

Immunoglobulin Isotype Profile of Tissue Transglutaminase Autoantibodies is Correlated with the Clinical Presentation of Coeliac Disease

J. Schilling*, U. Spiekerkoetter†, U. Wohlrab*, U. Wendel† & J. Seissler*

*German Diabetes Clinic, German Diabetes Center, Leibniz-Institute at the Heinrich-Heine-University; and †Department of Pediatrics, Heinrich-Heine-University Hospital, Duesseldorf, Germany

Received 14 October 2004; Accepted in revised form 22 November 2004

Correspondence to: J. Seissler, MD, German Diabetes Center, Leibniz Institute at the University of Dusseldorf, Auf'm Hennekamp 65, D-40225 Dusseldorf, Germany. E-mail: seissler@ddfi.uni-duesseldorf.de

Abstract

Coeliac disease (CD) is characterized by the appearance of autoantibodies against tissue transglutaminase (tTG-Ab). Immunoglobulin A (IgA) tTG-Ab have been described as excellent diagnostic markers, but the Ig subclass distribution and the importance of isotype tTG-Ab have not yet been established. In this study, using newly developed isotype- and subclass-specific radioligand assays, we examined anti-tTG IgA1, IgA2, IgG1, IgG4 and IgE antibodies in 30 symptomatic, untreated patients with CD and 22 subjects suspected to suffer from silent CD (sCD). Among 30 patients with CD, 27 (90.0%) were positive for IgA1 tTG-Ab, whereas only 12 (40.0%) had autoantibodies of the IgA2 subclass ($P < 0.001$). IgG1, IgG4 and IgE tTG-Ab were detected in 17 (56.6%), 0 and 3 (10.0%) individuals, respectively. IgA1 was also the predominant anti-tTG subclass in patients with sCD ($n = 20$, 90.1%), followed by IgA2 antibodies ($n = 7$, 31.8%), IgG1 antibodies ($n = 4$, 18.2%), IgG4 antibodies ($n = 1$, 4.5%) and IgE antibodies ($n = 1$, 4.5%). The comparison between both groups revealed a significantly higher prevalence of IgG1 antibodies in patients with symptomatic CD ($P < 0.01$). In 10 of 11 subjects undergoing an intestinal biopsy, the diagnosis of an sCD was confirmed. In this subgroup, there was a positive association between the presence of IgA2 and IgG1 tTG-Ab and severe (Marsh 2–3) mucosal abnormalities. In conclusion, patients with symptomatic and sCD predominantly have IgA1 tTG-Ab. IgG1 tTG-Ab are associated with symptomatic disease and, when present in patients with sCD, are correlated with a severe mucosal destruction. These data suggest that tTG-Ab subclasses could reflect inflammatory events associated with epithelial destruction.

Introduction

Coeliac disease (CD) is an inflammatory disorder of the upper small bowel triggered by an intolerance against the gluten component gliadin. The disease process is characterized by the intramucosal infiltration of dietary gluten-specific T lymphocytes, the activation of intestinal fibroblasts and the appearance of specific autoantibodies directed to the endomysium (EMA) [1]. After the identification of the enzyme tissue transglutaminase as the major target antigen of EMA [2, 3], autoantibodies against tTG (tTG-Ab) were described as useful diagnostic marker for silent CD (sCD) and overt CD. Using enzyme-linked immunosorbent assay (ELISA) or radioligand assays, researchers found that 95–99% of patients with untreated

CD were immunoglobulin A (IgA) tTG-Ab positive, with a specificity similar to the classical EMA [4–8]. In addition, disease-specific IgG1 subclass EMA and anti-tTG-Ab were described in about 50% of patients with CD that were associated with typical histological abnormalities [9, 10]. However, the presence and the importance of other anti-tTG Ig subclasses have not been studied thus far.

