OUTI KOSKINEN

Small-bowel Mucosal Transglutaminase-2-specific Autoantibody Deposits in Coeliac Disease

Usefulness in diagnostics and follow-up

ACADEMIC DISSERTATION
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To my family

ABSTRACT

The diagnosis of coeliac disease is made from a small-bowel mucosal biopsy specimen if villous atrophy with crypt hyperplasia is found on a gluten-containing diet and there is a response to a gluten-free diet. Positivity for serum coeliac antibodies supports the diagnosis. These autoantibodies are targeted to transglutaminase-2 (TG2) and produced in the small intestine. In coeliac disease, deposited autoantibodies have been found in the small-bowel mucosa. The diagnostics of coeliac disease is often problematic in that the quality of a small-bowel biopsy sample is sometimes compromised and villous atrophy is occasionally patchy. Further villous atrophy can also appear in other diseases such as giardiasis, viral infections, food allergies and autoimmune enteropathy. In a minority of coeliac disease patients, small-intestinal damage does not recover on a gluten-free diet. Serum coeliac autoantibodies usually give no additional support in the diagnosis of non-responsive coeliac disease, as most non-responsive patients are serum autoantibody-negative. Gluten-induced small-bowel mucosal villous atrophy is the end stage of the disease and develops gradually from lymphocytic infiltration of the epithelium to crypt hyperplasia and further to villous atrophy. Small-bowel mucosal abnormalities caused by early-stage coeliac disease are unspecific and diagnosis cannot rely on them. The present aim was to assess the value of determinating small-bowel mucosal TG2-specific IgA autoantibody deposits in coeliac disease diagnostics and follow-up, especially in problematic cases. The occurrence of mucosal autoantibody deposits was compared to villous atrophy (**I-IV**), CD3+ and γδ+ intraepithelial lymphocyte (IEL) densities (**I, III, IV**) and serum autoantibodies (**I-IV**).

In studies **I-IV,** a total of 261 patients had overt and 48 early-stage coeliac disease. In addition, 177 coeliac patients were examined after different periods on a gluten-free diet, and 27 patients evincing no histological response to the diet. Coeliac patients were compared to 86 non-coeliac controls. Small-bowel mucosal autoantibody deposits were found in all and serum autoantibodies in 91% of overt coeliac patients. Elevated densities of CD3+ and γδ+ IELs were present in 91% and

96% of overt coeliac patients, respectively (**I**). On a gluten-free diet, after recovery from villous atrophy, the densities of CD3+ IELs and the intensity of autoantibody deposits decreased, eventually also the density of γδ+ IELs, but remained constantly higher than in non-coeliac controls. None of the controls had serum autoantibodies, but 16% had weak autoantibody deposits in the small intestine.

In assessment of gluten dependence (**II**) ten patients underwent a gluten challenge with wheat, barley, rye and oats. Gluten caused villous atrophy and the appearance of small-intestinal autoantibody deposits and serum autoantibodies, in contrast to oats, which had no effect on any of the markers.

Of the 27 non-responsive coeliac patients 21 were on a strict gluten-free diet (**III**). Of these, 95% evinced small-bowel mucosal autoantibody deposits, although serum autoantibodies were found in only 24%. Increased densities of CD3+ and γδ+ IELs were found in 76% and 71%, respectively. None of the ten controls with other enteropathies had coeliac disease autoantibodies in the serum or deposited in the small-bowel mucosa. Five of the controls had villous atrophy and one third increased densities of IELs.

In early-stage coeliac disease (**IV**) small-bowel mucosal IgA deposit was found in 96%, serum autoantibodies in 73% and increased densities of CD3+ IELs in 62% and γδ+ IELs in 71%. In addition, twenty symptomatic early-stage patients with small-bowel mucosal TG2-specific IgA deposits started a gluten-free diet before villous atrophy developed. On a gluten-free diet the autoantibody deposits disappeared and symptoms were alleviated.

The findings showed that determination of small-bowel mucosal TG2-specific IgA deposits is a sensitive tool in finding overt and early-stage coeliac disease. Morphological analysis or IEL densities do not suffice to identify early-stage disease or distinguish different causes of villous atrophy, whereas small-bowel mucosal autoantibodies are accurate in detecting coeliac disease even in seronegative patients. These results together give an opportunity to ascertain or exclude coeliac disease in patients with small-bowel mucosal lesions compatible with coeliac disease but without serum autoantibodies.

TIIVISTELMÄ

Keliakian diagnoosiin vaaditaan ohutsuolen limakalvon villusatrofia ja kryptahyperplasia gluteenipitoisen ruokavalion aikana sekä vaste gluteenittomaan ruokavaliohoitoon. Positiiviset seerumin keliakiavasta-aineet tukevat diagnoosia. Nämä keliakia-autovasta-aineet kohdistuvat transglutaminaasi-2:ta (TG2) vastaan ja tuotetaan ohutsuolessa. Keliakiassa autovasta-aine kertymiä on löydetty ohutsuolen limakalvolta. Keliakian diagnostiikka on usein ongelmallista, sillä ohutsuolen koepalojen laatu on toisinaan puutteellinen ja joskus villusatrofia on läiskittäinen. Villusatrofiaa voi esiintyä myös muissa sairauksissa kuten giardiaasissa, virusinfektioissa, ruoka-allergioissa ja autoimmuunienteropatiassa. Pienellä osalla keliakiapotilaista ei vastetta gluteenittomaan ruokavalioon ole. Hoitoon reagoimatonta keliakiaa sairastavilla seerumin keliakia vasta-aineiden määrittämisestä ei ole apua, koska usein heillä ei ole vasta-aineita seerumissa. Gluteenin aiheuttama ohutsuolen villusatrofia on taudin myöhäisvaihe, joka kehittyy vähitellen lymfosyyttien kertymisestä epiteelille ja edelleen villusatrofiaan. Alkavan keliakian aiheuttamat ohutsuolen limakalvon muutokset ovat epäspesifisiä eikä niiden perusteella voi tautia diagnosoida. Tämän tutkimuksen tavoitteena oli tutkia ohutsuolen limakalvon TG2-spesifisten autovasta-ainekertymien määrittämisen hyötyä keliakian diagnostiikassa ja seurannassa, erityisesti ongelmatapauksissa. Autovasta-ainekertymien esiintymistä verrattiin villusatrofiaan (**I-IV**), CD3+ ja γδ+ epiteelinsisäisiin lymfosyyttitiheyksiin (IEL) (**I, III, IV**) sekä seerumin vasta-aineisiin (**I-IV**).

Osatöissä **I-IV** tutkittiin 261 hoitamatonta keliakiapotilasta sekä 48 potilasta, joilla oli alkava keliakia. Lisäksi tutkittiin 177 potilasta gluteenittoman ruokavalion aikana ja 27 potilasta, joiden suolivaurio ei parantunut gluteenittomalla ruokavaliolla. Keliakiapotilaita verrattiin 86 verrokkiin, joilla oli vatsavaivoja, muttei keliakiaa. Keliakia-autovasta-ainekertymiä löydettiin ohutsuolesta kaikilta ja vasta-aineita seerumista 91 %:lta hoitamattomista keliakiapotilaista. Kohonneet CD3+ ja γδ+ IEL-tiheydet löytyivät 91 %:lta ja 96 %:lta hoitamattomista keliakiapotilaista (**I**). Gluteenittomalla ruokavaliolla villusatrofian korjaantumisen

jälkeen laskivat sekä CD3+ IEL-tiheys että autovasta-ainekertymien intensiteetti ja lopulta myös γδ+ IEL-tiheys, joka kuitenkin jäi pysyvästi korkeammaksi kuin verrokeilla, joilla ei ollut keliakiaa. Kenelläkään verrokeista ei ollut seerumin keliakiavasta-aineita, mutta 16 %:lla oli heikot autovasta-ainekertymät ohutsuolessa.

Tutkittaessa gluteeniriippuvuutta (**II**) kymmenen potilasta altistettiin gluteenille vehnällä, ohralla, rukiilla ja kauralla. Gluteeni aiheutti villusatrofian ja vasta-aineiden ilmaantumisen sekä seerumiin, että kertymisen ohutsuoleen päinvastoin kuin kaura, jolla ei ollut mitään vaikutusta käytettyihin markkereihin.

Hoitoon reagoimattomista 27 potilaasta, 21 noudatti tarkasti gluteenitonta ruokavaliota (**III**). Näistä 21 potilaasta 95 %:lla oli ohutsuolen limakalvolla autovasta-ainekertymiä vaikka seerumissa autovasta-aineita oli vain 24 %:lla. Kohonneet CD3+ ja γδ+ IEL-tiheydet löytyivät 76 %:lta ja 71 %:lta. Kenelläkään kymmenestä verrokista, joilla oli muu enteropatia, ei ollut autovasta-aineita ohutsuolen limakalvolla eikä seerumissa. Viidellä enteropatiapotilaista oli villusatrofia ja kolmanneksella kohonneet IEL-tiheydet.

Alkavassa keliakiassa (**IV**) autovasta-aine kertymät löydettiin 96 %:lta ja seerumin vasta-aineet 73 %:lta sekä kohonneet CD3+ ja γδ+ IEL-tiheydet 62 %:lta ja 71 %:lta. Lisäksi kaksikymmentä oireista potilasta, joilla oli alkavaan keliakia ja TG2-spesifiset autovasta-ainekertymät ohutsuolen limakalvolla, aloitti gluteenittoman ruokavalion ennen villusatrofian kehittymistä. Gluteenittomalla ruokavaliolla autovasta-ainekertymät ja oireet hävisivät.

Tämä tutkimus osoittaa, että TG2-spesifisten autovasta-ainekertymien määrittäminen ohutsuolen limakalvolta on herkkä menetelmä keliakian sekä alkavan keliakian löytämisessä. Morfologinen analyysi tai IEL-tiheyksien määrittäminen ei löydä alkavaa keliakiaa tai kykene erottamaan villusatrofian syytä. Ohutsuolen keliakia-autovasta-ainekertymät sitä vastoin löytävät myös seronegatiivisen keliakian. Nämä tulokset mahdollistavat keliakian varmistamisen tai poissulkemisen potilailla joilla ohutsuolen vaurio sopii keliakiaan, mutta joilla ei ole seerumin vasta-aineita.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by Roman numerals **I-IV**:

I Koskinen O, Collin P, Lindfors K, Laurila K, Mäki M, Kaukinen K. Usefulness of small-bowel mucosal transglutaminase-2 specific IgA autoantibody deposits in celiac disease diagnosis and follow up. J Clin Gastroenterol. In press.
DOI 10.1097/MCG.0b013e3181b64557.

II Koskinen O, Villanen M, Korponay-Szabo I, Lindfors K, Mäki M, Kaukinen K. Oats do not induce systemic or mucosal autoantibody response in children with coeliac disease. J Pediatr Gastroenterol Nutr. 2009;48:559-65. (Reprinted with permission of Lippincott Williams & Wilkins.)

III Koskinen O, Lindfors K, Collin P, Peräaho M, Laurila K, Woolley N, Partanen J, Mäki M, Kaukinen K. Intestinal transglutaminase 2 specific antibody deposits in non-responsive coeliac disease. Dig Liver Dis. In Press.
DOI 10.1016/j.dld.2010.03.008. (Reprintend with permission of Elsevier Limited)

IV Koskinen O, Collin P, Korponay-Szabo I, Salmi T, Iltanen S, Haimila K, Partanen J, Mäki M, Kaukinen K. Gluten-dependent small-bowel mucosal transglutaminase 2-specific IgA deposits in overt and mild enteropathy coeliac disease. J Pediatr Gastroenterol Nutr. 2008;47:436-42. (Reprinted with permission of Lippincott Williams & Wilkins.)

ABBREVIATIONS

AGA	anti-gliadin antibody
AIDS	acquired immunodeficiency syndrome
APC	antigen-presenting cell
ARA	antireticulin antibody
CTLA-4	cytotoxic T-lymphocyte-associated- 4
DNA	deoxyribonucleic acid
EATL	enteropathy-associated T-cell lymphoma
ELISA	enzyme-linked immunosorbent assay
EmA	endomysial antibody
HLA	human leukocyte antigen
IEL	intraepithelial lymphocyte
Ig	immunoglobulin
IL	interleukin
LPL	lamina propria lymphocyte
MHC	major histocompability complex
MICA	MHC class I polypeptide-related sequence A
MMP	matrix metalloproteinase
NK	natural killer
NKG2D	natural killer group 2D, receptor of MICA
NSAID	non-steroidal anti-inflammatory drug
PCR	polymerase chain reaction
p-value	is the probability of obtaining a test statistic at least as extreme as that actually observed, assuming that the null hypothesis holds.
TCR	T- cell receptor
TG	transglutaminase
Th	T-helper cell
Vh/CrD	villous height-crypt depth ratio

INTRODUCTION

Classically coeliac disease has been described as a severe malabsorbtion syndrome with diarrhoea, steatorrhea, weight loss, growth retardation and anaemia in infancy caused by intolerance to dietary gluten (Gee 1888, Dicke 1950). Currently patients with coeliac disease belong to all age-groups and may evince only mild gastrointestinal symptoms (Mäki et al. 1988, Collin et al. 1997) or extra-intestinal symptoms such as dermatitis herpetiformis (Collin and Reunala 2003) and neurological problems (Luostarinen et al. 1999). Coeliac disease appears in genetically predisposed patients and almost all have either the HLA-DQ2 or the DQ8 genotype, which are also present in 30% of the healthy population (Sollid et al. 1989, Karell et al. 2003).

The current diagnostic criteria for coeliac disease require small-bowel villous atrophy with crypt hyperplasia during a gluten-containing diet and clinical or histological recovery on a gluten-free diet (Walker-Smith et al. 1990, Catassi et al. 2001). A typical feature of coeliac disease is the presence of serum autoantibodies, which can support diagnosis of coeliac disease (Walker-Smith et al. 1990, Catassi et al. 2001). These autoantibodies are targeted mainly towards transglutaminase-2 (TG2) (Dieterich et al. 1997) and are produced at the small-intestinal level (Marzari et al. 2001). In coeliac disease, deposited autoantibodies have been found in the small-intestinal mucosa (Korponay-Szabo et al. 2004). In addition, small-intestinal TG2-specific IgA deposits have in some cases preceded villous atrophy (Korponay-Szabo et al. 2004, Kaukinen et al. 2005, Salmi et al. 2006a).

Gluten-induced small-bowel mucosal villous atrophy with crypt hyperplasia is the end stage of the disease process and develops gradually from lymphocytic infiltration of the epithelium to crypt hyperplasia and further to villous atrophy (Marsh 1992). It has previously been noted that serum autoantibody positivity (Collin et al. 1993), increased densities of either intraepithelial lymphocytes (IELs) (Salmi et al. 2006a) or T-cell receptor γδ–bearing IELs (Mäki et al. 1991a, Iltanen et al. 1999c, Salmi et al. 2006a) in patients with normal villous structure can predict forthcoming villous atrophy and coeliac disease. However, some patients are

seronegative during the early stages of the disease (Salmi et al. 2006a). In addition, increased densities of IELs are not specific for coeliac disease, as they are also encountered in other conditions (Salmi et al. 2006a). Patients with early-stage coeliac disease may show symptoms and even develop osteoporosis without evidence of small-bowel mucosal villous atrophy (Kaukinen et al. 2001, Kaukinen et al. 2005). There is thus a need for new tools to diagnose coeliac disease before the development of villous atrophy.

The treatment of coeliac disease aims to eliminate symptoms and effect a complete recovery of the small-bowel mucosal villi. Currently, the only treatment for coeliac disease is a gluten-free diet, where wheat, rye and barley are forbidden (Walker-Smith et al. 1990). A strict gluten-free diet, though burdensome, improves the quality of life (Mustalahti et al. 2002a, Wagner et al. 2008). Patients with poor adherence to gluten-free diet often have small-bowel mucosal lesions leading to an increased risk of complications (Troncone et al. 1995, Kaukinen et al. 2007b).

A minority of coeliac patients do not respond to a gluten-free diet; the condition is called refractory coeliac disease (Wahab et al. 2002, Leffler et al. 2007). Diagnosing refractory coeliac disease is challenging in that small-bowel mucosal villous atrophy does not respond as it should to a gluten-free diet and villous atrophy is known to appear in conjunction of other diseases such as giardiasis, viral infections, food allergies and autoimmune enteropathy (Green and Cellier 2007). Moreover, serum coeliac autoantibodies usually give no additional support in the diagnosis of coeliac disease, as most patients with refractory disease are serum autoantibody-negative on a gluten-free diet (Kaukinen et al. 2007b, de Mascarel et al. 2008, O'Shea et al. 2008, Verbeek et al. 2008b).

The aim of the present study was to investigate the value of determining small-bowel mucosal TG2-specific IgA autoantibody deposits in the diagnostic work-up and follow-up of coeliac disease. A further the purpose was to ascertain whether there are advantages in determining small-bowel mucosal IgA deposits compared to conventional methods in the diagnosis of coeliac disease in patients who do not completely fulfil the current criteria (Walker-Smith et al. 1990, Catassi et al. 2001) i.e. these with non-responsive and early-stage coeliac disease.

REVIEW OF THE LITERATURE

1. COELIAC DISEASE

Coeliac disease is a permanent intolerance to gluten appearing in genetically predisposed individuals. The condition was first described by Samuel Gee (1888) as a disease of all ages characterized by diarrhoea, cachexia and distended abdomen. After the harmful effect of wheat and rye was noted (Dicke 1950), a gluten-free diet was adopted as treatment for coeliac disease (van de Kamer et al. 1953). The current diagnostics of coeliac disease are based on the finding of small-bowel mucosal damage as described by J.W. Paulley (1954) more than fifty years ago. At present, coeliac disease is a common chronic disorder and patients with the severe symptoms described by Gee are in many countries rarely found (Mäki and Collin 1997).

2. CLINICAL FEATURES

2.1 Classical coeliac disease

Classically coeliac disease has been described as a severe malabsorbtion syndrome with diarrhoea, steatorrhea, weight loss, growth retardation and anaemia in infancy (Young and Pringle 1971). In the past decades, the symptoms of the condition have become milder, severe malabsorbtion being nowadays rarely seen (Mäki et al. 1988, Collin et al. 1997). Patients may be overweight (Dickey and Kearney 2006) or have only mild abdominal symptoms (Mäki et al. 1988, Collin et al. 1997). Isolated malabsorbtion of iron or folic acid is a common finding in coeliac patients but does not necessarily lead to clinical manifestation (Tikkakoski et al. 2007). Currently, coeliac disease is found in all age-groups and its prevalence increases with age (Mäki et al. 2003, Lohi et al. 2007, Vilppula et al. 2009). In the majority of patients symptoms are alleviated and the small-bowel mucosa recovers

on a gluten-free diet, but a small minority develop refractory coeliac disease, where there is no response to a withdrawal of gluten (O'Mahony et al. 1996, Collin et al. 2004a).

2.2 Extra-intestinal symptoms

The symptoms of coeliac disease are not restricted to the intestine and the disorder can be regarded more as a disease of the whole organ system. The most common extra-intestinal symptom is dermatitis herpetiformis, an itching bullous dermatitis, typically situated in the extensor surfaces of the skin in the knees and elbows (Collin and Reunala 2003).These skin lesions disappear on a gluten-free diet (Reunala et al. 1984). Small-intestinal mucosal subtotal villous atrophy with crypt hyperplasia can be found in 60% of patients with dermatitis herpetiformis and the remainder have milder mucosal lesions in their small bowel (Reunala et al. 1984).

In 7% of coeliac disease patients neurological symptoms lead to the diagnosis (Luostarinen et al. 1999). Common neurological symptoms related to the disease are neuropathy, gluten ataxia (Hadjivassiliou et al. 1996), cerebellar atrophy (Hadjivassiliou et al. 1998), epilepsy (Gobbi et al. 1992, Peltola et al. 2009), migraine (Gabrielli et al. 2003) and memory impairment (Luostarinen et al. 1999). Patients with gluten ataxia or epilepsy related to coeliac disease may benefit from a gluten-free diet (Hadjivassiliou et al. 2003, Gobbi 2005).

Hypertransaminasaemia may be encountered in one third of coeliac patients (Farre et al. 2002). In contrast, about 9% of patients evincing unexplained hypertransaminasaemia also have coeliac disease (Volta et al. 2001a). Usually liver disease related to coeliac disease is mild and the liver enzymes normalize on a gluten-free diet. Severe liver failure which recovers on a gluten-free diet has also been described (Volta et al. 2001a, Kaukinen et al. 2002a).

In the mouth, e.g. dental enamel defects (Aine et al. 1990) or aphtous stomatitis (Ferguson et al. 1976) can be the sole markers of coeliac disease. Arthritis related to coeliac disease has also been described (Bourne et al. 1985). Infertility (Collin et al. 1996), abortions and foetal growth retardation during pregnancy (Martinelli et al. 2000) can be caused by untreated coeliac disease, but on a gluten-free diet the problems of pregnancy are reduced. In children and adolescents coeliac disease may

cause growth retardation and delayed puberty (Mäki et al. 1988). On a gluten-free diet, however, catch-up growth is rapid (Damen et al. 1994) and the final height of coeliac patients diagnosed in childhood does not differ from that of the general population (Weiss et al. 2008).

Osteoporosis is a common manifestation of coeliac disease; from 21% to 35% of untreated and 17% to 34% of treated adult coeliac disease patients have osteoporosis (Kemppainen et al. 1999a, Mustalahti et al. 1999, Meyer et al. 2001, Kaukinen et al. 2007b). In addition, over one third of all coeliac patients manifest osteopenia (Meyer et al. 2001). Particularly, in patients not responding to a strict gluten-free diet and in non-adherent patients osteoporosis is even more frequent (Valdimarsson et al. 1994, Kemppainen et al. 1999a, Kaukinen et al. 2007b). Among coeliac patients, an increased risk of fractures has been reported by Vasquez and associates (2000) especially before initiation of a gluten-free diet or in non-compliant patients. Similar findings have since been reported in several studies (Olmos et al. 2008).

Coeliac disease patients often have calcium malabsorbtion, leading to secondary hyperparathyroidism in almost one third of patients (Ciacci et al. 1995, Selby et al. 1999). Secondary hyperparathyroidism is related to loss of bone mineral density and additional calcium is recommended in addition to the gluten-free diet, though calcium absorption has been described to normalize on a gluten-free diet (Corazza et al. 1995b, Selby et al. 1999). Furthermore, although bone mineral density improves during a gluten-free diet, it does not completely normalize in all patients (Kemppainen et al. 1999b, Meyer et al. 2001, Kaukinen et al. 2007b).

2.3 Silent coeliac disease and autoimmunity

In some patients, despite gluten-induced small-bowel mucosal lesions, coeliac disease is clinically asymptomatic and is often referred to as silent coeliac disease (Ferguson et al. 1993). This condition is usually found by serological screening in coeliac disease risk groups and in the general population (Volta et al. 2001a, Fasano et al. 2003, Mäki et al. 2003, Tommasini et al. 2004). One risk group consists of coeliac disease patients' family members and first-degree relatives. Here the prevalence of coeliac disease varies between 2.6% and 11% in different studies

(Mäki et al. 1991b, Mustalahti et al. 2002b, Fasano et al. 2003, Bonamico et al. 2006, Rubio-Tapia et al. 2008). Another risk group comprises patients with selective IgA deficiency, whose risk of coeliac disease has been found to be increased ten-fold (Collin et al. 1992). Furthermore, the prevalence of coeliac disease is increased in several autoimmune disorders or chromosomal anomalies (Table 1).

Table 1. The prevalence of coeliac disease (CD) found by serological screening in certain coeliac disease risk groups.

Associated condition	Reference	Prevalence of CD (%)
Type 1 diabetes mellitus	Mäki et al. 1984a	2.3%
	Collin et al. 1989	4.1%
	Not et al. 2001	5.7%
	Hansen et al. 2006	12.3%
	Mankai et al. 2007	5.3%
	Remes-Troche et al. 2008	5.9%
Autoimmune thyroid disease	Collin et al. 1994b	4.8%
	Meloni et al. 2001	4.4%
	Volta et al. 2001b	3.2%
Addison's disease	O'Leary et al. 2002	12.2%
	Myhre et al. 2003	7.9%
	Biagi et al. 2006	5.6%
Primary Sjögren's syndrome	Iltanen et al. 1999a	14.7%
	Luft et al. 2003	10.0%
	Szodoray et al. 2004	4.5%
Primary biliary cirrhosis	Bardella et al. 1997	0%
	Dickey et al. 1997	7.0%
	Volta et al. 2002	4.0%
Autoimmune hepatitis	Volta et al. 1998	2.8%
	Villalta et al. 2005	6.4%
	Diamanti et al. 2008	12.5%
Down's syndrome	Bonamico et al. 2001a	4.6%
	Carnicer et al. 2001	6.3%
	Agardh et al. 2002	18.8%
	Nisihara et al. 2005	5.6%
Turner's syndrome	Bonamico et al. 2002	6.4%
	Frost et al. 2009	4.7%
Juvenile chronic/idiopathic arthritis	Lepore et al. 1996	2.5%
	Stagi et al. 2005	6.6%
IgA nephropathy	Collin et al. 2002	3.6%
Autoimmune myocarditis	Frustaci et al. 2002	4.4%
Alopecia areata	Corazza et al. 1995a	1.2%

In addition to the increased risk of coeliac disease in autoimmune disorders, patients with coeliac disease have an increased risk of developing other autoimmune disorder. In coeliac patients, the prevalence of type 1 diabetes mellitus varies between 3.8 and 5.4% (Collin et al. 1994a, Ventura et al. 1999, Sategna Guidetti et

al. 2001) and that of thyroid autoimmune disorders between 1.2 and 14% (Collin et al. 1994a, Ventura et al. 1999, Sategna Guidetti et al. 2001, Hadithi et al. 2007, Hakanen et al. 2001). Sjögren's syndrome is present in 3.3% of coeliac disease patients (Collin et al. 1994a). Other autoimmune disorders associated with coeliac disease include autoimmune hepatitis, alopecia areata, Addison's disease, pernicious anaemia, epilepsy with calcifications of brain and psoriasis (Ventura et al. 1999, Sategna Guidetti et al. 2001). In paediatric patients, the duration of gluten consumption seems to increase the risk of developing an autoimmune disorder later in life (Ventura et al. 1999), whereas studies in adults have not confirmed the protecting effect of a gluten-free diet in patients who have consumed gluten for decades before starting the diet (Sategna Guidetti et al. 2001, Viljamaa et al. 2005, Biagi et al. 2002). However, a contradictory finding in adults supporting the results of Ventura and colleagues (1999) in children has been published by Cosnes and associates (2008).

3. DIAGNOSTIC CRITERIA

The current European Society of Paediatric Gastroenterology, Hepatology and Nutrition criteria for coeliac disease from the year 1990 require small-bowel villous atrophy with crypt hyperplasia during a gluten-containing diet and either alleviation of symptoms or recovering of the small-bowel on a gluten-free diet. Positive serum antibody test results will support the diagnosis (Walker-Smith et al. 1990). For adults, the Amsterdam criteria entail similar demands for diagnosis (Catassi et al. 2001).

3.1 Differential diagnostics

Although small-intestinal mucosal villous atrophy with crypt hyperplasia is characteristic of coeliac disease and required for diagnosing the condition (Walker-Smith et al. 1990, Catassi et al. 2001), it is not a pathognomic finding only for coeliac disease. Similar lesions have been reported in patients with giardiasis, food allergies, tropical sprue, autoimmune-enteropathy, collagenous sprue, tuberculosis,

graft versus host reactions, idiopathic AIDS enteropathy and during viral gastrointestinal tract infections (Marsh 1992, Green and Cellier 2007, Cello and Day 2009).

Gluten challenge is not mandatory in the current diagnostic criteria. Nonetheless, it is recommended if the diagnosis of coeliac disease is obscure or in children diagnosed under the age of two years. In this age group, enteropathies such as cow's milk sensitive enteropathy, post-enteritis syndrome and giardiasis often occur. Differentiation between other enteropathies and coeliac disease may prove difficult (Walker-Smith et al. 1990). Serum autoantibodies help the diagnosis in the majority of coeliac patients, but some patients are seronegative and thus challenging in the diagnostic process (Salmi et al. 2006b).

4. SMALL-BOWEL MUCOSAL BIOPSY

4.1 Morphology of small-bowel mucosa

The diagnosis of coeliac disease is made from a small-bowel mucosal biopsy sample where villous atrophy and crypt hyperplasia are seen during a gluten-containing diet (Walker-Smith et al. 1990). The diagnosis of coeliac disease should be made from well-oriented high-quality specimens, but in about 10% of specimens the quality is not adequate for diagnosis, mainly due to poor orientation (Collin et al. 2005). The villous atrophy with crypt hyperplasia starts proximally and may be patchy and thus a single biopsy may miss it (Vogelsang et al. 2001, Bonamico et al. 2004, Hopper et al. 2008). Furthermore, as mentioned in section 3.1., small-bowel mucosal villous atrophy with crypt hyperplasia is not pathognomonic only to coeliac disease.

Gluten-induced small-bowel mucosal villous atrophy with crypt hyperplasia develops gradually (Figure 1) as Marsh has described. First, lymphocytes infiltrate the epithelium (Marsh I), whereafter the crypts become elongated (Marsh II) and finally villous atrophy develops (Marsh III) (Marsh 1992).

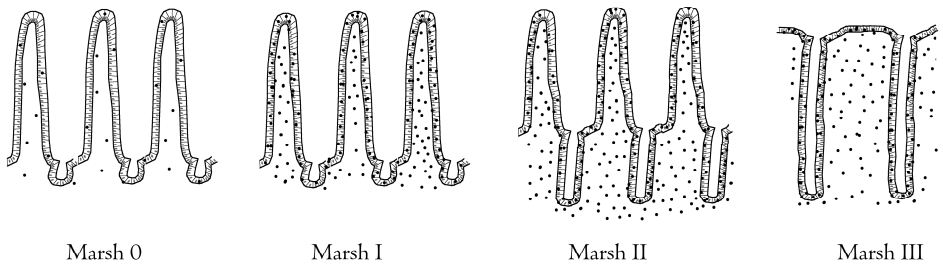

Figure 1. The development of coeliac small-bowel mucosal lesion and classification according to Marsh (1992).

4.2 Inflammation in small-bowel mucosa

The small-bowel mucosal epithelial cell layer contains not only epithelial cells, goblet cells, Paneth cells and enteroendocrine cells but also lymphocytes and occasionally other inflammatory cells. The majority of the intraepithelial lymphocytes (IELs) carry a certain type of surface molecule, CD3, on their surface and the density of CD3+ IELs has been shown to correlate well with total IEL density (Arranz et al. 1994). CD3+ IELs express T cell receptors (TCR) consisting of either α and β chains (αβ TCR) or γ and δ chains (γδ TCR) (Borst et al. 1988). Most CD3+ IELs express αβ TCR and CD8 (cytotoxic T-cells) or CD4 (helper T-cells), but a minority of cells express γδ TCR but neither CD8 nor CD4 (Selby et al. 1983, Borst et al. 1988, Verkasalo et al. 1990, Arato et al. 1998). The characteristics of CD3+ IELs in the small-bowel mucosa in coeliac disease patients and healthy controls are shown in Table 2.

Table 2. Characteristics of CD3+ intraepithelial lymphocytes according to different studies (Selby et al. 1983, Verkasalo et al. 1990, Arato et al. 1998, Halstensen et al. 1989, Jenkins et al. 1986)

	Active coeliac disease	**Control**
CD8+	59-93%	82-94%
CD4+	<10%	5-16%
CD8-CD4-	10-33%	0.4-9%
αβ+	50-80%	> 90%
γδ+	20-50%	< 10%

In coeliac disease the density of intraepithelial lymphocytes is increased (Ferguson and Murray 1971). On a gluten-free diet, the densities of IELs decrease

towards normal, but in many patients still remain elevated (Ferguson and Murray 1971, Iltanen et al. 1999b, Kaukinen et al. 1999). In detecting coeliac disease, it has been estimated that the sensitivity and specificity of CD3+ IELs are 93% and 73%, respectively (Järvinen et al. 2003). Nonetheless, there are also diseases other than coeliac disease, e.g. milk allergy, which could cause both small-bowel mucosal villous atrophy and an increased density of IELs (Kuitunen et al. 1982). The density of TCR αβ-bearing IELs is increased in active coeliac disease, but returns to normal levels after adoption of a gluten-free diet (Savilahti et al. 1990, Savilahti et al. 1992, Kutlu et al. 1993, Kaukinen et al. 1999). The sensitivity and specificity of increased density of αβ+ IELs have been is 83% and 66%, respectively, in detecting coeliac disease (Järvinen et al. 2003). TCR γδ-bearing lymphocytes in the small-bowel mucosal surface epithelium are increased in coeliac disease (Savilahti et al. 1990). The density of γδ+ IELs remains elevated during a gluten-free diet, although it decreases (Iltanen et al. 1999b, Kaukinen et al. 1999, Järvinen et al. 2003). The sensitivity and specificity of γδ+ IELs are 93% and 88%, respectively, in detecting coeliac disease (Järvinen et al. 2003).

An abnormal IEL population can be found in some refractory coeliac disease patients. These aberrant cells usually lack TCR and some surface molecules, being either CD3+CD8- or CD3-CD8-phenotype. A few patients also have CD30+ IELs in their small-intestinal mucosa; these aberrant lymphocytes have been associated with poor prognosis in refractory coeliac disease (Farstad et al. 2002, Verbeek et al. 2008b). It has been suggested that an abnormal IEL population could be an early manifestation of enteropathy-associated T-cell lymphoma (EATL) (Daum et al. 2001) and patients yielding such findings are at an increased risk of death or developing EATL (O'Shea et al. 2008). Refractory coeliac disease and EATL are discussed in sections 11.1 and 11.2, respectively.

In the lamina propria, situated under the epithelial cell layer of the small-bowel mucosa, T-cells constitute 50% of lymphocytes (Arato et al. 1998). In contrast to the epithelium, two thirds of T-lymphocytes are CD4+ and a smaller population are CD8+ in the lamina propria (Selby et al. 1983, Verkasalo et al. 1990). Almost all CD3+ lymphocytes in the lamina propria are αβ+, whereas γδ+ IELs are rare (Halstensen et al. 1989). In coeliac disease, changes in lamina propria lymphocyte

densities are relatively small and densities are similar to those in controls (Selby et al. 1983, Verkasalo et al. 1990).

In addition to T-lymphocytes, other inflammatory cells, e.g. B-lymphocytes and plasma cells, are present in the lamina propria (Arato et al. 1998). The number of immunoglobulin (Ig)- containing cells is increased in the lamina propria of the small intestinal mucosa and during gluten consumption a majority of cells in the lamina propria are plasma cells, indicating that the immune system producing antibodies is activated in coeliac disease (Savilahti 1972, Shiner 1973, Lancaster-Smith et al. 1976, Lancaster-Smith et al. 1977). The number of IgM-containing cells is constantly increased in patients with coeliac disease, whereas the number of IgA- and IgG- containing cells in the lamina propria returns to the same level as in non-coeliac subjects during a gluten-free diet (Lancaster-Smith et al. 1976). Similarly to the increased numbers of immunoglobulin-containing cells, extracellular immunoglobulin has also been found in the small-intestinal mucosal lamina propria during a gluten containing diet in coeliac patients (Shiner and Ballard 1972, Lancaster-Smith et al. 1977); this topic is reviewed more specifically in section 5.2.

5. COELIAC DISEASE ANTIBODIES

5.1 Serological tests

Serum autoantibodies targeted against transglutaminase-2 (TG2), as Dieterich and associates (1997) have shown, are a characteristic feature of coeliac disease; in the diagnostics serum antibodies have a supporting role (Walker-Smith et al. 1990, Catassi et al. 2001). These IgA class autoantibodies can be determined in the sera by indirect immunofluorescense using as antigen either rodent (R1-type antireticulin, ARA), primate or human tissues (anti-endomysium, EmA). In IgA-deficient coeliac patients IgG class autoantibodies against TG2 can be measured (Korponay-Szabo et al. 2003a). Levels of anti-TG2 antibodies can also be measured by enzyme-linked immunosorbent assay (ELISA) with human or guinea pig TG2 as antigen. These tests are highly sensitive and specific (Table 3). However, the EmA test is more accurate than anti-TG2 antibody tests and false-positive anti-TG2 tests have been

described in conjunction with chronic liver disease or Crohn's disease (Carroccio et al. 2002b). The accuracy of anti-TG2 antibody tests is better with human TG2 as antigen than those with guinea pig TG2 (Carroccio et al. 2002b). Regardless of the good sensitivity and specificity of these tests, some coeliac disease patients with villous atrophy and adequate response to a gluten-free diet have no autoantibodies against TG2 in the sera at the time of diagnosis (Salmi et al. 2006b). These seronegative patients are often older and have a more severe condition than seropositive patients (Salmi et al. 2006b). According to some studies, another defect of these tests is their inability to recognize partial villous atrophy (Rostami et al. 1999, Emami et al. 2008).

