

Short communication

IgA antiendomysium antibodies in human umbilical cord sections as a screening test in relatives of patients with celiac disease

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We performed the serum IgA antiendomysium antibody (EmA) assay by indirect immunofluorescence on human umbilical cord sections in 86 subjects with celiac disease, in 187 first-degree relatives of such patients, and in a control group of 68 unrelated subjects, to investigate the suitability of the method in the screening of populations at risk of gluten sensitivity. Conventional EmA assay using monkey esophagus sections was performed in parallel experiments. The results obtained showed a perfect correlation between the two methods. All the celiac patients and none of the controls were positive for EmA. EmA positivity was also observed in 11 apparently healthy relatives: intestinal biopsy performed in five of them invariably showed villous atrophy and increase of mucosal lymphocytes. Taking into account the low cost of EmA assay on human umbilical cord, especially when compared to monkey esophagus sections, the method is probably suitable and effective in identifying latent, asymptomatic gluten sensitivity in at-risk populations.

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Celiac disease (CD) is one of the most common forms of food intolerance, and is a health problem of increasing importance worldwide (1–3). Consequently, the search for reliable serologic markers of CD is of considerable interest to identify high-risk individuals in selected populations, or to estimate the prevalence of latent gluten sensitivity in unselected populations (1, 4–9).

Different serologic markers have been evaluated, first of all anti gliadin antibodies (AGA), especially of the IgA class, then IgA antireticulin (ARA) and IgA anti jejunal (JAB), and finally IgA antiendomysium antibodies (EmA) (2, 6–7, 10–13). The last is now considered the most sensitive and specific CD marker, and the only one of sufficiently high predictive value for CD in unselected populations with low disease prevalence (4, 7, 13–19). The presence of EmA in subjects with normal small intestine histology who showed an increase in γ/δ -T-cell receptor-bearing intraepithe-

lial lymphocytes has been regarded as predictive of CD development (20); of note, we previously reported that γ/δ -positive gut T-cell increase is not restricted to CD (21).

The high cost of the EmA IgA immunofluorescence (IF) assay performed on monkey esophagus sections has prevented its routine use. During the past 2 years, however, very good results have been reported with human umbilical cord sections as substrate for the IgA EmA IF assay (22, 23). The widespread use of such a substrate would result in a dramatic reduction of costs and in the suitability of the EmA assay as a screening procedure. In the present work, we used the EmA assay on human umbilical cord sections as a single screening method in a selected population at risk of CD; namely, first-degree relatives of CD patients (8). The results obtained indicate, in our opinion, a highly satisfactory cost/benefit ratio.

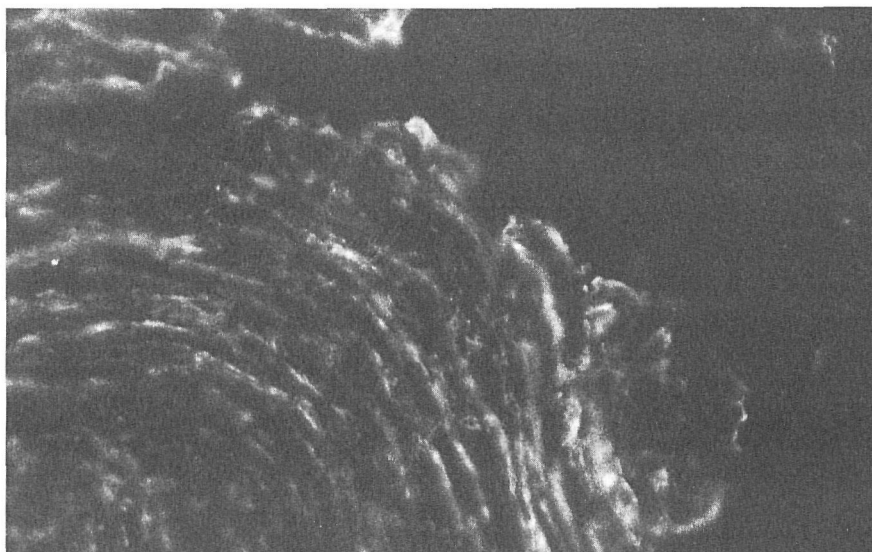


Fig. 1. Typical immunofluorescence picture of human umbilical cord section stained with serum containing antiendomysium antibodies.

Material and methods

Serum samples were obtained from 86 patients with CD (65 pediatric patients, age range 1–14 years; 21 adults, age range 15–65 years). Thirty-six of them were male, and 50 were female. The diagnosis was established on commonly accepted criteria, including typical histology on jejunal biopsy and clinical response to gluten-free diet (9). Serum samples were also obtained from 187 first-degree relatives of the above CD patients (95 males and 92 females, including 38 children and 149 adults). In some of them, intestinal biopsies were obtained (see Results). As controls, sera from 68 pediatric patients hospitalized for unrelated clinical problems were used.

Histologic abnormalities of intestinal mucosa were evaluated and graded according to standardized criteria (24).