The formation of antibodies to antigens in the gut is dependent on the interaction between antigen-presenting cells (APCs) with T and B cells in the Peyer's patches as well as cytokine secretion from lamina propria stromal cells. Under normal conditions, gut stromal cells as well as regulatory T helper (Th) 2 and Th3 cells maintain the tolerance to dietary antigens and indigenous microorganisms and favour

IgA class-switching [11–13]. In inflammatory bowel diseases, however, there is evidence for a disturbed T-cell immunity leading to an activation of cellular immunity and a shift towards Th1 cells. Several studies conclusively demonstrate that increased amounts of proinflammatory cytokines such as tumour necrosis factor- α (TNF- α), interferon- γ (IFN- γ) and interleukin-18 (IL-18) are expressed in the lamina propria and in CD-specific T-cell clones from patients with untreated CD [14–16]. Because proinflammatory cytokines induce the differentiation of B cells into IgG-producing plasma cells, it could be possible that the autoantibody isotype and subclass profile serve as surrogate marker for the activity of CD-associated autoimmunity and the severity of mucosal damage. Therefore, we studied the prevalence of anti-tTG IgA subclass antibodies as well as IgG1-, IgG4- and IgE-tTG-Ab in patients with different forms of CD. We demonstrate a heterogeneous Ig response in both symptomatic and sCD that is dominated by IgA1 and an association between IgG1 antibodies and mucosal damage in patients with silent disease.

Materials and methods

Subjects. Sera were tested from 30 patients (18 females and 12 males; age 6–72 years, median 15.5 years) with untreated CD. Diagnosis was based on jejunal biopsy with evidence for the typical mucosal lesions [17]. The second group of patients was recruited from a screening of 415 asymptomatic patients with type 1 diabetes [18–20] who were detected to be IgA tTG-Ab positive (13 females and 9 males; age 3–19 years, median 11.5 years). These subjects are at high risk of suffering from silent disease and were therefore designated as sCD in this study. Parents of 11 children agreed for a duodenal biopsy to be performed to confirm the diagnosis. Fifty-eight sera from healthy individuals (35 females and 23 males; age 8–40 years, median 27.0 years) served as controls. The study was approved by the ethics committee, and informed consent was obtained from all participants.

Detection of total IgA tTG antibodies. The assays for the detection of IgA tTG-Ab were performed as described previously [6, 20]. Briefly, full-length tTG was produced by *in vitro* transcription/translation followed by incubation of radiolabelled tTG (15,000 cpm per probe) with 5 μ l of serum diluted in 50 μ l of Tris buffer [20 mM Tris, pH 7.4, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA), 5 mM benzimidazole, 5 mM methionine and 0.5% Triton X-100] at 4°C. After 16 h, immunocomplexes were bound with 20 μ l of anti-human IgA-Agarose (Sigma, St. Louis, MO, USA) for 2 h, transferred to 96-well filtration plates (Millipore, Bedford, MA, USA), washed in Tris buffer and directly measured in a 96-well β -counter (Top Count, Canberra Packard, Groningen, Netherlands). All measurements were performed in dupli-

cates. In each experiment, the same positive (serum P1) and negative (serum C1) serum was used as internal control to calculate antibody levels, expressed in arbitrary units (AU), as follows: (cpm of test serum – cpm of C1)/(cpm of P1 – cpm of C1) $\times$ 100. At the cut-off of 8.3 AU, the assay has a diagnostic sensitivity of 95.6% and a specificity of 99.5% [6].

Determination of isotype- and subclass-specific tTG antibodies.