On a gluten-free diet serum autoantibodies return rapidly to normal levels after gluten withdrawal, regardless of mucosal condition. The sensitivity of serum autoantibody tests to recognize villous atrophy on a gluten-free diet has been reported to be as low as 26-60%, specificity 77-93% (Kaukinen et al. 2002b, Dipper et al. 2009, Vecsei et al. 2009).

In addition to anti-TG2 autoantibodies, many other autoantibodies have also been found in coeliac disease patients' sera. Some of these autoantibodies are associated with a certain extra-intestinal manifestation. For example, autoantibodies targeted to TG3 are found in some dermatitis herpetiformis patients (Sardy et al. 2002) and autoantibodies against TG6 in gluten ataxia patients (Hadjivassiliou et al. 2008). Gluten-dependent serum anti-actin antibodies are associated with severe small-bowel mucosal lesion (Clemente et al. 2000). Antibodies against desmin are present in some coeliac disease patients, but can be found in other diseases as well (Teesalu et al. 2001).

Antibodies in coeliac disease are not restricted to antibodies against self-antigens; antibodies against gliadin, an alcohol-soluble part of wheat gluten, are also found. In untreated coeliac disease elevated levels of anti-gliadin antibodies (AGA) can be found in both IgA and IgG class (Troncone and Ferguson 1991). The sensitivity of serum AGA by ELISA test varies between 31% and 95% and the specificity between 46% and 97% (Mäki et al. 1991b, Sulkanen et al. 1998, Mankai et al. 2005, Kaukinen et al. 2007a). Furthermore, elevated AGA levels have also been found in

Table 3. Sensitivities and specificities of serological tests in untreated coeliac disease

	Coeliac disease			EmA		ARA		Anti-TG2	
References	patients	Controls	Age group	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Mäki et al. 1984b	29	245	children	-	-	97%	98%	-	-
Hällström 1989	50	69	mixed	94%	100%	94%	100%	-	-
Mäki et al. 1991b	13	109	mixed	92%	95%	92%	95%	-	-
Ferreira et al. 1992	21	160	adults	100%	99%	90%	99%	-	-
Kolho and Savilahti 1997	88	114	children	94%	100%	-	-	-	-
Dieterich et al. 1998	106	114	mixed	100%	99%	-	-	98%	95%
Sulkanen et al. 1998	92	95	mixed	85%	100%	78%	100%	-	-
Rostami et al. 1999	85	16	mixed	60%	100%	-	-	-	-
Bonamico et al. 2001b	62	56	children	95%	-	-	-	90%	100%
Fabiani et al. 2001	25	3516	mixed	-	-	-	-	90%	96%
Carroccio et al. 2002b	24	183	adult	100%	100%	-	-	100%	97%
Burgin-Wolff et al. 2002	208	157	mixed	96%	100%	-	-	96%	99%
Fabiani et al. 2004	399	432	mixed	-	-	-	-	96%	100%
Collin et al. 2005	126	106	mixed	89%	98%	-	-	94%	99%
Mankai et al. 2005	143	74	mixed	-	-	87-94%	100%	86-87%	96%
Bazzigaluppi et al. 2006	143	64	children	98%	97%	-	-	96%	96%
Reeves et al. 2006	26	228	mixed	62-68%	80-98%	-	-	90-92%	81-83%
Kaukinen et al. 2007a	44	46	adults	80%	100%	-	-	89%	98%
Emami et al. 2008	21	329	mixed	-	-	-	-	38%	98%

EmA endomysial antibody, ARA antireticulin antibody, TG transglutaminase, - no data

conditions other than coeliac disease, for example in food allergies and in post-infectious sprue (Lindberg et al. 1985). Moreover, increased levels of AGAs have been found even in healthy subjects without coeliac-disease-type genetics (Mäki et al. 1991b). Hence conventional AGA testing is not recommended in the diagnostics of coeliac disease (Hill et al. 2005).

In contrast, antibodies against deamidated gliadin peptides, formed in the deamidation of gliadin by TG2, have a sensitivity between 84% and 91% and a specificity between 90% and 98% in detecting coeliac disease (Kaukinen et al. 2007a, Volta et al. 2008). Antibodies against deamidated gliadin peptides are thus more promising than those against whole gliadin.

5.2 Small-bowel mucosal antibodies

Immunoglobulin-containing cells, especially those with IgA and IgM, are increased in coeliac disease patients' small-bowel mucosa (Savilahti 1972). In the same way, the amount of immunoglobulins, which are produced in plasma cells of the lamina propria, is also elevated (Perkkiö et al. 1981). In addition to higher amounts of immunoglobulins in coeliac patients' small-bowel mucosa, total immunoglobulin secretion to the small-intestinal lumen is also elevated in coeliac these patients (O'Mahony et al. 1991).

Antibodies against gliadin (O'Mahony et al. 1990), reticulin (Mawhinney and Love 1975) and TG2 (Wahnschaffe et al. 2001) have been found in untreated coeliac disease patients' small-intestinal secretions *in vivo*. In contrast to serum AGA, the amounts of anti-gliadin antibodies secreted to the small-intestinal lumen do not decrease during a gluten-free diet (O'Mahony et al. 1991). Similarly to the secretion of autoantibodies in the small bowel *in vivo*, autoantibodies can be found in supernatants of *in vitro*-cultured gliadin challenged small-bowel mucosal biopsy samples (Picarelli et al. 1996, Carroccio et al. 2002a, Stenman et al. 2008). Furthermore, Marzari and colleagues (2001) were able to isolate TG2 antibodies from intestinal lymphocyte libraries only, but not from peripheral lymphocyte libraries by a phage display library technique and identified the small-intestinal mucosa as the production site for coeliac disease autoantibodies.

In the 1970s, it was observed that treated coeliac disease patients develop extracellular depositions of IgA after hours of gluten challenge (Shiner and Ballard 1972). Subsequently it was shown that there are strong depositions of extracellular IgA in untreated coeliac disease patients' small-bowel mucosa (Jos and Labbe 1976, Lancaster-Smith et al. 1976, Jos et al. 1979). These depositions of IgA are situated under the basement membrane of epithelial cells in the surface and crypt epithelium and around vessels (Jos and Labbe 1976, Lancaster-Smith et al. 1976, Jos et al. 1979). It was also noted that IgA deposits disappear on a gluten-free diet and rapidly reappear after gluten reintroduction, and that even a single dose of gluten is sufficient to induce their reappearance in coeliac patients (Lancaster-Smith et al. 1976, Lancaster-Smith et al. 1977). In 1980s Karpati and associates (1988) also found these deposits in the small intestine of patients with dermatitis herpetiformis.

After Dietrich and colleagues (1997) had identified TG2 as the main antigen of coeliac disease autoantibodies, Korponay-Szabo and associates (2004) showed that small-bowel mucosal IgA depositions target TG2. Extracellular IgA deposits disappeared from coeliac patients' small-bowel biopsy samples when TG2 binding to fibronectin was disrupted by chloroacetic acid. In addition, IgA which was eluted from tissues by chloroacetic treatment directed towards purified TG2 both in ELISA and in western blot (Korponay-Szabo et al. 2004). Furthermore, it has been shown that IgA deposits in the small-bowel of active coeliac disease patients have the ability to bind external TG2 added to the tissue *ex vivo* (Salmi et al. 2006b).

Determination of TG2-specific IgA deposits is made from frozen sections by double staining, a method developed by Korponay-Szabo and associates (2004). All IgA is stained by the direct immunofluorescense method and co-localization of IgA and TG2 is detected by double-staining both IgA and TG2. In coeliac disease, linear bands of subepithelial deposits are present along the villous and crypt epithelium and around vessels, whereas in non-coeliac samples endogenous IgA is found inside plasma cells and epithelial cells (Korponay-Szabo et al. 2004).

In previous studies with small numbers of patients, results have been promising in detecting coeliac disease by determining small-bowel mucosal TG2-specific IgA deposits (Korponay-Szabo et al. 2004, Kaukinen et al. 2005, Salmi et al. 2006a). TG2-specific IgA deposits are present in untreated coeliac patients' small-bowel mucosa also in a small minority of coeliac disease patients without serum antibodies

(Salmi et al. 2006b). TG2-specific IgA deposits have also been found in some coeliac patients with normal villi before the development of villous atrophy (Korponay-Szabo et al. 2004, Kaukinen et al. 2005, Salmi et al. 2006a). In contrast, IgA autoantibody deposits have been found neither in healthy persons nor in patients with other intestinal diseases (Salmi et al. 2006b).

6. EARLY-STAGE AND LATENT COELIAC DISEASE

Marsh pointed out that coeliac disease develops gradually (Marsh 1992). However, the diagnosis of coeliac disease requires a lesion which is the end-stage of the disease process (Walker-Smith et al. 1990, Marsh 1992). A patient is considered to have latent coeliac disease when evincing normal small-intestinal mucosal villous morphology while consuming a gluten-containing diet, but at some other time, previously or subsequently, may have a flat small-intestinal mucosal lesion which recovers on a gluten-free diet (Ferguson et al. 1993). Latent coeliac disease was first described by Weinstein (1974) in two patients with dermatitis herpetiformis who on a gluten-containing diet had normal mucosal architecture in their small bowel, but who during a gluten challenge developed villous atrophy and crypt hyperplasia.

Diagnosis of latent coeliac disease is often retrospective, when patients with minor abnormalities in small-bowel inflammation markers, i.e. early-stage coeliac disease patients, are followed up on a gluten-containing diet and subsequently develop small-bowel mucosal villous atrophy with crypt hyperplasia compatible with coeliac disease (Salmi et al. 2006a). In the literature, patients with normal villous morphology and some abnormality in coeliac disease-related markers are also called potential coeliac disease patients (Arranz et al. 1994). Some patients, diagnosed in childhood, have developed tolerance to gluten after some years on a gluten-free diet. During this tolerance they have neither symptoms nor ongoing small-bowel mucosal damage despite long-term gluten consumption (Shmerling and Franckx 1986, Matysiak-Budnik et al. 2007, Kurppa et al. 2008). Of coeliac disease patients diagnosed in childhood 6.6% to 20% develop tolerance to gluten, but the latency may be transient or the patient may even change the disease phenotype and

develop dermatitis herpetiformis after decades of a gluten-containing diet (Shmerling and Franckx 1986, Matysiak-Budnik et al. 2007, Kurppa et al. 2008). Latent coeliac disease has also been described in a patient with giardiasis and active coeliac disease, which recovered to the latent stage without a gluten-free diet when giardiasis was treated (Carroccio et al. 2001).

Patients having early-stage coeliac disease or latent coeliac disease may be symptomatic and even develop osteoporosis before they evidence small-bowel mucosal villous atrophy (Kaukinen et al. 2001, Kaukinen et al. 2005). Furthermore, it has also been recently observed that serum coeliac autoantibody-positive patients without villous atrophy benefit from a gluten-free diet similarly to those with villous atrophy and crypt hyperplasia compatible with coeliac disease (Kurppa et al. 2009). Even one case of malignancy associated with coeliac disease has been described as a first manifestation of early-stage coeliac disease (Freeman and Chiu 1986). For these reasons, developing coeliac disease would preferably be found earlier and several studies have been conducted to establish a diagnostic tool sufficiently sensitive and specific. Serum autoantibody positivity without villous atrophy may predict the development of coeliac disease, but some patients are initially seronegative with intact small-intestinal villous morphology, and autoantibodies appear later in the serum (Collin et al. 1993, Salmi et al. 2006a).

An increase in IELs is an early phenomenon during coeliac disease development (Marsh 1992), although densities of IELs may remain within normal range in early-stage coeliac disease (Iltanen et al. 1999b). Elevated densities of CD3+ IELs without villous atrophy may predict forthcoming coeliac disease, but they are not specific for this condition (Mahadeva et al. 2002, Salmi et al. 2006a). For example, Helicobacter pylori infection may increase IEL densities (Memeo et al. 2005). According to one recent study the sensitivity and specificity of an increased density of CD3+ IELs in predicting developing coeliac disease have been 59% and 57%, respectively (Salmi et al. 2006a). Elevated densities of γδ+ IELs can be regarded as a marker of coeliac disease without villous atrophy as they are also found in dermatitis herpetiformis patients with normal morphology in the small-bowel mucosa (Savilahti et al. 1992). High densities of γδ+ IELs have also been described in the early stage of coeliac disease (Mäki et al. 1991a, Iltanen et al. 1999c, Salmi et al. 2006a). Nonetheless, a raised density of γδ+ IELs is not specific for coeliac

disease– a similar increase has been observed in patients with small-intestinal bacterial overgrowth (Remes-Troche et al. 2008), cow milk-sensitive enteropathy, post-enteritis syndrome (Spencer et al. 1991) and other autoimmune disorders (Iltanen et al. 1999a). Furthermore, elevated densities of γδ+ IELs have shown no correlation with coeliac-type HLA-DQ genetics (Iltanen et al. 1999c). The sensitivity and specificity of increased densities of γδ+ IELs have been 76% and 60%, respectively, in a recent study (Salmi et al. 2006a).

In the normal small-bowel mucosa IELs are distributed in descrendo pattern, i.e. there are few scattered IELs in the upper third of the villi and the density of IELs increases downwards. In contrast, evenly distributed IELs are also to be found in coeliac disease. In addition, increased numbers of villous tip IELs have been reported to improve the accuracy of diagnostics in coeliac disease (Goldstein and Underhill 2001). Analysing villous tip IELs can also be used as a marker of forthcoming coeliac disease, its sensitivity and specificity being 84-88% and 71-88%, respectively, in detecting coeliac disease without villous atrophy (Järvinen et al. 2004).

In a small number of patients, determining small-bowel mucosal TG2-specific IgA deposits has proved a promising tool in detecting early-stage coeliac disease. The sensitivity and specificity of determining TG2-specific IgA autoantibody deposits have been 93% in patients with normal small-intestinal mucosal morphology who have subsequently developed villous atrophy with crypt hyperplasia (Korponay-Szabo et al. 2004, Kaukinen et al. 2005, Salmi et al. 2006a).

7. EPIDEMIOLOGY

During past decades coeliac disease was thought of as a rare condition with a prevalence varying from 1:600 (Mylotte et al. 1973) to 1:2000 (Logan et al. 1986). However, after population-based screening studies with serum autoantibodies became available, the biopsy proven prevalence of coeliac disease has risen to 1% in Europe, the Indian subcontinent and North America (Korponay-Szabo et al. 1999, Fasano et al. 2003, Mäki et al. 2003, Tommasini et al. 2004, Demirceken et al. 2008, Bhattacharya et al. 2009). Areas with lower prevalences (0.5-0.1%) of coeliac

disease have been reported in Brazil, Tunisia, Estonia, Russian Karelia and Greece (Akbari et al. 2006, Ben Hariz et al. 2007, Oliveira et al. 2007, Ress et al. 2007, Roka et al. 2007, Kondrashova et al. 2008). A higher prevalence of 3% has been reported in Swedish children (Myleus et al. 2009). The highest prevalence of coeliac disease has been found in Saharawi, where 5.6% of children are coeliac disease autoantibody-positive (Catassi et al. 1999).

It has recently been shown that the prevalence of coeliac disease in the population in general is increasing over time, indicating that the phenomenon is not due merely to better diagnostics (Lohi et al. 2007, Rubio-Tapia et al. 2009b). Furthermore, coeliac disease is more common in the elderly population than in the younger and the prevalence of coeliac disease in older patients is at present over two per cent in Finland (Vilppula et al. 2009).

8. GENETICS

The genetic background of coeliac disease is strong which means an increased risk of the disorder in family members of coeliac patients. Concordance is 80% between monozygotic twins and 20% between dizygotic twins (Greco et al. 2002, Fasano et al. 2003, Nistico et al. 2006). The majority of coeliac disease patients express the HLA DQ2 molecule, encoded by the alleles DQA1*05 and DQB1*02 either in cis or trans position and almost all the remainder have HLA DQ8 (DQA1*03 and DQB1*0302) (Sollid et al. 1989, Spurkland et al. 1997). The majority of coeliac patients with neither HLA DQ2 nor HLA DQ8 express half of the DQ2 molecule encoded by either DQA1*05 or DQB1*02 (Karell et al. 2003). Patients carrying two copies of DQB1*02 are at a greater risk of disease than those with only one copy, but the effect of the gene dosage of HLA DQ2-encoding genes on the severity of coeliac disease is uncertain (Vader et al. 2003b, Karinen et al. 2006, Murray et al. 2007). The HLA type (Celiac 1 locus) typical for coeliac disease is present in about 30% of the general population (Sollid et al. 1989). It is thus not sufficient genetic factor alone and many studies have been carried out to explain other genetic risk factors (Greco et al. 2001).

Several regions are associated with coeliac disease. The Celiac 2 locus (5q31-33) encodes many cytokines (Greco et al. 2001, Liu et al. 2002), Celiac 3 (2q23-32) regulators of T-lymphocyte activation, CTLA-4, CD28 and ICOS (Holopainen et al. 1999, Holopainen et al. 2004) and Celiac 4 (19p13) unconventional myosin 9B, which is involved in cellular permeability control (Van Belzen et al. 2003, Monsuur et al. 2005). It has recently been shown that certain alleles of myosin 9B together with HLA-DQ2 homozygosity may be involved in developing unresponsive coeliac disease (Wolters et al. 2007).

9. PATHOGENESIS

In the pathogenesis of coeliac disease both environmental factors and genetic factors exert a joint influence. The role of environmental factors other than gluten is uncertain, but it seems that some common viral infections such as adenovirus 12 and rotavirus infections in a genetically susceptible population might enter into coeliac disease development through molecular mimicry (Kagnoff et al. 1984, Stene et al. 2006). Also hepatitis C virus has been studied as a possible cause of an autoimmune process (Fine et al. 2001). The importance of infections during the mother's pregnancy and at the time of weaning is reflected in the variation of coeliac disease incidence according to birth season (Ivarsson et al. 2003). In addition to predisposing factors, protective factors such as breastfeeding during gluten introduction are also known (Ivarsson et al. 2002).

Gluten, gliadin from wheat and the related storage proteins hordein from barley and secalin from rye, is rich in proline and thus resistant to degradation of proteases in the gastrointestinal tract (Vader et al. 2003a, Kagnoff 2007). In coeliac disease patients gluten peptides increase the permeability of the small-bowel epithelium by affecting zonulin in tight junctions (Drago et al. 2006). In untreated coeliac disease, gluten peptides also cross the epithelial barrier of the small-intestinal mucosa via transcytosis. (Heyman and Menard 2009, Zimmer et al. 2009). However, after entrance to the lamina propria, gluten activates mechanisms of both the innate and adaptive immune systems in coeliac disease patients, as presented in Figure 2.

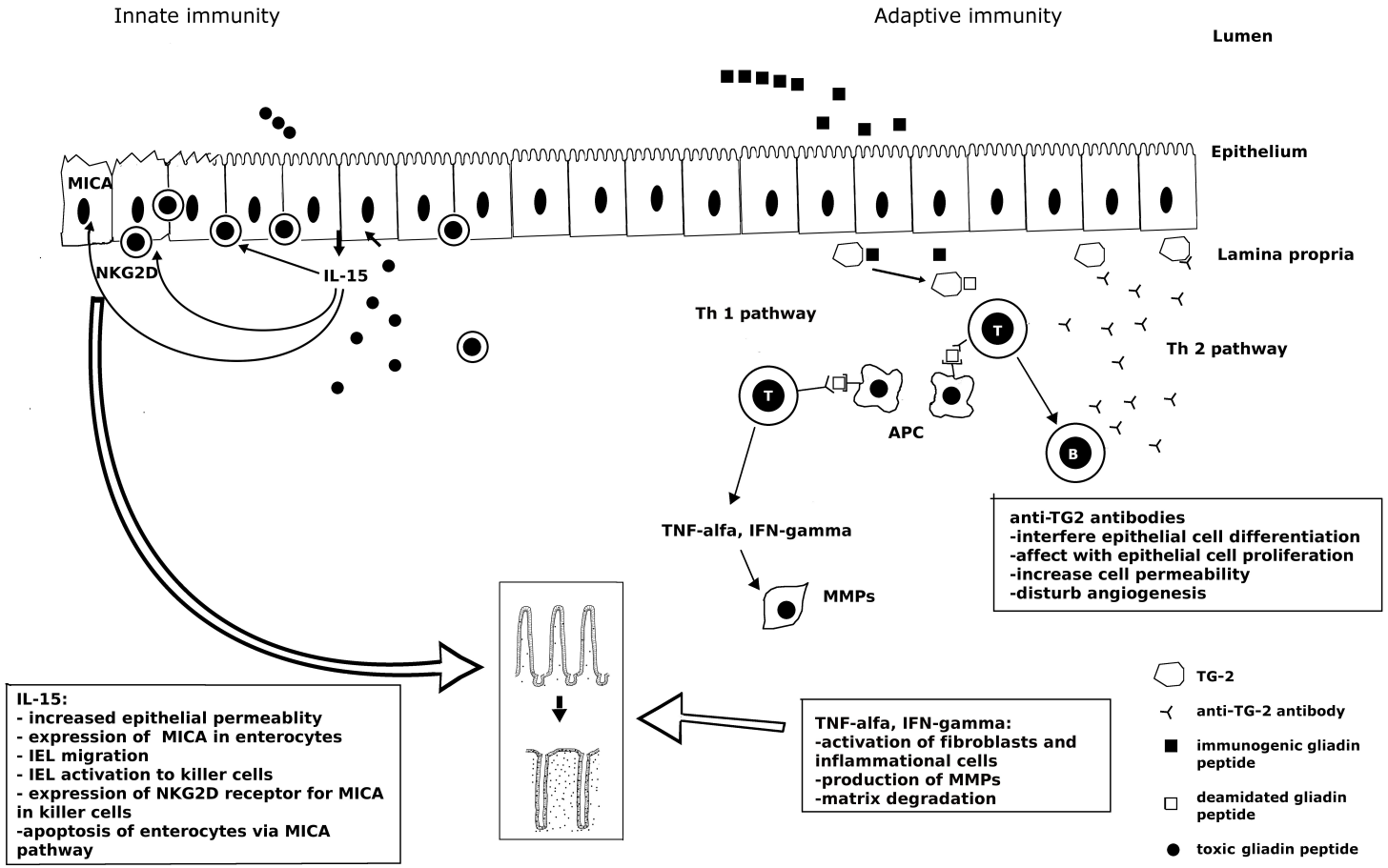

Figure 2. Summary of mechanisms of the innate and adaptive immune systems involved in the pathogenesis of coeliac disease. Th, T helper cell; TNF, tumor necrosis factor; IFN, interferon; TG, transglutaminase; IEL, intraepithelial lymphocyte; MMP, matrix metalloproteinase; MICA, major histocompability complex I class molecule A; IL, interleukin NKG2D, natural killer group 2D (receptor of MICA).

The so-called toxic gliadin peptide p31-43 causes activation of innate immune system. This peptide induces increased interleukin (IL)-15 production in both epithelium and lamina propria (Maiuri et al. 2003, Hue et al. 2004), causing migration and activation of IEL to killer cells (Maiuri et al. 2001, Mention et al. 2003, Meresse et al. 2004). IL-15 also induces expression of major histocompability complex class I molecule A (MICA) in enterocytes as well as expression of MICA receptor NKG2D in natural killer (NK) cells, αβ and γδ IELs (Roberts et al. 2001, Mention et al. 2003, Hue et al. 2004, Meresse et al. 2004). Activated IELs drive cells expressing MICA into apoptosis, causing intestinal damage (Hue et al. 2004).

Gliadin peptide p56-89 activates the adaptive immune system after being deamidated by TG2. After deamidation of glutamine to glutamic acid, gliadin peptide fits into grooves in HLA DQ2 or DQ8 molecules of antigen presenting cells (APCs) (Molberg et al. 1998). Deamidation by TG2 is thus essential for gliadin peptide to become immunogenic. APCs present peptide to T-cells, causing activation of both T-helper cell (Th) 1 and Th2 pathways. In the Th1 pathway T-cells produce proinflammatory cytokines such as TNF-α and IFN-γ (Nilsen et al. 1995), which activate fibroblasts and inflammatory cells to produce matrix metalloproteinases (MMP) responsible for matrix degradation and small-intestinal mucosal damage (Pender et al. 1997). In the Th2 pathway B-cells are activated to produce anti-TG2 antibodies (Sollid et al. 1997).

The effect of coeliac disease autoantibodies on TG2 activity itself is debated. Esposito and colleagues (2002) showed the inhibitory effect of coeliac autoantibodies on TG2, whereas it was recently shown that coeliac autoantibodies increases the activity of TG2 (Kiraly et al. 2006, Myrsky et al. 2009). *In vitro*, coeliac autoantibodies interfere with the differentiation of epithelial cells (Halttunen and Mäki 1999), have effects on epithelial cell proliferation (Barone et al. 2007), increase epithelial cell permeability (Zanoni et al. 2006) and disturb angiogenesis (Myrsky et al. 2008).

10. TREATMENT

Currently the only treatment for coeliac disease has been removal of the cause of the small intestinal lesion by omitting wheat, rye and barley from the daily diet (Walker-Smith et al. 1990, Catassi et al. 2001). A gluten-free diet is a lifelong treatment. Some patients diagnosed in childhood have, however, returned to gluten-containing foods after years on a gluten-free diet and they may tolerate gluten for years or decades before symptoms reappear (Matysiak-Budnik et al. 2007, Hopman et al. 2008, Kurppa et al. 2008).

A gluten-free diet may be difficult to adhere to; it may be restrictive, the availability of gluten-free products may be limited and these products may be expensive (Hall et al. 2009). A strict gluten-free diet, though it can be burdensome, nevertheless improves the quality of life of patients (Mustalahti et al. 2002a, Wagner et al. 2008). Furthermore, those patients with poor adherence to the diet have ongoing small-bowel mucosal lesions ranging from increased inflammation to villous atrophy (Troncone et al. 1995). In several studies adherence to a strict gluten-free diet has varied between 36% and 96% (Högberg et al. 2003, Viljamaa et al. 2005). Early onset and diagnosis of coeliac disease makes the diet easier to follow, whereas asymptomatic patients often show poor compliance (Fabiani et al. 2000, Högberg et al. 2003, Wagner et al. 2008).

In addition to relief of the classical symptoms of coeliac disease, a gluten-free diet increases bone mineral density (Kemppainen et al. 1999b) and reduces the risk of fractures (Olmos et al. 2008), reduces mortality (Corrao et al. 2001), and the risk of developing most cancers to the same level as in the population generally (Holmes et al. 1989) and lowers the incidence of amenorrhoea and abortions due to coeliac disease (Kotze 2004). The role of a gluten-free diet in preventing subsequent autoimmune disorders in coeliac disease patients remains controversial (Ventura et al. 1999, Sategna Guidetti et al. 2001, Biagi et al. 2002, Viljamaa et al. 2005, Cosnes et al. 2008).

Monitoring of the response to a gluten-free diet is important in ensuring adequate strictness of the diet. However, complete recovery of the small-intestinal mucosa usually requires more than one year on a gluten-free diet (Collin et al. 2004a). Serum autoantibodies disappear soon after gluten withdrawal and they are used to

reveal dietary transgressions, although serum autoantibodies may also be absent despite persisting small-bowel mucosal damage (Troncone et al. 1995, Dickey et al. 2000, Kaukinen et al. 2002b). Persistent villous atrophy despite diet increases the risks of complications, even in asymptomatic coeliac patients (Kaukinen et al. 2007b). Examination of the morphology of the small-bowel mucosal biopsy is thus the best method to follow-up the response to a gluten-free diet.

10.1 Oats in coeliac disease

Among the cereals oats is distinct, belonging to different a tribe than wheat, rye and barley its storage protein, avenin, contains less prolines than gliadin, secalin or hordein (Jabri et al. 2005). Traditionally oats has been forbidden in coeliac patients' gluten-free diet (Weijers and van de Kamer 1965).However, Janatuinen and associates (1995) showed that coeliac disease patients can tolerate oats. Since then several studies have found that ingested oats in coeliac patients in remission caused neither symptoms nor histological relapse (Srinivasan et al. 1996, Janatuinen et al. 2000, Janatuinen et al. 2002, Storsrud et al. 2003c, Peräaho et al. 2004, Holm et al. 2006). In addition, oats improves iron, fiber, thiamin and zinc intake in a gluten-free diet (Storsrud et al. 2003a). In newly diagnosed coeliac disease patients oats seems not to prevent improvement of the small-bowel mucosa (Janatuinen et al. 1995, Hoffenberg et al. 2000, Högberg et al. 2004). The suitability of oats has also been shown in dermatitis herpetiformis (Hardman et al. 1997, Reunala et al. 1998). Oats would not appear to evoke serum autoantibody formation or small-bowel mucosal damage in most coeliac patients (Picarelli et al. 2001, Kilmartin et al. 2003, Hollen et al. 2006, Srinivasan et al. 2006). Despite the tolerance to oats shown in a majority of studies, some patients develop symptoms and even small-bowel mucosal damage after ingestion of oats (Lundin et al. 2003, Arentz-Hansen et al. 2004, Peräaho et al. 2004). Moreover, T-cells reactive to proline-rich areas of avenin have been found in some coeliac disease patients' small-intestinal mucosa (Arentz-Hansen et al. 2004).

10.2 New treatments

Currently the only treatment for coeliac disease is a strict gluten-free diet (Walker-Smith et al. 1990) but new therapeutic options have been widely studied. *In vitro* studies on blocking of HLA DQ2-mediated gluten presentation to T-cells (Xia et al. 2007), inhibition of TG2 activity by gluten peptide analogs (Siegel et al. 2007) or inhibition of paracellular permeability (Paterson et al. 2007) have been performed to obtain new treatment options for coeliac disease. Also germinating wheat (Stenman et al. 2009) and barley enzymes together with bacterial endopeptidases (Gass et al. 2007) have had promising effects on gluten degradation, thus diminishing gluten toxicity. Some of these treatments have been tested in humans (Paterson et al. 2007), but in future, when more new clinical drug trials will be conducted, sensitive and gluten-specific tools to detect immunoreactions caused by gluten will be needed.

11. COMPLICATIONS

11.1 Non-responsive coeliac disease and refractory sprue

Among the criteria for coeliac disease, either a histological or a clinical response to a gluten-free diet is obligatory for diagnosis (Walker-Smith et al. 1990). However, about 1 to 8% of coeliac disease patients do not respond histologically to a gluten-free diet (O'Mahony et al. 1996, Kaukinen et al. 2007b). The main reason for an insufficient response is poor adherence to the diet or gluten contamination in the diet (Leffler et al. 2007). Gluten contamination can easily be suspected if serum autoantibodies are positive despite the gluten-free diet (Leffler et al. 2007). In addition, the diagnosis of coeliac disease may be incorrect, since small-bowel villous atrophy is not specific solely for coeliac disease (Green and Cellier 2007).

Only 7-18% per cent of patients with non-responsive coeliac disease have symptomatic refractory coeliac disease (Wahab et al. 2002, Leffler et al. 2007). Diagnosis of both non-responsive and refractory coeliac disease is challenging in

that in these cases small-bowel mucosal villous atrophy does not respond to a gluten-free diet as it should. Moreover, serum coeliac autoantibodies usually give no additional support, as most patients with refractory coeliac disease are serum autoantibody-negative during a gluten-free diet in spite of the failure of small-bowel mucosa to recover (Kaukinen et al. 2007b, de Mascarel et al. 2008, O'Shea et al. 2008, Verbeek et al. 2008b). The question thus arises as to whether these patients are really coeliac disease patients.

Refractory coeliac disease patients can be divided into two types: refractory coeliac disease type I with a normal IEL population and type II with an aberrant IEL population in the small-intestinal mucosa. Usually, the lymphocytes in an abnormal IEL population lack TCR-γδ (Verbeek et al. 2008b) and express neither CD3 nor CD8 molecules on their surface, but heterogeneity in the phenotypes of these cells is to be found (Farstad et al. 2002). In addition, expression of the CD30 molecule in IELs seems to be an indicator of poor prognosis (Farstad et al. 2002) as is lack of γδ+ IELs (Verbeek et al. 2008b). In consequence of the heterogeneity of IEL phenotypes, refractory coeliac disease patients cannot be straightforwardly divided into refractory coeliac disease types I and II only by expression of cell surface molecules. In addition, rearrangement of the TCR-γ gene is found in patients with refractory coeliac disease type II (de Mascarel et al. 2008).

In refractory coeliac disease type I the prognosis of patients is good and the five year survival rate varies from 80% to 96% (Al-Toma et al. 2007, Rubio-Tapia et al. 2009a). Patients with type II refractory disease, in contrast are at an increased risk of developing EATL; in a recent study 52% developed EATL during four to six years' follow-up (Al-Toma et al. 2007) and the overall five-year survival rate is between 45% and 58% in type II patients (Al-Toma et al. 2007, Rubio-Tapia et al. 2009a). It has in fact also been suggested that an abnormal IEL population in the small-intestinal mucosa is an early manifestation of EATL (Cellier et al. 2000, Daum et al. 2001).

Refractory coeliac disease is treated with immunosuppressive drugs in conjunction with a strict gluten-free diet (Al-Toma et al. 2007, Rubio-Tapia et al. 2009a). Refractory coeliac disease type I responds well to azathioprine after clinical remission is achieved by corticosteroids (Al-Toma et al. 2007). Case reports have also been published where patients have been treated with cyclosporine A,

infliximab or tacrolimus (Al-Toma et al. 2007). Refractory coeliac disease type II can be treated similarly to type I, but the response is often poor. Some cases have benefited from autologous stem cell transplantation (Al-Toma et al. 2007, Rubio-Tapia et al. 2009a).

11.2 Malignancies

In studies published years ago, coeliac disease patients were at an increased risk of developing a number of malignancies; cancers of mouth, pharynx and oesophagus, small-bowel adenocarcinomas and lymphomas, particularly non-Hodgkin lymphomas (Swinson et al. 1983, Holmes et al. 1989). Current studies indicate that the total risk of malignancies is not elevated, but in certain types of malignancies, i.e. non-Hodgkin lymphomas, the risk is higher in coeliac disease patients than in the general population (West et al. 2004). On a gluten-free diet, the risk of malignancies is reduced and after five years the risks of most cancers, except non-Hodgin lymphomas, have returned to the level of the population provided that the diet is strict (Holmes et al. 1989).

EATL is a rare type of non-Hodgkin lymphomas with an incidence of 0.1 per 100 000 (Verbeek et al. 2008a). It is situated usually in the proximal small-intestine and is found almost exclusively in coeliac disease patients (Brousse and Meijer 2005, Verbeek et al. 2008a).The prognosis is poor. In a recent study the 5-year survival rate was 8% despite of chemotherapy (Al-Toma et al. 2007). Another type of non-Hodgkin lymphoma occurring in coeliac disease patients is B-cell lymphoma, which is usually located outside the small intestine and is more common than EATL (Sigurgeirsson et al. 1994, Hervonen et al. 2005).

THE PRESENT STUDY

12. AIMS OF THE STUDY

The aim of this study was to establish, whether determination of TG2-specific IgA deposits in the small-bowel mucosa is an accurate tool in the diagnostic work-up and follow-up of coeliac disease when compared to conventional histology and serology.

