

Anti-actin IgA antibodies in severe coeliac disease

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(Accepted for publication 14 May 2004)

SUMMARY

Anti-actin IgA antibodies have been found in sera of coeliacs. Our aim was to define the prevalence and clinical significance of anti-actin IgA in coeliacs before and after gluten withdrawal. One hundred and two biopsy-proven coeliacs, 95 disease controls and 50 blood donors were studied. Anti-actin IgA were evaluated by different methods: (a) antimicrofilament positivity on HEp-2 cells and on cultured fibroblasts by immunofluorescence; (b) anti-actin positivity by enzyme-linked immuosorbent assay (ELISA); and (c) presence of the tubular/glomerular pattern of anti-smooth muscle antibodies on rat kidney sections by immunofluorescence. Antimicrofilament IgA were present in 27% of coeliacs and in none of the controls. Antimicrofilament antibodies were found in 25 of 54 (46%) coeliacs with severe villous atrophy and in three of 48 (6%) with mild damage ($P < 0.0001$). In the 20 patients tested, antimicrofilaments IgA disappeared after gluten withdrawal in accordance with histological recovery. Our study shows a significant correlation between antimicrofilament IgA and the severity of intestinal damage in untreated coeliacs. The disappearance of antimicrofilament IgA after gluten withdrawal predicts the normalization of intestinal mucosa and could be considered a useful tool in the follow-up of severe coeliac disease.

Keywords: anti-actin antibodies antimicrofilament antibodies coeliac disease intestinal villous atrophy mucosal recovery

INTRODUCTION

Coeliac disease (CD) is an immune-mediated enteropathy triggered by ingestion of gluten in genetically susceptible individuals and histologically characterized by different grades of small intestine damage that consist of villous atrophy, crypt hyperplasia and an increased number of intraepithelial lymphocytes [1]. IgA anti-endomysial antibodies (EmA) are considered the most reliable serological marker of CD [2]. After the recent identification of tissue transglutaminase (tTG) as the main, if not the sole endomysial antigen, a very sensitive enzyme-linked immunosorbent assay (ELISA), using human recombinant tissue transglutaminase (h-tTG) as antigen, has been set up [3]. Despite the high sensitivity and specificity of these serological tests for diagnosis of CD, anti-endomysial antibodies do not correlate with histopathological features [4]. Moreover, anti-tTG antibodies are known to be more correlated with dietetic compliance than

with intestinal biopsy morphology [5]. At present, intestinal biopsy with demonstration of gluten-dependent mucosal damage remains the gold standard procedure for a definitive diagnosis of coeliac disease [6].

Recently, Clemente *et al.* described a very high prevalence of IgA antibodies against actin filaments (AAA) in coeliac patients with severe or moderate intestinal villous atrophy, showing that the finding of these antibodies correlates with the degree of mucosal damage [7]. Actin is known as the major component of microfilaments of cell cytoskeleton and it is regarded as the target of smooth muscle antibodies with the tubular pattern (SMA-T), detected by indirect immunofluorescence (IFL) on kidney sections and closely associated with type 1 autoimmune hepatitis (AIH) [8].

On isolated cells (fibroblasts, HEp-2 cells, peripheral blood mononuclear cells), anti-actin antibodies react with the microfilaments giving the pattern of parallel 'stress fibres' [9].

The cytoskeleton network is the basis of the ultrastructural architecture of the enterocytes and it is known that gluten challenge in CD patients causes rapid distortion of microvillus structure with disorganization of actin network on the intestinal mucosa [10,11].

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The aim of this study was to define the prevalence of IgA AAA in CD patients before and after gluten withdrawal, using different methods for their detection. Moreover, we have correlated the presence of AAA with small intestinal mucosal morphology before and after gluten withdrawal to evaluate clinical significance of these antibodies.

MATERIALS AND METHODS

Study population

One hundred and two consecutive biopsy-proven CD patients, 69 adults (M/F 19/50, median age 32 years, range 15–74), 33 children (M/F 14/19, median age 6 years, range 2–14), were studied at the time of diagnosis. Intestinal biopsy specimens were graded according to Marsh's classification, modified by Oberhuber [12] as follows: increase in intra-epithelial lymphocytes (IEL) (grade 1), increase in IEL with crypt hyperplasia (grade 2), mild villous flattening (grade 3a), marked villous flattening (grade 3b) and total villous flattening (grade 3c). From grade 1 to grade 3a the histological damage was considered mild, grades 3b and 3c were considered severe.