Isotype antibodies were detected as described previously [21]. Aliquots of radiolabelled tTG (15,000 cpm) were incubated with 5 μ l of serum diluted in 50 μ l of Tris buffer. After 12 h at 4°C, probes were incubated with biotinylated mouse monoclonal anti-human IgG1 (4.0 μ g), IgG4 (2.0 μ g), IgE (5.0 μ g) (PharMingen, San Diego, CA, USA), IgA1 (2.5 μ g) or IgA2 (2.5 μ g) (Southern Biotechnology, Birmingham, AL, USA) for 2 h, followed by the addition of 50 μ l (50% v/v) of NeutrAvidin agarose (Pierce, Rockford, IL, USA) for an additional 2 h. Then, probes were transferred to 96-well filtration plates, washed in Tris buffer and measured as described above. All samples were measured in duplicates. Positive and negative standard sera were included in each assay. All sera were measured using the same tTG preparation. Values above mean + 3 standard deviation (SD) of normal controls were considered to be antibody positive: IgA1, 977 cpm; IgA2, 841 cpm; IgG1, 1144 cpm; IgG2, 518 cpm; IgE, 511 cpm. At these thresholds, one control serum was found to be positive for IgA1, IgA2, IgG4 and IgE tTG-Ab, whereas none of the sera exceeded the cut-off for IgG1 tTG-Ab.

Intestinal histology. Duodenal biopsies were analysed by a pathologist according to the Marsh scoring system [22]. Histopathologic changes were graded from normal (Marsh 0), increased amount of intraepithelial lymphocytes (IEL) (Marsh 1), increased numbers of IEL plus enlarged crypts (Marsh 2), flat mucosa and significant alteration of normal villous or crypt architecture (Marsh 3) and total villous atrophy (Marsh 4).

Statistical analyses. The significance of differences between observations was tested using the two-sided Fisher's exact test. Analysis of variance (ANOVA) was used for the comparison of autoantibody levels. A *P*-value below 0.05 was considered significant.

Results

Tissue TG antibodies in patients with symptomatic CD

Using the total IgA assay, we found that all patients with CD were positive for tTG-Ab. Among IgA subclasses, IgA1 was predominant and found in 27 (90.0%) cases, whereas IgA2 tTG-Ab were detected in only 12 (40.0%) subjects (*P* < 0.001) (Table 1). All sera with IgA2 tTG-Ab were also positive for antibodies to the IgA1 subclass (Fig. 1). Individual IgA1 tTG-Ab levels were higher in all but one of the patients as compared with IgA2 levels

Table 1 Frequency of tissue transglutaminase (tTG) isotype antibodies among study groups

Subjects	Number	Prevalence of tTG-Ab isotypes				
		IgA1	IgA2	IgG1	IgG4	IgE
Coeliac disease	30	27 (90.0%)*†	12 (40.0%)‡	17 (56.6%)§¶	0	3 (10.0%)
Females	18	17 (94.4%)	6 (33.3%)	10 (55.6%)	0	1 (5.6%)
Males	12	10 (83.3%)	6 (50.0%)	7 (58.3%)	0	2 (16.7%)
Silent coeliac disease	22	20 (90.1%)**	7 (31.8%)	4 (18.2%)	1 (4.5%)	1 (4.5%)
Female	13	12 (92.3%)	5 (38.5%)	3 (23.0%)	1 (7.7%)	1 (7.7%)
Males	9	8 (88.8%)	2 (22.2%)	1 (11.1%)	0	0

* $P < 0.001$ versus IgA2, IgG4 and IgE.

† $P < 0.01$ versus IgG1.

‡ $P < 0.02$ versus IgE and IgG4.

§ $P < 0.01$ versus IgG4 and IgE.

¶ $P < 0.01$ versus silent CD.

** $P < 0.01$ versus IgA2, IgG1, IgG4 and IgE.

(mean antibody level 2281 ± 1298 cpm for IgA1 versus 1009 ± 664 cpm for IgA2) ($P < 0.01$) (Fig. 2).

IgG1 tTG-Ab were detected in 17 (56.6%) patients (mean antibody level 1319 ± 666 cpm), but none of the patients had IgG4 antibodies and only three (10.0%) patients had IgE antibodies. All IgG1 antibody-positive sera were also positive for IgA1 tTG-Ab, and 10 (33.3%) sera had both IgA2 and IgG1 tTG-Ab (Fig. 1). Antibody levels did not differ significantly between IgA1/IgA2 double-positive sera, IgA1/IgG1 double-positive sera and sera with only IgA1 tTG-Ab. There was also no difference in isotype distribution and antibody levels with respect to age and gender (Table 1).