Specific aims were to ascertain:

1. The usefulness of small-bowel mucosal TG2-specific IgA autoantibody deposits in diagnosing overt coeliac disease (**I**)
2. The effect of a gluten-free diet (**I-IV)** and oats (**II**) on small-bowel mucosal IgA deposits.
3. The advantages of measurement of small-bowel mucosal IgA deposits in ascertaining the diagnosis of coeliac disease in patients who do not completely fulfil the current diagnostic criteria for coeliac disease i.e. non-responsive (**III**) and early-stage coeliac disease (**IV**)

13. PATIENTS

*13.1 Coeliac disease patients (**I-IV**)*

This study was carried out mainly retrospectively and small-bowel biopsy specimens analyzed in this study were taken between the years 1993 and 2007. Small-bowel mucosal biopsy specimens from 379 IgA-competent coeliac disease patients were analyzed during this study (see Table 4). Specimens were taken from 261 patients with overt coeliac disease either at the time of the diagnosis or during a gluten challenge (**I, II, IV**). Further, on a gluten-free diet of one year, a frozen follow-up biopsy was available from 72 of these 261 patients (**I-IV**). In addition, 105 coeliac disease patients with a median of 8.0 (range 2.0-41.0) years on a gluten-free diet were examined (**I**). The remaining patients recovered during the diet, but the use of frozen biopsy specimens is not routine practice in follow-up and thus these patients' specimens were not available for analysis. Diagnosis of all these patients was based on the presence of villous atrophy and crypt hyperplasia in the small-bowel mucosa on a gluten-containing diet, thus fulfilling the current diagnostic criteria (Walker-Smith et al. 1990, Catassi et al. 2001).

To reveal the gluten dependency of small-bowel mucosal TG2 specific IgA deposits, 23 children with coeliac disease in remission on a gluten-free diet were challenged with gluten-containing cereals in study **II**. Thirteen patients were randomized to add only oats to their otherwise gluten-free diet and ten patients also wheat, rye and barley (Figure 3). Follow-up biopsies were taken from these patients in the baseline situation, after six months and after 24 months of ingestion of oats. In addition, gluten-challenged patients were biopsied when relapse was suspected on the basis of symptoms or serology.

Small-bowel mucosal biopsy specimens were taken from 27 patients with non-responsive coeliac disease, including five with refractory sprue and six with enteropathy associated T-cell lymphoma (duration of gluten-free diet median of 6.0 (range 0.1-24.0) years and six patients with poor adherence to diet (median time of diet 13.5, range 6.0-18.0 years) (**III**).

Table 4. Summary of patients and controls in studies **I-IV**

Study	Untreated CD	Treated CD	Long-term treated CD	Challenged CD	Complicated CD	Early-stage CD	Non CD
I	261	72	105	-	-	-	78
II		-	-	23	-	-	-
III	28*	28*	-	-	27	-	10
IV	13*	13*	-	-	-	48	42*

* also included in study I; CD, coeliac disease

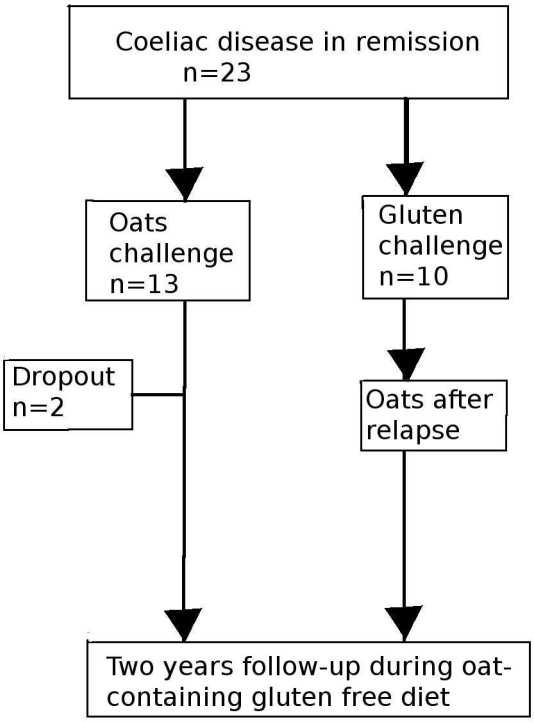

Figure 3. Study protocol of oat and gluten challenge study (II). Oats challenge= oats added to a gluten-free diet; gluten challenge= a diet containing wheat, rye, barley and oats.

A total of 48 patients with clinical suspicion of coeliac disease and intact villous morphology were biopsied (**IV**). Of these 48, 28 continued on a gluten-containing diet (median 1.7, range 0.2-7.4 years), developed overt coeliac disease and started a gluten-free diet (follow-up time median 1.4 range 0.8-5.9 years). These 28 are referred to as early-stage coeliac disease patients (**IV**). The remaining 20 symptomatic patients with small-bowel mucosal TG2-specific IgA autoantibody deposits started on a gluten-free diet prior to fulfilling the current criteria (Walker-Smith et al. 1990, Catassi et al. 2001) to find evidence for gluten dependency. As clinical improvement was clear, they were considered as coeliac disease patients and referred to as the potential coeliac disease group; from these patients follow-up specimens were also taken after one year's gluten withdrawal.

*13.2 Controls (**I, III, IV**)*

Small-bowel mucosal biopsy specimens were taken from 86 IgA-competent patients without coeliac disease (**I, III, IV**) (Table 4). Of these, 43 were initially suspected of having coeliac disease, but during follow-up on a normal, gluten containing-diet (median 6.0, range 0.8-10.0 years) they did not develop villous atrophy and coeliac disease was thus excluded. The final diagnoses of non-coeliac control patients were functional dyspepsia in 28, irritable bowel syndrome in 20, gastro-oesophageal reflux disease in 13, autoimmune enteropathy in 4, collagenous colitis in 3, inflammatory bowel disease in 3, lactose intolerance in 2 and aphtous stomatitis of unknown origin in 2. In the remaining 10 cases there were no special findings and during the follow-up their symptoms disappeared. They were considered healthy.

*13.3 Dietary intervention for coeliac disease patients (**II**)*

A total of 23 children in remission during a gluten-free diet, together with parents, were given instructions by a trained dietician on the oat-containing gluten-free diet and gluten challenge (Holm et al. 2006). A detailed dietary analysis was assessed repeatedly by means of interview and 4-day record of food intake. At the outset, thirteen children were randomized to undergo oats challenge. The rolled, uncontaminated oats (Melia Ltd, Raisio, Finland) was given to the patients free of charge. During a two-year trial, the median daily consumption of oats was 45 g/day (range 13-81 g/day) in the oats challenge group. One patient this group had transient dietary lapses at the six-month time-point; all the rest adhered to a strict gluten-free diet throughout the study (Holm et al. 2006).

A total of ten children were randomized to the gluten challenge group. In this group the median daily intake of gluten in the form of wheat-, rye-, barley- and oat-containing normal bread was 14 g (range 7-19 g/day) and after adopting an oat-containing gluten-free diet the median intake of oats was 41 g/day, range 24-59 g/day.

14. METHODS

*14.1 Small-bowel biopsy (**I-IV**)*

Seven forceps biopsies were taken upon upper gastrointestinal endoscopy or one specimen by adult-size Watson capsule from the distal duodenum or proximal jejunum. Five forceps biopsies or one piece of capsule biopsy were formalin-fixed and embedded in paraffin. The remaining two forceps biopsies and one piece of capsule biopsy was embedded in optimal cutting temperature compound (OCT; Tissue-Tec, Miles Inc, Elkhart, IN, USA), snap-frozen and stored in -70°C. Determination of the Vh/CrD ratio and the percentage of CD3+CD8- IELs was carried out by two investigators, determination of small-bowel mucosal TG-2-specific IgA deposits and CD3+ and γδ+ IEL densities by several investigators. All specimens were evaluated without prior knowledge of disease history or laboratory findings. Intra- and interobserver variations are given in the context of each method if known.

*14.1.1 Morphometrical studies (**I-IV**)*

Haematoxylin-eosin staining was carried out on well-oriented 2µm thick paraffin-embedded sections. From these specimens villous height crypt depth ratio (Vh/CrD) was determined as Kuitunen and colleagues (1982) have previously described. A Vh/CrD ratio below two was considered compatible with untreated coeliac disease or inadequate response to a gluten-free diet.

*14.1.2 Detection of small-bowel mucosal TG2-specific IgA deposits (**I-IV**)*

Frozen, unfixed, 5µm-thick sections were stained by direct immunofluorescence using fluorescein isothiocyanate-labelled rabbit antibody against human IgA (Dako

AS, Glostrup, Denmark). Sections were incubated in a humidified chamber for 30 minutes at room temperature in the antibody at a dilution of 1:40 in phosphate buffered saline (PBS). To ascertain whether there is co-localization of IgA and TG2, sections from all specimens were double-stained with anti-IgA antibody as described above and using monoclonal mouse antibodies against TG2 at a dilution 1:200 (CUB7402, NeoMarkers, Fremont, CA, USA), followed by rhodamine-conjugated antimouse immunoglobulin antibodies (DAKO) diluted 1:120 in PBS. In coeliac disease subepithelial deposition can be found along the villous and crypt epithelium and around vessels, in contrast to non-coeliac samples where endogenous IgA is found inside plasma cells and epithelial cells (Korponay-Szabo et al. 2004, Kaukinen et al. 2005). The intensity of the IgA autoantibody deposits was classified either strong (+++), moderate (++), weak (+) or negative. In some specimens the intensity of depositions varied and in these cases the mean of these areas was regarded as intensity of the whole sample. The evaluation of samples was carried out in blinded fashion without knowledge of the patient's disease history. Intra- and interobserver variation has been 0.98 in earlier studies (Salmi et al. 2006b). Henceforward small-bowel mucosal TG2-specific IgA deposits are referred to with the term mucosal autoantibody deposits.

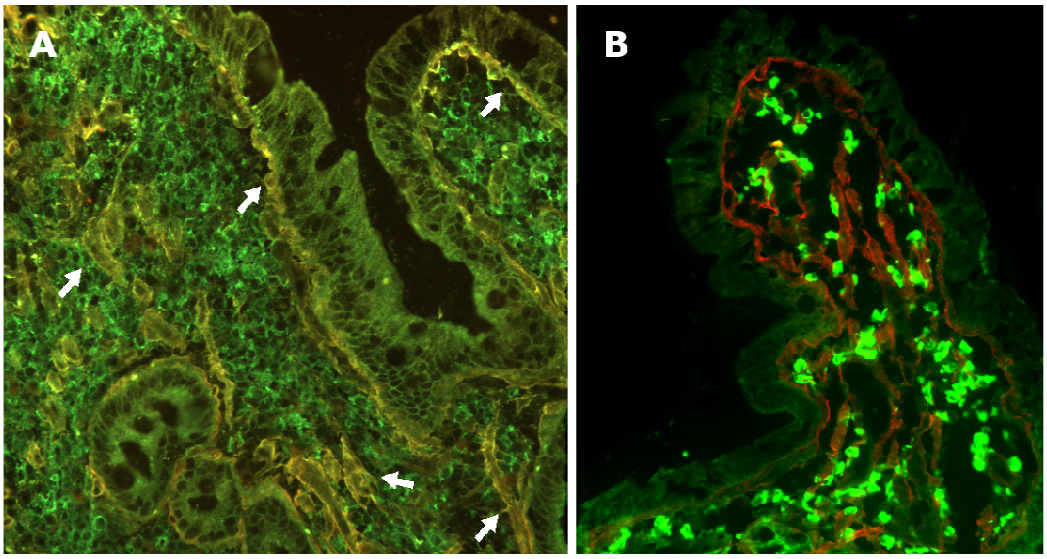

Figure 4. Immunofluorescense staining of transglutaminase-2-specific IgA deposits from small-bowel mucosa. IgA is stained in green, transglutaminase-2 in red and co-localization of them in yellow. In Figure A, strong (+++) transglutaminase-2-specific IgA depositions (arrows) can be seen under both villous and crypt epithelium and around blood vessels in a coeliac disease patient. In Figure B no co-localization of IgA and transglutaminase is found in a non-coeliac control.

*14.1.3 Intraepithelial lymphocytes (**I, III, IV**)*

Immunohistochemical staining of CD3+ and γδ+ IELs was carried out on frozen sections. CD3+ IELs were stained with monoclonal antibody Leu-4 (Becton Dickinson, San Jose, CA, USA) and γδ+ IELs with T-cell receptor -γ antibody (Endogen, Woburn, MA, USA) (**I, III, IV**). Positively stained CD3+ and γδ+ IELs were counted at a magnification x 100 throughout the surface epithelium. The result was expressed as IEL density/mm of epithelium and at least 30 fields were counted. Upper limits for normal values were set at 37 cells/mm of epithelium for CD3+ and 4.3 cells/mm of epithelium for γδ+ IELs (Järvinen et al. 2003) (**I, III, IV**). Intra- and interobserver variation for CD3+ IELs has been 0.95 and 0.92, respectively, and for γδ+ IELs 0.98 (Salmi et al. 2006b).

In addition, staining of CD3+, CD8+, CD30+ IELs was carried out on paraffin-embedded sections, applying the standard immunoperoxidase method (Cellier et al. 1998) with the following antibodies (all from Lab Vision Corporation, Fremont, CA, USA) CD3 (dilution 1:150), CD8 (1:50) and CD30 (1:60) (**IV**). Positively stained intraepithelial CD3+, CD8+, CD30+ lymphocytes were counted from randomly selected surface epithelium and expressed as IELs per 100 epithelial cells. The percentage of CD3+CD8- IELs was determined in patients with high numbers of IELs; a proportion above 52% was considered abnormal (Verkarre et al. 2003) (**IV**).

*14.2 Serology (**I-IV**)*

During the study period serum EmA (primate-type reticulin) replaced the ARA (rodent-type reticulin) test. Both of these antibody tests have been shown to measure anti-TG2 antibodies (Korponay-Szabo et al. 2000, Korponay-Szabo et al. 2003b), and the two tests have been shown to be virtually identical (Sulkanen et al. 1998). ARA and EmA were determined by an indirect immunofluorescence method, a serum dilution of 1:≥5 being considered positive (Sulkanen et al. 1998) (**I, III, IV**). In addition, serum IgA-class antibodies against TG2 were detected by enzyme-

linked immunosorbent assay (ELISA) using human recombinant TG2 as antigen (Celikey, Phadia, GmbH, Freiburg, Germany). Concentrations over 5.0 U/ml were considered abnormal (**I**, **II, III**). Serum autoantibody positivity means that either ARA/EmA or anti-TG2 or both autoantibody tests are positive.

*14.3 HLA-typing (**I, III, IV**)*

A total of 165 patients were genotyped either for HLA-DQB1*02 and *0302 alleles (corresponding to serological DQ2 and DQ8) using the DELFIA Celiac Disease Hybridization Assay (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) according to the manufacturer's instructions, or based on polymerase chain reaction with allele-specific primers identifying HLA DQ2 and DQ8 and performed with a Dynal DQ low-resolution SSp kit (Dynal AS. Oslo, Norway), or for HLA DQB1* allele groups were determined using the Olerup SSP DQ low-resolution kit (Olerup SSP AB, Saltsjobaden, Sweden). This method determines the HLA DQ2, DQ4, DQ5, DQ6, DQ7, DQ8 and DQ9 allele groups.

*14.4 Deoxyribonucleic acid (DNA) extraction and analysis of T-cell clonality (**IV**)*

DNA was extracted from paraffin-embedded biopsy samples with the Illustra DNA extraction nucleon HT kit (GE Healthcare, Buchinghamshire, UK) according to manufacturer’s instructions. One hundred nanograms of DNA were used as a template in two separate polymerase chain reactions (PCR) to detect the clonality of the TCR-γ gene as described elsewhere (Diss et al. 1995). DNA from T-cell line Jurkatt was used as a positive control for monoclonality. All PCRs were performed in duplicate.

*14.5 Ethical considerations (**I-IV**)*

The study protocols were approved by the Ethical Committee of Tampere University Hospital. All adult subjects and all children and their parents gave their written informed consent.

*14.6 Statistical analysis (**I-IV**)*

Sensitivities were calculated from the equation a/(a+c)*100, specificities d/(b+d)*100. In the equations, ‘‘a’’ denotes the number of biopsy-proven coeliac disease patients recognized by the test, ‘‘b’’ is the number of biopsy-proven non-coeliac disease controls with a positive test result, ‘‘c’’ denotes the number of coeliac disease patients misclassified by the test and ‘‘d’’ represents the non-coeliac disease controls negative for the test. The efficiency of test was calculated by dividing the number of patients correctly classified by the test as diseased and non-diseased by the number of all tested patients.

Quantitative data were expressed as medians and ranges. Statistical differences were evaluated using Mann-Whitney U test and Wilcoxon signed-rank test as appropriate and all testing was two-sided. Correlations were tested by Spearman’s correlation test. P- values < 0.05 were considered statistically significant. All statistical testing was performed using Statistical Package for the Social Sciences version 17 (SPSS Inc. Chicago, IL, USA).

15. RESULTS

*15.1 Diagnosing overt coeliac disease (**I**)*

A total of 261 (**I-IV**) small-bowel mucosal specimens from patients with overt coeliac disease were analyzed and compared to 86 specimens from controls without coeliac disease (**I, IV**). Demographic data and main symptoms leading to endoscopy in both groups are presented in Table 5. All patients with active coeliac disease had a Vh/CrD ratio under 2 by definition; in contrast, only five (6%) patients, with autoimmune enteropathy or Crohn's disease, out of 86 non-coeliac patients had villous atrophy. The Vh/CrD ratio was within normal limits in all the remaining non-coeliac controls (Table 7, Figure 6A).

Table 5. Demographic data on untreated coeliac patients and controls

	Coeliac disease n=261	**Controls** n=86
Age, median (range), years	47 (4-79)	49 (29-76)
Female, n (%)	167 (64)	54 (63)
Main symptom, n (%)		
Abdominal complaints	97 (37)	73 (85)
Malabsorbtion	21 (8)	7 (8)
Extra-intestinal symptoms*	38 (15)	6 (7)
Screening†	96 (37)	0
Other‡	9 (3)	0

* aphtous stomatitis, arthralgia, collagenosis, growth retardation, infertility, depression, memory disorders, ataxia, extra-pyramidal symptoms, renal failure, alopecia
† screening in risk groups (family members, autoimmune thyroid diseases, Sjögren's syndrome, IgA nephropathy, epilepsy, type 1 diabetes mellitus) and population screening
‡ after gluten challenge

Small-bowel mucosal TG2-specific IgA deposits were present in all active coeliac disease patients, being strong or moderate in 235 (90%) out of 261 cases. Of non-coeliac control subjects 14 (16%) out of 86 had weak IgA deposits in the small-bowel mucosa, giving a sensitivity and specificity of 100% and 84%, respectively for small-bowel mucosal IgA deposits in finding overt coeliac disease (Figure 5 and Table 14). Although all patients with overt coeliac disease had small-bowel mucosal

autoantibody deposits, serum EmA was negative in 24 (9%) out of the 261 cases. In all non-coeliac patients serum autoantibodies were negative.

Densities of CD3+ IELs were increased in 91% of coeliac disease patients with active disease and in 31% of the non-coeliac controls. Similarly, an increased density of γδ+ IELs was found in 96% of patients with active disease and in 27% of the non-coeliac controls. Medians and distribution of Vh/CrD and IELs are presented in Table 7 and Figure 6. Measuring the densities of IELs had lower sensitivity and specificity than determination of mucosal autoantibody deposits (Table 14).

*15.2 Follow-up on a gluten-free diet (**I**)*

At baseline, patients with available follow-up biopsies did not differ from those without in degree of small-bowel mucosal villous atrophy (p=0.982). During a gluten-free diet, clinical symptoms were alleviated and small-bowel mucosal atrophy completely recovered in 70% of the short-term treated and in 98% of the long-term treated patients. Demographic data on treated patients are shown in Table 6.

Table 6. Demographic data on coeliac disease patients examined during gluten-free diet.

	Short-term treated	**Long-term treated**
Age, median (range), years	47 (16-72)	54 (7-81)
Female, n(%)	48 (68)	70 (67)
Duration of gluten-free diet, median (range), years	1*	8 (2-41)

*duration of gluten-free diet was one year in all

The intensity of mucosal autoantibody deposits decreased statistically significantly (p<0.001) during a gluten-free diet; deposits were still present in 58 (82%) out of 71 short-term and in 59 (56%) of the 105 long-term treated patients (Figure 5). Serum autoantibodies normalized faster than mucosal autoantibody deposits, being positive in 15% of short-term treated patients and negative in all long-term treated patients, despite villous atrophy in two patients. After gluten withdrawal the small-bowel villous atrophy normalized more slowly than serum autoantibody levels, but faster

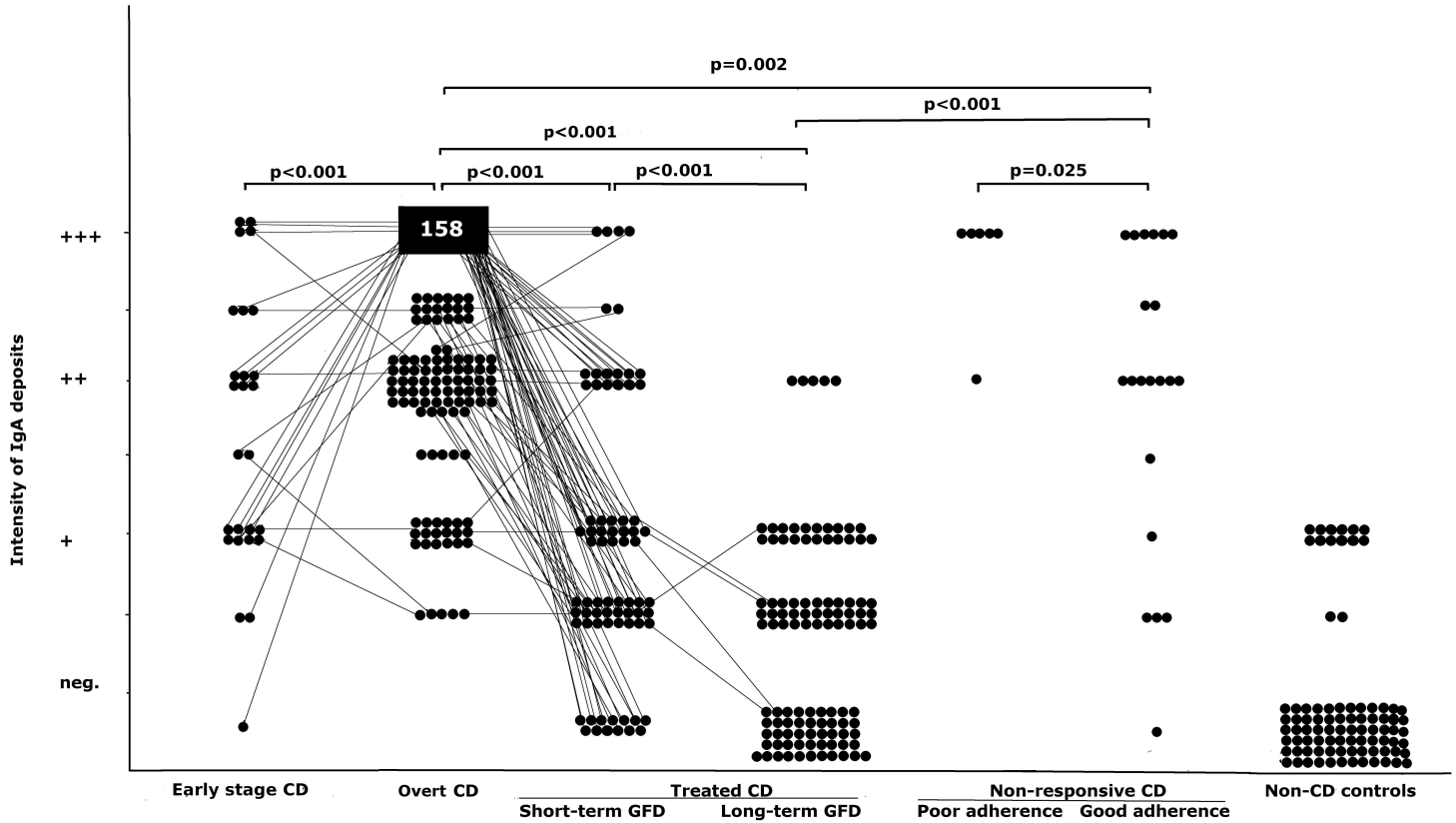

Figure 5. Intensity of small-bowel mucosal transglutaminase-2 specific IgA deposits in different study groups (**I, III, IV**) All coeliac groups differed statistically significantly from non-coeliac controls (p<0.001 in all). CD, coeliac disease; GFD, gluten-free diet.

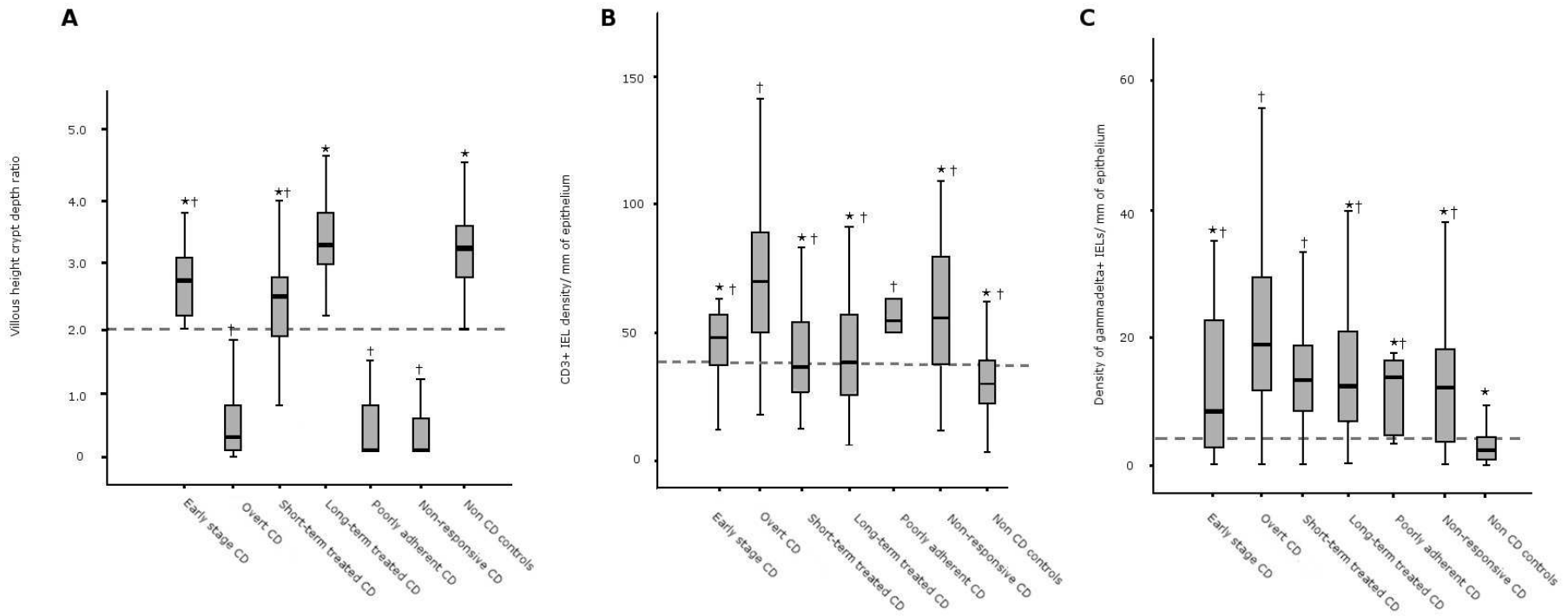

Figure 6. Villous height crypt depth ratio (A) densities of CD3+ (B) and γδ+ (C) IELs in different study groups; medians, quartiles and minimum and maximum values are shown. The dash line represents upper limit of normal range. Outliers, below or above more than 1.5x interquartile range are not shown. All statistically significant ($p<0.05$) p-values compared to either untreated coeliac disease (CD) or non-coeliac controls are given as follows * $p< 0.05$ compared to untreated CD and † $p< 0.01$ compared to non-coeliac controls.

than mucosal autoantibody deposits and the densities of CD3+ and γδ+ IELs. After long-term treatment, the increased density of γδ+ IELs persisted longer than other markers of coeliac disease. Medians and distribution of Vh/CrD and IELs are presented in Table 7 and Figure 6.

Table 7. Villous height crypt depth ratio, median (range) and densities of IELs per mm of epithelium, median (range) in coeliac patients with and without gluten in diet and in non-coeliac control patients.

	Vh/CrD	**CD3+ IELs**	**γδ+□□ IELs**
Active CD , n=261	0.3 (0.0-1.9) ‡	70 (18-170) ‡	18.7 (0-76.5) ‡
Treated CD			
Short GFD, n=71	2.5 (0.3-4.0)* ‡	37 (12-133)* ‡	13.1 (0-56.3)* ‡
Long GFD, n=105	3.3 (1.2-5.9)*†	38 (6-91)* ‡	12.1 (0-39.6)* †‡
Non CD controls, n=86	3.2 (0.1-4.9)*	31 (3-62)*	2.0 (0.7-24.8)*

* p<0.001 compared to active coeliac disease group,
† p< 0.001 compared to short-term gluten-free diet group
‡ p <0.001 compared to non-CD group
CD coeliac disease, GFD gluten-free diet, IEL intraepithelial lymphocyte, Vh/CrD villous height crypt depth ratio

*15.3 Exposure to gluten and oats (**II**)*

At baseline in this study, all treated coeliac disease children were in clinical remission and had normal villous architecture. Demographic data on the children are shown in Table 8. The Small-bowel mucosal median Vh/CrD ratio was 4.1 (range 3.0-5.1) in the oat challenge group and 4.4 (range 3.2-5.9) in the gluten challenge group (p=0.351). At baseline, weak to moderate TG2-specific IgA autoantibody deposits were present in the small-bowel mucosa in four out of 13 (31%) in the oat challenge group and in three out of 10 (30%) (p=0.544) in the gluten challenge group, although serum coeliac disease autoantibodies were negative in all patients.

Table 8. Demographic data on treated children in remission in oat and gluten challenge groups.

	Oats group n=13	**Gluten group** n=10
Age, median (range), years	11 (9-18)	13 (7-18)
Female, n (%)	6 (46)	9 (90)
Duration of gluten-free diet, median (range), years	7 (2-17)	5 (2-10)

In the oat challenge group there was no significant change in the intensity of mucosal IgA deposits during the two-year trial (p=0.276) (Figure 1B in original article **II**). In contrast, in the gluten challenge group all children developed moderate to strong mucosal IgA deposits in parallel to mucosa relapse (Figure 1A in original article **II**). Two patients were biopsied because of positive serum autoantibody tests before they developed small-intestinal mucosal villous atrophy during a gluten-containing diet, and both had clear mucosal TG2-specific IgA depositions despite intact small-intestinal mucosal morphology. When an oat-containing gluten-free diet was adopted after relapse of the disease, the intensity of the small-bowel mucosal IgA deposits decreased significantly within six months (p=0.01). After two years with oats only one out of seven (14%) had minor depositions left in the mucosa, this not differing significantly from the baseline situation (p=0.257). At the end of the two-year trial with oats all patients were again negative for serum autoantibodies and had a normal Vh/CrD ratio (Table 9).

Table 9. Villous height crypt depth ratio (Vh/CrD), median (range), presence of mucosal IgA deposits, n (%), and serum autoantibodies, median (range) in children challenged with cereals. All statistically significant p-values (p<0.05) are given.

	Vh/CrD	**Mucosal autoantibody deposit**	**Serum autoantibody**
Oat group n=13			
Baseline	4.1 (3.0-5.1)	4 (31%)	0.3 (0-1.0)
Oats 24 months	4.0 (2.9-5.2)	3 (23%)	0.5 (0-0.9)
Gluten* group n=10			
Baseline	4.3 (3.2-5.9)	3 (30%)	0.4 (0-1.3)
During gluten	0.6 (0.1-2.0)†	10 (100%)†	96.5 (9.5-100)†
Oats 6 months	4.0 (1.3-5.2) ‡	6 (75%)‡	1.9 (0.6-10.5) ‡
Oats 24 months	4.9 (3.3-5.2)	1 (14%)	1.3 (0.1-1.6)
Reference value	>2.0		<5.0

*Gluten means wheat, rye and barley, †p= 0.05 compared to baseline situation, ‡ p<0.05 compared to diet containing gluten

One child in the oat challenge group admitted passing dietary transgressions at six months. At that time, his serum anti-TG2 antibody titre increased from 0.4 to 3.1 U/ml, but still remained below the cut-off level of 5 U/ml. The intensity of small-bowel mucosal IgA deposits was slightly increased. After continuing with a strict oat-containing gluten-free diet both serum and mucosal autoantibodies decreased.

Two coeliac disease children experienced abdominal pain and vomiting immediately after intake of oats and within one month both withdrew prematurely

from the study. At the time of follow-up biopsies taken upon withdrawal, the small-bowel mucosal villous morphology was normal. Furthermore, serum anti-TG2 antibodies and small-bowel mucosal autoantibody deposits remained negative in both patients (Figure 4 in original article **II**).

*15.4 Differentiating non-responsive coeliac disease from other enteropathies (**III**)*

Non-responsive coeliac disease was found in 27 patients during a gluten-free diet. Demographic data are presented in Table 10. In six of these cases the reason for poor histological recovery was clearly dietary transgressions. All these non-adherent patients were asymptomatic, but one later died of malabsorption. In the remaining 21 patients adherence to the diet was strict; EATL was found in six and five had symptomatic refractory disease. The remaining ten were asymptomatic regardless of persistent villous atrophy. During follow-up, six of these non-responsive coeliac disease patients who adhered to the gluten-free diet died of coeliac disease complications; one with EATL is alive and the remaining fourteen are in remission with or without immunosuppressive drugs. As controls, ten patients with other enteropathies were examined. Of these, five had villous atrophy (four with autoimmune enteropathy and one with Crohn's disease) but not the HLA DQ2 or HLA DQ8 genotypes compatible with coeliac disease. During the follow-up control patients were all in remission with or without immunosuppressive medication.

Table 10. Demographic data on non-responsive coeliac disease patients and controls with other enteropathies

	Non-responsive coeliac disease		
	poor adherence	**good adherence**	**Other enteropathies**
Age, median (range), years	49 (30-77)	52 (21-76)	52 (23-64)
Female, n(%)	6 (100 %)	10 (48 %)	6 (60 %)
Duration of gluten-free diet, median (range), years	14 (6-18) *	5 (0.3-24)	0

* gluten contaminations repeatedly

Coeliac disease autoantibodies were both present in sera and deposited in the small bowel in all six coeliac disease patients with poor adherence to the gluten-free diet. In contrast, only four (19%) out of the 21 non-responsive coeliac disease patients with good adherence to the diet had autoantibodies in their sera while 20 (95%) of these 21 patients had TG2-specific IgA class autoantibodies deposited in the small-intestinal mucosa (Figure 5). There was no difference in the intensity of small-bowel mucosal TG2-specific IgA deposits in patients with EATL, refractory sprue, asymptomatic non-responsive coeliac disease or untreated coeliac disease patients with subsequent response to the diet. In contrast to clear IgA depositions in almost all non-responsive coeliac patients, none of the ten patients with other enteropathies had autoantibodies either in sera or deposited in their small-intestinal mucosa.

Increased densities of CD3+ and γδ+ IELs were present in 76% and 71% of patients with villous atrophy despite strict gluten-free diet and in 83% and 67% of patients with poor adherence to diet, respectively. As many as 30% of patients with other enteropathies had also elevated densities of IELs. Medians and distributions of markers are shown in Table 11 and Figure 6.

Table 11. Villous height crypt depth ratio (Vh/CrD) and densities of intraepithelial lymphocytes (IELs) and presence of abnormal IEL population in non-responsive coeliac disease patients and in patients with enteropathies of other type. Statistically significant p-values compared to other enteropathies are shown. Between coeliac disease patient groups there were no statistically significant differences.