As a control population we enrolled 20 patients with dyspepsia and normal intestinal biopsy, 15 patients with inflammatory bowel disease (seven ulcerative colitis (UC) and eight Crohn's disease), 11 with post-enteritis syndrome, 29 with type I AIH and 20 with primary biliary cirrhosis (PBC). We also tested 50 age- and sex-matched blood donors as normal controls. All sera were stored at -20°C until use.

This study was approved by the ethical committee of our hospital; in each case, informed consent was obtained before inclusion in the study.

Indirect immunofluorescence

Detection of IgA EmA. IgA EmA were detected by indirect immunofluorescence using human umbilical cord cryostat sections ($4\text{ }\mu\text{m}$) as substrate. Sera were tested at the initial dilution of 1 : 5 and, when positive, were titred up to the endpoint [2].

Detection of SMA on rat tissue sections. SMA were detected by IFL as described previously [13]. Briefly, sera diluted 1 : 40 in phosphate buffered saline (PBS) were tested on cryostatic frozen sections of rat liver, kidney and stomach. A fluorescein-conjugated secondary antibody, either polyspecific or IgA-specific (anti-human polyvalent immunoglobulins IgA, IgG, IgM and anti-IgA FITC conjugate, respectively, Sigma ImmunoChemicals, St Louis, MO, USA), was used diluted 1 : 100 in PBS. The positivity for SMA was assessed under a fluorescence microscope (Orthoplan, Leitz, Wetzlar, Germany).

The identification of the SMA patterns was made according to Bottazzo *et al.* [8]: (1) SMA-V (vessels): staining of small/medium-size vessel walls; (2) SMA-G (glomeruli): staining, in addition to vessels of glomerular mesangial cells also; (3) SMA-T (tubuli): staining of vessels, glomerular and peritubular structures. According to Bottazzo *et al.* sera were regarded as anti-actin positive only when SMA-T or SMA-G pattern was present.

Detection of SMA-T/G antibodies was performed blindly by two independent investigators (F.C. and A.G.)

Detection of antimicrofilament antibodies (anti-MF) on HEP-2 cells and on human fibroblasts. All samples were tested by indirect IFL on HEP-2 (Euroimmun, Labordiagnostika, Lubeck, Germany) for detection of IgA anti-MF antibodies; briefly, serum samples were diluted 1 : 5 in PBS and incubated in a humid chamber for 30 min; after three PBS washings, the secondary antibody (anti-human IgA FITC Conjugate, Sigma ImmunoChemicals) at a dilution of 1 : 100 was added.

IgA anti-MF antibodies were also assayed by indirect IFL on human fibroblasts kindly provided by Dr A. Ripalti (Department of Clinical and Experimental Medicine, Section of Microbiology, University of Bologna, Italy). Fibroblasts were grown to semiconfluence on coverslips in Dulbecco's solution with 10% fetal calf serum in 5% carbon dioxide atmosphere, and then fixed with acetone at -20°C for 10 min. Sample sera were diluted 1 : 5 in PBS and the secondary antibody (anti-human IgA FITC conjugate, Sigma ImmunoChemicals) was diluted 1 : 100. The positivity for anti-MF (staining of parallel 'stress fibres') was assessed independently and blinded by two investigators (F.C. and A.G.). Positive reactions were titred by the double dilution method.

Enzyme-linked immunosorbent assay (ELISA)

Detection of IgA h-tTG. IgA h-tTG were detected by ELISA using a commercial kit (Eurospital, Trieste, Italy) as reported previously [14]; the cut-off was fixed at seven arbitrary units (AU). The optical density (OD) was read at 450 nm.

Detection of anti-actin antibodies (AAA) by ELISA. ELISA was performed using as antigen lyophilized actin commercially available from rabbit muscle (Sigma Chemicals, St Louis, MO, USA).

Briefly, each well of microtitre plates (Dynatech Laboratories Inc., Chantilly, VA, USA) was coated overnight at 4°C with $100\text{ }\mu\text{l}$ ($10\text{ }\mu\text{g/ml}$) of actin diluted in bicarbonate buffer, pH 7.5. After three washings with PBS Tween 0.05%, $100\text{ }\mu\text{l}$ of blocking buffer (0.05 M Tris-HCL, 0.15 M NaCl, 0.01 M EDTA, 0.1% Tween-20, pH 7.4) was added to each well for 60 min at room temperature. One hundred μl of the each serum diluted 1 : 100 in PBS/powdered milk 3% were added to wells and incubated for 60 min at room temperature. After three washing steps with PBS Tween 0.05%, $100\text{ }\mu\text{l}$ of peroxidase-conjugated antibody antihuman IgA (Dako, Denmark) diluted 1 : 500 in PBS/powdered milk 3% was added to each well and incubated for 60 min at room temperature. After the washing steps the reaction was developed with o-phenylenediamine dihydrochloride (Sigma) and H_2O_2 in citrate buffer for 15 min, and the colourimetric reaction was measured at 492 nm by spectrophotometer. The cut-off was established as the mean optical density at $492\text{ nm} \pm 5$ standard deviations in 100 further healthy blood donors ($0.080 + 5 \times 0.004 = 0.100$).