Tissue TG antibodies in patients with sCD

Among the subjects with sCD, there was a significantly higher prevalence of IgA1 tTG-Ab (90.1%) as compared with IgA2 (31.8%) or IgG1 (18.2%) tTG-Ab ($P < 0.001$). Only one patient had IgG4 or IgE tTG-Ab. There was no serum that was positive for IgA2 or IgG1 antibodies in the absence of IgA1 tTG-Ab. One serum had both IgA2 and IgG1 antibodies (Fig. 1).

The highest antibody levels were observed for IgA1 (2858 ± 1079 cpm), followed by IgG1 (890 ± 373 cpm) and IgA2 (717 ± 347 cpm) ($P < 0.001$) (Fig. 2). In all sera, individual titres of IgA1 antibody were higher as compared with IgA2 tTG-Ab levels. IgG1 antibodies tend to be more frequent in young children (<10 years) (44.4% positive) compared to older individuals (0% positive) ($P =$ no significance). There was no correlation of any isotype or subclass antibody with gender.

Correlation of tTG isotype antibodies with the clinical presentation

Comparison between patients with symptomatic and sCD revealed no obvious difference in the prevalence of IgA1,

IgA2, IgG4 and IgE tTG-Ab. However, IgG1 tTG-Ab were significantly more prevalent in symptomatic CD (56.6%) as compared with patients with sCD (18.2%) ($P < 0.01$).

In the total population, the mean IgA1 antibody levels were higher in patients with sCD as compared with patients with symptomatic CD (2281 ± 1298 cpm versus 2858 ± 1079 cpm) ($P < 0.001$). IgA2 and IgG1 were not different among study groups (IgA2: 1009 ± 664 cpm versus 717 ± 347 cpm; IgG1: 1319 ± 666 cpm versus 890 ± 373 cpm). A similar pattern of IgA1, IgA2 and IgG1 tTG-Ab was observed when only patients with positive isotype antibodies were analysed [sCD/symptomatic CD: IgA1 3071 ± 875 cpm versus 2457 ± 1246 cpm ($P < 0.05$); IgA2 1571 ± 750 cpm versus 1121 ± 271 cpm; IgG1 1665 ± 704 cpm versus 1534 ± 311 cpm].

Association of isotype antibodies with histology in patients with sCD

In 11 of the 22 patients with sCD, a jejunal biopsy was performed. In all but one of the subjects, typical changes in mucosal histology were observed, confirming the presence of sCD. Comparison of the tTG-Ab isotype pattern with the CD severity score revealed that patients with positive IgA1 antibodies had mucosal changes ranging from normal mucosa (Marsh 0, $n = 1$), increased infiltration of IEL but normal mucosal architecture (Marsh 1, $n = 1$), to destructive mucosal lesions (Marsh 2, $n = 4$; Marsh 3, $n = 5$). IgG1 as well as IgA2 tTG-Ab were associated with medium (Marsh 2: $n = 1$ IgG1 positive, $n = 2$ IgA2 positive) or severe abnormalities (Marsh 3: $n = 2$ IgG1 positive, $n = 3$ IgA2 positive), suggesting that the appearance of these isotypes may reflect an increased inflammatory state of the immune response.