	Non-responsive coeliac disease patients		**Other enteropathies** n=10
	Poor Adherence n=6	**Good adherence** n=21	
Vh/CrD	0.1 (0.1-1.5)*	0.1 (0.1-1.8)**	2.3 (0.1-4.6)
CD3+IELs /mm of epithelium	55 (30-132)**	55 (11-109)**	28 (15-40)
γδ+ IELs /mm of epithelium	13.3 (3.2-17.2)*	11.9 (0-37.8)**	1.0 (0-14.0)
CD30+ IELs /100 epithelial cells	0 (0-4.6)	0 (0-14.4)	0 (0-28.3)
Abnormal IEL population, n (%)	0	6 (29%)	0

* $p < 0.05$
** $p < 0.01$

Densities of CD30+ IELs and CD8-/CD3+ IEL ratio were determined in all non-responsive coeliac patients on a strict gluten-free diet, in six coeliac disease patients with dietary transgressions and in four controls suffering from autoimmune enteropathy. An abnormal proportion of CD8-/CD3+ IELs was found in all coeliac patients with EATL, but not in coeliac patients without lymphoma or in controls. An increased density of CD30+ IELs was found in six (28%) out of 21 non-responsive but adherent coeliac disease patients and in one patient with dietary transgressions and one in the control group. Of these six adherent but non-responsive patients with CD30+ IELs four had EATL, one had refractory coeliac disease and one was asymptomatic. The TCR-γ gene rearrangement was found to be polyclonal in three patients, of whom two had EATL and one had symptomatic refractory sprue.

*15.5 Finding early-stage coeliac disease (**IV**)*

Specimens from 26 out of 28 early-stage coeliac disease patients were available for determination of TG2-specific IgA deposits from small-intestinal mucosa when villous atrophy was not present. Demographic data on these patients are presented in Table 12. Of these 26 patients 25 (96%) had small-bowel mucosal TG2-specific IgA deposits. Increased densities of CD3+ and γδ+ IELs were found in 13 (62%) and 15 (71%) out of 21 patients before villous atrophy developed. Medians and distributions of Vh/CrD and IELs are shown in Table 13 and Figure 6. Serum coeliac disease autoantibodies were present in 19 out of the 26 patients (73%). The sensitivity of mucosal IgA deposit was 96% in finding coeliac disease prior to the development of villous atrophy.

In parallel with the progression of small-bowel lesion, the intensity of mucosal IgA deposits and densities of intraepithelial cells increased (Figures 5 and 6). At the time of diagnosis of coeliac disease, when villous atrophy was evident, all coeliac disease patients evinced mucosal autoantibody deposits. All patients showed clinical and histological response to a gluten-free diet and the intensity of IgA deposits decreased. A total of seven patients in the early-stage coeliac disease group were initially serum autoantibody-negative; six of them still had positive small-bowel mucosal IgA deposits, two had a family history of coeliac disease and all (six) cases tested had coeliac-type HLA.

Table 12. Demographic data on mild enteropathy coeliac disease patients divided into early-stage coeliac disease (CD) with subsequent villous atrophy on a gluten-containing diet and the potential CD group who started a gluten-free diet without villous atrophy.

	Early stage CD n=28	**Potential CD** n=20
Age, years	37 (2-69)	46 (21-74)
Female, n (%)	21 (75)	16 (80)
Main symptom, n (%)		
Abdominal complaints	17 (61)	17 (85)
Malabsorbtion	2 (7)	1(5)
Extra-intestinal symptoms*	5 (18)	2 (10)
Screening†	4 (14)	0
HLA-DQ2 or DQ8 present	12/12	16/16

*aphtous stomatitis, growth retardation, epilepsy, atopic dermatitis, polyneuropathy, arthralgia

† type I diabetes mellitus, family history, autoimmune thyroid disorders, Sjögren's syndrome, and osteoporosis

Table 13. Villous height crypt depth (Vh/CrD) ratio, median (range) and densities of intraepithelial lymphocytes (IELs) per mm of epithelium, median (range) in early-stage and in potential coeliac disease. Statistically significant p-values are shown. Between early-stage and potential coeliac disease groups there were no statistically significant differences at the time of the first biopsies.

	Vh/CrD	**CD3+ IEL**	**γδ+ IELs**
Early-stage CD, n=28			
early-stage situation	2.8 (2.0-4.5) †	46 (12-185) ††	8.8 (0-34.8) ††
at diagnosis‡	1.0 (0.3-2.0)* †	68 23-138)** †	16.8 (1.4-58.7)** †
after GFD	2.9 (2.1-3.5)*** ††	36 (21-82)***	7.8 (1.4-56.3)*** †
Potential CD, n=20			
early-stage situation	2.6 (2.2-3.6) †	52(22-118) †	13.4 (4.2-34.8) †
after GFD	2.9 (2.1-3.8) ††	29 (19- 66)	8.6 (3.2-25.5) †
Controls n=76	3.3 (2.1-4.9)	30 (3.2-62)	2.0 (0-24.8)

‡ villous atrophy with crypt hyperplasia present in all
* p<0.001 compared to first biopsy specimens
** p<0.05 compared to first biopsy specimens
*** p <0.01 compared to biopsy specimen at diagnosis
† p<0.001 compared to control group
†† p<0.05 compared to control group

In the remaining twenty patients (Table 12) with small-intestinal mucosal TG2-specific IgA deposits but intact villous morphology, serum autoantibodies were found in 14 (74%) out of 19 tested patients before starting a gluten-free diet. Intraepithelial CD3+ and γδ+ lymphocyte densities were increased in 15 (75%) and

in 18 (90%) out of the twenty patients prior to commencing the diet, respectively. Sixteen out of the 20 patients having TG2-specific small-bowel mucosal IgA deposits in intact villi (potential coeliac disease) consented to a follow-up biopsy after one year on a gluten-free diet. The intensity of the deposits decreased in all but one during the diet. Parallel to these changes, abdominal symptoms and signs of malabsorption were resolved in 13 (65%) and improved in five (25%) of the patients during the gluten-free diet. Two patients with extra-intestinal symptoms experienced no change in their condition during the diet.

15.6 Comparison of coeliac disease-related markers

Small-bowel mucosal TG2- specific IgA deposits were proved to be the most efficient of the coeliac disease-related markers investigated in this study (Table 14). In overt coeliac disease its sensitivity was 100%, whereas serum autoantibodies, which have the highest specificity, were not present in 9% of patients with overt coeliac disease. Small-bowel mucosal densities of IELs had both lower sensitivities and specificities compared to mucosal autoantibody deposits in detecting overt coeliac disease (Table 14). In differentiating coeliac disease patients without response to a gluten-free diet from non-coeliac disease subjects only small intestinal-mucosal TG2-specific IgA deposits showed high sensitivity, whereas increased densities of IELs and in particularly serum autoantibodies failed to find non-responsive coeliac disease patients (Table 14). In detecting early-stage coeliac disease, before villous atrophy developed, small-bowel mucosal autoantibody deposits turned out to be superior in predicting forthcoming coeliac disease and had the highest sensitivity, 96%, while the sensitivities of increased densities of IELs and serology were between 62% and 73% (Table 14).

In overt coeliac disease, there was a weak inverse correlation ($r=-0.149$, $p=0.016$, Spearman's correlation test) between small-bowel mucosal TG2-specific IgA deposits and Vh/CrD ratio, whereas the intensity of mucosal autoantibody deposits and serum coeliac autoantibodies did not correlate ($r=0.061$, $p=0.324$, Spearman's correlation test) in patients with overt coeliac disease. Similarly, mucosal autoantibody deposits were also found in seronegative patients on a gluten-free diet

with both good and poor response and even in early-stage coeliac disease patients without villous atrophy but subsequently developing coeliac disease on a gluten-containing diet. It is of note that irrespective of diet, all serum autoantibody-positive patients also had TG2-specific IgA deposits present in the small-bowel mucosa.

Table 14. Sensitivities and specificities of coeliac disease-related markers in differentiating active, non-responsive (during gluten-free diet) and early-stage coeliac disease (CD) from non-coeliac patients, including patients with enteropathies of other cause than coeliac disease

	Villous atrophy	**Mucosal auto-antibody deposits**	**CD3+ IELs**	**γδ+ IELs**	**Serum auto-antibodies**
Sensitivity					
Overt CD	100%*	100%	91%	96%	91%
Non-responsive CD	100%*	95%	76%	71%	24%
Early-stage CD	0%*	96%	62%	71%	73%
Specificity	94%	84%	69%	73%	100%
Efficiency of test **	91%	96%	85%	90%	92%

*by definition
** calculated on coeliac disease patients before starting a gluten-free diet and all non-coeliac controls

16. DISCUSSION

16.1 Diagnosing overt coeliac disease

The current criteria for coeliac disease require villous atrophy with crypt hyperplasia during a gluten-containing diet and recovery of the small-bowel mucosa on a gluten-free diet, while positive serum antibodies have a supporting role (Walker-Smith et al. 1990, Catassi et al. 2001). In addition, gluten challenge is recommended in special cases (Walker-Smith et al. 1990). Although villous atrophy is a diagnostic criterion for coeliac disease, it is not exclusively pathognomic for this condition (Marsh 1992, Green and Cellier 2007, Cello and Day 2009) as also seen in this patient material, where villous atrophy was encountered in five control patients without coeliac disease. Serum autoantibodies are highly sensitive and specific (Table 3) in detecting coeliac disease. However, in some patients fulfilling the current criteria serum autoantibodies are never found (Salmi et al. 2006b), which makes the diagnostics of coeliac disease even more challenging.

In this study, all coeliac disease patients evincing villous atrophy during a gluten-containing diet had TG2-specific IgA deposits in their small-bowel mucosa, this including 26 seronegative coeliac patients, similarly to the situation recently described in adults by Salmi and colleagues (2006b). In contrast to the present findings, in a recent study 27% of children with untreated coeliac disease below the age of two years had no autoantibody deposits in their small-bowel mucosa (Maglio et al. 2010). During the disease process the avidity of autoantibodies increases (Westerlund et al. 2007). Thus the lower sensitivity of mucosal autoantibody deposits in detecting coeliac disease in the youngest age groups may be due to the lower avidity of autoantibodies, which would promote autoantibody spillover into circulation rather than depositing in the small-intestinal mucosa.

TG2-specific IgA deposits were as sensitive as villous atrophy in detecting overt coeliac disease, but their specificity was lower. This may be due to the fact that patients in the control group were symptomatic patients. They were all negative for coeliac disease serum autoantibodies, but elevated densities of IELs were seen in

approximately 30% of them. Whether these “false-positive” TG2-specific mucosal autoantibody deposits in 16% of the control patients predict forthcoming coeliac disease and the lower specificity turns out to be a consequence of the higher sensitivity of IgA deposits than villous atrophy, remains to be seen. Nonetheless, this group with symptoms is a relevant control group in that in clinical work it has to be determined whether symptomatic patients have coeliac disease or not.

16.2 Gluten dependency of markers

On a gluten-free diet symptoms should disappear and the small-bowel mucosal villi should recover (Walker-Smith et al. 1990). In this study changes in small-bowel mucosal markers during a gluten-free diet as well as gluten and oat challenge were investigated and compared to coeliac serology and the clinical picture. The current findings indicate that the coeliac disease markers disappear in a sequential order during a gluten-free diet. Serum autoantibodies normalize rapidly after gluten withdrawal and disappear completely after strict long-term treatment. This seems to be followed by the recovery of the small-bowel mucosal villous morphology, as also previously reported (Dickey et al. 2000, Kaukinen et al. 2002b). Thereafter, the densities of CD3+ IELs decline, although considerably more slowly, being still elevated in half of the patients even after a long-term gluten-free diet (Figure 6b). Subsequently, TG2-specific IgA deposits begin to disappear, but remnants of deposits persist for a fairly long time after commencement of a gluten-free diet (Figure 5). Eventually, the last marker, which remains abnormal, is the density of TCR γδ+ IELs, which does decrease but does not reach normal values (Figure 6c), as has been described previously (Savilahti et al. 1990, Iltanen et al. 1999b, Kaukinen et al. 1999).

In the present study, ingestion of oats during two years had no any adverse effects in most patients, as also shown in numerous previous studies (Janatuinen et al. 1995, Srinivasan et al. 1996, Janatuinen et al. 2000, Janatuinen et al. 2002, Storsrud et al. 2003c, Peräaho et al. 2004, Holm et al. 2006). Only two patients discontinued eating oats due to symptoms and returned to a gluten-free diet. During an oat-containing diet there was no change in coeliac disease markers, including small-bowel mucosal TG2-specific IgA deposits, not even in those two patients who

discontinued the diet. These results are compatible with those in many studies, where oats has had no immunogenic effect on coeliac disease patients (Picarelli et al. 2001, Kilmartin et al. 2003, Hollen et al. 2006, Srinivasan et al. 2006). In contrast to oats in a gluten-free diet, when wheat, rye and barley were added to the diet together with oats, villous atrophy developed in all patients, densities of IELs increased and autoantibodies were deposited in the small-bowel mucosa and reappeared in the serum. During an oat-containing gluten-free diet their small-bowel mucosa healed completely in two years.

The disappearance of small-bowel mucosal TG2-specific IgA deposits during a gluten-free diet and reappearance during gluten challenge but not during oat ingestion shows that small-bowel mucosal IgA deposits are gluten-dependent as well as independent of oat consumption. These promising results on gluten dependency and the gluten restricted appearance of small-bowel mucosal TG2-specific IgA deposits would imply that mucosal IgA deposits can be used to help in diagnostics. For example, in those cases where patients have started a gluten-free diet on their own and the villous architecture in the small-intestine has normalized before the intestinal biopsy, small-bowel mucosal autoantibody deposits allow reliable confirmation of the diagnosis.

16.3 Differentiating non-responsive coeliac disease from other enteropathies

A strict gluten-free diet improves the quality of life, even though keeping the diet strictly gluten-free may be burdensome (Mustalahti et al. 2002a, Wagner et al. 2008). In contrast, patients with poor adherence to a gluten-free diet have persisting small-bowel mucosal lesions leading from an increased density of IELs to villous atrophy (Troncone et al. 1995). Persistent villous atrophy, even asymptomatic, increases the risks of complications in coeliac disease patients (Kaukinen et al. 2007b). Follow-up of mucosal healing is thus particularly important.

Dietary transgressions are a common reason for non-responsive coeliac disease (Leffler et al. 2007). As also previously shown these patients can be differentiated from other non-responsive patients by serum autoantibody titres (Leffler et al.

2007). Similarly to the situation in gluten-challenged patients these poorly adherent patients have high serum autoantibody titres, while most non-responsive patients with a strict diet are seronegative (Kaukinen et al. 2007b, de Mascarel et al. 2008, O'Shea et al. 2008, Verbeek et al. 2008b). This renders the diagnostics of non-responsive coeliac disease challenging and obscure.

A major finding in studying non-responsive coeliac disease was the high sensitivity and specificity of small-bowel mucosal TG2-specific IgA deposits in detecting coeliac disease among seronegative enteropathies; autoantibody deposits in the small-intestinal mucosa were present in 95% of all non-responsive coeliac disease patients during a strict gluten-free diet, also in patients with EATL, compared to none among patients with other enteropathies (i.e. autoimmune enteropathy and Crohn's disease). Determination of small-bowel mucosal TG2-specific IgA deposits is thus of assistance in diagnosing serum coeliac disease autoantibody-negative enteropathies in that it detects coeliac disease with high sensitivity and thus excludes coeliac disease in patients with no coeliac autoantibody deposits in the small-bowel mucosa. Nonetheless, determination of small-bowel mucosal IgA deposits is not able to distinguish patients with good or poor adherence to a gluten-free diet, whereas serum autoantibodies are.

Why do TG2-specific IgA deposits remain in the small-bowel mucosa during a strict gluten-free diet? One explanation is local production of autoantibodies due to traces of gluten in gluten-free products (Storsrud et al. 2003b, Collin et al. 2004b). A case has been described where a minimal amount of gluten sufficed to maintain autoantibody production and small-bowel mucosal lesion (Biagi et al. 2004). Another reason might be that during the disease process avidity of coeliac autoantibodies increases (Westerlund et al. 2007), leading to stronger antibody-antigen binding, so that the autoantibodies remain in the gut over a long period (Salmi et al. 2006b).

The monoclonal TCR-gamma gene is often found in EATL; the monoclonality of TCR-γ gene has been found in the duodenal mucosa in 38% of EATL patients and in 17% of refractory coeliac disease patients (Daum et al. 2001). In the present study, similar TCR-gamma gene monoclonality was observed; 33% of EATL patients and 25% of refractory coeliac disease patients without EATL evinced a monoclonal TCR-gamma gene rearrangement. In this study, the best marker to

differentiate patients with EATL from those without was an abnormal proportion of CD3+CD8- IELs; this abnormal IEL population was found in all EATL patients, but in none without EATL. Similarly to these results, a previous study also found immunophenotyping of IELs to be a more accurate method to find patients with EATL than determination of TCR-γ gene clonality (Daum et al. 2001).

The present results indicate that the typical markers of abnormal IEL phenotype distinguish the most severe conditions of coeliac disease from the less severe, while determination of small-bowel mucosal IgA deposits distinguishes all coeliac disease patients. To detect the reason for non-responsive coeliac disease these tests should thus be used together to confirm the diagnosis of coeliac disease and to reveal abnormal immunophenotypes of IELs. It is important to find an abnormal IEL population early so that treatment can be initiated before EATL develops, as the prognosis of EATL is poor, only 8% to 20% surviving for 5 years, (Gale et al. 2000, Al-Toma et al. 2007). In the present study, five EATL patients died within a few years after diagnosis and only one (17%) patient out of six with EATL is still alive.

16.4 Finding early-stage coeliac disease

Coeliac disease without small-bowel mucosal villous atrophy does not fulfil the current criteria for coeliac disease (Walker-Smith et al. 1990), but the fact is that patients may be symptomatic and even develop osteoporosis without evidence of small-bowel mucosal villous atrophy (Kaukinen et al. 2001, Kaukinen et al. 2005). Furthermore, it has also recently been observed that coeliac patients benefit from a gluten-free diet regardless of the stage of villous atrophy (Kurppa et al. 2009). For these reasons, several studies have been made to establish a diagnostic tool sensitive and specific enough to detect early-stage coeliac disease before villous atrophy develops.

Serum autoantibody positivity without villous atrophy may predict the development of coeliac disease (Collin et al. 1993, Salmi et al. 2006a), but serum autoantibodies are not sensitive enough to reveal early-stage coeliac disease. In the current study only 73% of early stage coeliac disease patients without villous atrophy had serum autoantibodies as has previously been reported (Goldstein and Underhill 2001, Tursi and Brandimarte 2003). However, TG2-specific IgA

autoantibodies were deposited in the small-bowel mucosa in 96% of patients before the emergence of villous atrophy. Similar high accuracy has been reported by Korponay-Szabo and associates (2004), Kaukinen and associates (2005) and Salmi and colleagues (2006a). A slightly lower sensitivity of 79% of IgA autoantibody deposits was recently reported by Tosco and associates (2008) in children. Similarly to early-stage coeliac disease patients without villous atrophy, small-bowel mucosal autoantibody deposits have also been found in patients with gluten ataxia even without symptoms of gastrointestinal tract or villous atrophy (Hadjivassiliou et al. 2006).

In the present study, patients who were positive for serum autoantibodies also had deposited antibodies in the small-bowel mucosa, regardless of the diet. This is in contrast to the findings of Tosco and colleagues (2008) on children with early-stage coeliac disease, where fifteen percent of seropositive coeliac patients did not have autoantibodies deposited in the small-intestinal mucosa. In another study on small-bowel mucosal IgA deposits in diabetes mellitus type I patients with normal small-bowel mucosal morphology, the results showed that 21% of patients having coeliac disease autoantibodies in their sera evinced no deposits in the small-bowel mucosa (Maglio et al. 2009). In addition to the reasons discussed in section 16.1 the discrepancy may also be dependent on technical issues involved in visualizing the IgA deposits in histological specimens.

Despite the good accuracy of IEL densities in detecting early-stage coeliac disease (Iltanen et al. 1999c, Salmi et al. 2006a), their sensitivity was lower than that of mucosal autoantibody deposits in this study; before the development of villous atrophy CD3+ and γδ+ IEL densities were elevated in 62% and 71%, respectively.

Small-bowel mucosal TG2-specific IgA deposits are more sensitive than other markers tested in predicting forthcoming coeliac disease, but this prompt the question, whether these patients should be placed on a gluten-free diet before diagnostic criteria are fulfilled. Kurppa and associates (2009) showed that patients with positive coeliac disease serology benefit on gluten-free diet, regardless of the degree of villous atrophy. In this current study, twenty symptomatic patients with small-bowel mucosal IgA autoantibodies voluntarily started a gluten-free diet before villous atrophy developed and the clinical response was clear – at the same time IgA

deposits disappeared from the gut mucosa. These results together would imply that symptomatic patients with autoantibodies either in the sera or deposited in the small-intestinal mucosa would benefit from treatment, but further randomized controlled studies are required.

16.5 Strengths and shortcomings of the present study

The results of the present study show the high sensitivity of small-bowel mucosal TG2-specific IgA deposits in the diagnosis of coeliac disease in a large group of untreated coeliac disease patients. The specificity of determining small-bowel mucosal autoantibody deposits was, however, lower, 86%. The non-coeliac control group, where 16% were found to have deposits, consisted of symptomatic patients. For ethical reasons it was not possible to recruit completely healthy persons to undergo upper gastrointestinal endoscopy. Symptomatic patients may not be the ideal non-coeliac control group, since they may develop coeliac disease later in life, especially those proving positive for deposits.

This study was carried out mainly retrospectively on stored biopsy specimens and for example HLA DQ-typing was thus not available for all patients. Nonetheless a strength of the study was the opportunity to evaluate a series of biopsies from the same patients at different time-points and on different diets. This makes for the possibility to draw conclusions as to what happens during disease development and treatment. However, biopsy specimens during a gluten-free diet were not available from all untreated patients, as the use of frozen biopsy specimens is not routine in clinical practice. The frozen biopsy specimens were available from patients who participated in scientific studies. The remainder recovered during gluten-free diet.

This study showed for the first time that TG2-specific IgA autoantibody deposits are dependent on gluten consumption, but that consumption of pure oats has no effect on the intensity of autoantibody deposits in the small-bowel mucosa. This study also showed the presence of coeliac disease autoantibody deposits in six EATL patients, supporting the findings of Salmi and associates (2006b). Taking into account that refractory coeliac disease and especially EATL are rare conditions, with a prevalence of 0.1 per 100 000 (Verbeek et al. 2008a), several patients with these conditions were encountered in this study. However, such patients are

recognized later, not at the time of diagnosis of coeliac disease, and for this reason their first biopsies or serum samples were not available, though it would be both interesting and of scientific value to establish whether there are some differences in these patients already at the time when coeliac disease is diagnosed as compared to patients with responsive coeliac disease. The number of patients with other enteropathies is small and conclusions regarding the prevalence of TG2-specific IgA autoantibodies in for example Crohn's disease or ulcerative colitis patients' small-bowel mucosa cannot be drawn based on this study; further investigations among these patient groups are called for.

The wide variation in patients' ages is a further strength of this study. Here small-bowel mucosal TG2-specific IgA deposits were studied in both adults and children over two years of age. One shortcoming of the study is absence of children below the age of two. In that youngest age group, food allergies are more common causes of small-bowel mucosal villous atrophy than in older children and adults, and this could lead to wrong diagnoses of coeliac disease (Walker-Smith et al. 1990). In a recent study by Maglio and associates (2010), the authors showed that in the group of children under two years only 73% of coeliac disease patients had small-bowel mucosal autoantibody deposits present, while 88% of them had serum autoantibodies.

In the current study, only patients and controls with normal levels of serum IgA were included. However, coeliac disease is common in selective IgA deficiency (Korponay-Szabo et al. 2003a) and it is clear that small-bowel mucosal TG2-specific IgA deposits are not able to detect these patients, while autoantibody depositions can be determined with IgG and IgM immunoglobulin classes (Korponay-Szabo et al. 2004, Borrelli et al. 2009).

16.6 Re-evaluation of diagnostic criteria for coeliac disease

The current diagnostic criteria for coeliac disease do not take into account forms of the disease without villous atrophy with minor inflammatory changes in the small-bowel mucosa (Walker-Smith et al. 1990, Catassi et al. 2001); likewise

refractory coeliac disease without response to a gluten-free diet does not fulfil these criteria. It has been reported that positivity for serum autoantibodies predicts the development of coeliac disease (Collin et al. 1993). Such patients with early-stage coeliac disease often have symptoms and risks of osteoporosis (Kaukinen et al. 2005) and a recent study has shown that all symptomatic serum autoantibody-positive patients, despite the degree of villous atrophy, benefit from a gluten-free diet (Kurppa et al. 2009). It seems that a gluten-free diet should be considered for serum autoantibody-positive patients even in the absence of villous atrophy. Some coeliac patients with either early-stage or more severe disease are seronegative (Salmi et al. 2006a) and thus subjects with a clinical suspicion of coeliac disease should undergo gastroscopy and duodenal biopsies should be taken for routine and if necessary specialized histological examinations.

According to the present findings, IgA deposits should be determined in seronegative patients suspected of having coeliac disease. If villous atrophy and small-bowel mucosal TG2-specific IgA deposits are present the subject can be regarded as a coeliac disease patient and placed on a gluten-free diet. Equally in the situation where deposits are present without manifest mucosal lesion and the patients are symptomatic, a gluten-free diet should be prescribed. In patients without small-intestinal TG2-specific IgA deposits other causes of symptoms and possible small-intestinal lesion should be taken into consideration.

Altogether, there is clearly call for new diagnostic criteria which on the one hand specify the role of positive autoantibody findings in both sera and small-intestinal mucosa even without villous atrophy, and on the other hand give a diagnosis of refractory coeliac disease even without dietary response.

17. CONCLUSIONS AND FUTURE ASPECTS

This study showed that determination of small-bowel mucosal TG2-specific IgA deposit is as sensitive a tool in finding overt coeliac disease as the conventional villous morphological analysis. Morphological analysis fails to distinguish between different causes of villous atrophy, whereas small-bowel mucosal autoantibodies are accurate in detecting coeliac disease even in seronegative and EATL patients. Coeliac patients on a gluten-containing diet had small-bowel mucosal autoantibody deposits irrespective of their serological finding, in contrast to patients with other enteropathies, of whom none had autoantibody deposits in the small-intestinal mucosa. These results together give an opportunity to ascertain or exclude coeliac disease in patients with small-bowel mucosal lesions compatible for coeliac disease but without serum autoantibodies. Furthermore, TG2-specific IgA deposits remain in the small-intestinal mucosa for a fairly long time, even years, after gluten withdrawal and thus can be used as an instrument to diagnose coeliac disease in patients who have by themselves started a gluten-reduced diet without proper diagnosis.

In addition, TG2-specific IgA deposits in the small-intestinal mucosa were a more sensitive marker than determination of serum autoantibodies or densities of CD3+ or γδ+ IELs in detecting early-stage coeliac disease. Densities of IELs seemed to be unspecific and indicate rather inflammation in the small intestine than coeliac disease. These findings also indicated that determining autoantibodies from the small-intestinal mucosa is more sensitive than determining them from sera. In seronegative, potential coeliac patients with small-bowel mucosal autoantibody deposits, symptoms were alleviated during a gluten-free diet. Thus, in future, prospective controlled studies are warranted to establish whether subjects with small-bowel mucosal IgA deposits will benefit from a gluten-free diet regardless of serum autoantibodies or stage of mucosal lesion.

In clinical practice, determination of small-bowel mucosal TG2-specific IgA deposits could be available in a centralized framework as a special tool used in problematic cases but not in routine diagnostics, because the procedure requires

frozen sections and interpretation needs practice even if the staining itself is easy to perform. In research, determination of small-bowel mucosal autoantibody deposits offers a sensitive tool to detect the harmfulness of gluten in clinical studies where new treatments for coeliac disease are being developed.

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Outi Koskinen

REFERENCES

Agardh D, Nilsson A, Carlsson A, Kockum I, Lernmark A and Ivarsson SA. (2002) Tissue transglutaminase autoantibodies and human leucocyte antigen in Down's syndrome patients with coeliac disease. Acta Paediatr. 91:34-38.

Aine L, Mäki M, Collin P and Keyriläinen O. (1990) Dental enamel defects in celiac disease. J Oral Pathol Med. 19:241-245.

Akbari MR, Mohammadkhani A, Fakheri H, Javad Zahedi M, Shahbazkhani B, Nouraie M, Sotoudeh M, Shakeri R and Malekzadeh R. (2006) Screening of the adult population in Iran for coeliac disease: comparison of the tissue-transglutaminase antibody and anti-endomysial antibody tests. Eur J Gastroenterol Hepatol. 18:1181-1186.

Al-Toma A, Verbeek WH, Hadithi M, von Blomberg BM and Mulder CJ. (2007) Survival in refractory coeliac disease and enteropathy-associated T-cell lymphoma: retrospective evaluation of single-centre experience. Gut. 56:1373-1378.

Arato A, Hacsek G and Savilahti E. (1998) Immunohistochemical findings in the jejunal mucosa of patients with coeliac disease. Scand J Gastroenterol Suppl. 228:3-10.

Arentz-Hansen H, Fleckenstein B, Molberg O, Scott H, Koning F, Jung G, Roepstorff P, Lundin KE and Sollid LM. (2004) The molecular basis for oat intolerance in patients with celiac disease. PLoS Med. 1:e1.

Arranz E, Bode J, Kingstone K and Ferguson A. (1994) Intestinal antibody pattern of coeliac disease: association with gamma/delta T cell receptor expression by intraepithelial lymphocytes, and other indices of potential coeliac disease. Gut. 35:476-482.

Bardella MT, Quatrini M, Zuin M, Podda M, Cesarini L, Velio P, Bianchi P and Conte D. (1997) Screening patients with celiac disease for primary biliary cirrhosis and vice versa. Am J Gastroenterol. 92:1524-1526.

Barone MV, Caputo I, Ribecco MT, Maglio M, Marzari R, Sblattero D, Troncone R, Auricchio S and Esposito C. (2007) Humoral immune response to tissue transglutaminase is related to epithelial cell proliferation in celiac disease. Gastroenterology. 132:1245-1253.

Bazzigaluppi E, Roggero P, Parma B, Brambillasca MF, Meroni F, Mora S, Bosi E and Barera G. (2006) Antibodies to recombinant human tissue-transglutaminase in coeliac disease: diagnostic effectiveness and decline pattern after gluten-free diet. Dig Liver Dis. 38:98-102.

Ben Hariz M, Kallel-Sellami M, Kallel L, Lahmer A, Halioui S, Bouraoui S, Laater A, Sliti A, Mahjoub A, Zouari B, Makni S and Maherzi A. (2007) Prevalence of celiac disease in Tunisia: mass-screening study in schoolchildren. Eur J Gastroenterol Hepatol. 19:687-694.

Bhattacharya M, Dubey AP and Mathur NB. (2009) Prevalence of Celiac Disease in North Indian Children. Indian Pediatr. 46:415-417.

Biagi F, Pezzimenti D, Campanella J and Corazza GR. (2002) Gluten exposure and risk of autoimmune disorders. Gut. 51:140-141.

Biagi F, Campanella J, Martucci S, Pezzimenti D, Ciclitira PJ, Ellis HJ and Corazza GR. (2004) A milligram of gluten a day keeps the mucosal recovery away: a case report. Nutr Rev. 62:360-363.

Biagi F, Campanella J, Soriani A, Vailati A and Corazza GR. (2006) Prevalence of coeliac disease in Italian patients affected by Addison's disease. Scand J Gastroenterol. 41:302-305.

Bonamico M, Mariani P, Danesi HM, Crisogianni M, Failla P, Gemme G, Quartino AR, Giannotti A, Castro M, Balli F, Lecora M, Andria G, Guariso G, Gabrielli O, Catassi C, Lazzari R, Balocco NA, De Virgiliis S, Culasso F, Romano C and SIGEP (Italian Society of Pediatric Gastroenterology and Hepatology) and Medical Genetic Group. (2001a) Prevalence and clinical picture of celiac disease in italian down syndrome patients: a multicenter study. J Pediatr Gastroenterol Nutr. 33:139-143.

Bonamico M, Tiberti C, Picarelli A, Mariani P, Rossi D, Cipolletta E, Greco M, Tola MD, Sabbatella L, Carabba B, Magliocca FM, Strisciuglio P and Di Mario U. (2001b) Radioimmunoassay to detect antitransglutaminase autoantibodies is the most sensitive and specific screening method for celiac disease. Am J Gastroenterol. 96:1536-1540.