Absorption studies

Five sera positive for anti-tTG, AAA by ELISA and for anti-MF by IFL were incubated overnight at 4°C , under agitation, with increasing amounts ($10\text{ }\mu\text{g}$, $50\text{ }\mu\text{g}$, $250\text{ }\mu\text{g}$, $500\text{ }\mu\text{g}$) of rabbit muscle actin diluted in bicarbonate buffer (pH 7.5) and guinea-pig liver transglutaminase (Sigma) diluted in 0.05 M Tris-HCL, 0.15 M NaCl, 5 mM CaCl_2 buffer (pH 7.5). Antibody-antigen complexes were separated from sera by centrifugation at $100\text{--}000\text{ g}$ for 30 min. ELISA for AAA (sera diluted 1 : 100 were added in duplicate to wells, peroxidase-conjugated antibody antihuman IgA diluted 1 : 500 in PBS/powdered milk 3% was used as secondary antibody, as described previously) and anti-tTG (sera diluted 1 : 26 were added in duplicate to wells, using commercial Eurospital kit as described previously) and IFL on HEP-2 cells and fibroblasts (sera diluted 1 : 5 as described previously) were then performed with an unabsorbed and absorbed serum.

Follow-up study

Coeliac patients positive for IgA AAA at the time of diagnosis were reevaluated after 12 months of gluten-free diet (GFD). Follow-up study included detection of AAA, EmA and anti-tTG antibodies together with intestinal biopsy. An accurate dietetic interview was used to evaluate the compliance to gluten free diet.

Statistical analysis

The comparison of categorical variables was performed using χ^2 and Fisher's exact test when applicable. Nominal variables were correlated by contingency table. A probability (*P*) value less than 0.05 was considered significant.

RESULTS

Anti-MF IgA antibodies on fibroblasts (Fig. 1a) were found in 28 (27%) of the 102 CD patients studied (median titre 1 : 160, range 1 : 40–1 : 1280). Of these 28 cases, 26 (25% of total CD patients) were also positive for anti-MF IgA on HEp-2 cells (Fig. 1b) and 15 (14.7% of total CD patients) for IgA AAA by ELISA. EmA and anti-tTG were found positive, respectively, in 101 (99%) and 100 (98%) coeliac patients. All the 145 control sera were negative for EmA, anti-tTG and both IFL-detected anti-MF and ELISA-detected AAA. SMA with the T/G pattern were found in 10.7% (11/102) coeliac patients and in 82% (24/29) AIH patients. By IFL with an IgA-specific secondary conjugate, the SMA T/G was due to IgA antibodies in five (4.9%) CD patients and never in AIH patients. Table 1 summarizes the results obtained with the different methods. Table 2 shows the detailed spectrum of reactivities of the 28 CD sera positive for anti-MF antibodies on fibroblasts.

Taking into account the mucosal histology, anti-MF antibodies were found in 25 (46%) of the 54 CD patients with severe villous atrophy (grades 3b–c) and in only three (6%) of the 48 with mild intestinal damage (grades 1–3a) ($P < 0.0001$). Among childhood CD patients, anti-MF antibodies were positive in nine (47%) of the 19 patients with severe villous atrophy but in none of those with mild intestinal damage ($P = 0.0039$). Sixteen (45.7%) of the 35 adult CD patients with severe villous atrophy and only three (8.8%) of the 34 with grades 1–3a of mucosal damage were anti-MF positive ($P = 0.0009$) (Table 3).

As shown in Fig. 2, a progressive decrease of anti-actin reactivity by ELISA and MF-pattern positivity by IFL was seen after absorption with actin, but not with tTG.

After preincubation with 500 μ g of rabbit actin, anti-actin reactivity by ELISA decreased $> 75\%$ and anti-MF antibodies on fibroblasts and HEp-2 (Fig. 1c) cells disappeared almost completely. On the contrary, absorption with guinea-pig liver transglutaminase did not modify anti-actin reactivity by ELISA and anti-MF antibodies by IFL, which remained unaltered, but abolished anti-tTG reactivity selectively by ELISA (Fig. 3).