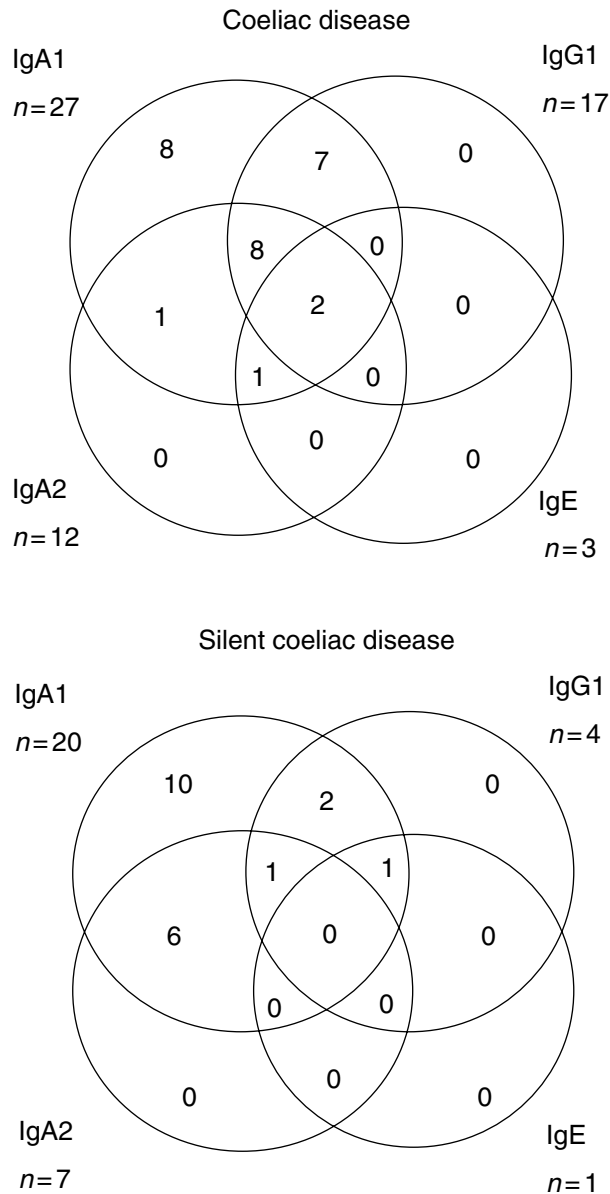


Figure 1 Correlation of tissue transglutaminase (tTG) isotype and subclass antibodies in 30 patients with symptomatic coeliac disease (CD) and 22 patients with silent CD who were positive for immunoglobulin A (IgA) tTG antibodies.

Discussion

CD is an autoimmune disorder where the enzyme tissue transglutaminase has been identified as the major autoantigen. In this study, we provide the first data that the tTG autoantibody response in CD is not restricted to a specific Ig subclass. We demonstrate a heterogeneous humoral response with a predominance of IgA1 autoantibodies and the presence of IgA2 and IgG1 tTG-Ab in a subgroup of patients. Our data are novel in that we demonstrate for the first time that IgG1 tTG antibodies were found more frequently in patients with symptomatic CD, suggesting

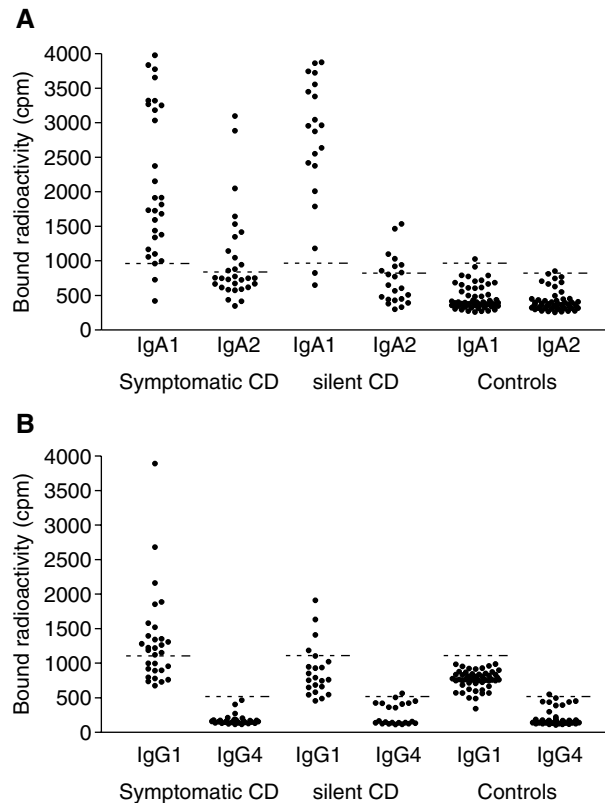


Figure 2 Distribution of immunoglobulin A (IgA) (A) and IgG subclass tissue transglutaminase (tTG) antibodies (B) among study groups. Antibody levels are expressed as bound radioactivity (cpm). The dotted lines represent the cut-offs of the assays.