Bonamico M, Pasquino AM, Mariani P, Danesi HM, Culasso F, Mazzanti L, Petri A, Bona G, Italian Society Of Pediatric Gastroenterology Hepatology (SIGEP) and Italian Study Group for Turner

Syndrom (ISGTS). (2002) Prevalence and clinical picture of celiac disease in Turner syndrome. J Clin Endocrinol Metab. 87:5495-5498.
Bonamico M, Mariani P, Thanasi E, Ferri M, Nenna R, Tiberti C, Mora B, Mazzilli MC and Magliocca FM. (2004) Patchy villous atrophy of the duodenum in childhood celiac disease. J Pediatr Gastroenterol Nutr. 38:204-207.
Bonamico M, Ferri M, Mariani P, Nenna R, Thanasi E, Luparia RP, Picarelli A, Magliocca FM, Mora B, Bardella MT, Verrienti A, Fiore B, Uccini S, Megiorni F, Mazzilli MC and Tiberti C. (2006) Serologic and genetic markers of celiac disease: a sequential study in the screening of first degree relatives. J Pediatr Gastroenterol Nutr. 42:150-154.
Borrelli M, Maglio M, Agnese M, Paparo F, Gentile S, Colicchio B, Tosco A, Auricchio R and Troncone R. (2009) High density of intraepithelial gammadelta lymphocytes and deposits of immunoglobulin (Ig)M anti-tissue transglutaminase antibodies in the jejunum of coeliac patients with IgA deficiency. Clin Exp Immunol. DOI:10.1111/j.1365-2249.2009.04077.x:
Borst J, van Dongen JJ, Bolhuis RL, Peters PJ, Hafler DA, de Vries E and van de Griend RJ. (1988) Distinct molecular forms of human T cell receptor gamma/delta detected on viable T cells by a monoclonal antibody. J Exp Med. 167:1625-1644.
Bourne JT, Kumar P, Huskisson EC, Mageed R, Unsworth DJ and Wojtulewski JA. (1985) Arthritis and coeliac disease. Ann Rheum Dis. 44:592-598.
Brousse N and Meijer JW. (2005) Malignant complications of coeliac disease. Best Pract Res Clin Gastroenterol. 19:401-412.
Burgin-Wolff A, Dahlbom I, Hadziselimovic F and Petersson CJ. (2002) Antibodies against human tissue transglutaminase and endomysium in diagnosing and monitoring coeliac disease. Scand J Gastroenterol. 37:685-691.
Carnicer J, Farre C, Varea V, Vilar P, Moreno J and Artigas J. (2001) Prevalence of coeliac disease in Down's syndrome. Eur J Gastroenterol Hepatol. 13:263-267.
Carroccio A, Cavataio F, Montalto G, Paparo F, Troncone R and Iacono G. (2001) Treatment of giardiasis reverses "active" coeliac disease to "latent" coeliac disease. Eur J Gastroenterol Hepatol. 13:1101-1105.
Carroccio A, Iacono G, D'Amico D, Cavataio F, Teresi S, Caruso C, Di PL, Colombo A, D'Arpa F, Florena A, Notarbartolo A and Montalto G. (2002a) Production of anti-endomysial antibodies in cultured duodenal mucosa: usefulness in coeliac disease diagnosis. Scand J Gastroenterol. 37:32-38.
Carroccio A, Vitale G, Di Prima L, Chifari N, Napoli S, La Russa C, Gulotta G, Averna MR, Montalto G, Mansueto S and Notarbartolo A. (2002b) Comparison of anti-transglutaminase ELISAs and an anti-endomysial antibody assay in the diagnosis of celiac disease: a prospective study. Clin Chem. 48:1546-1550.
Catassi C, Ratsch IM, Gandolfi L, Pratesi R, Fabiani E, El Asmar R, Frijia M, Bearzi I and Vizzoni L. (1999) Why is coeliac disease endemic in the people of the Sahara? Lancet. 354:647-648.
Catassi C, Cellier C, Cerf-Bensussan N, Ciclitira PJ, Collin P, Corazza GR, Dickey W, Fasano A, Holmes GK, Klincewicz P, Mearin ML, Mulder CJS, Murray JA, Pena AS, Schuppan D, Sollid LM, Uil JJ, Wahab PJ, Walker-Smith JA and Watson P. (2001) When is a coeliac a coeliac? Report of a working group of the United European Gastroenterology Week in Amsterdam, 2001. Eur J Gastroenterol Hepatol. 13:1123-1128.
Cellier C, Patey N, Mauvieux L, Jabri B, Delabesse E, Cervoni JP, Burtin ML, Guy-Grand D, Bouhnik Y, Modigliani R, Barbier JP, Macintyre E, Brousse N and Cerf-Bensussan N. (1998) Abnormal intestinal intraepithelial lymphocytes in refractory sprue. Gastroenterology. 114:471-481.
Cellier C, Delabesse E, Helmer C, Patey N, Matuchansky C, Jabri B, Macintyre E, Cerf-Bensussan N and Brousse N. (2000) Refractory sprue, coeliac disease, and enteropathy-associated T-cell lymphoma. French Coeliac Disease Study Group. Lancet. 356:203-208.
Cello JP and Day LW. (2009) Idiopathic AIDS enteropathy and treatment of gastrointestinal opportunistic pathogens. Gastroenterology. 136:1952-1965.
Ciacci C, Cirillo M, Mellone M, Basile F, Mazzacca G and De Santo NG. (1995) Hypocalciuria in overt and subclinical celiac disease. Am J Gastroenterol. 90:1480-1484.
Clemente MG, Musu MP, Frau F, Brusco G, Sole G, Corazza GR and De Virgiliis S. (2000) Immune reaction against the cytoskeleton in coeliac disease. Gut. 47:520-526.
Collin P and Reunala T. (2003) Recognition and management of the cutaneous manifestations of celiac disease: a guide for dermatologists. Am J Clin Dermatol. 4:13-20

Collin P, Salmi J, Hällström O, Oksa H, Oksala H, Mäki M and Reunala T. (1989) High frequency of coeliac disease in adult patients with type-I diabetes. Scand J Gastroenterol. 24:81-84.
Collin P, Mäki M, Keyriläinen O, Hällström O, Reunala T and Pasternack A. (1992) Selective IgA deficiency and coeliac disease. Scand J Gastroenterol. 27:367-371.
Collin P, Helin H, Mäki M, Hällström O and Karvonen AL. (1993) Follow-up of patients positive in reticulin and gliadin antibody tests with normal small-bowel biopsy findings. Scand J Gastroenterol. 28:595-598.
Collin P, Reunala T, Pukkala E, Laippala P, Keyriläinen O and Pasternack A. (1994a) Coeliac disease--associated disorders and survival. Gut. 35:1215-1218.
Collin P, Salmi J, Hällström O, Reunala T and Pasternack A. (1994b) Autoimmune thyroid disorders and coeliac disease. Eur J Endocrinol. 130:137-140.
Collin P, Vilska S, Heinonen PK, Hällström O and Pikkarainen P. (1996) Infertility and coeliac disease. Gut. 39:382-384.
Collin P, Reunala T, Rasmussen M, Kyrönpalo S, Pehkonen E, Laippala P and Mäki M. (1997) High incidence and prevalence of adult coeliac disease. Augmented diagnostic approach. Scand J Gastroenterol. 32:1129-1133.
Collin P, Syrjänen J, Partanen J, Pasternack A, Kaukinen K and Mustonen J. (2002) Celiac disease and HLA DQ in patients with IgA nephropathy. Am J Gastroenterol. 97:2572-2576..
Collin P, Mäki M and Kaukinen K. (2004a) Complete small intestine mucosal recovery is obtainable in the treatment of celiac disease. Gastrointest Endosc. 59:158-9; author reply 159-60.
Collin P, Thorell L, Kaukinen K and Mäki M. (2004b) The safe threshold for gluten contamination in gluten-free products. Can trace amounts be accepted in the treatment of coeliac disease? Aliment Pharmacol Ther. 19:1277-1283.
Collin P, Kaukinen K, Vogelsang H, Korponay-Szabo I, Sommer R, Schreier E, Volta U, Granito A, Veronesi L, Mascart F, Ocmant A, Ivarsson A, Lagerqvist C, Burgin-Wolff A, Hadziselimovic F, Furlano RI, Sidler MA, Mulder CJ, Goerres MS, Mearin ML, Ninaber MK, Gudmand-Hoyer E, Fabiani E, Catassi C, Tidlund H, Alainentalo L and Mäki M. (2005) Antiendomysial and antihuman recombinant tissue transglutaminase antibodies in the diagnosis of coeliac disease: a biopsy-proven European multicentre study. Eur J Gastroenterol Hepatol. 17:85-91.
Corazza GR, Andreani ML, Venturo N, Bernardi M, Tosti A and Gasbarrini G. (1995a) Celiac disease and alopecia areata: report of a new association. Gastroenterology. 109:1333-1337.
Corazza GR, Di Sario A, Cecchetti L, Tarozzi C, Corrao G, Bernardi M and Gasbarrini G. (1995b) Bone mass and metabolism in patients with celiac disease. Gastroenterology. 109:122-128.
Corrao G, Corazza GR, Bagnardi V, Brusco G, Ciacci C, Cottone M, Sategna Guidetti C, Usai P, Cesari P, Pelli MA, Loperfido S, Volta U, Calabro A, Certo M and Club del Tenue Study Group. (2001) Mortality in patients with coeliac disease and their relatives: a cohort study. Lancet. 358:356-361.
Cosnes J, Cellier C, Viola S, Colombel JF, Michaud L, Sarles J, Hugot JP, Ginies JL, Dabadie A, Mouterde O, Allez M, Nion-Larmurier I and Groupe D'Etude et de Recherche Sur la Maladie Coeliaque. (2008) Incidence of autoimmune diseases in celiac disease: protective effect of the gluten-free diet. Clin Gastroenterol Hepatol. 6:753-758.
Damen GM, Boersma B, Wit JM and Heymans HS. (1994) Catch-up growth in 60 children with celiac disease. J Pediatr Gastroenterol Nutr. 19:394-400.
Daum S, Weiss D, Hummel M, Ullrich R, Heise W, Stein H, Riecken EO, Foss HD and Intestinal Lymphoma Study Group. (2001) Frequency of clonal intraepithelial T lymphocyte proliferations in enteropathy-type intestinal T cell lymphoma, coeliac disease, and refractory sprue. Gut. 49:804-812.
de Mascarel A, Belleannee G, Stanislas S, Merlio C, Parrens M, Laharie D, Dubus P and Merlio JP. (2008) Mucosal intraepithelial T-lymphocytes in refractory celiac disease: a neoplastic population with a variable CD8 phenotype. Am J Surg Pathol. 32:744-751.
Demirceken FG, Kansu A, Kuloglu Z, Girgin N, Guriz H and Ensari A. (2008) Human tissue transglutaminase antibody screening by immunochromatographic line immunoassay for early diagnosis of celiac disease in Turkish children. Turk J Gastroenterol. 19:14-21.
Diamanti A, Basso MS, Pietrobattista A and Nobili V. (2008) Prevalence of celiac disease in children with autoimmune hepatitis. Dig Liver Dis. 40:965.
Dicke WK. (1950) Coeliakie.
Dickey W, McMillan SA and Callender ME. (1997) High prevalence of celiac sprue among patients with primary biliary cirrhosis. J Clin Gastroenterol. 25:328-329.

Dickey W, Hughes DF and McMillan SA. (2000) Disappearance of endomysial antibodies in treated celiac disease does not indicate histological recovery. Am J Gastroenterol. 95:712-714.
Dickey W and Kearney N. (2006) Overweight in celiac disease: prevalence, clinical characteristics, and effect of a gluten-free diet. Am J Gastroenterol. 101:2356-2359.
Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO and Schuppan D. (1997) Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med. 3:797-801.
Dieterich W, Laag E, Schopper H, Volta U, Ferguson A, Gillett H, Riecken EO and Schuppan D. (1998) Autoantibodies to tissue transglutaminase as predictors of celiac disease. Gastroenterology. 115:1317-1321.
Dipper C, Maitra S, Thomas R, Lamb C, McLean-Tooke A, Ward R, Smith D, Spickett G and Mansfield J. (2009) Anti-tissue transglutaminase antibodies in the follow-up of adult coeliac disease. Aliment Pharmacol Ther. 30:236-244.
Diss TC, Watts M, Pan LX, Burke M, Linch D and Isaacson PG. (1995) The polymerase chain reaction in the demonstration of monoclonality in T cell lymphomas. J Clin Pathol. 48:1045-1050.
Drago S, El Asmar R, Di Pierro M, Grazia Clemente M, Tripathi A, Sapone A, Thakar M, Iacono G, Carroccio A, D'Agate C, Not T, Zampini L, Catassi C and Fasano A. (2006) Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand J Gastroenterol. 41:408-419.
Emami MH, Karimi S, Kouhestani S, Hashemi M and Taheri H. (2008) Diagnostic accuracy of IgA anti-tissue transglutaminase in patients suspected of having coeliac disease in Iran. J Gastrointestin Liver Dis. 17:141-146.
Esposito C, Paparo F, Caputo I, Rossi M, Maglio M, Sblattero D, Not T, Porta R, Auricchio S, Marzari R and Troncone R. (2002) Anti-tissue transglutaminase antibodies from coeliac patients inhibit transglutaminase activity both in vitro and in situ. Gut. 51:177-181.
Fabiani E, Taccari LM, Ratsch IM, Di Giuseppe S, Coppa GV and Catassi C. (2000) Compliance with gluten-free diet in adolescents with screening-detected celiac disease: a 5-year follow-up study. J Pediatr. 136:841-843.
Fabiani E, Catassi C and International Working Group. (2001) The serum IgA class anti-tissue transglutaminase antibodies in the diagnosis and follow up of coeliac disease. Results of an international multi-centre study. International Working Group on Eu-tTG. Eur J Gastroenterol Hepatol. 13:659-665.
Fabiani E, Peruzzi E, Mandolesi A, Garbuglia G, Fanciulli G, D'Appello AR, Gasparin M, Bravi E, Bearzi I, Galeazzi R and Catassi C. (2004) Anti-human versus anti-guinea pig tissue transglutaminase antibodies as the first-level serological screening test for coeliac disease in the general population. Dig Liver Dis. 36:671-676.
Farre C, Esteve M, Curcoy A, Cabre E, Arranz E, Amat LL and Garcia-Tornel S. (2002) Hypertransaminasemia in pediatric celiac disease patients and its prevalence as a diagnostic clue. Am J Gastroenterol. 97:3176-3181.
Farstad IN, Johansen FE, Vlatkovic L, Jahnsen J, Scott H, Fausa O, Bjorneklett A, Brandtzaeg P and Halstensen TS. (2002) Heterogeneity of intraepithelial lymphocytes in refractory sprue: potential implications of CD30 expression. Gut. 51:372-378.
Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PH, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wasserman SS, Murray JA and Horvath K. (2003) Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med. 163:286-292.
Ferguson A and Murray D. (1971) Quantitation of intraepithelial lymphocytes in human jejunum. Gut. 12:988-994.
Ferguson A, Arranz E and O'Mahony S. (1993) Clinical and pathological spectrum of coeliac disease--active, silent, latent, potential. Gut. 34:150-151.
Ferguson R, Basu MK, Asquith P and Cooke WT. (1976) Jejunal mucosal abnormalities in patients with recurrent aphthous ulceration. Br Med J. 1:11-13.
Ferreira M, Davies SL, Butler M, Scott D, Clark M and Kumar P. (1992) Endomysial antibody: is it the best screening test for coeliac disease? Gut. 33:1633-1637.
Fine KD, Ogunji F, Saloum Y, Beharry S, Crippin J and Weinstein J. (2001) Celiac sprue: another autoimmune syndrome associated with hepatitis C. Am J Gastroenterol. 96:138-145.
Freeman HJ and Chiu BK. (1986) Multifocal small bowel lymphoma and latent celiac sprue. Gastroenterology. 90:1992-1997.

Frost AR, Band MM and Conway GS. (2009) Serological screening for coeliac disease in adults with Turner's syndrome: prevalence and clinical significance of endomysium antibody positivity. Eur J Endocrinol. 160:675-679.

Frustaci A, Cuoco L, Chimenti C, Pieroni M, Fioravanti G, Gentiloni N, Maseri A and Gasbarrini G. (2002) Celiac disease associated with autoimmune myocarditis. Circulation. 105:2611-2618.

Gabrielli M, Cremonini F, Fiore G, Addolorato G, Padalino C, Candelli M, De Leo ME, Santarelli L, Giacovazzo M, Gasbarrini A, Pola P and Gasbarrini A. (2003) Association between migraine and Celiac disease: results from a preliminary case-control and therapeutic study. Am J Gastroenterol. 98:625-629.

Gale J, Simmonds PD, Mead GM, Sweetenham JW and Wright DH. (2000) Enteropathy-type intestinal T-cell lymphoma: clinical features and treatment of 31 patients in a single center. J Clin Oncol. 18:795-803.

Gass J, Bethune MT, Siegel M, Spencer A and Khosla C. (2007) Combination enzyme therapy for gastric digestion of dietary gluten in patients with celiac sprue. Gastroenterology. 133:472-480.

Gee S. (1888) On the coeliac affection. St Bart Hosp Rep. 24:17-20.

Gobbi G. (2005) Coeliac disease, epilepsy and cerebral calcifications. Brain Dev. 27:189-200.

Gobbi G, Bouquet F, Greco L, Lambertini A, Tassinari CA, Ventura A and Zaniboni MG. (1992) Coeliac disease, epilepsy, and cerebral calcifications. The Italian Working Group on Coeliac Disease and Epilepsy. Lancet. 340:439-443.

Goldstein NS and Underhill J. (2001) Morphologic features suggestive of gluten sensitivity in architecturally normal duodenal biopsy specimens. Am J Clin Pathol. 116:63-71.

Greco L, Babron MC, Corazza GR, Percopo S, Sica R, Clot F, Fulchignoni-Lataud MC, Zavattari P, Momigliano-Richiardi P, Casari G, Gasparini P, Tosi R, Mantovani V, De Virgiliis S, Iacono G, D'Alfonso A, Selinger-Leneman H, Lemainque A, Serre JL and Clerget-Darpoux F. (2001) Existence of a genetic risk factor on chromosome 5q in Italian coeliac disease families. Ann Hum Genet. 65:35-41.

Greco L, Romino R, Coto I, Di Cosmo N, Percopo S, Maglio M, Paparo F, Gasperi V, Limongelli MG, Cotichini R, D'Agate C, Tinto N, Sacchetti L, Tosi R and Stazi MA. (2002) The first large population based twin study of coeliac disease. Gut. 50:624-628.

Green PH and Cellier C. (2007) Celiac disease. N Engl J Med. 357:1731-1743.

Hadithi M, de Boer H, Meijer JW, Willekens F, Kerckhaert JA, Heijmans R, Pena AS, Stehouwer CD and Mulder CJ. (2007) Coeliac disease in Dutch patients with Hashimoto's thyroiditis and vice versa. World J Gastroenterol. 13:1715-1722.

Hadjivassiliou M, Gibson A, Davies-Jones GA, Lobo AJ, Stephenson TJ and Milford-Ward A. (1996) Does cryptic gluten sensitivity play a part in neurological illness? Lancet. 347:369-371.

Hadjivassiliou M, Grunewald RA, Chattopadhyay AK, Davies-Jones GA, Gibson A, Jarratt JA, Kandler RH, Lobo A, Powell T and Smith CM. (1998) Clinical, radiological, neurophysiological, and neuropathological characteristics of gluten ataxia. Lancet. 352:1582-1585.

Hadjivassiliou M, Davies-Jones GA, Sanders DS and Grunewald RA. (2003) Dietary treatment of gluten ataxia. J Neurol Neurosurg Psychiatry. 74:1221-1224.

Hadjivassiliou M, Mäki M, Sanders DS, Williamson CA, Grunewald RA, Woodroofe NM and Korponay-Szabo IR. (2006) Autoantibody targeting of brain and intestinal transglutaminase in gluten ataxia. Neurology. 66:373-377.

Hadjivassiliou M, Aeschlimann P, Strigun A, Sanders DS, Woodroofe N and Aeschlimann D. (2008) Autoantibodies in gluten ataxia recognize a novel neuronal transglutaminase. Ann Neurol. 64:332-343.

Hakanen M, Luotola K, Salmi J, Laippala P, Kaukinen K and Collin P. (2001) Clinical and subclinical autoimmune thyroid disease in adult celiac disease. Dig Dis Sci. 46:2631-2635.

Hall NJ, Rubin G and Charnock A. (2009) Systematic review: Adherence to a gluten-free diet in adult patients with coeliac disease. Aliment Pharmacol Ther. 30:315-30.

Halstensen TS, Scott H and Brandtzaeg P. (1989) Intraepithelial T cells of the TcR gamma/delta+ CD8- and V delta 1/J delta 1+ phenotypes are increased in coeliac disease. Scand J Immunol. 30:665-672.

Halttunen T and Mäki M. (1999) Serum immunoglobulin A from patients with celiac disease inhibits human T84 intestinal crypt epithelial cell differentiation. Gastroenterology. 116:566-572.

Hansen D, Brock-Jacobsen B, Lund E, Bjorn C, Hansen LP, Nielsen C, Fenger C, Lillevang ST and Husby S. (2006) Clinical benefit of a gluten-free diet in type 1 diabetic children with screening-detected celiac disease: a population-based screening study with 2 years' follow-up. Diabetes Care. 29:2452-2456.

Hardman CM, Garioch JJ, Leonard JN, Thomas HJ, Walker MM, Lortan JE, Lister A and Fry L. (1997) Absence of toxicity of oats in patients with dermatitis herpetiformis. N Engl J Med. 337:1884-1887.

Hervonen K, Vornanen M, Kautiainen H, Collin P and Reunala T. (2005) Lymphoma in patients with dermatitis herpetiformis and their first-degree relatives. Br J Dermatol. 152:82-86.

Heyman M and Menard S. (2009) Pathways of gliadin transport in celiac disease. Ann N Y Acad Sci. 1165:274-278.

Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, Hoffenberg EJ, Horvath K, Murray JA, Pivor M, Seidman EG and North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. (2005) Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr. 40:1-19.

Hoffenberg EJ, Haas J, Drescher A, Barnhurst R, Osberg I, Bao F and Eisenbarth G. (2000) A trial of oats in children with newly diagnosed celiac disease. J Pediatr. 137:361-366.

Hollen E, Holmgren Peterson K, Sundqvist T, Grodzinsky E, Högberg L, Laurin P, Stenhammar L, Falth-Magnusson K and Magnusson KE. (2006) Coeliac children on a gluten-free diet with or without oats display equal anti-avenin antibody titres. Scand J Gastroenterol. 41:42-47.

Holm K, Mäki M, Vuolteenaho N, Mustalahti K, Ashorn M, Ruuska T and Kaukinen K. (2006) Oats in the treatment of childhood coeliac disease: a 2-year controlled trial and a long-term clinical follow-up study. Aliment Pharmacol Ther. 23:1463-1472.

Holmes GK, Prior P, Lane MR, Pope D and Allan RN. (1989) Malignancy in coeliac disease--effect of a gluten free diet. Gut. 30:333-338.

Holopainen P, Arvas M, Sistonen P, Mustalahti K, Collin P, Mäki M and Partanen J. (1999) CD28/CTLA4 gene region on chromosome 2q33 confers genetic susceptibility to celiac disease. A linkage and family-based association study. Tissue Antigens. 53:470-475.

Holopainen P, Naluai AT, Moodie S, Percopo S, Coto I, Clot F, Ascher H, Sollid L, Ciclitira P, Greco L, Clerget-Darpoux F, Partanen J and Members of the European Genetics Cluster on Coeliac Disease. (2004) Candidate gene region 2q33 in European families with coeliac disease. Tissue Antigens. 63:212-222.

Hopman EG, von Blomberg ME, Batstra MR, Morreau H, Dekker FW, Koning F, Lamers CB and Mearin ML. (2008) Gluten tolerance in adult patients with celiac disease 20 years after diagnosis? Eur J Gastroenterol Hepatol. 20:423-429.

Hopper AD, Cross SS and Sanders DS. (2008) Patchy villous atrophy in adult patients with suspected gluten-sensitive enteropathy: is a multiple duodenal biopsy strategy appropriate? Endoscopy. 40:219-224.

Hue S, Mention JJ, Monteiro RC, Zhang S, Cellier C, Schmitz J, Verkarre V, Fodil N, Bahram S, Cerf-Bensussan N and Caillat-Zucman S. (2004) A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. Immunity. 21:367-377.

Hällström O. (1989) Comparison of IgA-class reticulin and endomysium antibodies in coeliac disease and dermatitis herpetiformis. Gut. 30:1225-1232.

Högberg L, Grodzinsky E and Stenhammar L. (2003) Better dietary compliance in patients with coeliac disease diagnosed in early childhood. Scand J Gastroenterol. 38:751-754.

Högberg L, Laurin P, Falth-Magnusson K, Grant C, Grodzinsky E, Jansson G, Ascher H, Browaldh L, Hammersjo JA, Lindberg E, Myrdal U and Stenhammar L. (2004) Oats to children with newly diagnosed coeliac disease: a randomised double blind study. Gut. 53:649-654.

Iltanen S, Collin P, Korpela M, Holm K, Partanen J, Polvi A and Mäki M. (1999a) Celiac disease and markers of celiac disease latency in patients with primary Sjogren's syndrome. Am J Gastroenterol. 94:1042-1046.

Iltanen S, Holm K, Ashorn M, Ruuska T, Laippala P and Mäki M. (1999b) Changing jejunal gamma delta T cell receptor (TCR)-bearing intraepithelial lymphocyte density in coeliac disease. Clin Exp Immunol. 117:51-55.

Iltanen S, Holm K, Partanen J, Laippala P and Mäki M. (1999c) Increased density of jejunal gammadelta+ T cells in patients having normal mucosa--marker of operative autoimmune mechanisms? Autoimmunity. 29:179-187.

Ivarsson A, Hernell O, Stenlund H and Persson LA. (2002) Breast-feeding protects against celiac disease. Am J Clin Nutr. 75:914-921.

Ivarsson A, Hernell O, Nystrom L and Persson LA. (2003) Children born in the summer have increased risk for coeliac disease. J Epidemiol Community Health. 57:36-39.

Jabri B, Kasarda DD and Green PH. (2005) Innate and adaptive immunity: the yin and yang of celiac disease. Immunol Rev. 206:219-231.
Janatuinen EK, Pikkarainen PH, Kemppainen TA, Kosma VM, Järvinen RM, Uusitupa MI and Julkunen RJ. (1995) A comparison of diets with and without oats in adults with celiac disease. N Engl J Med. 333:1033-1037.
Janatuinen EK, Kemppainen TA, Pikkarainen PH, Holm KH, Kosma VM, Uusitupa MI, Mäki M and Julkunen RJ. (2000) Lack of cellular and humoral immunological responses to oats in adults with coeliac disease. Gut. 46:327-331.
Janatuinen EK, Kemppainen TA, Julkunen RJ, Kosma VM, Mäki M, Heikkinen M and Uusitupa MI. (2002) No harm from five year ingestion of oats in coeliac disease. Gut. 50:332-335.
Järvinen TT, Kaukinen K, Laurila K, Kyrönpalo S, Rasmussen M, Mäki M, Korhonen H, Reunala T and Collin P. (2003) Intraepithelial lymphocytes in celiac disease. Am J Gastroenterol. 98:1332-1337.
Järvinen TT, Collin P, Rasmussen M, Kyrönpalo S, Mäki M, Partanen J, Reunala T and Kaukinen K. (2004) Villous tip intraepithelial lymphocytes as markers of early-stage coeliac disease. Scand J Gastroenterol. 39:428-433.
Jenkins D, Goodall A and Scott BB. (1986) T-lymphocyte populations in normal and coeliac small intestinal mucosa defined by monoclonal antibodies. Gut. 27:1330-1337.
Jos J and Labbe F. (1976) Ultrastructural localization of IgA globulins in normal and coeliac intestinal mucosa using immunoenzymatic methods. Biomedicine. 24:425-434.
Jos J, Labbe F, Geny B and Griscelli C. (1979) Immunoelectron-microscopic localization of immunoglobulin A and secretory component in jejunal mucosa from children with coeliac disease. Scand J Immunol. 9:441-450.
Kagnoff MF, Austin RK, Hubert JJ, Bernardin JE and Kasarda DD. (1984) Possible role for a human adenovirus in the pathogenesis of celiac disease. J Exp Med. 160:1544-1557.
Kagnoff MF. (2007) Celiac disease: pathogenesis of a model immunogenetic disease. J Clin Invest. 117:41-49.
Karell K, Louka AS, Moodie SJ, Ascher H, Clot F, Greco L, Ciclitira PJ, Sollid LM, Partanen J and European Genetics Cluster on Celiac Disease. (2003) HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. Hum Immunol. 64:469-477.
Karinen H, Kärkkäinen P, Pihlajamäki J, Janatuinen E, Heikkinen M, Julkunen R, Kosma VM, Naukkarinen A and Laakso M. (2006) Gene dose effect of the DQB1*0201 allele contributes to severity of coeliac disease. Scand J Gastroenterol. 41:191-199.
Karpati S, Kosnai I, Torok E and Kovacs JB. (1988) Immunoglobulin A deposition in jejunal mucosa of children with dermatitis herpetiformis. J Invest Dermatol. 91:336-339.
Kaukinen K, Collin P, Holm K, Rantala I, Vuolteenaho N, Reunala T and Mäki M. (1999) Wheat starch-containing gluten-free flour products in the treatment of coeliac disease and dermatitis herpetiformis. A long-term follow-up study. Scand J Gastroenterol. 34:163-169.
Kaukinen K, Mäki M, Partanen J, Sievänen H and Collin P. (2001) Celiac disease without villous atrophy: revision of criteria called for. Dig Dis Sci. 46:879-887.
Kaukinen K, Halme L, Collin P, Färkkilä M, Mäki M, Vehmanen P, Partanen J and Hockerstedt K. (2002a) Celiac disease in patients with severe liver disease: gluten-free diet may reverse hepatic failure. Gastroenterology. 122:881-888.
Kaukinen K, Sulkanen S, Mäki M and Collin P. (2002b) IgA-class transglutaminase antibodies in evaluating the efficacy of gluten-free diet in coeliac disease. Eur J Gastroenterol Hepatol. 14:311-315.
Kaukinen K, Peräaho M, Collin P, Partanen J, Woolley N, Kaartinen T, Nuutinen T, Halttunen T, Mäki M and Korponay-Szabo I. (2005) Small-bowel mucosal transglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: a prospective and randomized clinical study. Scand J Gastroenterol. 40:564-572.
Kaukinen K, Collin P, Laurila K, Kaartinen T, Partanen J and Mäki M. (2007a) Resurrection of gliadin antibodies in coeliac disease. Deamidated gliadin peptide antibody test provides additional diagnostic benefit. Scand J Gastroenterol. 42:1428-1433.
Kaukinen K, Peräaho M, Lindfors K, Partanen J, Woolley N, Pikkarainen P, Karvonen AL, Laasanen T, Sievänen H, Mäki M and Collin P. (2007b) Persistent small bowel mucosal villous atrophy without symptoms in coeliac disease. Aliment Pharmacol Ther. 25:1237-1245.

Kemppainen T, Kroger H, Janatuinen E, Arnala I, Kosma VM, Pikkarainen P, Julkunen R, Jurvelin J, Alhava E and Uusitupa M. (1999a) Osteoporosis in adult patients with celiac disease. Bone. 24:249-255.

Kemppainen T, Kroger H, Janatuinen E, Arnala I, Lamberg-Allardt C, Kärkkäinen M, Kosma VM, Julkunen R, Jurvelin J, Alhava E and Uusitupa M. (1999b) Bone recovery after a gluten-free diet: a 5-year follow-up study. Bone. 25:355-360.

Kilmartin C, Lynch S, Abuzakouk M, Wieser H and Feighery C. (2003) Avenin fails to induce a Th1 response in coeliac tissue following in vitro culture. Gut. 52:47-52.

Kiraly R, Vecsei Z, Demenyi T, Korponay-Szabo IR and Fesus L. (2006) Coeliac autoantibodies can enhance transamidating and inhibit GTPase activity of tissue transglutaminase: dependence on reaction environment and enzyme fitness. J Autoimmun. 26:278-287.

Kolho KL and Savilahti E. (1997) IgA endomysium antibodies on human umbilical cord: an excellent diagnostic tool for celiac disease in childhood. J Pediatr Gastroenterol Nutr. 24:563-567.

Kondrashova A, Mustalahti K, Kaukinen K, Viskari H, Volodicheva V, Haapala AM, Ilonen J, Knip M, Mäki M, Hyoty H and Epivir Study Group. (2008) Lower economic status and inferior hygienic environment may protect against celiac disease. Ann Med. 40:223-231.

Korponay-Szabo IR, Kovacs JB, Czinner A, Goracz G, Vamos A and Szabo T. (1999) High prevalence of silent celiac disease in preschool children screened with IgA/IgG antiendomysium antibodies. J Pediatr Gastroenterol Nutr. 28:26-30.

Korponay-Szabo IR, Sulkanen S, Halttunen T, Maurano F, Rossi M, Mazzarella G, Laurila K, Troncone R and Mäki M. (2000) Tissue transglutaminase is the target in both rodent and primate tissues for celiac disease-specific autoantibodies. J Pediatr Gastroenterol Nutr. 31:520-527.

Korponay-Szabo IR, Dahlbom I, Laurila K, Koskinen S, Woolley N, Partanen J, Kovacs JB, Mäki M and Hansson T. (2003a) Elevation of IgG antibodies against tissue transglutaminase as a diagnostic tool for coeliac disease in selective IgA deficiency. Gut. 52:1567-1571.

Korponay-Szabo IR, Laurila K, Szondy Z, Halttunen T, Szalai Z, Dahlbom I, Rantala I, Kovacs JB, Fesus L and Mäki M. (2003b) Missing endomysial and reticulin binding of coeliac antibodies in transglutaminase 2 knockout tissues. Gut. 52:199-204.

Korponay-Szabo IR, Halttunen T, Szalai Z, Laurila K, Kiraly R, Kovacs JB, Fesus L and Mäki M. (2004) In vivo targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. Gut. 53:641-648.

Kotze LM. (2004) Gynecologic and obstetric findings related to nutritional status and adherence to a gluten-free diet in Brazilian patients with celiac disease. J Clin Gastroenterol. 38:567-574.

Kuitunen P, Kosnai I and Savilahti E. (1982) Morphometric study of the jejunal mucosa in various childhood enteropathies with special reference to intraepithelial lymphocytes. J Pediatr Gastroenterol Nutr. 1:525-531.

Kurppa K, Koskinen O, Collin P, Mäki M, Reunala T and Kaukinen K. (2008) Changing phenotype of celiac disease after long-term gluten exposure. J Pediatr Gastroenterol Nutr. 47:500-503.

Kurppa K, Collin P, Viljamaa M, Haimila K, Saavalainen P, Partanen J, Laurila K, Huhtala H, Paasikivi K, Mäki M and Kaukinen K. (2009) Diagnosing mild enteropathy celiac disease: a randomized, controlled clinical study. Gastroenterology. 136:816-823.

Kutlu T, Brousse N, Rambaud C, Le Deist F, Schmitz J and Cerf-Bensussan N. (1993) Numbers of T cell receptor (TCR) alpha beta+ but not of TcR gamma delta+ intraepithelial lymphocytes correlate with the grade of villous atrophy in coeliac patients on a long term normal diet. Gut. 34:208-214.

Lancaster-Smith M, Packer S, Kumar PJ and Harries JT. (1976) Immunological phenomena in the jejunum and serum after reintroduction of dietary gluten in children with treated coeliac disease. J Clin Pathol. 29:592-597.

Lancaster-Smith M, Joyce S and Kumar P. (1977) Immunoglobulins in the jejunal mucosa in adult coeliac disease and dermatitis herpetiformis after the reintroduction of dietary gluten. Gut. 18:887-891.

Leffler DA, Dennis M, Hyett B, Kelly E, Schuppan D and Kelly CP. (2007) Etiologies and predictors of diagnosis in nonresponsive celiac disease. Clin Gastroenterol Hepatol. 5:445-450.

Lepore L, Martelossi S, Pennesi M, Falcini F, Ermini ML, Ferrari R, Perticarari S, Presani G, Lucchesi A, Lapini M and Ventura A. (1996) Prevalence of celiac disease in patients with juvenile chronic arthritis. J Pediatr. 129:311-313.

Lindberg T, Nilsson LA, Borulf S, Cavell B, Fallstrom SP, Jansson U, Stenhammar L and Stintzing G. (1985) Serum IgA and IgG gliadin antibodies and small intestinal mucosal damage in children. J Pediatr Gastroenterol Nutr. 4:917-922.

Liu J, Juo SH, Holopainen P, Terwilliger J, Tong X, Grunn A, Brito M, Green P, Mustalahti K, Mäki M, Gilliam TC and Partanen J. (2002) Genomewide linkage analysis of celiac disease in Finnish families. Am J Hum Genet. 70:51-59.

Logan RF, Rifkind EA, Busuttil A, Gilmour HM and Ferguson A. (1986) Prevalence and "incidence" of celiac disease in Edinburgh and the Lothian region of Scotland. Gastroenterology. 90:334-342.

Lohi S, Mustalahti K, Kaukinen K, Laurila K, Collin P, Rissanen H, Lohi O, Bravi E, Gasparin M, Reunanen A and Mäki M. (2007) Increasing prevalence of coeliac disease over time. Aliment Pharmacol Ther. 26:1217-1225.

Luft LM, Barr SG, Martin LO, Chan EK and Fritzler MJ. (2003) Autoantibodies to tissue transglutaminase in Sjogren's syndrome and related rheumatic diseases. J Rheumatol. 30:2613-2619.

Lundin KE, Nilsen EM, Scott HG, Loberg EM, Gjoen A, Bratlie J, Skar V, Mendez E, Lovik A and Kett K. (2003) Oats induced villous atrophy in coeliac disease. Gut. 52:1649-1652.

Luostarinen L, Pirttila T and Collin P. (1999) Coeliac disease presenting with neurological disorders. Eur Neurol. 42:132-135.

Maglio M, Florian F, Vecchiet M, Auricchio R, Paparo F, Spadaro R, Zanzi D, Rapacciuolo L, Franzese A, Sblattero D, Marzari R and Troncone R. (2009) The great majority of children with type 1 diabetes produce and deposit anti-tissue transglutaminase antibodies in the small intestine. Diabetes. 58:1578-1584.

Maglio M, Tosco A, Paparo F, Auricchio R, Granata V, Colicchio B, Indolfi V, Miele E and Troncone R. (2010) Serum and Intestinal Celiac Disease-associated Antibodies in Children With Celiac Disease Younger Than 2 Years of Age. J Pediatr Gastroenterol Nutr. 50:43-8.

Mahadeva S, Wyatt JI and Howdle PD. (2002) Is a raised intraepithelial lymphocyte count with normal duodenal villous architecture clinically relevant? J Clin Pathol. 55:424-428.

Maiuri L, Ciacci C, Vacca L, Ricciardelli I, Auricchio S, Quaratino S and Londei M. (2001) IL-15 drives the specific migration of CD94+ and TCR-gammadelta+ intraepithelial lymphocytes in organ cultures of treated celiac patients. Am J Gastroenterol. 96:150-156.

Maiuri L, Ciacci C, Ricciardelli I, Vacca L, Raia V, Auricchio S, Picard J, Osman M, Quaratino S and Londei M. (2003) Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease. Lancet. 362:30-37.

Mankai A, Sakly W, Landolsi H, Gueddah L, Sriha B, Ayadi A, Sfar MT, Skandrani K, Harbi A, Essoussi AS, Korbi S, Fabien N, Jeddi M and Ghedira I. (2005) Tissue transglutaminase antibodies in celiac disease, comparison of an enzyme linked immunosorbent assay and a dot blot assay. Pathol Biol (Paris). 53:204-209.

Mankai A, Ben Hamouda H, Amri F, Ghedira-Besbes L, Harbi A, Tahar Sfar M, Sahloul Essoussi A, Jeddi M and Ghedira I. (2007) Screening by anti-endomysium antibodies for celiac disease in Tunisian children with type 1 diabetes mellitus. Gastroenterol Clin Biol. 31:462-466.

Marsh MN. (1992) Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). Gastroenterology. 102:330-354.

Martinelli P, Troncone R, Paparo F, Torre P, Trapanese E, Fasano C, Lamberti A, Budillon G, Nardone G and Greco L. (2000) Coeliac disease and unfavourable outcome of pregnancy. Gut. 46:332-335.