EmA IgA antibodies were tested on umbilical cord sections essentially as described by Ladinser et al. (22), with minor modifications. Human umbilical cords obtained at delivery were transversely sectioned in 3–4-g pieces, quick-frozen in 2-methylbutane, and stored at -80°C until sectioned. Cryostatic sections 5- μm -thick were prepared with a Reichert-Jung (LeicaCryocut 1800) cryotome on clean glass slides (Superfrost color, Menzel). The slides were immediately used or stored at -20°C for up to 1 month. Dilutions of 1 : 10 or more (see below) of sera were incubated with the slides for 30 min at room temperature. Thereafter, the slides were extensively washed with phosphate-buffered saline (PBS), pH 7.4. The second incubation was carried out

with FITC-labeled anti-human IgA rabbit anti-serum (Dakopatts, Glostrup, Denmark) at 1 : 40 dilution. This working dilution was chosen after preliminary titration experiments, on the basis of optimum specific fluorescence/background ratio. After washing in PBS, the sections were mounted in buffered glycerol and examined under a Leitz Laborlux microscope (Wild Microscopes, Rockleigh, NJ, USA). Each section was read by two investigators masked to the identity of the samples.

Pools of known positive and negative sera were assayed in each run for control. The typical IF pattern surrounding the perivascular smooth-muscle fibers was regarded as positive (Fig. 1).

In parallel experiments, EmA IgA was assayed with a commercially available kit employing monkey esophagus sections (BIOS, Grafelfing). The protocol suggested by the manufacturer, very similar to that described above, was followed.

Results

As expected, a very high sensitivity and specificity of the EmA IgA assay on umbilical cord sections in the diagnosis of CD was observed: all the 86 patients with CD, but none of the control pediatric patients, exhibited EmA positivity. Antibody titers ranged from 1 : 10 (four patients, 4.3%) to 1 : 320 (seven patients, 8.7%); in more than 80% of patients, antibody titers ranged between 1 : 40 and 1 : 160. However, no definite correlation between histologic variables (such as degree of atrophy) and EmA titers was apparent (not shown). The EmA IgA assay was performed on 100

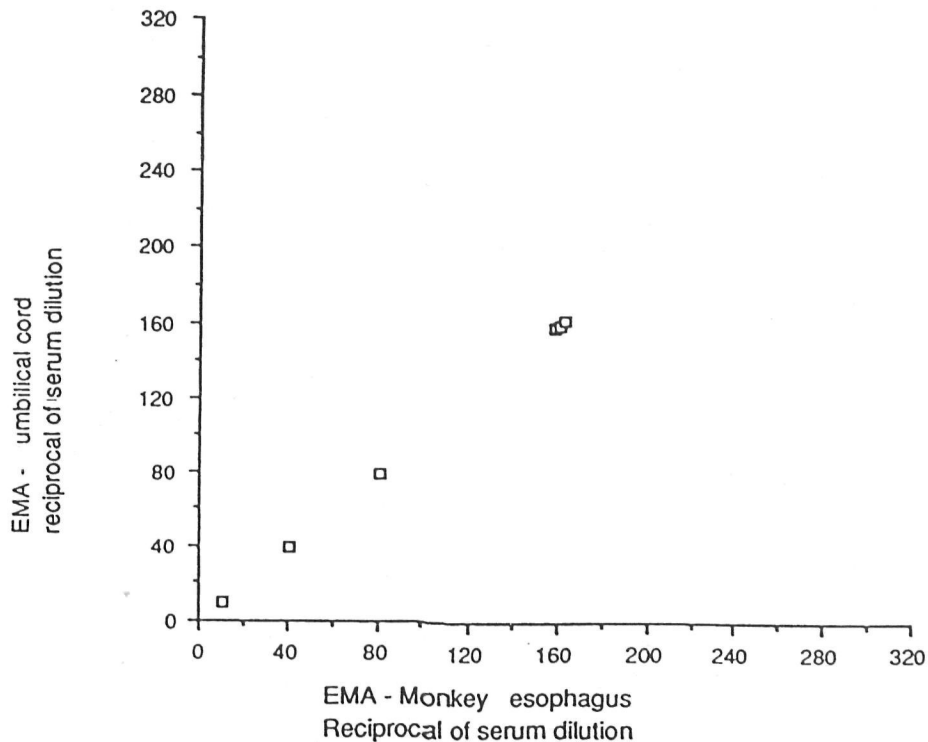


Fig. 2. Parallel titration of six representative sera from patients with celiac disease, simultaneously assayed for antiendomysium antibodies with human umbilical cord and monkey esophagus as substrates. Results obtained are superimposable.

sera (32 from CD patients and the 68 controls) with both umbilical cord and monkey esophagus sections. The results obtained were fully superimposable (32 positive, 68 negative with both methods). Parallel titration of six positive sera with both methods gave perfectly parallel results (Fig. 2).

