

Antibodies to Tissue Transglutaminase and *Saccharomyces cerevisiae* in Ankylosing Spondylitis and Psoriatic Arthritis

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ABSTRACT. *Objective.* Subclinical gut inflammation has been described in patients with ankylosing spondylitis (AS) or psoriatic arthritis (PsA). Joint involvement has also been reported related to celiac disease. We investigated IgA antibodies to bovine tissue transglutaminase (tTg) and IgA and IgG antibodies to human tTg and to *Saccharomyces cerevisiae* (ASCA) in patients with AS and PsA.

Methods. We evaluated the frequency of IgA antibodies to bovine tTg, and of IgA and IgG antibodies to human tTg and to ASCA in 43 patients with AS and 75 with PsA. As control groups we considered 79 patients with rheumatoid arthritis (RA) and 78 healthy blood donors.

Results. We detected antibodies as follows: IgA antibodies to bovine tTg in 1/43 patients with AS, 3/75 with PsA, 1/79 with RA, and in 9/78 healthy controls; IgA antibodies to human tTg in 1/43 patients with AS, 1/75 with PsA, 1/79 with RA, and in 3/78 healthy controls; IgG antibodies to human tTg in 1/43 patients with AS, 4/75 with PsA, 5/79 with RA, and in 7/78 healthy controls. IgA ASCA were confirmed in 10/43 patients with AS, 7/75 with PsA, 14/79 with RA, and in 7/78 healthy controls; IgG ASCA were present in 5/43 patients with AS, 4/75 with PsA, 8/79 with RA, and in 8/78 healthy controls. No statistically significant difference was observed in the prevalence of IgA or IgG antibodies to bovine and human tTg and in the frequency and in mean level of IgA or IgG ASCA between the studied groups or between each group and healthy controls.

Conclusion. Our data fail to show an increased prevalence of autoantibodies associated with celiac and Crohn's disease in patients with AS and PsA. (J Rheumatol 2004;31:920-4)

Key Indexing Terms:

CELIAC DISEASE
PSORIATIC ARTHRITIS

ANKYLOSING SPONDYLITIS
AUTOANTIBODIES

Many studies have elucidated the relationship between gut and synovium in spondyloarthropathies (SpA). It is well known that enteritis caused by some bacteria, such as *Campylobacter jejunii*, *Yersinia*, *Shigella*, and *Salmonella*, may precipitate synovitis^{1,2}. Moreover ileocolonoscopy studies have shown inflammatory changes, even in the absence of clinical intestinal symptoms, in 70% of patients with ankylosing spondylitis (AS)^{3,4} and, more recently, in 60% of patients with psoriatic arthritis (PsA)⁵. Conversely, arthritis is an extraintestinal manifestation of ulcerative colitis (UC) and Crohn's disease (CD)⁶, 2 chronic inflammatory disorders of the intestine in which a variety of immune abnormalities have been described at both the systemic and the intestinal level. In these disorders, 2 candidate antibodies for use in clinical diagnosis have been iden-

tified, perinuclear antineutrophil cytoplasmic antibodies (pANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA)⁷.

Arthritis has also been described as an extraintestinal clinical manifestation of such disorders as Whipple's disease⁸, intestinal bypass, and gluten-sensitive enteropathy⁹. Definitive data regarding the prevalence of articular involvement in celiac disease are still lacking, but it is known that rheumatic diseases occur in patients with gluten-enteropathy more frequently than in the general population¹⁰⁻¹³. Moreover, the striking response of the joint manifestations to a gluten-free diet supports the connection between this disorder and arthritis. Thus, the relationship between gut pathology and articular involvement appears as one of the most intriguing problems in rheumatology. Patients with celiac disease can have few or no symptoms and so the detection of anti-gliadin and anti-endomysial antibodies represents an important clue for early diagnosis and treatment¹⁴. Tissue transglutaminase (tTg) is the target of anti-endomysial antibodies, and IgA anti-tTg is now considered the most sensitive marker in celiac enteropathy^{15,16}.

We evaluated the frequency of autoantibodies associated

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with celiac disease (IgA antibodies to tTg) and CD (ASCA) in patients with AS and PsA.