Marzari R, Sblattero D, Florian F, Tongiorgi E, Not T, Tommasini A, Ventura A and Bradbury A. (2001) Molecular dissection of the tissue transglutaminase autoantibody response in celiac disease. J Immunol. 166:4170-4176.

Matysiak-Budnik T, Malamut G, de Serre NP, Grosdidier E, Seguier S, Brousse N, Caillat-Zucman S, Cerf-Bensussan N, Schmitz J and Cellier C. (2007) Long-term follow-up of 61 coeliac patients diagnosed in childhood: evolution toward latency is possible on a normal diet. Gut. 56:1379-1386.

Mawhinney H and Love AH. (1975) Anti-reticulin antibody in jejunal juice in coeliac disease. Clin Exp Immunol. 21:394-398.

Meloni GF, Tomasi PA, Bertoncelli A, Fanciulli G, Delitala G and Meloni T. (2001) Prevalence of silent celiac disease in patients with autoimmune thyroiditis from Northern Sardinia. J Endocrinol Invest. 24:298-302.

Memeo L, Jhang J, Hibshoosh H, Green PH, Rotterdam H and Bhagat G. (2005) Duodenal intraepithelial lymphocytosis with normal villous architecture: common occurrence in H. pylori gastritis. Mod Pathol. 18:1134-1144.

Mention JJ, Ben Ahmed M, Begue B, Barbe U, Verkarre V, Asnafi V, Colombel JF, Cugnenc PH, Ruemmele FM, McIntyre E, Brousse N, Cellier C and Cerf-Bensussan N. (2003) Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. Gastroenterology. 125:730-745.

Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T, Ebert EC, Green PH and Jabri B. (2004) Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity. 21:357-366.

Meyer D, Stavropolous S, Diamond B, Shane E and Green PH. (2001) Osteoporosis in a north american adult population with celiac disease. Am J Gastroenterol. 96:112-119.

Molberg O, Mcadam SN, Korner R, Quarsten H, Kristiansen C, Madsen L, Fugger L, Scott H, Noren O, Roepstorff P, Lundin KE, Sjostrom H and Sollid LM. (1998) Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. Nat Med. 4:713-717.

Monsuur AJ, de Bakker PI, Alizadeh BZ, Zhernakova A, Bevova MR, Strengman E, Franke L, van't Slot R, van Belzen MJ, Lavrijsen IC, Diosdado B, Daly MJ, Mulder CJ, Mearin ML, Meijer JW, Meijer GA, van Oort E, Wapenaar MC, Koeleman BP and Wijmenga C. (2005) Myosin IXB variant increases the risk of celiac disease and points toward a primary intestinal barrier defect. Nat Genet. 37:1341-1344.

Murray JA, Moore SB, Van Dyke CT, Lahr BD, Dierkhising RA, Zinsmeister AR, Melton LJ,3rd, Kroning CM, El-Youssef M and Czaja AJ. (2007) HLA DQ gene dosage and risk and severity of celiac disease. Clin Gastroenterol Hepatol. 5:1406-1412.

Mustalahti K, Collin P, Sievänen H, Salmi J and Mäki M. (1999) Osteopenia in patients with clinically silent coeliac disease warrants screening. Lancet. 354:744-745.

Mustalahti K, Lohiniemi S, Collin P, Vuolteenaho N, Laippala P and Mäki M. (2002a) Gluten-free diet and quality of life in patients with screen-detected celiac disease. Eff Clin Pract. 5:105-113.

Mustalahti K, Sulkanen S, Holopainen P, Laurila K, Collin P, Partanen J and Mäki M. (2002b) Coeliac disease among healthy members of multiple case coeliac disease families. Scand J Gastroenterol. 37:161-165.

Myhre AG, Aarsetoy H, Undlien DE, Hovdenak N, Aksnes L and Husebye ES. (2003) High frequency of coeliac disease among patients with autoimmune adrenocortical failure. Scand J Gastroenterol. 38:511-515.

Myleus A, Ivarsson A, Webb C, Danielsson L, Hernell O, Högberg L, Karlsson E, Lagerqvist C, Norstrom F, Rosen A, Sandstrom O, Stenhammar L, Stenlund H, Wall S and Carlsson A. (2009) Celiac Disease Revealed in 3% of Swedish 12-year-olds Born During an Epidemic. J Pediatr Gastroenterol Nutr. 49:170-176.

Mylotte M, Egan-Mitchell B, McCarthy CF and McNicholl B. (1973) Incidence of coeliac disease in the West of Ireland. Br Med J. 1:703-705.

Myrsky E, Kaukinen K, Syrjänen M, Korponay-Szabo IR, Mäki M and Lindfors K. (2008) Coeliac disease-specific autoantibodies targeted against transglutaminase 2 disturb angiogenesis. Clin Exp Immunol. 152:111-119.

Myrsky E, Caja S, Simon-Vecsei Z, Korponay-Szabo IR, Nadalutti C, Collighan R, Mongeot A, Griffin M, Mäki M, Kaukinen K and Lindfors K. (2009) Celiac disease IgA modulates vascular permeability in vitro through the activity of transglutaminase 2 and RhoA. Cell Mol Life Sci. 66:3375-3385.

Mäki M and Collin P. (1997) Coeliac disease. Lancet. 349:1755-1759.

Mäki M, Hällström O, Huupponen T, Vesikari T and Visakorpi JK. (1984a) Increased prevalence of coeliac disease in diabetes. Arch Dis Child. 59:739-742.

Mäki M, Hällström O, Vesikari T and Visakorpi JK. (1984b) Evaluation of a serum IgA-class reticulin antibody test for the detection of childhood celiac disease. J Pediatr. 105:901-905.

Mäki M, Kallonen K, Lahdeaho ML and Visakorpi JK. (1988) Changing pattern of childhood coeliac disease in Finland. Acta Paediatr Scand. 77:408-412.

Mäki M, Holm K, Collin P and Savilahti E. (1991a) Increase in gamma/delta T cell receptor bearing lymphocytes in normal small bowel mucosa in latent coeliac disease. Gut. 32:1412-1414.

Mäki M, Holm K, Lipsanen V, Hällström O, Viander M, Collin P, Savilahti E and Koskimies S. (1991b) Serological markers and HLA genes among healthy first-degree relatives of patients with coeliac disease. Lancet. 338:1350-1353.

Mäki M, Mustalahti K, Kokkonen J, Kulmala P, Haapalahti M, Karttunen T, Ilonen J, Laurila K, Dahlbom I, Hansson T, Hopfl P and Knip M. (2003) Prevalence of Celiac disease among children in Finland. N Engl J Med. 348:2517-2524.

Nilsen EM, Lundin KE, Krajci P, Scott H, Sollid LM and Brandtzaeg P. (1995) Gluten specific, HLA-DQ restricted T cells from coeliac mucosa produce cytokines with Th1 or Th0 profile dominated by interferon gamma. Gut. 37:766-776.

Nisihara RM, Kotze LM, Utiyama SR, Oliveira NP, Fiedler PT and Messias-Reason IT. (2005) Celiac disease in children and adolescents with Down syndrome. J Pediatr (Rio J). 81:373-376.

Nistico L, Fagnani C, Coto I, Percopo S, Cotichini R, Limongelli MG, Paparo F, D'Alfonso S, Giordano M, Sferlazzas C, Magazzu G, Momigliano-Richiardi P, Greco L and Stazi MA. (2006) Concordance, disease progression, and heritability of coeliac disease in Italian twins. Gut. 55:803-808.

Not T, Tommasini A, Tonini G, Buratti E, Pocecco M, Tortul C, Valussi M, Crichiutti G, Berti I, Trevisiol C, Azzoni E, Neri E, Torre G, Martelossi S, Soban M, Lenhardt A, Cattin L and Ventura A. (2001) Undiagnosed coeliac disease and risk of autoimmune disorders in subjects with Type I diabetes mellitus. Diabetologia. 44:151-155.

O'Leary C, Walsh CH, Wieneke P, O'Regan P, Buckley B, O'Halloran DJ, Ferriss JB, Quigley EM, Annis P, Shanahan F and Cronin CC. (2002) Coeliac disease and autoimmune Addison's disease: a clinical pitfall. QJM. 95:79-82.

Oliveira RP, Sdepanian VL, Barreto JA, Cortez AJ, Carvalho FO, Bordin JO, de Camargo Soares MA, da Silva Patricio FR, Kawakami E, de Morais MB and Fagundes-Neto U. (2007) High prevalence of celiac disease in Brazilian blood donor volunteers based on screening by IgA antitissue transglutaminase antibody. Eur J Gastroenterol Hepatol. 19:43-49.

Olmos M, Antelo M, Vazquez H, Smecuol E, Maurino E and Bai JC. (2008) Systematic review and meta-analysis of observational studies on the prevalence of fractures in coeliac disease. Dig Liver Dis. 40:46-53.

O'Mahony S, Vestey JP and Ferguson A. (1990) Similarities in intestinal humoral immunity in dermatitis herpetiformis without enteropathy and in coeliac disease. Lancet. 335:1487-1490.

O'Mahony S, Arranz E, Barton JR and Ferguson A. (1991) Dissociation between systemic and mucosal humoral immune responses in coeliac disease. Gut. 32:29-35.

O'Mahony S, Howdle PD and Losowsky MS. (1996) Review article: management of patients with non-responsive coeliac disease. Aliment Pharmacol Ther. 10:671-680.

O'Shea U, Abuzakouk M, O'Morain C, O'Donoghue D, Sheahan K, Watson P, O'Briain S, Alexander D, Catherwood M, Jackson J, Kelly J and Feighery C. (2008) Investigation of molecular markers in the diagnosis of refractory coeliac disease in a large patient cohort. J Clin Pathol. 61:1200-1202.

Paterson BM, Lammers KM, Arrieta MC, Fasano A and Meddings JB. (2007) The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: a proof of concept study. Aliment Pharmacol Ther. 26:757-766.

Paulley JW. (1954) Observation on the aetiology of idiopathic steatorrhoea; jejunal and lymph-node biopsies. Br Med J. 2:1318-1321.

Peltola M, Kaukinen K, Dastidar P, Haimila K, Partanen J, Haapala AM, Mäki M, Keränen T and Peltola J. (2009) Hippocampal sclerosis in refractory temporal lobe epilepsy is associated with gluten sensitivity. J Neurol Neurosurg Psychiatry. 80:626-630.

Pender SL, Tickle SP, Docherty AJ, Howie D, Wathen NC and MacDonald TT. (1997) A major role for matrix metalloproteinases in T cell injury in the gut. J Immunol. 158:1582-1590.

Peräaho M, Kaukinen K, Mustalahti K, Vuolteenaho N, Mäki M, Laippala P and Collin P. (2004) Effect of an oats-containing gluten-free diet on symptoms and quality of life in coeliac disease. A randomized study. Scand J Gastroenterol. 39:27-31.

Perkkiö M, Savilahti E and Kuitunen P. (1981) Semi-quantitative analysis of immunoglobulins and complement fractions 3 and 4 in the jejunal mucosa in coeliac disease and in food allergy in childhood. Immunohistochemical study by light and electron microscopy. Acta Pathol Microbiol Scand A. 89:343-350.

Picarelli A, Maiuri L, Frate A, Greco M, Auricchio S and Londei M. (1996) Production of antiendomysial antibodies after in-vitro gliadin challenge of small intestine biopsy samples from patients with coeliac disease. Lancet. 348:1065-1067.

Picarelli A, Di Tola M, Sabbatella L, Gabrielli F, Di Cello T, Anania MC, Mastracchio A, Silano M and De Vincenzi M. (2001) Immunologic evidence of no harmful effect of oats in celiac disease. Am J Clin Nutr. 74:137-140.

Reeves GE, Squance ML, Duggan AE, Murugasu RR, Wilson RJ, Wong RC, Gibson RA, Steele RH and Pollock WK. (2006) Diagnostic accuracy of coeliac serological tests: a prospective study. Eur J Gastroenterol Hepatol. 18:493-501.

Remes-Troche JM, Rios-Vaca A, Ramirez-Iglesias MT, Rubio-Tapia A, Andrade-Zarate V, Rodriguez-Vallejo F, Lopez-Maldonado F, Gomez-Perez FJ and Uscanga LF. (2008) High prevalence of celiac disease in Mexican Mestizo adults with type 1 diabetes mellitus. J Clin Gastroenterol. 42:460-465.

Ress K, Harro M, Maaroos HI, Harro J, Uibo R and Uibo O. (2007) High prevalence of coeliac disease: need for increasing awareness among physicians. Dig Liver Dis. 39:136-139.

Reunala T, Kosnai I, Karpati S, Kuitunen P, Torok E and Savilahti E. (1984) Dermatitis herpetiformis: jejunal findings and skin response to gluten free diet. Arch Dis Child. 59:517-522.

Reunala T, Collin P, Holm K, Pikkarainen P, Miettinen A, Vuolteenaho N and Mäki M. (1998) Tolerance to oats in dermatitis herpetiformis. Gut. 43:490-493.

Roberts AI, Lee L, Schwarz E, Groh V, Spies T, Ebert EC and Jabri B. (2001) NKG2D receptors induced by IL-15 costimulate CD28-negative effector CTL in the tissue microenvironment. J Immunol. 167:5527-5530.

Roka V, Potamianos SP, Kapsoritakis AN, Yiannaki EE, Koukoulis GN, Stefanidis I, Koukoulis GK and Germenis AE. (2007) Prevalence of coeliac disease in the adult population of central Greece. Eur J Gastroenterol Hepatol. 19:982-987.

Rostami K, Kerckhaert J, Tiemessen R, von Blomberg BM, Meijer JW and Mulder CJ. (1999) Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice. Am J Gastroenterol. 94:888-894.

Rubio-Tapia A, Van Dyke CT, Lahr BD, Zinsmeister AR, El-Youssef M, Moore SB, Bowman M, Burgart LJ, Melton LJ,3rd and Murray JA. (2008) Predictors of family risk for celiac disease: a population-based study. Clin Gastroenterol Hepatol. 6:983-987.

Rubio-Tapia A, Kelly DG, Lahr BD, Dogan A, Wu TT and Murray JA. (2009a) Clinical staging and survival in refractory celiac disease: a single center experience. Gastroenterology. 136:99-107;.

Rubio-Tapia A, Kyle RA, Kaplan EL, Johnson DR, Page W, Erdtmann F, Brantner TL, Kim WR, Phelps TK, Lahr BD, Zinsmeister AR, Melton LJ,3rd and Murray JA. (2009b) Increased prevalence and mortality in undiagnosed celiac disease. Gastroenterology. 137:88-93.

Salmi TT, Collin P, Järvinen O, Haimila K, Partanen J, Laurila K, Korponay-Szabo IR, Huhtala H, Reunala T, Mäki M and Kaukinen K. (2006a) Immunoglobulin A autoantibodies against transglutaminase 2 in the small intestinal mucosa predict forthcoming coeliac disease. Aliment Pharmacol Ther. 24:541-552.

Salmi TT, Collin P, Korponay-Szabo IR, Laurila K, Partanen J, Huhtala H, Kiraly R, Lorand L, Reunala T, Mäki M and Kaukinen K. (2006b) Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. Gut. 55:1746-1753.

Sardy M, Karpati S, Merkl B, Paulsson M and Smyth N. (2002) Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. J Exp Med. 195:747-757.

Sategna Guidetti C, Solerio E, Scaglione N, Aimo G and Mengozzi G. (2001) Duration of gluten exposure in adult coeliac disease does not correlate with the risk for autoimmune disorders. Gut. 49:502-505.

Savilahti E. (1972) Intestinal immunoglobulins in children with coeliac disease. Gut. 13:958-964.

Savilahti E, Arato A and Verkasalo M. (1990) Intestinal gamma/delta receptor-bearing T lymphocytes in celiac disease and inflammatory bowel diseases in children. Constant increase in celiac disease. Pediatr Res. 28:579-581.

Savilahti E, Reunala T and Mäki M. (1992) Increase of lymphocytes bearing the gamma/delta T cell receptor in the jejunum of patients with dermatitis herpetiformis. Gut. 33:206-211.

Selby PL, Davies M, Adams JE and Mawer EB. (1999) Bone loss in celiac disease is related to secondary hyperparathyroidism. J Bone Miner Res. 14:652-657.

Selby WS, Janossy G, Bofill M and Jewell DP. (1983) Lymphocyte subpopulations in the human small intestine. The findings in normal mucosa and in the mucosa of patients with adult coeliac disease. Clin Exp Immunol. 52:219-228.

Shiner M. (1973) Ultrastructural changes suggestive of immune reactions in the jejunal mucosa of coeliac children following gluten challenge. Gut. 14:1-12.

Shiner M and Ballard J. (1972) Antigen-antibody reactions in jejunal mucosa in childhood coeliac disease after gluten challenge. Lancet. 1:1202-1205.
Shmerling DH and Franckx J. (1986) Childhood celiac disease: a long-term analysis of relapses in 91 patients. J Pediatr Gastroenterol Nutr. 5:565-569.
Siegel M, Xia J and Khosla C. (2007) Structure-based design of alpha-amido aldehyde containing gluten peptide analogues as modulators of HLA-DQ2 and transglutaminase 2. Bioorg Med Chem. 15:6253-6261.
Sigurgeirsson B, Agnarsson BA and Lindelof B. (1994) Risk of lymphoma in patients with dermatitis herpetiformis. BMJ. 308:13-15.
Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F and Thorsby E. (1989) Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. J Exp Med. 169:345-350.
Sollid LM, Molberg O, McAdam S and Lundin KE. (1997) Autoantibodies in coeliac disease: tissue transglutaminase--guilt by association? Gut. 41:851-852.
Spencer J, Isaacson PG, MacDonald TT, Thomas AJ and Walker-Smith JA. (1991) Gamma/delta T cells and the diagnosis of coeliac disease. Clin Exp Immunol. 85:109-113.
Spurkland A, Ingvarsson G, Falk ES, Knutsen I, Sollid LM and Thorsby E. (1997) Dermatitis herpetiformis and celiac disease are both primarily associated with the HLA-DQ (alpha 1*0501, beta 1*02) or the HLA-DQ (alpha 1*03, beta 1*0302) heterodimers. Tissue Antigens. 49:29-34.
Srinivasan U, Leonard N, Jones E, Kasarda DD, Weir DG, O'Farrelly C and Feighery C. (1996) Absence of oats toxicity in adult coeliac disease. BMJ. 313:1300-1301.
Srinivasan U, Jones E, Carolan J and Feighery C. (2006) Immunohistochemical analysis of coeliac mucosa following ingestion of oats. Clin Exp Immunol. 144:197-203.
Stagi S, Giani T, Simonini G and Falcini F. (2005) Thyroid function, autoimmune thyroiditis and coeliac disease in juvenile idiopathic arthritis. Rheumatology (Oxford). 44:517-520.
Stene LC, Honeyman MC, Hoffenberg EJ, Haas JE, Sokol RJ, Emery L, Taki I, Norris JM, Erlich HA, Eisenbarth GS and Rewers M. (2006) Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. Am J Gastroenterol. 101:2333-2340.
Stenman SM, Lindfors K, Korponay-Szabo IR, Lohi O, Saavalainen P, Partanen J, Haimila K, Wieser H, Mäki M and Kaukinen K. (2008) Secretion of celiac disease autoantibodies after in vitro gliadin challenge is dependent on small-bowel mucosal transglutaminase 2-specific IgA deposits. BMC Immunol. 9:6.
Stenman SM, Venalainen JI, Lindfors K, Auriola S, Mauriala T, Kaukovirta-Norja A, Jantunen A, Laurila K, Qiao SW, Sollid LM, Mannisto PT, Kaukinen K and Mäki M. (2009) Enzymatic detoxification of gluten by germinating wheat proteases: implications for new treatment of celiac disease. Ann Med. 41:390-400.
Storsrud S, Hulthen LR and Lenner RA. (2003a) Beneficial effects of oats in the gluten-free diet of adults with special reference to nutrient status, symptoms and subjective experiences. Br J Nutr. 90:101-107.
Storsrud S, Malmheden Yman I and Lenner RA. (2003b) Gluten contamination in oat products and products naturally free from gluten. Eur Food Res Technol. 317:481-485.
Storsrud S, Olsson M, Arvidsson Lenner R, Nilsson LA, Nilsson O and Kilander A. (2003c) Adult coeliac patients do tolerate large amounts of oats. Eur J Clin Nutr. 57:163-169.
Sulkanen S, Collin P, Laurila K and Mäki M. (1998) IgA- and IgG-class antihuman umbilical cord antibody tests in adult coeliac disease. Scand J Gastroenterol. 33:251-254.
Swinson CM, Slavin G, Coles EC and Booth CC. (1983) Coeliac disease and malignancy. Lancet. 1:111-115.
Szodoray P, Barta Z, Lakos G, Szakall S and Zeher M. (2004) Coeliac disease in Sjogren's syndrome--a study of 111 Hungarian patients. Rheumatol Int. 24:278-282.
Teesalu K, Uibo O, Kalkkinen N, Janmey P and Uibo R. (2001) Increased levels of IgA antibodies against desmin in children with coeliac disease. Int Arch Allergy Immunol. 126:157-166.
Tikkakoski S, Savilahti E and Kolho KL. (2007) Undiagnosed coeliac disease and nutritional deficiencies in adults screened in primary health care. Scand J Gastroenterol. 42:60-65.
Tommasini A, Not T, Kiren V, Baldas V, Santon D, Trevisiol C, Berti I, Neri E, Gerarduzzi T, Bruno I, Lenhardt A, Zamuner E, Spano A, Crovella S, Martellossi S, Torre G, Sblattero D, Marzari R, Bradbury A, Tamburlini G and Ventura A. (2004) Mass screening for coeliac disease using antihuman transglutaminase antibody assay. Arch Dis Child. 89:512-515.

Tosco A, Maglio M, Paparo F, Rapacciuolo L, Sannino A, Miele E, Barone MV, Auricchio R and Troncone R. (2008) Immunoglobulin A anti-tissue transglutaminase antibody deposits in the small intestinal mucosa of children with no villous atrophy. J Pediatr Gastroenterol Nutr. 47:293-298.

Troncone R and Ferguson A. (1991) Anti-gliadin antibodies. J Pediatr Gastroenterol Nutr. 12:150-158.

Troncone R, Mayer M, Spagnuolo F, Maiuri L and Greco L. (1995) Endomysial antibodies as unreliable markers for slight dietary transgressions in adolescents with celiac disease. J Pediatr Gastroenterol Nutr. 21:69-72.

Tursi A and Brandimarte G. (2003) The symptomatic and histologic response to a gluten-free diet in patients with borderline enteropathy. J Clin Gastroenterol. 36:13-17.

Vader LW, Stepniak DT, Bunnik EM, Kooy YM, de Haan W, Drijfhout JW, Van Veelen PA and Koning F. (2003a) Characterization of cereal toxicity for celiac disease patients based on protein homology in grains. Gastroenterology. 125:1105-1113.

Vader W, Stepniak D, Kooy Y, Mearin L, Thompson A, van Rood JJ, Spaenij L and Koning F. (2003b) The HLA-DQ2 gene dose effect in celiac disease is directly related to the magnitude and breadth of gluten-specific T cell responses. Proc Natl Acad Sci U S A. 100:12390-12395.

Valdimarsson T, Toss G, Ross I, Lofman O and Strom M. (1994) Bone mineral density in coeliac disease. Scand J Gastroenterol. 29:457-461.

Van Belzen MJ, Meijer JW, Sandkuijl LA, Bardoel AF, Mulder CJ, Pearson PL, Houwen RH and Wijmenga C. (2003) A major non-HLA locus in celiac disease maps to chromosome 19. Gastroenterology. 125:1032-1041.

van de Kamer JH, Weijers HA and Dicke WK. (1953) Coeliac disease. IV. An investigation into the injurious constituents of wheat in connection with their action on patients with coeliac disease. Acta Paediatr. 42:223-231.

Vasquez H, Mazure R, Gonzalez D, Flores D, Pedreira S, Niveloni S, Smecuol E, Maurino E and Bai JC. (2000) Risk of fractures in celiac disease patients: a cross-sectional, case-control study. Am J Gastroenterol. 95:183-189.

Vecsei AK, Graf UB and Vogelsang H. (2009) Follow-up of adult celiac patients: which noninvasive test reflects mucosal status most reliably? Endoscopy. 41:123-128.

Ventura A, Magazzu G and Greco L. (1999) Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease. SIGEP Study Group for Autoimmune Disorders in Celiac Disease. Gastroenterology. 117:297-303.

Verbeek WH, Van De Water JM, Al-Toma A, Oudejans JJ, Mulder CJ and Coupe VM. (2008a) Incidence of enteropathy--associated T-cell lymphoma: a nation-wide study of a population-based registry in The Netherlands. Scand J Gastroenterol. 43:1322-1328.

Verbeek WH, von Blomberg BM, Scholten PE, Kuik DJ, Mulder CJ and Schreurs MW. (2008b) The presence of small intestinal intraepithelial gamma/delta T-lymphocytes is inversely correlated with lymphoma development in refractory celiac disease. Am J Gastroenterol. 103:3152-3158.

Verkarre V, Asnafi V, Lecomte T, Patey Mariaud-de Serre N, Leborgne M, Grosdidier E, Le Bihan C, Macintyre E, Cellier C, Cerf-Bensussan N and Brousse N. (2003) Refractory coeliac sprue is a diffuse gastrointestinal disease. Gut. 52:205-211.

Verkasalo MA, Arato A, Savilahti E and Tainio VM. (1990) Effect of diet and age on jejunal and circulating lymphocyte subsets in children with coeliac disease: persistence of CD4-8- intraepithelial T cells through treatment. Gut. 31:422-425.

Viljamaa M, Kaukinen K, Huhtala H, Kyrönpalo S, Rasmussen M and Collin P. (2005) Coeliac disease, autoimmune diseases and gluten exposure. Scand J Gastroenterol. 40:437-443.

Villalta D, Girolami D, Bidoli E, Bizzaro N, Tampoia M, Liguori M, Pradella M, Tonutti E and Tozzoli R. (2005) High prevalence of celiac disease in autoimmune hepatitis detected by anti-tissue tranglutaminase autoantibodies. J Clin Lab Anal. 19:6-10.

Vilppula A, Kaukinen K, Luostarinen L, Krekela I, Patrikainen H, Valve R, Mäki M and Collin P. (2009) Increasing prevalence and high incidence of celiac disease in elderly people: a population-based study. BMC Gastroenterol. 9:49.

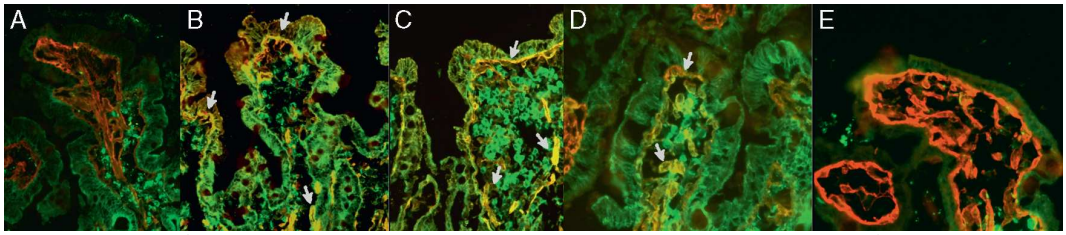
Vogelsang H, Hanel S, Steiner B and Oberhuber G. (2001) Diagnostic duodenal bulb biopsy in celiac disease. Endoscopy. 33:336-340.

Volta U, De Franceschi L, Molinaro N, Cassani F, Muratori L, Lenzi M, Bianchi FB and Czaja AJ. (1998) Frequency and significance of anti-gliadin and anti-endomysial antibodies in autoimmune hepatitis. Dig Dis Sci. 43:2190-2195.

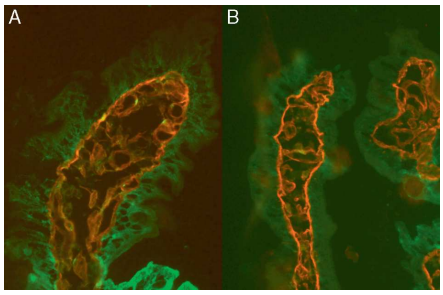
Volta U, Granito A, De Franceschi L, Petrolini N and Bianchi FB. (2001a) Anti tissue transglutaminase antibodies as predictors of silent coeliac disease in patients with hypertransaminasaemia of unknown origin. Dig Liver Dis. 33:420-425.
Volta U, Ravaglia G, Granito A, Forti P, Maioli F, Petrolini N, Zoli M and Bianchi FB. (2001b) Coeliac disease in patients with autoimmune thyroiditis. Digestion. 64:61-65.
Volta U, Rodrigo L, Granito A, Petrolini N, Muratori P, Muratori L, Linares A, Veronesi L, Fuentes D, Zauli D and Bianchi FB. (2002) Celiac disease in autoimmune cholestatic liver disorders. Am J Gastroenterol. 97:2609-2613.
Volta U, Granito A, Fiorini E, Parisi C, Piscaglia M, Pappas G, Muratori P and Bianchi FB. (2008) Usefulness of antibodies to deamidated gliadin peptides in celiac disease diagnosis and follow-up. Dig Dis Sci. 53:1582-1588.
Wagner G, Berger G, Sinnreich U, Grylli V, Schober E, Huber WD and Karwautz A. (2008) Quality of life in adolescents with treated coeliac disease: influence of compliance and age at diagnosis. J Pediatr Gastroenterol Nutr. 47:555-561.
Wahab PJ, Meijer JW and Mulder CJ. (2002) Histologic follow-up of people with celiac disease on a gluten-free diet: slow and incomplete recovery. Am J Clin Pathol. 118:459-463.
Wahnschaffe U, Ullrich R, Riecken EO and Schulzke JD. (2001) Celiac disease-like abnormalities in a subgroup of patients with irritable bowel syndrome. Gastroenterology. 121:1329-1338.
Walker-Smith JA, Guandalini S, Schmitz J, Schmerling DH and Visakorpi JK. (1990) Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. Arch Dis Child. 65:909-911.
Weijers HA and van de Kamer JH. (1965) Some Considerations of Celiac Disease. Am J Clin Nutr. 17:51-54.
Weinstein WM. (1974) Latent celiac sprue. Gastroenterology. 66:489-493.
Weiss B, Skourikhin Y, Modan-Moses D, Broide E, Fradkin A and Bujanover Y. (2008) Is adult height of patients with celiac disease influenced by delayed diagnosis? Am J Gastroenterol. 103:1770-1774.
West J, Logan RF, Smith CJ, Hubbard RB and Card TR. (2004) Malignancy and mortality in people with coeliac disease: population based cohort study. BMJ. 329:716-719.
Westerlund A, Ankelo M, Simell S, Ilonen J, Knip M, Simell O and Hinkkanen AE. (2007) Affinity maturation of immunoglobulin A anti-tissue transglutaminase autoantibodies during development of coeliac disease. Clin Exp Immunol. 148:230-240.
Wolters VM, Verbeek WH, Zhernakova A, Onland-Moret C, Schreurs MW, Monsuur AJ, Verduijn W, Wijmenga C and Mulder CJ. (2007) The MYO9B gene is a strong risk factor for developing refractory celiac disease. Clin Gastroenterol Hepatol. 5:1399-405, 1405.e1-2.
Xia J, Bergseng E, Fleckenstein B, Siegel M, Kim CY, Khosla C and Sollid LM. (2007) Cyclic and dimeric gluten peptide analogues inhibiting DQ2-mediated antigen presentation in celiac disease. Bioorg Med Chem. 15:6565-6573.
Young WF and Pringle EM. (1971) 110 Children with Coeliac Disease, 1950-1969. Arch Dis Child. 46:421-436.
Zanoni G, Navone R, Lunardi C, Tridente G, Bason C, Sivori S, Beri R, Dolcino M, Valletta E, Corrocher R and Puccetti A. (2006) In celiac disease, a subset of autoantibodies against transglutaminase binds toll-like receptor 4 and induces activation of monocytes. PLoS Med. 3:e358.
Zimmer KP, Fischer I, Mothes T, Weissen-Plenz G, Schmitz M, Wieser H, Mendez E, Buening J, Lerch MM, Ciclitira PC, Weber P and Naim HY. (2009) Endocytotic Segregation of Gliadin Peptide 31-49 in Enterocytes. Gut 59:300-310.

APPENDIX

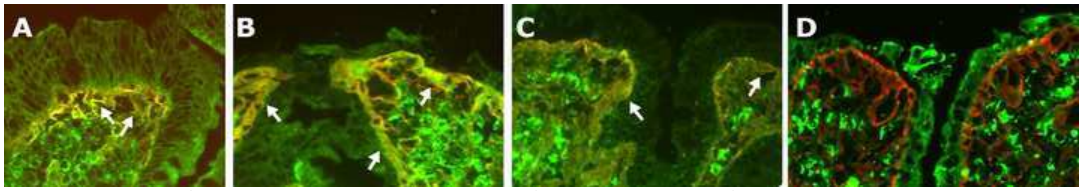
Colour figures from original articles (II-IV)



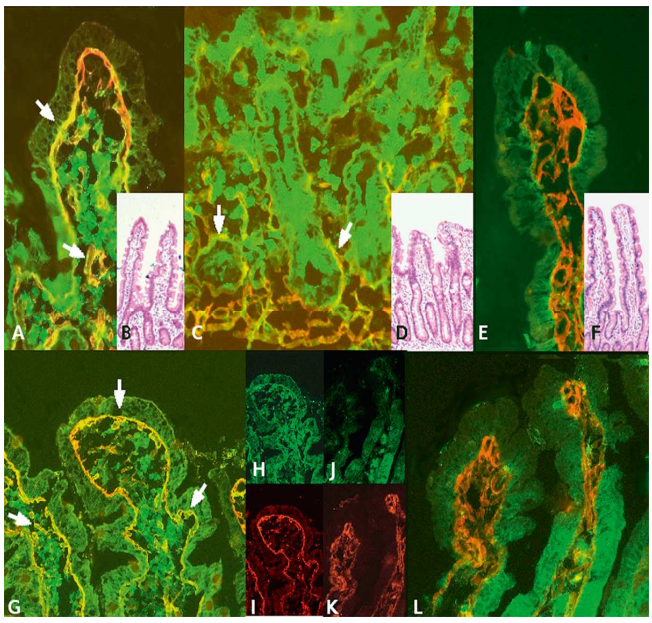
Original article II, figure 2: At baseline, this 13-year old boy had been on a conventional gluten-free diet avoiding wheat, rye, barley, and oats for 2 years andevinced normal small-bowel mucosa villous morphology and negative mucosal IgA deposits (A). After 6 months' gluten (wheat, rye, barley,and oats) challenge he still had normal villi, but clear IgA deposits were already present (B). The small-bowel mucosa relapsed after12 months with gluten, and strong IgA deposits can be seen (C). After adopting an oat-containing gluten-free diet the mucosal IgA deposits slowly disappeared following villous recovery (D), after 6 months with oats; (E), after 2 years with oats. IgA is stained in green andtransglutaminase 2 (TG2) in red; yellow (arrow) in composite figures indicates colocalization of coeliac-type IgA deposits and TG2.



Original article II, figure 4: A 16-year-old boy developed abdominal symptoms immediately after ingestion of oats; small-bowel mucosal villous morphology remained normal and densities of CD3þ intraepithelial lymphocytes even decreased (from 86 cells/mm to 51 cells/mm). Mucosal IgA deposits were negative both before (A) and immediately after (B) oats challenge. IgA is labelled green, transglutaminase 2 in red; IgA deposition or co-localization of IgA and TG2 (yellow) was not detected.



Original article III, figure 2: Small-bowel mucosal immunofluorescence staining of IgA (green) and transglutaminase-2 (red) from patients having untreated coeliac disease which subsequently responded well to a gluten-free diet (A), refractory coeliac disease patient with abnormal immunophenotype of small-bowel mucosal intraepithelial lymphocytes (B), a refractory coeliac patient with enteropathy-associated T cell lymphoma (C) and a patient with autoimmune enteropathy (D). Colocalization of IgA and TG2 is shown in yellow (arrow).