After 1 year of gluten-free diet 20 of the 28 patients positive for IgA anti-MF antibodies (12 of these also positive for IgA AAA by ELISA) accepted the follow-up re-evaluation, eight patients declined participation. The anti-actin reactivity disappeared by both ELISA and IFL in all of them, whereas IgA EmA and anti-tTG persisted positive in six of these 20 cases; tTG and EmA were not significantly modified, respectively, in four and five of these six patients. Dietetic interview revealed strict compliance to a gluten-free diet in all 20 patients. In all patients a second intestinal biopsy showed complete regrowth of intestinal mucosa.

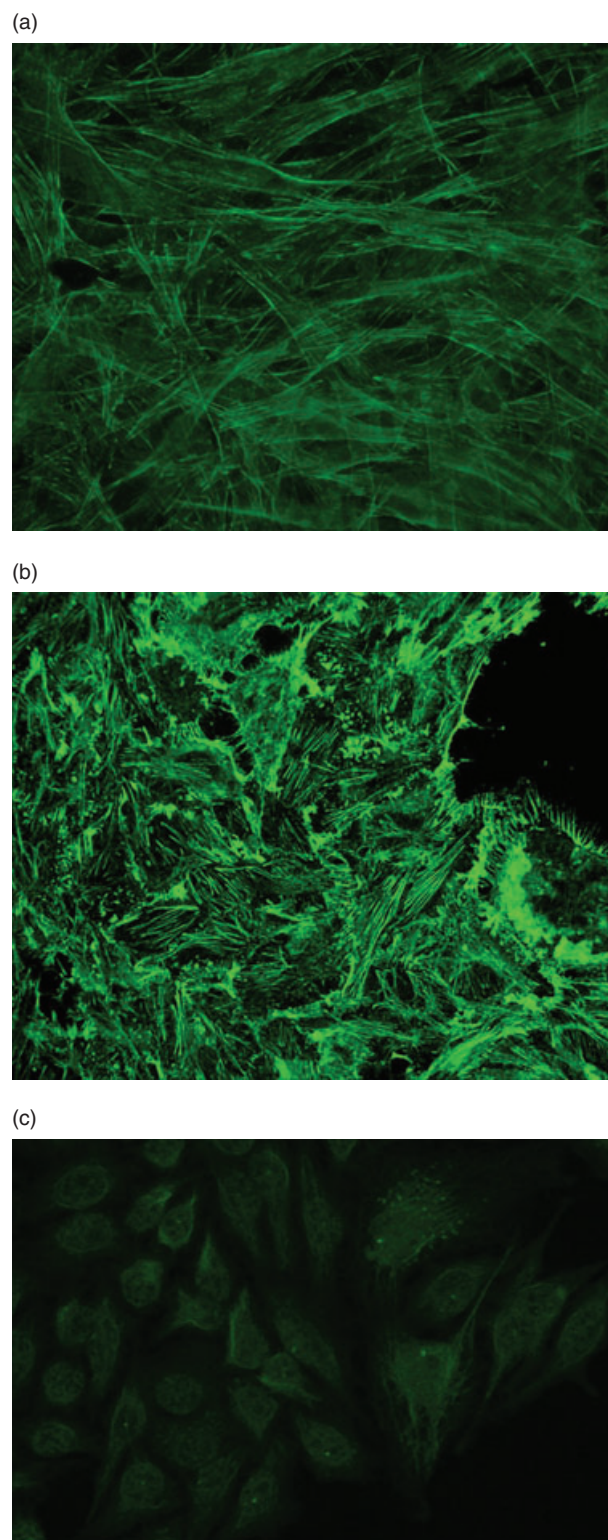


Fig. 1. Case no. 4. Anti-microfilaments antibodies (titre 1 : 160) on fibroblasts (a, magnification 40 $\times$) and HEp-2 cells (b, magnification 40 $\times$) at initial dilution of 1 : 5 with antihuman IgA FITC conjugate as secondary antibodies. Typical positive actin stress fibres (microfilaments) appear as cytoplasmic parallel filaments. Negative effect on microfilaments staining on HEp-2 cells after absorption of serum with 500 μ g of rabbit muscle actin. One hundred μ l of absorbed serum at dilution of 1 : 5 was used (c, magnification 40 $\times$).