that the isotype pattern can provide indirect information on the immunoregulation of the mucosal CD-specific inflammation.

IgA, by far the most abundant Ig in humans, is mainly produced by the mucosal immune system of the gastrointestinal and respiratory tracts. In the gut, antigens are presented by M cells or local dendritic cells (DCs), followed by the activation of T and B cells. The local regulation of cytokine secretion in mucosal tissues normally favours the generation of IgA-producing plasma cells [9, 23]. In line with this concept are the findings of numerous studies that have shown that CD-specific humoral autoimmunity, e.g. EMA and tTG-Ab, is predominantly of the IgA isotype [4–8]. We here demonstrate for the first time that the IgA reactivity mainly consists of the IgA1 subclass, whereas IgA2 tTG-Ab were present in subgroups of both patients with symptomatic and sCD. These findings are in agreement with those of a study on IgA subclass distribution of EMA demonstrating positive IgA2 antibodies in only four of 26 EMA IgA1-positive sera [24].

In this context, it is important that serum IgA2 has a much higher clearance rate as compared with that of IgA1 [25]. Therefore, serum measurements may underestimate the

proportion of mucosal, tTG-reactive plasma cells producing IgA2. There was no obvious difference between anti-tTG IgA2-positive and -negative subjects with respect to age and gender, suggesting that the induction of IgA2 antibodies may be due to differences in thus-far unknown genetic or immunoregulatory factors. Because it is known that IgA2 subclass-switch is induced by the combined activation of B cells with IL-10 and transforming growth factor- β (TGF- β) together with CD40 ligation on specialized APCs, the DCs [12, 26], it can be speculated that differences in the mucosal cytokine milieu and DC activation may be involved in the appearance of IgA2 tTG-Ab.

An interesting finding in several autoimmune diseases such as type 1 diabetes and bullous pemphigoid was that the isotype and subclass distribution of autoantibodies can provide indirect information on the Th1/Th2 balance and the activation state of T and B cells [21, 27, 28]. Therefore, we here also studied the relation between IgG4/IgE and IgG1 subclass autoantibodies, to further characterize the regulation of the B-cell response in CD. The strictly IL-4-dependent IgE and IgG4 formation, believed to be associated with mucosal immunity and/or a predominant Th2 response [29–31], was found in only a minority of patients with CD. In contrast, tTG IgG1 were present in most patients (57% positive), suggesting a polarization of the tTG-specific T-cell response along the Th1 pathway. The observed frequency of IgG1 antibodies is in accordance with that observed in studies where IgG1 endomysium antibodies and IgG1 tTG-Ab were described in about half of the coeliac patients with positive IgA antibodies [9, 10]. The frequency of IgG1 tTG antibodies was significantly lower in patients with sCD (18%), suggesting major differences in lymphocyte activation between patients with symptomatic and sCD. Although longitudinal studies on changes of the mucosal cytokine-expression patterns during the natural history of CD are missing, previous studies in patients with untreated and treated CD have shown that the severity of morphological abnormalities is associated with the mucosal lymphocyte-activation grade [15, 32]. The observed increase of pro-inflammatory cytokines such as IL-18, TNF- α and IFN- γ in biopsies from patients with CD may contribute one key microenvironmental signal for B cells to produce IgG1 antibodies [14–16, 23]. In this study, we did not observe a significant correlation between a specific isotype and the mucosal damage. In patients with sCD, there was a trend towards a more severe mucosal injury when IgG1 and IgA2 tTG-Ab were present, whereas two patients with positive IgA1 tTG-Ab had a normal histology and a mild inflammation. All patients with sCD suffer from type 1 diabetes, which could have some influence on the regulation of the mucosal immune response and isotype switching. To answer the question whether or not IgG1 tTG-Ab correlate with histology, we require larger studies including follow-up investigations in nondiabetic patients with sCD.