Original article IV, figure 2: A–F, Small bowel mucosal immunoglobulin (Ig)A deposits in a 48-year-old woman having latent coeliac disease. The first small bowel mucosal biopsy (B) showed normal villous morphology, but (D) 2 years later when she continued on a normal gluten-containing diet villous atrophy with crypt hyperplasia developed. Villous morphology (F) recovered on a gluten-free diet. Serum celiac autoantibodies were negative throughout, but (A) strong subepithelial tissue transglutaminase (TG2)-targeted IgA autoantibody deposits (yellow, arrow) already were present in the small bowel mucosa in the first biopsy, and (C) also when villous atrophywas detected. The deposits disappeared (E) with a gluten-free diet. G-L, Mucosal IgA deposits are shown from a 21-year-old woman with potential coeliac disease having (G) normal small bowel mucosal architecture and strong subepithelial tissue TG2-specific IgA deposits. After 1 year on a gluten-free diet her symptoms recovered and IgA deposits disappeared (L). IgA is stained with green (H, J), TG2 with red (I,K), and colocalization of IgA and TG2 is shown in yellow (A,C,G).

ORIGINAL PUBLICATIONS

Journal of Pediatric Gastroenterology and Nutrition
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Oats Do Not Induce Systemic or Mucosal Autoantibody Response in Children With Coeliac Disease

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ABSTRACT

Objectives: A gluten-free diet omitting wheat, rye, and barley is the only effective treatment for coeliac disease. The necessity of excluding oats from the diet has remained controversial. We studied the toxicity of oats in children with coeliac disease during a 2-year follow-up by investigating jejunal transglutaminase 2 (TG2)-targeted IgA-class autoantibody deposits, a potentially more sensitive disease marker than serum antibodies or conventional histology.

Patients and Methods: Twenty-three coeliac children in remission were randomized to undergo oat or gluten challenge with wheat, rye, barley, and oats. When jejunal histological relapse was evident after gluten challenge, patients excluded wheat, rye, and barley but continued with oats. Mucosal morphology and TG2-targeted autoantibody deposits were studied in jejunal biopsies taken at baseline and after 6 and 24 months. Furthermore, serum IgA-class TG2 antibodies were measured.

Results: At baseline, serum TG2 antibodies were negative in all 23 patients, but 7 of them had minor mucosal deposits. In the oats group, there was no significant change in the intensity of the deposits within 2 years. In contrast, during the gluten challenge, the intensity of the deposits clearly increased and decreased again when wheat, rye, and barley were excluded but consumption of oats was continued; this was in line with serum autoantibodies. The intensity of the mucosal deposits correlated well with both villous morphology and serum autoantibody levels.

Conclusions: Consumption of oats does not induce TG2 autoantibody production at mucosal level in children with coeliac disease. Measurement of small-intestinal mucosal autoantibody deposits is suitable for monitoring treatment in coeliac patients. ***JPGN 48:559–565, 2009.*** **Key Words:** Coeliac disease—Gluten challenge—IgA deposit—Oat challenge.

The only effective treatment for coeliac disease is a lifelong gluten-free diet that excludes all food products containing wheat, rye, and barley. However, the necessity of avoiding oats remains controversial (1). Early small-scale reports on coeliac disease patients suggested intestinal malabsorption and exacerbating abdominal symptoms after ingestion of oats (2,3), but more recent in vitro and in vivo studies have questioned its toxicity (4–11). There is now a large body of evidence to support the safe consumption of oats in the vast majority of both children and adults having coeliac disease and dermatitis herpetiformis (4–11). Nonetheless, some concerns persist regarding recommending oats to all coeliac patients. The purity of oat products cannot always be guaranteed and contamination of oats with other gluten-containing cereals during harvesting and milling is known to occur (12,13). Furthermore, there would appear to be a small subset of coeliac patients who experience more abdominal symptoms while consuming an oat-containing diet as against the conventional gluten-free diet (14,15). There are studies showing that the symptoms induced by the consumption of oats are not associated with small-bowel mucosal damage (15,16). It was recently demonstrated that 3 of 9 oat-intolerant patients with coeliac disease had

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avenin-reactive T cells in the small-bowel mucosa (17). Oats have sequence similarities to wheat at the amino acid level (18), and in in vitro studies oat avenin has been shown to stimulate gliadin-reactive T cell lines (17,19). The clinical relevance of these somewhat discrepant findings remains obscure, and more investigations on the toxicity and immunogenicity of oats in coeliac disease are thus called for.

In addition to gluten-induced small-bowel mucosal inflammatory and morphological changes, humoral response to transglutaminase-2 (TG2) is highly pathognomic for active coeliac disease. These circulating coeliac disease-specific autoantibodies disappear during a gluten-free diet but reappear if gluten is reintroduced (20). Recent evidence shows that anti-TG2 autoantibodies are produced locally in the small-bowel mucosa (21,22), where they can be found deposited extracellularly already early in the disease process before manifest mucosal lesion with villus atrophy has set in and before autoantibodies are detectable in the circulation (23–25). Interestingly, in an earlier study by Shiner et al (26) it was suggested that in treated coeliac disease patients small-bowel mucosal subepithelial IgA deposits appear rapidly after gluten challenge—even hours before marked mucosal lymphocytosis is detectable. This prompted us to hypothesize that detection of TG2-targeted IgA-class autoantibody deposits in the small-bowel mucosa may offer a useful tool for revealing early gluten-induced minor mucosal changes in coeliac disease. We used this method to investigate the toxicity of oats in children with coeliac disease during a 2-year follow-up.

METHODS

Patients and Study Design

This study is part of a randomized controlled trial, whose details have been presented elsewhere (27). In brief, 23 consecutive children aged 7 years or older with previously diagnosed coeliac disease (median age 13 years, range 7–18 years, 7 female) were enrolled. At the time of diagnosis all evinced positive serum IgA-class endomysial antibodies and villous atrophy with crypt hyperplasia in the small-bowel mucosa. After maintaining a conventional gluten-free diet avoiding wheat, rye, barley, and oats for at least 2 years all had experienced a good clinical, serological, and histological response. At the baseline of the study, 13 children (median age 11 years, range 9–18 years, 6 female) in remission were randomized to undergo open oats challenge and 10 (median age 13 years, range 7–8 years, 1 female) a gluten challenge allowing the consumption of wheat, rye, and barley in addition to oats. When clear small-bowel mucosal relapse was verified during the gluten challenge, patients reverted to a gluten-free diet, avoiding wheat, rye, and barley but continuing oat consumption. Small-bowel mucosal biopsies and serum samples were taken at baseline and after 6 and 24 months from the patients ingesting only oats during the entire study period. In patients undergoing a gluten challenge, the first follow-up examination was carried out when clinical symptoms suggested a relapse or coeliac antibodies seroconverted positive. After the relapse and commencement of an oat-containing gluten-free diet, follow-up examinations were carried out in the same way at 6 and 24 months as in the oats challenge group. During the 2-year trial, oats had no detrimental effect on intestinal mucosal villous morphology, densities of CD3+, $\alpha\beta$+ and $\gamma\delta$+ intraepithelial lymphocytes (IEL) or HLA DR expression. In contrast, the gluten challenge group relapsed after 3 to 12 months, but complete recovery from the disease was accomplished in all on an oat-containing gluten-free diet during the 2-year follow-up (27).

Oat Product and Dietary Assessment

At baseline, children and their parents were given instructions by a trained dietician on the oat-containing gluten-free diet and gluten challenge (27). A detailed dietary analysis was assessed repeatedly by means of interview and 4-day record of food intake. The rolled, uncontaminated oats (1 single batch; Melia Ltd, Raisio, Finland; details of the cultivar were not provided by the manufacturer) were given to the patients free of charge. During the gluten challenge, patients bought their wheat-, rye-, barley-, and oats-containing products freely from grocery stores, and thus no specific cultivar was used. During a 2-year trial, the median daily consumption of oats was 45 g/day (range 13–81 g/day) in the oats challenge group. In the gluten challenge group, the median daily intake of gluten in the form of wheat-, rye-, barley-, and oat-containing normal bread was 14 g (range 7–19 g/day), and after adopting an oat-containing gluten-free diet the median intake of oats was 41 g/day, range 24 to 59 g/day. One patient in the oats challenge group had transient dietary lapses at the 6-month time point; all of the rest adhered to a strict gluten-free diet throughout the study (27).

Small-bowel Mucosal Morphology and TG2-specific IgA Deposits

All small-bowel intestinal biopsies were obtained by an adult Watson capsule in X-ray control next to the ligamentum of Treitz in jejunum. One part of the biopsy specimen was paraffin embedded and stained with hematoxylin and eosin to study small-bowel mucosal morphology and to determinate the villous height–crypt depth ratios (Vh/CrD) (28). From well-oriented biopsy sections Vh/CrD < 2 was considered characteristic for active coeliac disease.

The other part of the biopsy was snap-frozen and embedded in optimal cutting temperature compound (OCT, Tissue-Tec, Miles Inc, Elkhart, IN). In each case, altogether 6 unfixed, 5-μm-thick sections from the frozen small-bowel specimens were processed, 3 for investigation of IgA deposits and 3 for double-colour labelling for both IgA and TG2 by direct immunofluorescence. IgA was detected using fluorescein isothiocyanate-labelled rabbit antibody against human IgA (Dako AS, Glostrup, Denmark) at a dilution of 1:40 in phosphate-buffered saline (PBS), pH 7.4. For the double labelling, sections were stained for human IgA (as above, green) and for TG2 (red) using monoclonal mouse antibodies against TG2 (CUB7402, NeoMarkers, Fremont, CA), followed by rhodamine-conjugated anti-mouse immunoglobulin antibodies (Dako), both diluted 1:200 in PBS. In normal small-intestinal mucosal samples IgA is detected only inside the plasma and epithelial cells. In contrast, it has been shown that in active coeliac disease on a

gluten-containing diet a clear TG2-targeted subepithelial IgA deposition can be found below the basement membrane along the villous and crypt epithelium and around mucosal vessels. In earlier studies it has been shown that such small-intestinal mucosal IgA deposition targets against TG2 (23,29). In short, when this IgA was eluted from the tissues, it targeted purified TG2 both in enzyme-linked immunosorbent assay (ELISA) and Western blot (23). In addition, when TG2 binding to fibronectin was disrupted by chloroacetic acid, disappearance of extracellular IgA deposits was demonstrated in coeliac small-bowel samples. Furthermore, we have shown that IgA deposits in the small bowel of active coeliac disease patients have the ability to bind external TG2 added to the tissue (29). The method used here was based on our previous experiments to detect TG2-specific antibodies in situ in tissue sections by their colocalization with TG2 when double-labelled by immunofluorescence. In this study the IgA deposits were graded semiquantitatively according to their intensity along the basement membrane in the villous-crypt area as follows: negative (−), weak positive (+), moderate positive (++), and strong positive (+++). In cases in which the intensity of the staining was patchy, the intensity of IgA deposits was graded from different areas and a mean value of the staining was given. All evaluations were carried out blindly without knowledge of disease history or laboratory findings. In our laboratory inter- and intraobserver variation have both been 98% in the detection of IgA deposits (29).

Serology

Serum IgA-class antibodies against TG2 were detected by ELISA using human recombinant TG2 as antigen, with a cutoff line of 5.0 U/mL (Celikey, Phadia, GmbH, Freiburg, Germany).

Statistics

Quantitative data were expressed as medians and ranges, and qualitative data as percentage of abnormal values. Statistical differences were evaluated using the Wilcoxon test, as appropriate. Correlations were tested by Spearman correlation test. All testing was 2-sided, and P values <0.05 were considered statistically significant. All calculations were performed with the Statistical Package for Social Sciences version 14.0 (SPSS, Inc, Chicago, IL).

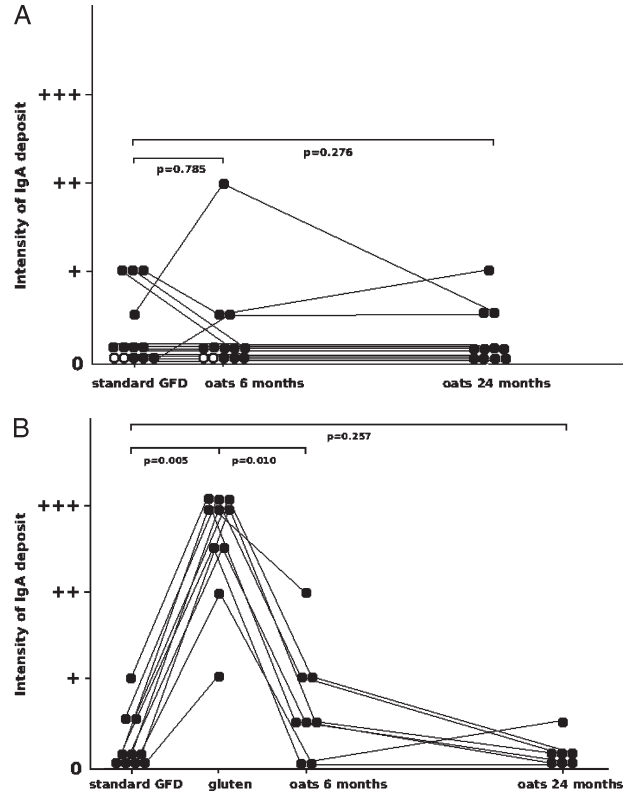

FIG. 1. Intensity of small-bowel mucosal transglutaminase 2-specific IgA deposits in patients randomized to oats (A) or gluten challenge (B) groups. Two patients in the oats challenge group (A) who prematurely withdrew from the study were rebiopsied immediately after symptoms occurred (within 1 month after oat challenge was started), and these cases are indicated with open circles.

Ethical Considerations

The study protocol was approved by the Ethics Committee of Tampere University Hospital. All children and their parents gave their written informed consent.

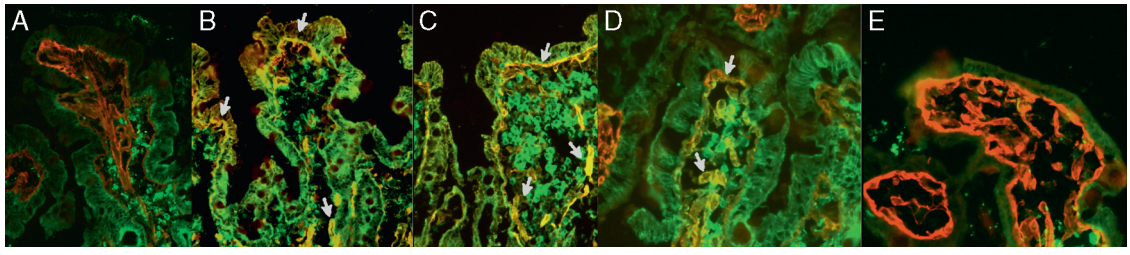

FIG. 2. At baseline, this 13-year old boy had been on a conventional gluten-free diet avoiding wheat, rye, barley, and oats for 2 years and evinced normal small-bowel mucosa villous morphology and negative mucosal IgA deposits (A). After 6 months' gluten (wheat, rye, barley, and oats) challenge he still had normal villi, but clear IgA deposits were already present (B). The small-bowel mucosa relapsed after 12 months with gluten, and strong IgA deposits can be seen (C). After adopting an oat-containing gluten-free diet the mucosal IgA deposits slowly disappeared following villous recovery (D), after 6 months with oats; (E), after 2 years with oats. IgA is stained in green and transglutaminase 2 (TG2) in red; yellow (arrow) in composite figures indicates colocalization of coeliac-type IgA deposits and TG2.

RESULTS

At baseline, all treated children with coeliac disease were in clinical remission and had normal villous architecture at histology (27). Small-bowel mucosal median Vh/CrD was 4.1 (range 3.0–5.1) in the oat challenge group and 4.4 (range 3.2–5.9) in the gluten challenge group. In addition, serum IgA-class anti-TG2 antibodies were negative in all. When autoantibodies were sought at the small-bowel mucosal level, weak-to-moderate TG2-specific IgA deposits were present in 4 of 13 (31%) in the oat challenge group and in 3 of 10 (30%) in the gluten challenge group (Fig. 1A and B).

In the oat challenge group, there was no significant change in the intensity of mucosal IgA deposits during the 2-year trial (Fig. 1A). In contrast, in the gluten challenge group the intensity of IgA deposits increased parallel with the development of small-bowel mucosal villous atrophy with crypt hyperplasia, and at the time of relapse all had moderate-to-severe IgA depositions present in the mucosa (Fig. 1B and 2). During the gluten challenge, 2 patients were also biopsied before they developed small-bowel mucosal villous atrophy, and interestingly, both already evinced clear TG2-specific IgA deposits even if the villous morphology was still intact (Fig. 2B). When an oat-containing gluten-free diet was adopted after relapse of the disease, the intensity of small-bowel mucosal IgA deposits again decreased significantly within 6 months, and after 2 years with oats only 1 of 7 (14%) had minor depositions left in the mucosa (Fig. 1B and 2). At the end of the 2-year trial with oats all patients again had negative serum anti-TG2 antibodies and Vh/CrD was normal in all (median Vh/CrD 4.2, range 2.9–5.2).

When data from both study groups and all time points were collated, it was noted that the intensity of small-bowel mucosal IgA deposits correlated well with serum TG2-antibody levels (Spearman test $r = 0.694$, $P < 0.001$) (Fig. 3A), and the severity of small-bowel mucosal villous damage ($r = -0.550$, $P < 0.001$) (Fig. 3B).

One patient in the oat challenge group admitted passing dietary transgressions at 6 months. At that time, his serum anti-TG2 antibody titre increased from 0.4 to 3.1 U/mL, still, however, remaining below the cutoff level, and the intensity of small-bowel mucosal IgA deposits was slightly increased. After continuing with a strict oat-containing gluten-free diet both serum and mucosal autoantibodies again decreased (Fig. 1A).

Two children with coeliac disease experienced abdominal pain and vomiting immediately after intake of oats, and within 1 month both withdrew prematurely from the study. The follow-up biopsies were taken upon withdrawal immediately after symptoms occurred, and they showed that the small-bowel mucosa villous morphology was normal and the densities of IELs were even lower than at the beginning of the study (27). Furthermore, at follow-up serum anti-TG2 antibodies and small-bowel mucosal autoantibody deposits remained negative in both patients (Fig. 4A and B).

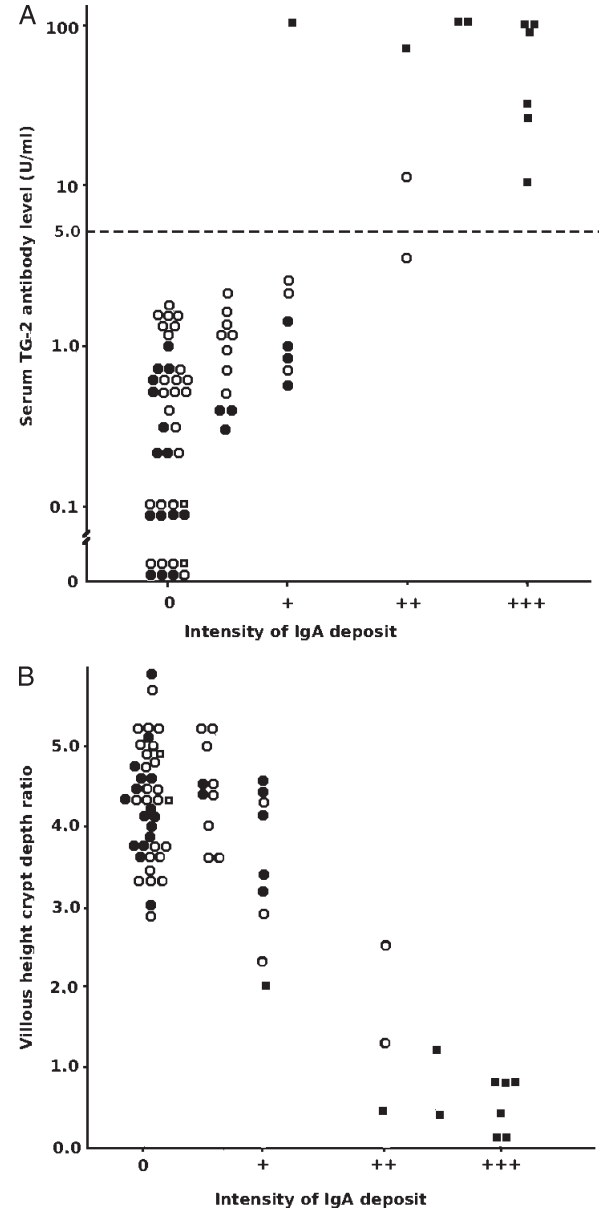

FIG. 3. Correlation between the intensity of small-bowel mucosal transglutaminase 2-specific IgA deposits and serum IgA-class TG2 antibodies (A), (Spearman correlation coefficient was $r = 0.694$, $P < 0.001$), and between small-bowel mucosal IgA deposits and villous height-crypt depth ratios (B), (Spearman correlation coefficient was $r = -0.550$, $P < 0.001$). Dotted line indicates the cutoff value for positivity in TG2 antibody measurements from serum. Cases having standard gluten-free diet omitting wheat, rye, barley, and oats (n = 23) are shown in black circles, cases having gluten challenge (n = 10) in black squares, cases with oat containing diet (n = 39) in open circles, and 2 patients in oat group with premature withdrawals in open squares.

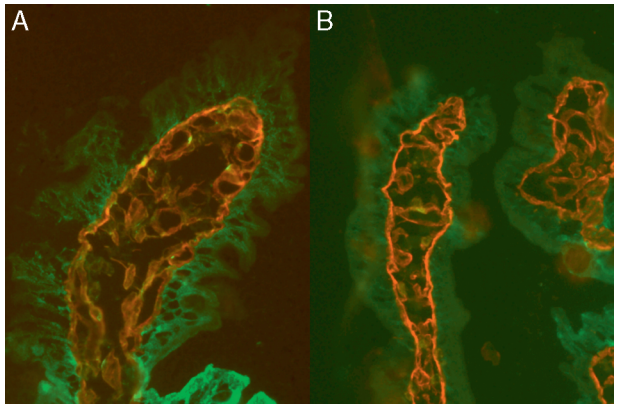

FIG. 4. A 16-year-old boy developed abdominal symptoms immediately after ingestion of oats; small-bowel mucosal villous morphology remained normal and densities of CD3+ intraepithelial lymphocytes even decreased (from 86 cells/mm to 51 cells/mm). Mucosal IgA deposits were negative both before (A) and immediately after (B) oats challenge. IgA is labelled green, transglutaminase 2 in red; IgA deposition or co-localization of IgA and TG2 (yellow) was not detected.

DISCUSSION

We demonstrated by measuring the intensities of small-bowel mucosal TG2-specific autoantibody deposits that the ingestion of oats during a gluten-free diet for 2 years does not result in humoral immune activation in children with coeliac disease. In contrast, when wheat, rye, and barley are consumed in addition to oats, a marked small-bowel mucosal antibody response occurs parallel with small-bowel mucosal damage within 3 to 12 months. If oats were disease inducing per se, then the small-bowel mucosa would not have recovered after the relapse on a diet excluding wheat, rye, and barley but containing oats. The present findings are in accord with those in earlier studies in children (Table 1) and adults with coeliac disease or dermatitis herpetiformis, showing that oats have neither toxic nor immunogenic effects on the small-bowel mucosa and are tolerated by the majority of patients with coeliac disease (4–8,16).

Although several studies have suggested that oats can be safely added to the diet of patients with coeliac disease, reports that some develop more abdominal symptoms or may even have avenin-reactive T cells in the small-bowel mucosa while consuming oats raise some concern (15,17). Furthermore, some studies have reported somewhat higher withdrawal frequencies from oats challenge groups when compared with standard gluten-free diet groups also omitting oats (11,15). Most patients who dropped out of these studies were not properly followed up to study whether oat intolerance was due to relapse of the disease or whether it was related to something else in oats, for example, high fibre content (16). In the present study, both children with coeliac disease who developed dramatic abdominal symptoms

TABLE 1. *Previous oat trials in children with coeliac disease*

References	n	Study design	Oats (g/day)	Duration of oats	Outcome variables	Safety of oats
Hoffenberg et al (30)	10 UNTR	Uncontrolled	24	6 mo	Small-bowel biopsy (Marsh score, IELs) TG2-ab, haemoglobin, iron, zinc levels, growth charts, symptom score	Yes
Hogberg et al (11)	116 UNTR	Randomized, double blind, controlled	5–40	12 mo	Small-bowel biopsy (Marsh score, IELs) TG2-ab, AGA, EmA, symptoms	Yes, majority
Hollen et al* (31)	86 UNTR	Randomized, double blind, controlled	5–40	12 mo	Avenin-antibodies urinary nitric oxide	Yes, majority
Hollen et al* (32)	87 UNTR	Randomized, double blind, controlled	5–40	12 mo	Urinary nitric oxide	Yes, majority
Holm et al (27)	9 UNTR 23 TR	Randomized controlled	50	2 y (biopsies) up to 7 y (serology, clinical data)	Small-bowel biopsy (Vh/CrD, CD3+, αβ+ and γδ+ IELs, HLA DR expression) TG2-ab, AGA, EmA, haemoglobin, growth charts, symptoms	Yes
Koskinen et al,† current study	23 TR	Randomized controlled	50	2 y	Small-bowel biopsy (IgA-deposits), TG2-ab	Yes

AGA = gliadin antibodies, EmA = endomysial antibodies, IEL = intraepithelial lymphocyte, TG2-ab = transglutaminase-2 antibodies, TR = treated coeliac disease, UNTR = untreated coeliac disease.

* Part of the study by Hogberg et al (11).

† Part of the study by Holm et al (27).

immediately after ingesting oats were biopsied, but no signs of immune activation or relapse of coeliac disease were found. This notwithstanding, the possibility that some patients with coeliac disease may be truly oat intolerant and should thus avoid oats to remain in remission should be kept in mind.

In our challenge study, small-bowel mucosal TG2-specific IgA deposits proved to be clearly gluten-sensitive and they were, in fact, present in the mucosa even before the onset of villous atrophy (Fig. 2). Furthermore, in 1 case, the intensity of the IgA deposition transiently increased when the patient had advertent dietary lapses even if serum anti-TG2 antibody levels remained normal (Fig. 1A). The specificity for TG2 of IgA deposits have been shown in earlier studies by ELISA, Western blot, and in situ (23,29). Gluten sensitivity of mucosal IgA deposits has been also shown in studies concerning early-stage coeliac disease; mucosal IgA deposits have been detected in patients still evincing normal small-bowel mucosal villous architecture but who subsequently developed mucosal damage when gluten consumption was continued (23–25). Interestingly, IgA deposits can also be detected in the small-bowel mucosa of such patients with coeliac disease who do not have the autoantibodies in their serum (23,29). Because the coeliac disease–specific autoantibodies against TG2 are produced in the mucosa of the small intestine, it would appear that after gluten ingestion the autoantibodies are first sequestered in the bowel and that only later ''spilling over'' from the gut leads to their appearance in the serum.

In conclusion, this study showed that consumption of oats is safe for the majority of children with coeliac disease. Lifelong follow-up is recommended for children wishing to consume a diet containing oats. The detection of mucosal IgA-deposits is a potent means of monitoring treatment of coeliac disease, although not needed in routine surveillance. However, new treatment options in coeliac disease are under study, meaning that reliable and sensitive diagnostic tools to detect minor gluten-induced small-bowel mucosal changes are warranted. The detection of mucosal IgA deposits could provide such a tool and be useful also in the follow-up of clinically problematic cases.

REFERENCES

1. Haboubi NY, Taylor S, Jones S. Coeliac disease and oats: a systematic review. *Postgrad Med J* 2006;82:672–8.
2. Moulton ALC. The place of oats in the coeliac diet. *Arch Dis Child* 1959;34:51–5.
3. Baker PG, Read AE. Oats and barley toxicity in coeliac patients. *Postgrad Med J* 1976;52:264–8.
4. Janatuinen EK, Pikkarainen PH, Kemppainen TA, et al. A comparison of diets with and without oats in adults with celiac disease. *N Engl J Med* 1995;333:1033–7.
5. Janatuinen EK, Kemppainen TA, Julkunen RJK, et al. No harm from five year ingestion of oats in coeliac disease. *Gut* 2002; 50:332–5.
6. Srinivasan U, Leonard N, Jones E, et al. Absence of oats toxicity in adult coeliac disease. *BMJ* 1996;313:1300–1.
7. Hardman CM, Garioch JJ, Leonard JN, et al. Absence of toxicity of oats in patients with dermatitis herpetiformis. *New Engl J Med* 1997;337:1884–7.
8. Reunala T, Collin P, Holm K, et al. Tolerance to oats in dermatitis herpetiformis. *Gut* 1998;43:490–3.
9. Picarelli A, Di Tola M, Sabbatella F, et al. Immunologic evidence of no harmful effect of oats in celiac disease. *Am J Clin Nutr* 2001;74:137–40.
10. Kilmartin C, Lynch S, Abuzakouk M, et al. Avenin fails to induce a Th1 response in coeliac tissue following in vitro culture. *Gut* 2003;52:47–52.
11. Hogberg L, Laurin P, Falth-Magnusson K, et al. Oats to children with newly diagnosed coeliac disease: a randomised double blind study. *Gut* 2004;53:649–54.
12. Thompson T. Gluten contamination of commercial oat products in the United States. *N Engl J Med* 2004;351:2021–2.
13. Hernando A, Mujico JR, Juanas D, et al. Confirmation of the cereal type in oat products highly contaminated with gluten. *J Am Diet Assoc* 2006;106:665.
14. Lundin KEA, Nilsen EM, Scott HG, et al. Oats induced villous atrophy in coeliac disease. *Gut* 2003;52:1649–52.
15. Peräaho M, Kaukinen K, Mustalahti K, et al. Effect of an oats-containing gluten-free diet on symptoms and quality of life in coeliac disease. A randomized study. *Scand J Gastroenterol* 2004;39:27–31.
16. Storsrud S, Olsson M, Arvidsson Lenner R, et al. Adult coeliac patients do tolerate large amounts of oats. *Eur J of Clin Nutr* 2003;57:163–9.
17. Arentz-Hansen H, Fleckenstein B, Molberg O, et al. The molecular basis for oat intolerance in patients with celiac disease. *PLoS Med* 2004;1:e1.
18. Vader LW, Stepniak DT, Bunnik EM, et al. Characterization of cereal toxicity for celiac disease patients based on protein homology in grains. *Gastroenterology* 2003;125: 1105–13.
19. Kilmartin C, Wieser H, Abuzakouk M, et al. Intestinal T cell responses to cereal proteins in celiac disease. *Dig Dis Sci* 2006;51:2002–9.
20. Sulkanen S, Halttunen T, Laurila K, et al. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. *Gastroenterology* 1998;115:1322–8.
21. Marzari R, Sblattero D, Florian F, et al. Molecular dissection of tissue transglutaminase autoantibody response in celiac disease. *J Immunol* 2001;166:4170–6.
22. Picarelli A, Maiuri L, Frate A, et al. Production of antiendomysial antibodies after in-vitro gliadin challenge of small intestine biopsy samples from patients with coeliac disease. *Lancet* 1996;348: 1065–7.
23. Korponay-Szabo IR, Halttunen T, Szalai Z, et al. In vivo targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. *Gut* 2004;53:641–8.
24. Kaukinen K, Peräaho M, Collin P, et al. Small bowel mucosal transglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: a prospective and randomized study. *Scand J Gastroenterol* 2005;40:564–72.
25. Salmi TT, Collin P, Järvinen O, et al. Immunoglobin A autoantibodies against transglutaminase 2 in the small intestinal mucosa predict forthcoming coeliac disease. *Aliment Pharmacol Ther* 2006;24:541–52.
26. Shiner M, Ballard J. Antigen-antibody reactions in jejunal mucosa in childhood coeliac disease after gluten challenge. *Lancet* 1972;1:1202–5.
27. Holm K, Mäki M, Vuolteenaho N, et al. Oats in the treatment of childhood coeliac disease: a two-year controlled and a long-term clinical follow-up study. *Aliment Pharmacol Ther* 2006;23: 1463–72.

28. Kuitunen P, Kosnai I, Savilahti E. Morphometric study of the jejunal mucosa in various childhood enteropathies with special reference to intraepithelial lymphocytes. *J Pediatr Gastroenterol Nutr* 1982;1:525–31.
29. Salmi TT, Collin P, Korponay-Szabo I, et al. Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. *Gut* 2006;55:1746–53.
30. Hoffenberg EJ, Haas J, Drescher A, et al. A trial of oats in children with newly diagnosed celiac disease. *J Pediatr* 2000;137:361–6.
31. Hollen E, Holmgren Peterson K, Sundqvist T, et al. Coeliac children on a gluten-free diet with or without oats display equal anti-avenin antibody titres. *Scand J Gastroenterol* 2006;41: 42–7.
32. Hollen E, Forslund T, Högberg L, et al. Urinary nitric oxide during one year of gluten-free diet with or without oats in children with coeliac disease. *Scand J Gastroenterol* 2006;41: 1272–8.

Intestinal transglutaminase 2 specific antibody deposits in non-responsive coeliac disease

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Disclosures

The authors declare no conflicts of interest

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Abbreviations

EATL enteropathy-associated T-cell lymphoma, ELISA enzyme-linked immunosorbent assay, EmA endomysial antibody, IEL intraepithelial lymphocyte, PCR polymerase chain reaction, TG transglutaminase, TCR T-cell receptor, Vh/CrD villous height/ crypt depth ratio,

Abstract

Background and aims: The diagnosis of coeliac disease is problematic in individuals not responding to a gluten free diet. Small-bowel villous atrophy occurs in other enteropathies and non-responsive patients are often seronegative. We investigated whether small-bowel mucosal transglutaminase-2 specific autoantibody deposits distinguish non-responsive coeliac disease from other enteropathies.

Methods: Small bowel mucosal autoantibody deposits were determined in 27 non-responsive, 28 responsive coeliac patients and ten controls with other enteropathies. Of the non-responsive coeliac patients six were adhering poorly and 21 strictly to the diet; six of the 21 had enteropathy-associated lymphoma, five refractory coeliac disease and ten otherwise persistent villous atrophy. The presence of mucosal autoantibody deposits was compared to serology, villous morphology, densities of intraepithelial lymphocytes (IELs) and markers of refractory coeliac disease.

Results: Twenty out of 21 well-adhering, all six poorly adhering non-responsive and all 28 untreated responsive coeliac patients had small-bowel mucosal autoantibody deposits present, while controls with other enteropathies were negative. Small-bowel mucosal autoantibody deposits were more accurate in detecting coeliac disease than serology or IEL densities. Refractory coeliac markers revealed only cases with the most severe condition.

Conclusions: Small-bowel mucosal autoantibody deposits differentiate coeliac disease from other enteropathies, enabling the design of appropriate therapeutic strategies.

Introduction

The basis for the treatment of coeliac disease is life-long adherence to a gluten-free diet during which the gluten-induced small-bowel mucosal villous atrophy with crypt hyperplasia generally resolves within 1-2 years. In cases with poor histological response to diet further investigations are always required. The most common reason for persistent villous atrophy is continuous intentional or unintentional gluten consumption [1,2], which may be difficult to reveal even with careful dietary assessment. Moreover, as villous atrophy sometimes also occurs in conjunction with other enteropathies such as autoimmune enteropathy and Crohn's disease [3], the initial diagnosis has to be ascertained especially if coeliac-specific serum endomysial (EmA) and transglutaminase 2 antibodies (TG2-ab) have been negative at the time of diagnosis. In addition, sustained severe mucosal damage might be due to refractory coeliac disease. Refractory coeliac disease fortunately affects less than 5% of coeliac patients, but is a serious condition with the potential to develop to ulcerative jejunitis and further to enteropathy-associated T-cell lymphoma (EATL) [4,5]. A subset of patient suffering from refractory coeliac disease (type II) present with an abnormal immunophenotype of small-bowel mucosal intraepithelial lymphocytes (IELs) and with clonal proliferation of these cells, both regarded as poor prognostic markers of the condition [4-8].