Table 1. Prevalence of different reactivities in CD patients and in pathological controls

	IgA anti-MF on fibroblasts	IgA anti-MF on Hep-2 cells	IgA AAA by ELISA	SMA T/G†	IgA SMA T/G
CD total cases (<i>n</i> = 102)	28 (27%)	26 (25%)	15 (14.7%)	11 (10.7%)	5 (4.9%)
CD grades 1–3a (<i>n</i> = 48)	3 (6%)*	3 (6%)	3 (6%)	5 (10.4%)	2 (4%)
CD grades 3b–c (<i>n</i> = 54)	25 (46%)*	23 (42%)	12 (22%)	6 (11%)	3 (5.5%)
Dyspepsia (<i>n</i> = 20)	0	0	0	0	0
IBD (<i>n</i> = 15)	0	0	0	0	0
Post-enteritis syndrome (<i>n</i> = 11)	0	0	0	0	0
AIH type 1 (<i>n</i> = 29)	0	0	0	24 (82%)	0
PBC (<i>n</i> = 20)	0	0	0	0	0

*Grades 3b–c versus 1–3a, $P < 0.0001$ (Fisher's test). †IFL on rat tissue with antihuman total Ig as secondary antibodies.

Table 2. Reactivities of the 28 coeliac patients anti-MF positive

Patient no.	Anti-MF on fibroblasts*	Anti-MF on HEp-2*	IgA AAA ELISA OD	SMA T/G	IgA SMA T/G	Anti-tTg AU†	EmA*
3	1 : 40	1 : 40	neg	neg	neg	14	1:160
4	1 : 160	1 : 160	0.224	pos	pos	19	1 : 80
6	1 : 640	1 : 320	neg	pos	neg	32	1 : 320
9	1 : 320	1 : 320	neg	neg	pos	20	1 : 80
11	1 : 320	1 : 320	neg	neg	neg	11	1:160
23	1 : 640	1 : 320	0.254	pos	neg	12	1 : 160
25	1 : 1280	1 : 1280	0.542	neg	neg	15	1 : 320
30	1 : 40	1 : 40	neg	pos	pos	13	1 : 80
45	1 : 320	1 : 320	neg	neg	neg	16	1 : 80
47	1 : 320	1 : 320	neg	pos	neg	neg	1 : 160
49	1 : 320	1 : 160	0.264	neg	neg	20	1 : 320
50	1 : 80	1 : 80	neg	neg	neg	15	1 : 160
51	1 : 40	1 : 40	0.188	pos	neg	26	1 : 320
54	1 : 320	1 : 320	neg	neg	neg	25	1 : 320
58	1 : 40	1 : 20	0.136	pos	pos	21	1 : 320
63	1 : 320	1 : 160	0.246	neg	neg	neg	1 : 320
68	1 : 640	1 : 640	0.288	pos	neg	9	1 : 80
69	1 : 160	1 : 160	0.214	neg	neg	22	1 : 40
74	1 : 40	1 : 10	neg	neg	neg	15	1 : 160
75	1 : 40	1 : 40	neg	pos	neg	14	1 : 80
77	1 : 160	1 : 160	0.256	neg	neg	13	1 : 80
78	1 : 80	1 : 80	0.198	neg	neg	16	1 : 40
81	1 : 80	1 : 80	0.208	neg	neg	11	1 : 40
82	1 : 640	1 : 640	0.274	neg	neg	19	1 : 320
87	1 : 40	1 : 20	neg	pos	neg	12	1 : 160
91	1 : 160	1 : 80	0.236	neg	neg	16	1 : 160
96	1 : 40	neg	neg	neg	neg	10	1 : 40
99	1 : 40	neg	0.148	pos	pos	12	1 : 40

*Anti-MF and EmA values are expressed as titres. †AU: arbitrary units.

Table 4 shows details of the serological and histological features of 20 anti-MF positive coeliacs reevaluated after 1 year of GFD.

DISCUSSION

Smooth muscle antibodies of the IgG class with anti-actin specificity are regarded commonly as markers of type I autoimmune hepatitis, and different techniques have been used for their identification [9,15]. SMA-T/G and anti-MF antibodies are present in

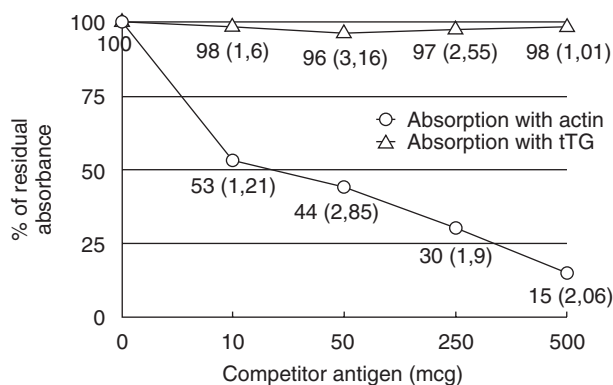
about 80% patients with AIH-1, where the two reactivities are strictly associated, both being regarded as anti-actin antibodies [16].