In conclusion, this study provides the first data on a heterogeneous IgA and IgG antibody isotype distribution directed against the self-antigen tTG in CD. Our data suggest a link between tTG IgA2 and IgG1 subclass antibodies and the clinical presentation. The autoantibody subclass-switching thus seems to reflect the type of T-cell activation and can therefore be useful as a diagnostic marker for risk estimation and the monitoring of intervention studies.

Acknowledgments

The study was supported by a grant from the Deutsche Zöliakie Gesellschaft (J.S.).

References

- 1 Schuppan D. Current concepts of celiac disease pathogenesis. *Gastroenterology* 2000;119:234–42.
- 2 Dieterich W, Ehnis T, Bauer M *et al.* Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* 1997;3:797–801.
- 3 Korponay-Szabo IR, Laurila K, Szondy Z *et al.* Missing endomysial and reticulon binding of coeliac antibodies in transglutaminase 2 knockout tissues. *Gut* 2003;52:199–204.
- 4 Sulkanen S, Halttunen T, Laurila K *et al.* Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. *Gastroenterology* 1998;115:1322–8.
- 5 Dieterich W, Laag E, Schopper H *et al.* Autoantibodies to tissue transglutaminase as predictors of celiac disease. *Gastroenterology* 1998;115:1317–21.
- 6 Seissler J, Boms S, Wohlrab U *et al.* Antibodies to human recombinant tissue transglutaminase: Evidence for a high diagnostic sensitivity for coeliac disease. *Horm Metab Res* 1999;31:375–9.
- 7 Williams A, Annis P, Lock R, Unsworth D, Gale E, Bingley P. Evaluation of a high-throughput second antibody radiobinding assay for measuring IgA antibodies to human tissue transglutaminase. *J Immunol Methods* 1999;228:81–5.
- 8 Bazzigaluppi E, Lampasona V, Barera G *et al.* Comparison of tissue transglutaminase-specific antibody assays with established antibody measurements for coeliac disease. *J Autoimmun* 1999;12:51–6.
- 9 Picarelli A, di Tola M, Sabbatella L *et al.* Identification of a new coeliac disease subgroup: Antiendomysial and anti-transglutaminase antibodies of IgG class in the absence of selective IgA deficiency. *J Intern Med* 2001;249:181–8.
- 10 Agardh D, Borulf S, Lernmark A, Ivarsson SA. Tissue transglutaminase immunoglobulin isotypes in children with untreated and treated celiac disease. *J Pediatr Gastroenterol Nutr* 2002;36:77–82.
- 11 Fagarasan S, Honjo T. Intestinal IgA synthesis: Regulation of front-line body defences. *Nat Rev Immunol* 2003;3:63–72.
- 12 Fayette J, Dubois B, Vandenabeele S *et al.* Human dendritic cells skew isotype switching of CD40-activated naive B cells towards IgA1 and IgA2. *J Exp Med* 1997;185:1909–18.
- 13 Levings MK, Bacchetta R, Schulz U, Roncarolo MG. The role of IL-10 and TGF- β in the differentiation and effector function of T regulatory cells. *Int Arch Allergy Immunol* 2002;29:263–76.
- 14 Breese EJ, Kumar P, Farthing MJ, MacDonald TT. Interleukin-2 and interferon-gamma producing cells in the lamina propria in celiac disease. *Dig Dis Sci* 1994;39:2243.
- 15 Nilsen EM, Lundin KE, Krajci P, Scott H, Sollid LM, Brandtzaeg P. Gluten specific, HLA-DQ restricted T cells from coeliac mucosa