MATERIALS AND METHODS

Patients. Forty-three patients with AS and 75 with PsA from the Rheumatology Unit of the University of Pisa were enrolled in the study. As control groups we considered 79 patients with rheumatoid arthritis (RA) being treated at the same rheumatology unit, and a group of 78 healthy blood donors from the Blood Bank of Pisa. Diagnosis of AS was according to the revised New York criteria¹⁷, while PsA was diagnosed according to criteria of Vasey and Espinoza¹⁸. Mean age was 37 years (range 21–59) in the AS group (female-male ratio 9:34), 51 years (range 19–78) in the PsA group (female-male ratio 36:39), and 62 years in patients with RA (female-male ratio 60:19). No relevant history of abdominal pain or diarrhea or weight loss was elicited from any subject.

Methods. With subjects' informed consent, serum samples were taken and immediately stored at –20°C until tested. IgA antibodies to bovine tTg, IgA, and IgG antibodies to human tTg and IgA and IgG ASCA were measured in all sera.

Detection of anti-tTg antibodies and ASCA. Anti-bovine tTg antibodies were detected in sera by an ELISA assay, according to the method described by Dieterich, *et al*, with minor modifications¹⁹. Briefly, we used bovine tTg (Sigma Chemical Co., St. Louis, MO, USA) as antigen for the coating of flat-bottom-well microtiter plates (Greiner Laboratory; Greiner, Germany) at 10 µg/ml in Tris buffered saline, pH 7.3, CaCl₂ 5 mM in overnight incubation. Saturation was carried out with 3% bovine serum albumin (BSA) in phosphate buffered saline (PBS). After 1 h the sera diluted 1:250 in PBS, 1% BSA, 0.05% Tween 20 were added, and the plates were incubated 3 h at room temperature. The plates were then washed once with PBS, 1% Tween and twice with PBS. Alkaline phosphatase-conjugated goat anti-human IgA F(ab')₂ (Sigma) 1:5000 in diluting buffer was added, and the plates were incubated 3 h at room temperature or overnight at 4°C. After washing, the bound enzymatic activity was measured with p-nitrophenyl-phosphate-based substrate. The results of the assay are expressed as percentage of a positive control that was run in each assay; the cutoff value for sera positivity is 15% (mean + 2 standard deviations, SD, of a group of healthy laboratory personnel, comprising 44 subjects).

Anti-human tTg IgG and IgA antibodies were detected with a commercial kit (Eu-tTG[®] IgG and Eu-tTG[®] IgA, Eurospital SpA, Trieste, Italy). According to manufacturer's instructions, sera were considered positive when their IgA and IgG antibody content was higher, respectively, than 7 AU/ml and 30 AU/ml.

ASCA IgG and IgA were detected with a commercial kit (Quanta Lite[™] ASCA IgG and Quanta Lite[™] ASCA IgA; Inova Diagnostics Inc., San Diego, CA, USA). According to the manufacturer's instructions, sera were considered positive when their antibody content was more than 25 U/ml.

Statistical analysis. Differences in the prevalence of the antibodies under study were analyzed by Fisher's exact test in the disease groups and in controls. Mean antibody levels in the different groups were compared by means of Student's *t* test.

RESULTS

IgA antibodies to bovine tTg were detected in 1/43 (2.3%) AS patients, 3/75 (4.0%) PsA, 1/79 (1.3%) RA, and in 9/78 (11%) controls.

IgA antibodies to human tTg were present in 1/43 (2.3%) AS patients, 1/75 (1.3%) PsA, 1/79 (1.3%) RA, and in 3/78 (3.8%) controls; IgG antibodies to human tTg were detected in 1/43 (2.3%) AS patients, 4/75 (5.3%) PsA, 5/79 (6.3%) RA, and in 7/78 (8.9%) controls. Of note, only 3 healthy

controls out of the 9 that reacted with bovine tTg had IgA antibodies to human tTg. The 3 PsA patients that reacted with bovine tTg had no antibodies to human tTg. No statistically significant difference was observed in the prevalence of IgA or IgG antibodies to human tTg between the AS, PsA, and RA groups, or between each of these groups and the healthy controls.

IgA ASCA were detected in 10/43 (23.2%) AS patients, in 7/75 (9.3%) PsA, 14/79 (17.7%) RA, and in 7/78 (8.9%) controls; while IgG ASCA were present in 5/43 (11.6%) AS patients, 4/75 (5.3%) PsA, 8/79 (10.1%) RA, and in 8/78 (10.2%) controls. No statistically significant difference was observed in the frequency or in the mean level of IgA or IgG ASCA between the studied groups or between each group and controls (Table 1 and 2, Figure 1 and 2).