Similar anti-MF antibodies belonging, however, to the IgA class, have also been reported in CD [7]. This study confirms that in patients with untreated CD IgA anti-MF antibodies are a common finding. In our hands IFL on fibroblasts was the most sensitive method for the detection of anti-actin antibodies in CD. By IFL on kidney sections, the prevalence of SMA T/G was definitely

Table 3. Prevalence of IgA anti-MF in the two groups of CD patients and correlation with histological grade

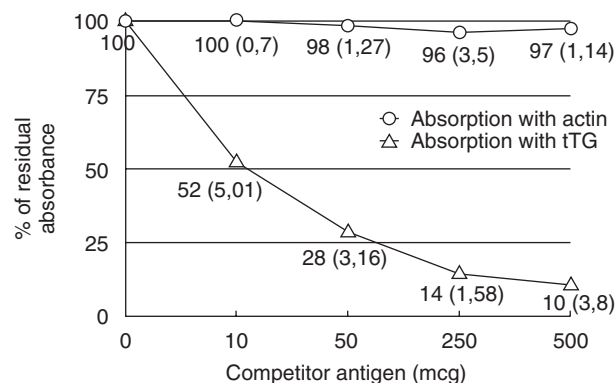
CD patients	IgA anti-MF on fibroblasts
Adult CD (<i>n</i> = 69)	19 (27.5%)
(a) Grades 1–3a (<i>n</i> = 34)	3 (8.8%)*
(b) Grades 3b–c (<i>n</i> = 35)	16 (45.7%)*
Childhood CD (<i>n</i> = 33)	9 (27.2%)
(c) Grades 1–3a (<i>n</i> = 14)	0†
(b) Grades 3b–c (<i>n</i> = 19)	9 (47%)†

*Grades 3b–c versus 1–3a. *P* = 0.0009 (Fisher's test); †grades 3b–c versus 1–3a. *P* = 0.0039 (Fisher's test).

**Fig. 2.** IgA Anti-actin reactivity by ELISA of five anti-tTG/AAA/anti-MF positive sera. Mean of percentage ($\pm$ standard deviation) of residual absorbance (optical density at 492 nm) is on the y-axis. Absorbance was reduced by preincubation with increasing amounts of actin, whereas it remained virtually unaltered by preincubation with tTG.

lower, possibly because of the masking effect of the concomitant positivity for antireticulin antibodies, detected in 41% (data not shown) of our CD patients and in 16 (57%) of the 28 coeliacs anti-MF positive; moreover, different sera dilution were used (1:40 on kidney sections vs. 1:5 on cultured fibroblasts). With regard to AAA IgA, the lower sensitivity of ELISA in comparison with IFL on fibroblasts could be explained by the use of monomeric actin (G-actin) as antigen for ELISA. In CD patients, as already speculated for AIH, anti-actin antibodies probably recognize filamentous or polymerized actin (F-actin), which offers a higher number of potential epitopes than unpolymerized actin (G-actin) [17–19]. The polymerization of monomeric actin in high amounts [20] may explain why G-actin successfully removed the MF-pattern in absorption experiments.

It has been shown a co-localization of tissue transglutaminase with stress fibres in vascular smooth muscle cells and immunoprecipitation experiments have demonstrated that transglutaminase co-immunoprecipitated with myosin, suggesting that this pattern of distribution was due to cross-linking of transglutaminase with myosin but not with actin [21]. The results of absorption experiments suggest that actin is the target of the anti-MF pattern; our results also suggest that anti-actin and anti-tTG are separate reactivities, giving different morphological patterns in IFL experiments.

**Fig. 3.** IgA Anti-tTG reactivity by ELISA of five anti-tTG/AAA/anti-MF positive sera. Mean of percentage ($\pm$ standard deviation) of residual absorbance (optical density at 450 nm) is on the y-axis. Preincubation with actin did not modify absorbance, which remained unaltered.

Antibodies to cytoskeleton components such as anti-intermediate filaments (IMF) and anti-MF antibodies of IgA class have been also found in inflammatory bowel disease. However, their occurrence in these conditions is less frequent than in CD and lack clinical significance, probably being the effect only of antigen leakage in the course of mucosal damage [22]. Instead, in CD setting the appearance of AAA IgA could be enhanced by cross-linking of cytoskeleton components with tTG as actin is a good substrate of tTG [23]. Generation of novel neopeptides by tTG-catalysed cross-linking reactions or post-translational modifications of cryptic self-antigen, unmasked during cell injury, is supposed as a hypothetical mechanism of CD-related autoimmune disorders [24–26]. Immune reaction towards cytoskeleton components with appearance of specific AAA of the IgA class, resulting from actin-network involvement, could be included with the secondary autoimmune phenomena observed in CD.

