosal damage. In the present study we used an ELISA method and found IgA AGA in 60% and IgG AGA in 40% of our DH patients. We showed that a good correlation exists between high IgA class AGA and the occurrence of severe jejunal damage, i.e. SVA. Patients with PVA or normal jejunal mucosa had significantly lower IgA AGA values than patients with SVA. The individual variation was remarkable but only 2 of the 13 patients with SVA had normal IgA AGA levels. The highest IgG AGA values occurred also in patients with SVA, although the mean antibody level did not differ significantly from the level in patients with PVA. Therefore, the occurrence of high levels of either IgA or IgG class AGA in a patient with DH seems to be a good indicator of mucosal damage.

It is of interest to follow individual AGA levels during the GFD treatment. In this study the striking observation was the rapid decrease of IgA AGA levels after gluten withdrawal whereas IgG AGA levels seemed to fall more slowly (Figs. 2 and 3). All patients who had increased IgA AGA levels at the beginning and then adhered to a strict GFD showed a decrease of IgA AGA to normal levels during the follow-up. Only half of the IgG AGA positive patients showed normal levels of these antibodies after gluten withdrawal, a figure which is similar to that found previously by Kieffer et al. (18). In contrast to our study Kieffer et al. detected only low IgA AGA titers in their DH patients. This discrepancy is difficult to explain but it could be due to differences in ELISA techniques or the labelled second antibodies used. However, we have recently with our method shown a good correlation between falling IgA AGA and mucosal healing in children with CD (9) suggesting that the falling IgA levels in the present DH patients are also relevant. In one patient increasing AGA values revealed faults in her diet, which otherwise would have remained undetected, because she felt clinically well and was able to stop dapsone. In contrast to patients on GFD, patients on normal diet had increasing AGA levels, which again supports the idea that AGA are dependent on gluten intake.

Granular IgA deposits occurring in papillary dermis are pathognomonic to DH, but the antigen to this tissue-bound IgA remains unknown (1). That IgA class AGA observed in the present patients would have some pathogenetic importance for the rash seems improbable. The occurrence of these antibodies also in patients with CD, who do not have skin symptoms, and the rapid fall of IgA AGA before DH patients on GFD could discontinue the dapsone therapy suggest that IgA class AGA are only good markers of small intestinal damage in DH.

CMPA have been found in various percentages in patients with DH and CD but also in normal controls (19–21). Bürgin-Wolff et al. (20) proposed that CMPA are of no value in the diagnosis or treatment of CD. In contrast Scott et al. (22) suggested that monitoring of antibody response to such dietary antigens would help to follow the mucosal improvement. In our study we found increased levels of both IgA and IgG class CMPA in DH patients with highest AGA values. However, fluctuation in CMPA levels occurred both in patients adhering to GFD and in those taking normal diet opposite to the results of Scott et al. Evidently the significance of these antibodies remains to be established.

In conclusion, determinations of class-specific AGA with ELISA in patients with DH

revealed a good correlation between increased IgA AGA levels and subtotal villous atrophy. The observed rapid fall of AGA levels after gluten withdrawal indicates that this method is useful when following patients on GFD.

ACKNOWLEDGEMENTS

We are grateful to Mrs Maija Salonen for her skilful technical assistance. This study was supported by grants from the Paulo Foundation and the Research Foundation of Medica. The milk protein discs were a generous gift provided by Pharmacia Diagnostics.

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