Using the EmA IgA assay on human umbilical cord sections, we examined 187 first-degree relatives of children with CD. None of them, at a first examination, had a clinical history suggestive of gluten sensitivity, or were suffering from other major health problems. The results obtained are summarized in Table 1. As shown, we found serum EmA in 11 of them (six males, five females, including four children and seven adults). They were totally asymptomatic, but one woman complained of slight weight loss, anemia, and osteoporosis, although this did not affect her quality of life.

To date, five of these 11 EmA-positive subjects have consented to undergo jejunal biopsy. In four of them, third-degree, and in one, second-degree villous atrophy was observed. Intraepithelial lymphocytes comprised 10–50% of epithelial cells; moreover, an increase of lamina propria mononuclear cells and eosinophils (although to a variable extent) was observed (not shown). All the 11 subjects were told to follow a gluten-free diet (GFD). To date, 6/11 subjects have been re-evaluated after 4–10 months of GFD: in all of them, EmA positivity had disappeared. Moreover, the symptoms complained of by the above subject disappeared after a few months of GFD.

Finally, in Table 2 are compared the costs of the EmA IgA assay using umbilical cord sections prepared in the laboratory itself, and commercial monkey esophagus substrate.

Table 1. EmA prevalence in groups of subjects studied

	<i>n</i>	IgA EmA on umbilical cord	%	Jejunal biopsy	Villous atrophy degree	Intraepithelial lymphocytes % <i>a</i>
CD patients	86	86	100	86	I–III	10–60
Relatives	187	11	5.8	5	II–III	10–50
Controls	68	0	0	0	–	–

^a As percentage of epithelial cells.

Table 2. Costs of EmA assay by different methods

	Materials/ test		Time of work <i>c</i>	Cost of work/test		Total cost/test <i>b</i>
	<i>a</i>	<i>b</i>		<i>d</i>	<i>b</i>	
IgA EmA on umbilical cord	ITL 720 USD 0.48		2 h 40 min	ITL 5008 USD 3.28		ITL 5730 USD 3.76
IgA EmA on monkey esophagus kit	ITL 18125 USD 11.90		2 h 30 min	ITL 4607 USD 3.02		ITL 22732 USD 14.92

^a Calculated as cost of separate reagents and materials for human umbilical cord and as cost of commercial kit in Italy. ^b ITL = Italian lire; USD = US dollars.

^c Calculated on basis of 16 tests simultaneously. ^d Calculated on basis of cost/h in Italy of trained operator.

Discussion

The results of the present study confirm the suitability of the EmA IgA assay using human umbilical cord sections as IF substrate in the serologic screening of CD. In fact, the results obtained correlate perfectly with the IF assay using monkey esophagus sections: 100% sensitivity and specificity for CD were obtained by both methods. Moreover, parallel titration using both substrates gave identical results. No sensitivity improvement was observed with human substrate, at variance with a previous report (22), but consistently with another one (23). Of note, superimposable variations of EmA IgA positivity with both methods have been observed during GFD (23). Thus, it is expected that human umbilical cord sections will ultimately replace monkey esophagus as substrate for EmA testing, with the major advantages of overcoming the ethical problem of primate killing, and of consistent reduction of costs. The high cost of EmA determination has been the major obstacle to its use as a first-line screening for CD: this has probably contributed to the underdiagnosis of CD in at-risk populations, due to the lower sensitivity of the cheaper and more widely used screening with serum AGA (8, 17, 25).

As shown in Table 2, the reduction of costs with human substrate is indeed remarkable: its easy availability enables many laboratories to set up the assay without the need to purchase a commercial kit. At any rate, the cost of the unique commercial kit for the EmA assay using umbilical cord sections is far lower than for kits using monkey esophagus. These considerations prompted us to use it as a unique screening procedure in a population at risk of CD; namely, a large sample of first-degree relatives of CD patients. As mentioned above, accurate screening for CD, or, more generally speaking, for gluten sensitivity (1), is a major health problem and has relevant impact on clinical practice, in that gluten-sensitive subjects can benefit

from an early beginning of GFD, which may reduce the risk of malignancies (26).

We found EmA positivity in 11 of 187 relatives of CD patients. The proportion (about 6%) is comparable with that expected on the basis of literature data (18). The single 1 : 10 serum dilution was selected on the basis of the results obtained in CD patients and controls, and taking into account the lack of correlation between EmA titers and histologic grading of CD in basal conditions.

All five subjects who consented to undergo intestinal biopsy had abnormal histology. Note that all of them were asymptomatic but one, who complained of minor previously undiagnosed problems, although these problems were quite consistent with the suspected gluten sensitivity. Thus, all these subjects may be presumed to have had "compensated-silent" or initially symptomatic forms of gluten sensitivity, although biopsy would be required in each of them to establish the diagnosis of CD (27). In fact, all the 11 EmA-positive subjects are now following a GFD. In conclusion, we think that the results obtained in the present study by EmA assay on human umbilical cord sections are encouraging, and that this low-cost and low-discomfort procedure can be proposed for the screening of compensated/latent CD among high-risk populations.

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