In our study, a similar prevalence of anti-MF antibodies was observed between childhood and adult CD patients. A significant difference was found in the prevalence of anti-MF IgA between patients with different degrees of mucosal damage. In fact, about half of our CD patients with marked or total villous flattening resulted positive for anti-MF IgA, in contrast with 6% of those with mild intestinal mucosal damage. The predictive positive value of anti-MF IgA antibodies for severe villous atrophy (grades 3b and c) is estimated to be 89%. None of the pathological and normal controls had anti-MF IgA, indicating a very high specificity for CD.

Compared with the original report by Clemente *et al.* [7], we found a lower prevalence of anti-MF antibodies in CD patients; this discrepancy could be due both to selection criteria (a higher prevalence of cases with severe mucosal damage in Clemente's study) and to differences in the tests used (different commercial kits of HEP-2 cells and different secondary antibody used). Although Clemente *et al.* described the disappearance of AAA after GFD, the correlation between AAA and mucosal morphology was investigated before GFD, while no correlation analysis was performed after GFD. Our results indicate that IgA anti-MF antibodies disappeared totally in all 20 cases tested after 1 year of GFD, showing a high correlation with histological recovery, assessed by second intestinal biopsy; on the other hand, both Ema and anti-tTG antibodies persisted in about 30% of the 20

Table 4. Serological and histological features of the 20 anti-MF positive coeliacs reevaluated after 1 year of GFD

Patient no.	Age (years)	Histology at diagnosis	EmA at diagnosis*	Anti-tTG at diagnosis†	IgA Anti-MF at diagnosis	Histology after GFD	EmA after GFD*	Anti-tTG after GFD†	IgA anti-MF after GFD
3	33	2	1:160	14 AU	1:40	normal‡	neg	neg	neg
4	30	3c	1:80	19 AU	1:160	normal	1:80	18 AU	neg
6	43	3c	1:320	32 AU	1:640	normal	1:320	9 AU	neg
9	32	3c	1:80	20 AU	1:320	normal	1:80	21 AU	neg
11	53	3b	1:160	11 AU	1:320	normal	1:160	11 AU	neg
23	43	3b	1:160	12 AU	1:640	normal	neg	neg	neg
25	29	3b	1:320	15 AU	1:1280	normal	1:40	15 AU	neg
30	28	3c	1:80	13 AU	1:40	normal	1:80	9 AU	neg
45	30	3c	1:80	16 AU	1:320	normal	neg	neg	neg
47	29	3b	1:160	neg	1:320	normal	neg	neg	neg
49	47	2	1:320	20 AU	1:320	normal	neg	neg	neg
50	37	1	1:160	15 AU	1:80	normal	neg	neg	neg
51	40	3c	1:320	26 AU	1:40	normal	neg	neg	neg
54	36	3b	1:320	25 AU	1:320	normal	neg	neg	neg
58	21	3c	1:320	21 AU	1:40	normal	neg	neg	neg
63	25	3c	1:320	neg	1:320	normal	neg	neg	neg
68	39	3b	1:80	9 AU	1:640	normal	neg	neg	neg
69	41	3b	1:40	22 AU	1:160	normal	neg	neg	neg
87	48	3c	1:160	12 AU	1:40	normal	neg	neg	neg
96	14	3c	1:40	10 AU	1:40	normal	neg	neg	neg

*Cut-off > 1:5. †Cut-off > 7 AU. ‡Intraepithelial lymphocytes < 40 per 100 epithelial cells, absence of crypt hyperplasia and normal villous architecture.

patients. Despite the limited number of cases, almost entirely concerning adult patients, our results, obtained by a complete follow-up study, show for the first time a significant correlation between disappearance of anti-MF IgA and the normalization of intestinal mucosa, suggesting that these antibodies could be helpful for monitoring the response to GFD in severe coeliac disease, especially when anti-tTG or EmA antibodies after GFD are still present.

In conclusion, our study shows that anti-MF IgA antibodies with anti-actin specificity are present in a significant proportion of patients with untreated CD.

Although anti-MF IgA antibodies, due to their global low sensitivity, cannot replace EmA and anti-tTG in the diagnostic algorithm of CD, their finding is associated strictly with flat mucosa and is predictive of a severe gluten-sensitive enteropathy; moreover, they could be considered a further tool for the follow-up of severe coeliac disease in addition to traditional markers.