- produce cytokines with Th1 or Th0 profile dominated by interferon gamma. *Gut* 1995;37:766–76.
- 16 Salvati VM, MacDonald TT, Bajaj-Elliott M *et al.* Interleukin 18 and associated markers of T helper cell type 1 activity in coeliac disease. *Gut* 2002;50:186–90.
- 17 Walker-Smith J. Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition (ESPGAN). *Arch Dis Child* 1990;65:909–11.
- 18 Seissler J, Schott M, Boms S *et al.* Autoantibodies to human tissue transglutaminase identify silent coeliac disease in type 1 diabetes. *Diabetologia* 1999;42:1440–1.
- 19 Spiekerkoetter U, Seissler J, Wendel U. General screening for coeliac disease is advisable in children with type 1 diabetes. *Horm Metab Res* 2002;34:192–5.
- 20 Seissler J, Wohlrab U, Wuensche C, Scherbaum WA, Boehm BO. Autoantibodies from patients with coeliac disease recognize distinct functional domains of the autoantigen tissue transglutaminase. *Clin Exp Immunol* 2001;125:216–21.
- 21 Seissler J, Eikamp K, Schott M, Scherbaum WA. IA-2 autoantibodies restricted to the IgG4 subclass are associated with protection from type 1 diabetes. *Horm Metab Res* 2002;34:186–91.
- 22 Marsh M. Gluten, major histocompatibility complex, and the small intestine. *Gastroenterology* 1992;102:330–54.
- 23 Schultz CL, Coffman RL. Control of isotype switching by T cells and cytokines. *Curr Opin Immunol* 1991;3:350–4.
- 24 Osman AA, Richter T, Stern M, Mothes T. The IgA subclass distributions of endomysium and gliadin antibodies in human sera are different. *Clin Chim Acta* 1996;255:145–52.
- 25 Rifai A, Fadden K, Morrison SL, Chintalacharuvu KR. The N-glycans determine the differential blood clearance and hepatic uptake of human immunoglobulin (Ig)A1 and IgA2 isotypes. *J Exp Med* 2000;191:2171–82.
- 26 Dubois B, Barthelemy C, Durand I, Liu YJ, Caux C, Briere F. Toward a role of dendritic cells in the germinal center reaction: Triggering of B cell proliferation and isotype switching. *J Immunol* 1999;162:3428–36.
- 27 Bonifacio E, Scirpoli M, Kredel K *et al.* Early autoantibody responses in prediabetes are IgG1 dominated and suggest antigen-specific regulation. *J Immunol* 1999;163:525–32.
- 28 Laffitte E, Skaria M, Jaunin F *et al.* Autoantibodies to the extracellular and intracellular domain of bullous pemphigoid 180, the putative key autoantigen in bullous pemphigoid, belong predominantly to the IgG1 and IgG4 subclasses. *Br J Dermatol* 2001;144:760–8.
- 29 Lundgren M, Persson U, Larsson P *et al.* Interleukin 4 induces synthesis of IgE and IgG4 in human B cells. *Eur J Immunol* 1989;19:1311–5.
- 30 Yazdanbakhsh M, Paxton WA, Brandenburg A *et al.* Differential antibody isotype reactivity to specific antigens in human lymphatic filariasis: gp15/400 preferentially induces immunoglobulin E (IgE), IgG4, and IgG2. *Infect Immun* 1995;63:3772–9.
- 31 King CL, Nutman TB. IgE and IgG subclass regulation by IL-4 and IFN-gamma in human helminth infections. Assessment by B cell precursor frequencies. *J Immunol* 1993;151:458–65.
- 32 Kontakou M, Przemioslo RT, Sturgess RP, Limb AG, Ciclitira PJ. Expression of tumour necrosis factor-alpha, interleukin-6, and interleukin-2 mRNA in the jejunum of patients with coeliac disease. *Scand J Gastroenterol* 1995;30:456–63.