Regarding the association of the 2 antibody specificities, one patient with AS showed IgA antibodies to bovine and human tTg and to ASCA, and another AS patient showed both IgA and IgG ASCA. One subject with PsA and one with RA were also positive for both IgA and IgG ASCA, while only one patient with RA was positive for IgA antibodies to bovine and human tTg and for IgA and IgG ASCA.

DISCUSSION

Celiac disease is characterized by inflammation of small intestine mucosa due to intolerance to gluten, a protein derived from wheat. A genetically based disorder, it typically causes malabsorption syndrome with diarrhea, steatorrhea, and weight loss. However, several studies have shown that gluten enteropathy is often atypical in presentation or even symptomless, so that its prevalence in the general population is probably widely underestimated¹⁴. Serological tests such as antibodies to gliadin, endomysium, reticulin, and tTg have provided very useful tools for screening patients with silent or latent celiac disease. In particular, the determination of IgA antibodies to tTg showed a high specificity and sensitivity for gluten enteropathy^{15,16}. According to Koop, *et al* IgA anti-tTg, especially when in high titer, is closely associated with celiac disease, while in the low titer range overlap exists with liver diseases, inflammatory bowel disease (IBD), and diabetes type I²⁰. It is known that a proportion of patients with celiac disease may develop arthritis, mostly involving hip, knee, shoulder, and lumbar spine, and that autoimmune diseases such as thyroiditis²¹ and in particular rheumatic diseases¹³ occur more frequently in patients with celiac disease than in the general population. In 2002 Lindqvist, *et al* reported 5 cases of celiac disease in a group of 114 PsA patients (4.4%) and an increased prevalence of raised serum IgA anti-gliadin antibodies associated to a more aggressive arthritis²².

Patients with IBD, such as CD and UC, may also develop arthritis involving the axial skeleton and/or peripheral joints, so-called enteropathic arthropathy. The etiology of IBD is still unclear and the mechanism by which a patho-

Table 1. Prevalence of antibodies to bovine and human tissue transglutaminase (tTg) and to *Saccharomyces cerevisiae* in disease groups and controls.

	Bovine tTg	Human tTg		ASCA	
	IgA N (%)	IgA N (%)	IgG N (%)	IgA N (%)	IgG N (%)
AS	1/43 (2.3)	1/43 (2.3)	1/43 (2.3)	10/43 (23.2)	5/43 (11.6)
PsA	3/75 (4)	1/75 (1.3)	4/75 (5.3)	7/75 (9.3)	4/75 (5.3)
RA	1/79 (1.27)	1/79 (1.27)	5/79 (6.3)	14/79 (17.7)	8/79 (10.1)
Controls	9/78 (11)	3/78 (3.8)	7/78 (8.9)	7/78 (8.9)	8/78 (10.2)

AS: ankylosing spondylitis; PsA: psoriatic arthritis; tTg: tissue transglutaminase; ASCA: anti-*Saccharomyces cerevisiae* antibodies.

Table 2. Levels (mean \pm SD) of antibodies to bovine and human tissue transglutaminase and to *Saccharomyces cerevisiae* in disease groups and controls.

	Bovine tTg	Human tTg		ASCA	
	IgA	IgA	IgG	IgA	IgG
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
AS	6.07 \pm 4.27	1.58 \pm 1.42	9.16 \pm 7.79	18.23 \pm 14.61	10.63 \pm 10.03
PsA	6.48 \pm 4.82	2.05 \pm 2.87	8.28 \pm 7.57	13.88 \pm 11.15	8.70 \pm 7.24
RA	3.64 \pm 3.56	2.89 \pm 3.70	12.58 \pm 9.45	22.72 \pm 55.82	10.64 \pm 14.39
Controls	8.11 \pm 9.74	2.71 \pm 3.22	17.55 \pm 8.73	12.51 \pm 7.18	12.92 \pm 12.14

AS: ankylosing spondylitis; PsA: psoriatic arthritis; tTg: tissue transglutaminase; ASCA: anti-*Saccharomyces cerevisiae* antibodies.