REFERENCES

- 1 Trier JS. Celiac sprue. *N Engl J Med* 1991; **325**:1709–19.
- 2 Volta U, Molinaro N, de Franceschi L *et al.* IgA anti-endomysial antibodies on human umbilical cord tissue for celiac disease screening. Save both money and monkeys. *Dig Dis Sci* 1995; **40**:1902–5.
- 3 Sblattero D, Berti I, Trevisiol C *et al.* Human recombinant tissue transglutaminase ELISA: an innovative diagnostic assay for celiac disease. *Am J Gastroenterol* 2000; **95**:1253–7.
- 4 Dickey W, Hughes DF, McMillan SA. Disappearance of endomysial antibodies in treated celiac disease does not indicate histological recovery. *Am J Gastroenterol* 2000; **95**:712–4.
- 5 Ciacci C, Cavallaro R, della Valle N *et al.* The use of serum tTG-ab assay in patients on gluten-free diet as a measure of dietetic compliance. *Gastroenterology* 2002; **122**:588.
- 6 Farrell RJ, Kelly CP. Celiac sprue. *N Engl J Med* 2002; **346**:180–8.
- 7 Clemente MG, Musu MP, Frau F *et al.* Immune reaction against the cytoskeleton in coeliac disease. *Gut* 2000; **47**:520–6.
- 8 Bottazzo GF, Florin-Christensen A, Fairfax A *et al.* Classification of smooth muscle autoantibodies detected by immunofluorescence. *J Clin Pathol* 1976; **29**:403–10.
- 9 Fusconi M, Cassani F, Zauli D *et al.* Anti-actin antibodies: a new test for an old problem. *J Immunol Meth* 1990; **130**:1–8.
- 10 Bailey DS, Freedman AR, Price SC *et al.* Early biochemical responses of the small intestine of coeliac patients to wheat gluten. *Gut* 1989; **30**:78–85.
- 11 Holmgren Peterson K, Magnusson KE, *et al.* Confocal laser scanning microscopy of small-intestinal mucosa in celiac disease. *Scand J Gastroenterol* 1995; **30**:228–34.
- 12 Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol* 1999; **11**:1185–94.
- 13 Cassani F, Cataleta M, Valentini P *et al.* Serum autoantibodies in chronic hepatitis C: comparison with autoimmune hepatitis and impact on the disease profile. *Hepatology* 1997; **26**:561–6.
- 14 Volta U, Rodrigo L, Granito A *et al.* Celiac disease in autoimmune cholestatic liver disorders. *Am J Gastroenterol* 2002; **97**:2609–13.
- 15 Zamanou A, Samiotaki M, Panayotou G *et al.* Fine specificity and subclasses of IgG anti-actin autoantibodies differ in health and disease. *J Autoimmun* 2003; **20**:333–44.
- 16 Muratori P, Muratori L, Agostinelli D *et al.* Smooth muscle antibodies and type 1 autoimmune hepatitis. *Autoimmunity* 2002; **35**:497–500.
- 17 Kurki P. Determination of anti-actin antibodies by a solid-phase immunoenzymatic assay and by indirect immunofluorescence technique. *Clin Immunol Immunopathol* 1978; **11**:328–38.
- 18 Andersen P, Small JV, Andersen HK *et al.* Reactivity of smooth-muscle antibodies with F- and G-actin. *Immunology* 1979; **37**:705–9.

- 19 Cancado EL, Abrantes-Lemos CP, Vilas-Boas LS *et al.* Thermolabile and calcium-dependent serum factor interferes with polymerized actin, and impairs anti-actin antibody detection. *J Autoimmun* 2001; **17**:223–8.
- 20 Cooper JA, Buhle EL Jr, Walker SB *et al.* Kinetic evidence for a monomer activation step in actin polymerization. *Biochemistry* 1983; **26**:2193–202.
- 21 Chowdhury ZA, Barsigian C, Chalupowicz GD *et al.* Colocalization of tissue transglutaminase and stress fibers in human vascular smooth muscle cells and human umbilical vein endothelial cells. *Exp Cell Res* 1997; **231**:38–49.
- 22 Zauli D, Crespi C, Dall'Amore P *et al.* Antibodies to cytoskeleton components and other autoantibodies in Inflammatory bowel disease. *Digestion* 1985; **32**:140–4.
- 23 Huang YP, Seguro K, Motoki M *et al.* Cross-linking of contractile proteins from skeletal muscle by treatment with microbial transglutaminase. *J Biochem* 1992; **112**:229–34.
- 24 Molberg O, McAdam SN, Korner R *et al.* Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat Med* 1998; **4**:713–7.
- 25 Schuppan D. Current concepts of celiac disease pathogenesis. *Gastroenterology* 2000; **119**:234–42.
- 26 Schuppan D, Ciccocioppo R. Coeliac disease and secondary autoimmunity. *Dig Liver Dis* 2002; **34**:13–5.