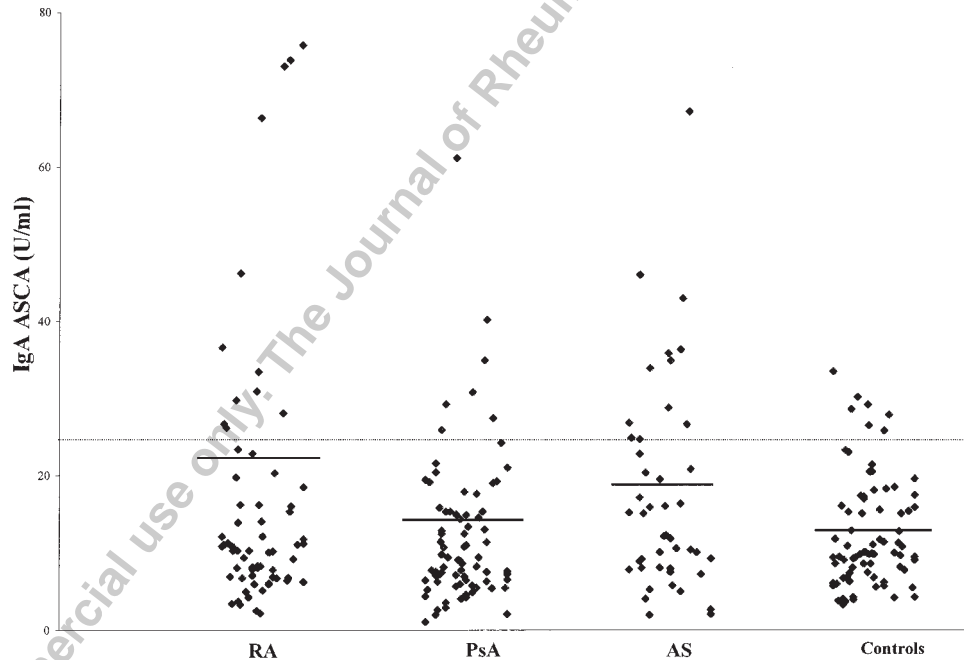


Figure 1. IgA ASCA levels in patients with rheumatoid arthritis (RA), ankylosing spondylitis (AS), and psoriatic arthritis (PsA) and in healthy controls.

logical process in the gut leads to the development of arthropathy is largely unknown. Increased permeability of the gut wall to antigenic materials associated to defective local immune responses is probably sufficient to initiate

articular inflammation in genetically predisposed individuals. ASCA are considered specific for CD, with prevalence varying from 39% to 76%. However, some recent studies suggest a specificity of ASCA lower than previously

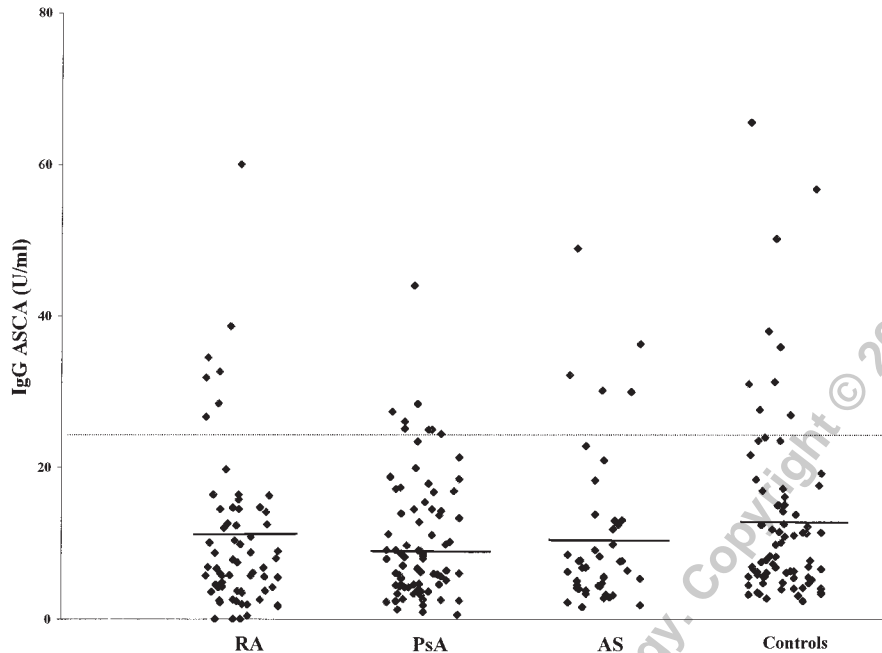


Figure 2. IgG ASCA levels in patients with rheumatoid arthritis (RA), ankylosing spondylitis (AS), and psoriatic arthritis (PsA) and in healthy controls.

reported: for example, a high prevalence has been described in celiac disease²³ and in autoimmune liver disease²⁴. The clinical value of the ASCA test is bound to the possibility to differentiate CD and UC in both adult and pediatric patients. Indeed, it is now accepted that a positive ASCA test with negative pANCA is strongly associated with CD, while the reverse is considered associated with UC⁷.

Thus, a battery of serological tests is now available to study patients with symptoms suggestive of autoimmune gut disorders. Using these tests, we screened patients with 2 frequent SpA (AS and PsA). However, we could not identify a subgroup of subjects in whom antibodies usually associated with disorders involving the gut, such as celiac disease or CD, are present. In fact, our results fail to show any significant difference in the prevalence of antibodies to human tTg or ASCA between patients with AS or PsA and healthy subjects. To detect the antibodies to tTg we used as antigen both bovine tTg and the recombinant human tTg. The results we obtained are not completely concordant, mainly in the group of healthy controls. Thus, while the prevalence observed in disease groups is identical using bovine or human tTg, in controls IgA anti-bovine tTg are more frequently detected than antibodies to human tTg (11% vs 3.8%).

These data suggest that caution must be used when analyzing and comparing results obtained with tTg of different origins. As reported for guinea-pig tTg, it is possible also that positive results for bovine tTg may be false positive rather than a latent or silent form of celiac

disease. Only antibodies reacting with human tTg may be considered bona fide autoantibodies. It is of interest that antibodies recognizing species-specific epitopes are detected only in healthy subjects, while in patients with autoimmune disorders anti-tTg antibodies, like most autoantibodies, recognize epitopes conserved across different species. However, even considering only data from tests employing human tTg, a very high frequency (3.8%) of antibodies to tTg is detected in controls.

In the past the reported prevalence of celiac disease ranged from 1:1000 to 1:4000, but many recent studies with new serological screening methods showed a significantly higher prevalence. In 1999 Trevisiol, *et al* studied concentrations of serum IgA anti-endomysial antibodies by immunofluorescence in 4000 healthy blood donors at 2 immunotransfusion centers in Italy²⁵. Intestinal biopsy was performed in all anti-endomysium positive subjects. Celiac disease was diagnosed in 10/4000 (0.25%) patients. Presently, only 2 published studies report the prevalence of antibodies to tTg in Italian healthy subjects^{21,26}. In the first study, one of 250 (0.4%) blood donors was positive for IgA to guinea-pig tTg. This subject was found to have celiac disease by subsequent investigations. In the second study, none of 100 healthy subjects showed reactivity to guinea-pig or human tTg.

The high frequency of anti-tTg antibodies in our group of healthy blood donor controls cannot be easily explained. It is possible that undisclosed symptomless autoimmune diseases, such as autoimmune thyroiditis, may be present in

our control group and may have influenced the results of our study. To clarify the true frequency of anti-tTg antibodies in healthy subjects, a larger group of healthy subjects should be tested and HLA typed to verify if DQ2, the HLA allele strongly associated with celiac disease, is overrepresented in our population.

As far as ASCA are concerned, our results differ from those reported by Hoffmann, *et al*²⁷. These authors detected higher IgA ASCA in patients with AS and undifferentiated SpA as compared to RA, PsA, or healthy subjects. In our study, in contrast, ASCA IgA or IgG levels are similar in patients with SpA, in healthy controls, and in disease controls. The assays used to measure ASCA, however, are not identical and presently it cannot be excluded that 2 methods, both commercially available, perform differently in different disease groups. Although the patients included in the 2 studies fulfilled the same diagnostic criteria, the different genetic background may influence their ability to produce antibodies of a given specificity. However, the predictive value of raised ASCA levels in SpA seems questionable. Indeed, in the patients studied by Hoffman, *et al* the authors point out that ASCA levels are not related to gut involvement and do not differ in patients with or without histologically proven bowel inflammation.

Our data fail to show an increased prevalence of autoantibodies associated with celiac disease and CD in patients with AS and PsA. The relation between gut and articular inflammation in the spondyloarthropathies is a matter of intense research, in particular since animal models have confirmed the strong link between synovitis and gastrointestinal involvement. Further studies on larger populations of various ethnic backgrounds are necessary to draw more definitive conclusions.

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