As patients suffering from refractory coeliac disease are often seronegative while adhering to a gluten-free diet, differential diagnostics between unresponsive coeliac disease and other causes of enteropathy can be laborious. It is well known that the serum coeliac-specific antibodies normalize fairly rapidly after commencement of a gluten-free diet, even before full recovery of the small-bowel mucosal villous morphology [9,10], which further limits the usefulness of coeliac serology during the follow-up of the disease. Evidence shows that in coeliac disease, the TG2-specific autoantibodies are produced at small-bowel mucosal level, where they are also sequestered below the basement membrane along the villous and crypt epithelium and around the blood vessels [9,11-14]. These autoantibody deposits can be found even in newly diagnosed coeliac disease patients having no serum autoantibodies [11], and interestingly these depositions seem to disappear slowly during a gluten free diet [15]. As simple immunofluorescent

staining can reveal the presence of such TG2-specific coeliac disease type IgA autoantibody deposits, we hypothesized that this method could be used in distinguishing non-responsive, often seronegative, coeliac disease from other types of enteropathy. We addressed this issue by studying altogether 27 non-responsive coeliac disease patients evincing persistent villous atrophy despite a gluten-free diet, and ten disease control patients suffering from other intestinal disorders.

Methods

Patients and study design

Our study cohort comprised altogether 27 consecutive adults having non-responsive coeliac disease and referred to the Department of Gastroenterology and Alimentary Tract surgery of Tampere University Hospital. Non-responsive coeliac disease was considered to be refractory coeliac disease when symptoms due to villous atrophy persisted after gluten withdrawal or recurred after a former good response on a gluten free diet [4,7,16]. A thorough dietary assessment revealed that six of these patients were adhering poorly to a gluten-free diet, whereas the remaining 21 had good adherence (Table 1). Of these latter 21 coeliac patients, six developed EATL, five had refractory coeliac disease and ten persistent villous atrophy despite being apparently asymptomatic. Clinical data on some of these patients have been presented in detail in a previous study [17].

For comparison, 28 adults with responsive coeliac disease were studied at diagnosis and after one year on a gluten-free diet (Table 1). Further, ten patients with enteropathy other than coeliac disease served as disease controls. Of these ten, four had autoimmune enteropathy with small bowel-mucosal villous atrophy, one had Crohn's disease with patchy, mild villous atrophy, and the remaining five had normal villous architecture (two with ulcerative colitis or Crohn's disease and three with collagenous colitis) (Table 1). All disease controls were diagnosed according to published criteria [18-20]. At least six small-intestinal mucosal biopsy specimens were taken from the distal duodenum upon gastroscopy or enteroscopy. Three of the biopsies were embedded in paraffin and the remaining three freshly in optimal cutting temperature compound (OCT; Tissue-Tec, Miles Inc, Elkhart, IN), snap-frozen and stored at -70°C until used. Serum coeliac antibodies were measured at the time of endoscopy.

Small-bowel mucosal transglutaminase 2 (TG2)-specific IgA deposit

Unfixed small-bowel mucosal frozen sections (5µm) were stained by direct immunofluorescence using fluorescein isothiocyanate-labelled rabbit antibody against human IgA (Dako AS, Glostrup, Denmark) at a dilution of 1:40 in phosphate-buffered saline (PBS). To establish whether there is co-localization of IgA and TG2, sections were double-stained with anti-IgA antibody as described above and using monoclonal mouse antibodies against TG2 (CUB7402, NeoMarkers, Fremont, CA), followed by rhodamine-conjugated anti-mouse immunoglobin antibodies (Dako) diluted 1:200 in PBS. In coeliac disease subepithelial deposition can be found along the villous and crypt epithelium and around vessels, in contrast to that observed in non-coeliac samples, where endogenous IgA is found inside plasma cells and epithelial cells [12,21]. It has previously been shown that these mucosal IgA deposits are targeted specifically against TG2 [11,12]. The occurrence of IgA deposits was graded semi-quantitatively according to their intensity along the basement membrane in the villous-crypt area as follows: negative, weak (+), moderate (++) and strong positive (+++). In cases where the intensity of the staining was patchy, it was graded from different areas and the mean value given. All evaluations were carried out in blinded fashion without knowledge of disease history or laboratory findings. In our laboratory the inter- and intraobserver variations have both been 0.98 in the detection of IgA deposits [11].

Small-bowel mucosal histology and intraepithelial lymphocytes

Hematoxylin-eosin staining was carried out on processed paraffin-embedded sections and the villous height/ crypt depth ratio (Vh/CrD) determined as previously described [22]. Vh/CrD below two was considered abnormal.

Sections of paraffin-embedded tissue from the duodenum were used for immunohistochemistry, applying the standard immunoperoxidase method [5]. The following antibodies (all from Lab Vision Corporation, Fremont, CA) were used: anti-CD3 and anti-CD8 (both at a dilution of 1:30) and anti-CD30 (1:50). Positively stained intraepithelial lymphocytes were counted from randomly selected surface epithelium and expressed as IELs per 100 epithelial cells. The percentage of CD3+CD8-IELs was

determined in patients with high numbers of IELs as previously described and a proportion above 52% was considered abnormal [23].

In addition, immunohistochemical staining of CD3+ and γδ + IELs was carried out on frozen sections. CD3+ IELs were stained with monoclonal antibody Leu-4 (Becton Dickinson, San Jose, CA) and γδ+ IELs with T-cell receptor -γ antibody (Endogen, Woburn, MA). Positive IELs were counted with a magnification x100 throughout the surface epithelium and at least 30 fields were counted; the result was given as IEL density cells/mm of epithelium. IEL densities over 37 cells/mm of epithelium for CD3+ IELs and 4.3 cells /mm of epithelium for γδ + IELs were considered abnormal [24].

DNA extraction and analysis of T-cell clonality

DNA was extracted from paraffin-embedded biopsy samples with the Illustra DNA extraction nucleon HT kit (GE Healthcare, Buckinghamshire, UK) according to manufacturer's instructions. One hundred nanograms of DNA were used as template in two separate polymerase chain reactions (PCR) to detect the clonality of the TCR- γ gene as previously described [25]. All PCR reactions were performed in duplicate.

Serum autoantibodies

EmA was determined by an indirect immunofluorescence method, a serum dilution of 1:≥5 being considered positive. In addition, serum TG2 antibody titers were measured by enzyme-linked immunosorbent assay (ELISA) (Celikey, Pharmacia Diagnostics, GmbH, Freiburg, Germany) using human recombinant TG2 as antigen, unit values over 5 being considered positive [26]. When either EmA or TG2 antibodies were above the cut-off level, the subject was regarded as coeliac autoantibody-positive.

HLA-typing

HLA DQB1* allele groups were determined using the Olerup SSP DQ low-resolution kit (Olerup SSP AB, Saltsjobaden, Sweden). This method determines the HLA DQ2, DQ4, DQ5, DQ6, DQ7, DQ8 and DQ9 allele groups. In coeliac disease 90–95% of patients carry the HLA DQ2-haplotype and most of the rest HLA DQ8 [27].

Statistical methods

Quantitative data were expressed as medians and ranges. Statistical differences were evaluated using Mann-Whitney test and Wilcoxon's test as appropriate and all testing was two-sided. P- values < 0.005 were considered statically significant. All statistical testing was performed using the Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL).

Ethical considerations

The study protocol was approved by the Ethical Committee of Tampere University Hospital. All subjects gave written informed consent.

Results

Of our study group of 27 non-responsive coeliac disease patients having a manifest small-intestinal mucosal lesion, all six with poor adherence to gluten-free diet had coeliac disease autoantibodies present in both sera and small-bowel mucosa as deposits (Figure 1, Table 2). In contrast, of the remaining 21 non-responsive coeliac disease patients with good adherence to gluten-free diet, only four (19%) had autoantibodies in the serum while twenty (95%) had autoantibodies deposited in the small-bowel mucosa, this including the five patients with EATL (Figure 2). All 28 coeliac disease control patients who responded to a gluten-free diet had TG2-specific IgA deposits in their small intestinal mucosa before treatment, two (7%) of them being however initially seronegative. During a gluten-free diet of one year the serum autoantibody levels normalized in all, but 21 of the 28 (75%) responsive coeliac disease patients still evinced autoantibodies deposited in the small-intestinal mucosa, although their intensity significantly declined (Figure 1). In contrast, none of the patients with enteropathies other than coeliac disease had autoantibodies in their small-intestinal mucosa or in sera (Figures 1 and 2, Table 2). Thus, the sensitivity and specificity of TG2-specific mucosal IgA autoantibody deposits in distinguishing non-responsive coeliac disease patients adhering to a gluten-free diet from those with other types of enteropathy was 95% and 100%, respectively.

Small-bowel mucosal IgA deposits were found to differentiate non-responsive coeliac patients regardless of the strictness of the diet from controls with other enteropathies better than the densities of CD3+ or γδ+ IELs (Table 2). Increased densities of both types of IELs could also be found in one third of the disease control patients with other enteropathies.

Typical markers of refractory coeliac disease (abnormal CD8/CD3 ratio and TCR γ -gene rearrangement) were found only in patients with refractory coeliac disease with or without EATL (Table 2). CD30+ IELs were found in six out of 21 non-responsive adhering coeliac disease patients, in one non-adhering coeliac patient and in one with autoimmune enteropathy.

Discussion

The major finding in this study was that small-bowel mucosal TG2-specific IgA autoantibody deposits can distinguish non-responsive coeliac disease from other non-coeliac-related enteropathies (e.g. autoimmune enteropathy) better than other markers utilized in coeliac disease diagnostics (Figure 1, Table 2). Serum TG2-autoantibodies have proved to be highly sensitive and specific in finding untreated coeliac disease patients and as they normalize fairly quickly on a gluten-free diet they have been used in monitoring dietary response in clinical practice [10,28,29]. A positive serum autoantibody test result during a gluten free diet most often points to dietary transgressions. However, as also shown in this study, the serum autoantibodies often fail to detect non-responsive coeliac disease and ongoing coeliac-type small-bowel mucosal damage, as non-responsive coeliac disease patients tend to be seronegative [10,11,17]. Often diagnosis of coeliac disease is established years before non-responsiveness is perceived, thus initial serum autoantibody results at the time of diagnosis may not be available anymore. Consequently, serum TG2-autoantibodies are not applicable in differential diagnostics between coeliac-type small-bowel mucosal lesion and other causes of enteropathy. Furthermore, although increased densities of CD3+ and γδ+ IELs would strongly suggest coeliac disease [24,30], they are not sensitive or specific enough to be relied on for a definitive coeliac disease diagnosis in problematic cases (Table 2). Moreover, the widely used markers of aberrant phenotype and T cell clonality of IELs in

detecting refractory coeliac disease patients [4,5] were able to identify only a subset of our non-responsive coeliac disease patients having the most severe condition. In addition, immunophenotyping requires access to molecular laboratory facilities. Taken together, the value of these refractory markers is limited in the diagnostic workup of seronegative small-bowel villous atrophy. As small bowel mucosa does not normalize in all (especially in adults) coeliac patients in whom clinical remission is achieved [31] the differential diagnostics between histologically unresponsive celiac disease and other enteropathies remains a challenge in clinical practice.

An intriguing question is why non-responsive patients adhering strictly to a gluten-free diet evince TG2-specific IgA deposits in their small-bowel mucosa even after long-term dietary treatment. Since coeliac disease autoantibodies are produced in the small-bowel mucosa [32], one explanation could lie in minor gluten contaminations in gluten-free products [33,34], these possibly sustaining a local immunoresponse in the small intestine. Furthermore, it has been shown that during the progression of coeliac disease the *in vitro* avidity of the autoantibodies increases [35]. Similarly, in an earlier study of the target specificity of the deposited autoantibodies in the small-intestine we found that the coeliac autoantibodies were bound to intestinal TG2 *in situ* with considerably high avidity [11]. Such high avidity in antigen-antibody binding might prevent the spilling over of the autoantibodies from the gut mucosa into the circulation and thus result in seronegativity [11].

Patients with IgA deficiency often tend have coeliac disease [36], and in this group determining small-bowel mucosal IgA deposits can not be used; deposits must be determined in other Ig-class autoantibodies (IgG or IgM) instead [12,37]. HLA DQ2 or DQ8 haplotypes are present in up to 40% in the general population, therefore the presence of these HLA-types does not necessarily confirm the diagnosis of coeliac disease but their absence speaks in favour of other disease entities [38].

In our earlier paper [9] our control group consisted of 78 nonceliac subjects who underwent endoscopy because of indigestion or suspicion of celiac disease, the condition being excluded by normal small-bowel mucosal villous architecture. Of these, 18% had at most weak, often patchy deposits. However, as these controls were patients suffering from indigestion or suspected but excluded for celiac disease, it remains to be seen

whether the subjects with minor IgA deposits but normal mucosal architecture have early-stage celiac disease and will develop villous atrophy later in life while continuing consumption of gluten. In our current study we wanted to have disease controls suffering from other enteropathies than celiac disease to obtain certainty whether the determination of intestinal IgA deposits is reliable in differential diagnostics between celiac disease and other enteropathies. Furthermore, it is important to note that the majority of the nonresponsive celiac disease patient in the current study evinced moderate or strong IgA deposits which clearly differs from the week and patchy deposits of the control subjects in the previous study [9].

Although the investigation of intestinal IgA deposits is fairly simple and easy to perform, it is a special method requiring frozen small-bowel biopsy specimens and the interpretation of results requires practice. The method is thus probably not appropriate in routine coeliac disease diagnostics and surveillance but in a certain subset of patients it can help the diagnostic work-up. As the diagnostic workup of problematic cases often requires repeated endoscopies and multiple biopsies, it might be advisable to take a few extra biopsies for the determination of small-bowel mucosal TG2-specific IgA deposits. In conclusion, small-bowel mucosal TG2-specific IgA autoantibody deposits offer a valuable tool in the differential diagnostics of seronegative enteropathies, enabling the design of appropriate therapeutic strategies.

REFERENCES

[1] Abdulkarim AS, Burgart LJ, See J, et al. Etiology of nonresponsive celiac disease: results of a systematic approach. Am.J.Gastroenterol. 2002; 97: 2016-21.

[2] Hall NJ, Rubin G, Charnock A. Systematic review: Adherence to a gluten-free diet in adult patients with coeliac disease. Aliment.Pharmacol.Ther. 2009; 30: 315-30.

[3] Freeman HJ. Adult celiac disease and the severe "flat" small bowel biopsy lesion. Dig.Dis.Sci. 2004; 49: 535-45.

[4] Verbeek WH, von Blomberg BM, Scholten PE, et al. The presence of small intestinal intraepithelial gamma/delta T-lymphocytes is inversely correlated with lymphoma development in refractory celiac disease. Am.J.Gastroenterol. 2008; 103: 3152-8.

[5] Cellier C, Patey N, Mauvieux L, et al. Abnormal intestinal intraepithelial lymphocytes in refractory sprue. Gastroenterology 1998; 114: 471-81.

[6] Daum S, Weiss D, Hummel M, et al. Frequency of clonal intraepithelial T lymphocyte proliferations in enteropathy-type intestinal T cell lymphoma, coeliac disease, and refractory sprue. Gut 2001; 49: 804-12.

[7] Farstad IN, Johansen FE, Vlatkovic L, et al. Heterogeneity of intraepithelial lymphocytes in refractory sprue: potential implications of CD30 expression. Gut 2002; 51: 372-8.

[8] Rubio-Tapia A, Kelly DG, Lahr BD, et al. Clinical staging and survival in refractory celiac disease: a single center experience. Gastroenterology 2009; 136: 99-107.

[9] Koskinen O, Collin P, Lindfors K, et al. Usefulness of small-bowel mucosal transglutaminase-2 specific autoantibody deposits in the diagnosis and follow-up of celiac disease . J.Clin.Gastroenterol. DOI: 10.1097/MCG.0b013e3181b64557.
[10] 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.
[11] Salmi TT, Collin P, Korponay-Szabo IR, et al. Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. Gut 2006; 55: 1746-53.
[12] Korponay-Szabo IR, Halttunen T, Szalai Z, et al. In vivo targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. Gut 2004; 53: 641-8.
[13] Koskinen O, Collin P, Korponay-Szabo I, et al. Gluten-dependent small bowel mucosal transglutaminase 2-specific IgA deposits in overt and mild enteropathy coeliac disease. J.Pediatr.Gastroenterol.Nutr. 2008; 47: 436-42.
[14] Tosco A, Maglio M, Paparo F, et al. Immunoglobulin A anti-tissue transglutaminase antibody deposits in the small intestinal mucosa of children with no villous atrophy. J.Pediatr.Gastroenterol.Nutr. 2008; 47: 293-8.
[15] Koskinen O, Villanen M, Korponay-Szabo I, et al. Oats do not induce systemic or mucosal autoantibody response in children with coeliac disease. J.Pediatr.Gastroenterol.Nutr. 2009; 48: 559-65.
[16] Patey-Mariaud De Serre N, Cellier C, Jabri B, et al. Distinction between coeliac disease and refractory sprue: a simple immunohistochemical method. Histopathology 2000; 37: 70-7.
[17] Kaukinen K, Peraaho M, Lindfors K, et al. Persistent small bowel mucosal villous atrophy without symptoms in coeliac disease. Aliment.Pharmacol.Ther. 2007; 25: 1237-45.
[18] Nikolaus S, Schreiber S. Diagnostics of inflammatory bowel disease. Gastroenterology 2007; 133: 1670-89.
[19] Akram S, Murray JA, Pardi DS, et al. Adult autoimmune enteropathy: Mayo Clinic Rochester experience. Clin.Gastroenterol.Hepatol. 2007; 5: 1282-90.
[20] Freeman HJ. Collagenous mucosal inflammatory diseases of the gastrointestinal tract. Gastroenterology 2005; 129: 338-50.
[21] Kaukinen K, Peraaho M, Collin P, et al. Small-bowel mucosal transglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: a prospective and randomized clinical study. Scand.J.Gastroenterol. 2005; 40: 564-72.
[22] Kuitunen P, Kosnai I, Savilahti E. Morphometric study of the jejunal mucosa in various childhood enteropathies with special reference to intraepithelial lymphocytes. J.Pediatr.Gastroenterol.Nutr. 1982; 1: 525-31.
[23] Verkarre V, Asnafi V, Lecomte T, et al. Refractory coeliac sprue is a diffuse gastrointestinal disease. Gut 2003; 52: 205-11.
[24] Jarvinen TT, Kaukinen K, Laurila K, et al. Intraepithelial lymphocytes in celiac disease. Am.J.Gastroenterol. 2003; 98: 1332-7.
[25] Diss TC, Watts M, Pan LX, et al. The polymerase chain reaction in the demonstration of monoclonality in T cell lymphomas. J.Clin.Pathol. 1995; 48: 1045-50.
[26] Sulkanen S, Halttunen T, Laurila K, et al. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology 1998; 115: 1322-8.
[27] Karell K, Louka AS, Moodie SJ, et al. HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. Hum.Immunol. 2003; 64: 469-77.
[28] Kaukinen K, Sulkanen S, Maki M, et al. IgA-class transglutaminase antibodies in evaluating the efficacy of gluten-free diet in coeliac disease. Eur.J.Gastroenterol.Hepatol. 2002; 14: 311-5.
[29] Rostami K, Kerckhaert J, Tiemessen R, et al. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice. Am.J.Gastroenterol. 1999; 94: 888-94.
[30] Iltanen S, Holm K, Ashorn M, et al. Changing jejunal gamma delta T cell receptor (TCR)-bearing intraepithelial lymphocyte density in coeliac disease. Clin.Exp.Immunol. 1999; 117: 51-5.
[31] Bardella MT, Velio P, Cesana BM, et al. Coeliac disease: a histological follow-up study. Histopathology 2007; 50: 465-71.
[32] Marzari R, Sblattero D, Florian F, et al. Molecular dissection of the tissue transglutaminase autoantibody response in celiac disease. J.Immunol. 2001; 166: 4170-6.

[33] Collin P, Thorell L, Kaukinen K, et al. The safe threshold for gluten contamination in gluten-free products. Can trace amounts be accepted in the treatment of coeliac disease?. Aliment.Pharmacol.Ther. 2004; 19: 1277-83.
[34] Storsrud S, Malmheden Yman I, Lenner RA. Gluten contamination in oat products and products naturally free from gluten. Eur Food Res Technol 2003; 317: 481-5.
[35] Westerlund A, Ankelo M, Simell S, et al. Affinity maturation of immunoglobulin A anti-tissue transglutaminase autoantibodies during development of coeliac disease. Clin.Exp.Immunol. 2007; 148: 230-40.
[36] Korponay-Szabo IR, Dahlbom I, Laurila K, et al. Elevation of IgG antibodies against tissue transglutaminase as a diagnostic tool for coeliac disease in selective IgA deficiency. Gut 2003; 52: 1567-71.
[37] Borrelli M, Maglio M, Agnese M, et al. High density of intraepithelial gammadelta lymphocytes and deposits of immunoglobulin (Ig)M anti-tissue transglutaminase antibodies in the jejunum of coeliac patients with IgA deficiency. Clin.Exp.Immunol. DOI:10.1111/j.1365-2249.2009.04077.x.
[38] Sollid LM, Markussen G, Ek J, et al. Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. J.Exp.Med. 1989; 169: 345-50.

FIGURE LEGENDS

Figure 1 Intensity of transglutaminase-2 specific small-bowel mucosal IgA deposits in different study groups. Patients with refractory coeliac disease (CD) are marked with open squares, and enteropathy associated T cell lymphoma patients have been marked with black squares. A p value less than 0.05 was considered significant.

Figure 2 Small-bowel mucosal immunofluorescence staining of IgA (green) and transglutaminase-2 (red) from patients having untreated coeliac disease which subsequently responded well to a gluten-free diet (A), refractory coeliac disease patient with abnormal immunophenotype of small-bowel mucosal intraepithelial lymphocytes (B), a refractory coeliac patient with enteropathy-associated T cell lymphoma (C) and a patient with autoimmune enteropathy (D). Colocalization of IgA and TG2 is shown in yellow (arrow).

Table 1. Demographic data on the study subjects

		Non responsive CD		
	Responsive CD n=28	Poor adherence n=6	Good adherence n=21	Other enteropathy n=10
Female, n (%)	22 (79%)	6 (100%)	10 (48%)	6 (60%)
Age, median (range), years	43 (18-63)	49 (30-77)	52 (21-76)	52 (23-64)
Duration of GFD, median (range), years	0	14 (6-18) [a]	5 (0.3-24)	0
Symptoms at endoscopy, n (%)				
Abdominal symptoms	19 (68%)	0	7 (33%)	8 (80%)
Malabsorbtion	3 (11%)	1 (17%)	2 (10 %)	1(10%)
Extraintestinal symptoms [b]	1 (4%)	0	2 (10%)	1 (10%)
Asymptomatic	5 (18%)	5 (83%)	10 (47%)	0
Outcome	All asymptomatic	1 died of malabsorption 5 asymptomatic	5 died of EATL 1 died of refractory CD 1 EATL alive 4 refractory CD asymptomatic with or without immuno-suppressive drugs 10 asymptomatic	5 on immunosuppressive drugs, asymptomatic 5 mild symptoms

EATL enteropathy-associated T-cell lymphoma, CD celiac disease, GFD gluten-free diet,

[a] gluten contaminations repeatedly

[b] dermatitis herpetiformis, aphtous stomatitis of unknown origin, articular problems

Table 2. Abnormality of celiac markers in study groups.

	Responsive CD		Non-responsive CD		
	Untreated n=28	Treated[a] n=28	Poor adherence n=6	Good adherence n=21	Other enteropathies n=10
Villous atrophy	28 (100%)	0	6 (100%)	21 (100%)	5 (50%)
Mucosal IgA deposit+	28 (100%)	21 (75%)	6 (100%)	20 (95%)	0
Serum autoantibody+	26 (93%)	0	5 (100%)	4 (20%)	0
Increased density of CD3+ IELs	27 (100%)	14 (50%)	5 (83%)	16 (76%)	3 (30%)
Increased density of γδ + IELs	27 (100%)	27 (96%)	4 (67%)	15 (71%)	3 (30%)
Increased density of CD30+ IELs	nd	nd	2 (33%)	6 (28%)	1 (25%)[b]
Abnormal CD3/CD8 ratio	nd	nd	0	5 (24%)[c]	0[b]
TCR γ gene rearrangement	nd	nd	0	3 (14%)	0[b]
HLA-type	19 DQ2 1 DQ8 8 nd	19 DQ2 1 DQ8 8 nd	4 DQ2 2 nd	12 DQ2 3 DQ 2 and DQ8 6 nd	3 DQ2 5 other[d] 2 nd

CD celiac disease, IEL intraepithelial lymphocyte, nd not determined, TCR T-cell receptor, HLA human leukocyte antigen

[a] same patients as in the untreated CD group after one year on gluten-free diet

[b] four autoimmune enteropathy samples analyzed

[c] all had enteropathy-associated t-cell lymphoma

[d] none of the patients with small-bowel mucosal villous atrophy had HLA DQ2

Figure 1.

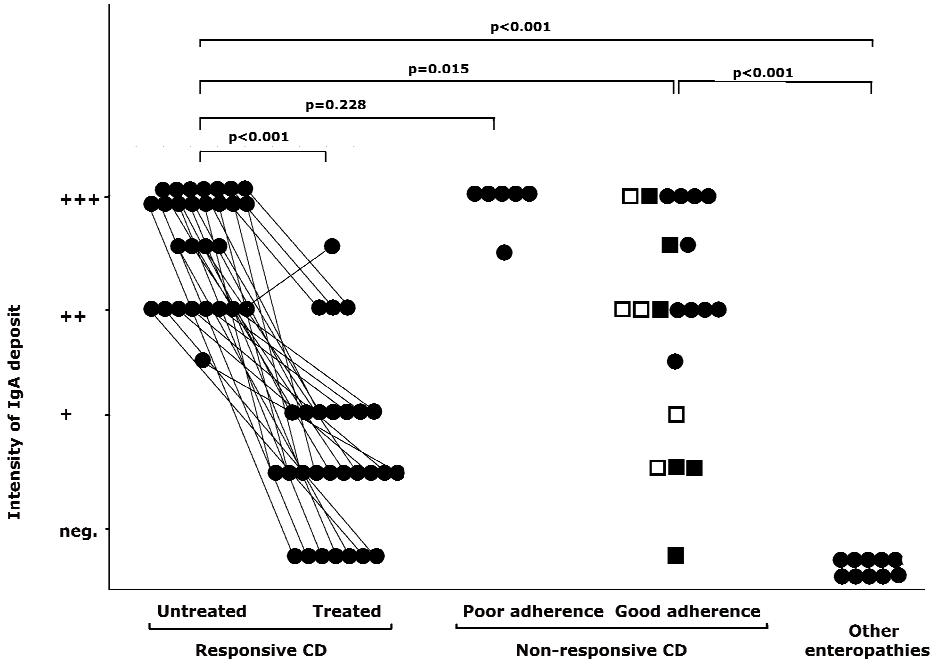
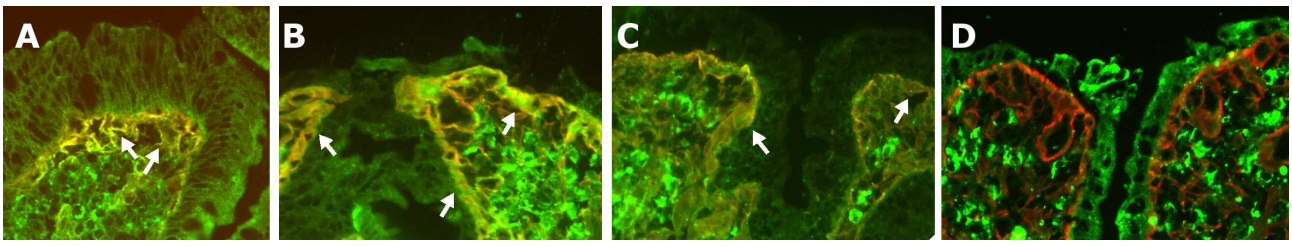


Figure 2.



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Gluten-dependent Small Bowel Mucosal Transglutaminase 2–specific IgA Deposits in Overt and Mild Enteropathy Coeliac Disease

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ABSTRACT

Objectives: In coeliac disease, immunoglobulin (Ig)A–class autoantibodies against transglutaminase-2 are produced in the small intestinal mucosa, where they are deposited extracellularly. It remains unclear whether positive intestinal transglutaminase-2-targeted IgA deposits in subjects having normal small bowel mucosal morphology are signs of early-stage coeliac disease. We evaluated the gluten dependency of these deposits in overt and mild enteropathy coeliac disease.

Patients and Methods: All together 48 subjects suspected of coeliac disease but having normal small bowel mucosal villi were enrolled; 28 of them had latent coeliac disease. The remaining 20 having positive intestinal IgA deposits adopted a gluten-free diet before villous atrophy had developed. For comparison, 13 patients with overt coeliac disease and 42 noncoeliac controls were studied. Small bowel mucosal transglutaminase-2–specific autoantibodies were compared with villous morphology, intraepithelial lymphocyte densities, and serum coeliac autoantibodies.

Results: Intestinal IgA deposits were seen in all but 1 of the patients with latent coeliac disease, when the morphology was still intact; the intensity of these deposits increased as villous atrophy developed and decreased again on a gluten-free diet. In 20 patients with intestinal IgA deposits in normal villi, the intensity of the deposits decreased with the diet similarly to that seen in patients with overt coeliac disease. Mucosal IgA deposits were seen initially only in 5% of noncoeliac controls and in 8% after extended gluten consumption.

Conclusions: The response of small bowel mucosal transglutaminase-2–specific IgA deposits for dietary intervention was similar in overt and mild enteropathy coeliac disease. Detection of such IgA deposits thus offers a good diagnostic tool to uncover early-stage coeliac disease. ***JPGN 47:436–442, 2008.*** **Key Words:** Latent coeliac disease—Immunoglobulin A deposit—Gluten dependency. © 2008 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

The current diagnostic criteria for coeliac disease are based on small bowel mucosal villous atrophy with crypt hyperplasia (1). The gluten-triggered mucosal lesion develops gradually from mucosal inflammation to elongation of crypts and finally to overt villous atrophy (2,3), the atrophy thus covering only the end stage of the disease. Evidence suggests that coeliac patients may suffer from gluten-sensitive symptoms and signs even before villous atrophy has developed (4–7). Minor small bowel mucosal morphological and inflammatory changes such as increased densities of CD3+ and γδ+ intraepithelial lymphocytes (IELs) are unspecific and subject to false diagnosis (8–11), and new reliable tools to detect mild enteropathy coeliac disease are therefore warranted. A specific test also should encompass proof of gluten dependency when patients are placed on gluten-free dietary treatment.

Apart from mucosal changes, a typical feature of untreated coeliac disease is the presence of serum

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The authors report no conflicts of interest.

TABLE 1. *Demographic data on study patients*

	Latent coeliac disease, n=28	Potential coeliac disease, n=20	Overt coeliac disease, n=13	Noncoeliac controls, n=42
Female, %	75	80	77	71
Median age (range), y	37 (2–69)	46 (21–74)	47 (28–68)	41 (17–68)
Children, n (%)	10 (36)	0	0	0
Main indication for biopsy, n (%)				
Abdominal complaints*	17 (61)	17 (85)	7 (54)	30 (71)
Malabsorbtion†	2 (7)	1 (5)	1 (8)	7 (17)
Atypical symptoms‡	5 (18)	2 (10)	2 (15)	3 (7)
Screening of risk groups§	4 (14)	0 (0)	3 (23)	2 (5)
Family history of coeliac disease, n (%)	11 (41)	4 (20)	7 (54)	5 (12)

* Abdominal pain, flatulence, diarrhoea.
† Weight loss, anaemia.
‡ Aphthous stomatitis, growth retardation, epilepsy, polyneuropathy, atopic dermatitis, arthralgia.
§ Type 1 diabetes mellitus, family history, autoimmune thyroid disease, Sjögrens syndrome, osteoporosis.

immunoglobulin (Ig)A–class autoantibodies against transglutaminase-2 (TG2) (12,13), which offer high sensitivity and specificity (14,15). In fact, positive autoantibodies in patients having normal small bowel mucosal villous morphology do not necessarily constitute a false positive finding because they may be a predictive sign of forthcoming mucosal villous atrophy and coeliac disease (9,16–18). These autoantibodies are produced in the small intestinal mucosa (19,20), where they may be present even when there are no measurable levels in the sera (5,21,22). Intestinal TG2-specific IgA deposits may offer a diagnostic tool for detecting early-stage coeliac disease without villous atrophy (9). These intestinal antibodies (with absence in serum) also have been found in patients with diabetes mellitus and in first-degree relatives of coeliac patients, without full certainty as to whether these antibodies are gluten induced or related to coeliac disease (23–25). The demonstration of gluten dependency in intestinal TG2-specific IgA deposits would further enhance the specificity of the test.

Our aim was to evaluate the gluten dependency and diagnostic value of small bowel mucosal TG2-specific IgA autoantibody deposits in overt and mild enteropathy coeliac disease. Our series included children and adults with latent coeliac disease, who initially showed normal small bowel mucosal villous architecture but subsequently developed mucosal villous atrophy and celiac disease while continuing on a normal gluten-containing diet. In addition, we found individuals evincing small bowel mucosal TG2-specific IgA autoantibody deposits in intact villi who were directly placed on an experimental gluten-free diet to find evidence of gluten sensitivity. The data were compared with those from patients with overt coeliac disease and noncoeliac controls.

PATIENTS AND METHODS

The study group comprised 48 patients suspected of coeliac disease but found to have normal small bowel mucosal villous architecture. Of these, 28 had latent coeliac disease because they developed mucosal villous atrophy during the follow-up when continuing on a gluten-containing diet (Table 1). In these patients, the median follow-up time from normal small bowel mucosal architecture to villous atrophy was 1.7 years (range 0.2–7.4 years). After the mucosal deterioration, the patients were placed on a gluten-free diet, the median duration of the diet being 1.4 years (range 0.8–5.9 years). The remaining 20 patients in the study group were found to have small bowel mucosal IgA deposits in intact villi (defined in this study as potential coeliac disease) (Table 1); to find evidence for their gluten dependency, these individuals were placed on an experimental gluten-free diet even if they did not fullfil the current diagnostic criteria for coeliac disease. After 1 year, the response to the dietary treatment was evaluated.

The controls comprised 13 subjects with overt coeliac disease with small bowel mucosal villous atrophy and crypt hyperplasia and 42 patients with suspicion of coeliac disease but no evidence of villous atrophy in 2 successive biopsy samples on a gluten-containing diet with a median interval of 6.0 years (range 0.8–10.0 years) (Table 1). The study protocol was approved by the Ethical Committee of Tampere University Hospital. All of the subjects gave written informed consent.

Small Bowel Mucosal Morphology and Intraepithelial Lymphocytes

Small bowel mucosal biopsies were taken upon upper gastrointestinal endoscopy and in small children with a Watson capsule. For morphometrical analysis, the samples were paraffin embedded, processed, and stained with hematoxylin-eosin. The villous height–crypt depth ratio was counted in well-oriented biopsy samples as previously described (26) and a ratio <2.0 was considered compatible with coeliac disease.

One piece of capsule biopsy or 2 forceps biopsy specimens were freshly embedded in optimal cutting temperature compound (OTC, Tissue-Tec, Miles, Elkhart, IN), frozen in liquid nitrogen, and stored at -70°C. Immunohistochemical studies were carried out on 5-μm-thick frozen sections. CD3+ IELs were stained with monoclonal antibody Leu-4 (Becton Dickinson, San Jose, CA) and $\gamma\delta$+ IELs with T-cell receptor bearing cell γ antibody (T Cell Diagnostics, Woburn, MA). Positive

IELs were counted from immunohistochemically stained specimens with a ×100 light microscope objective as described elsewhere (27). The reference value for CD3+ IELs was set at 37 cells per millimeter of epithelium and for γδ+ cells at 4.3 cells per millimeter of epithelium (27).

Small bowel Mucosa TG2-specific IgA Deposits

Small bowel mucosal TG2-specific IgA deposits were investigated in unfixed, 5-μm-thick, frozen sections of small bowel specimens by direct immunofluorescence using fluorescein isothiocyanate–labelled rabbit antibody against human IgA (Dako, Glostrup, Denmark) at a dilution of 1:40 in phosphate-buffered saline, pH 7.4. In coeliac disease a clear subepithelial IgA deposition can be found below the basement membrane along the villous and crypt epithelium and around mucosal vessels; this is in contrast to normal small bowel samples, in which IgA is detected only inside the plasma and epithelial cells (21). The IgA deposits were graded semiquantitatively from 0 to 3 according to their intensity along the basement membrane in the villous crypt area, as previously described (5). It has been shown that these mucosal IgA deposits are specifically targeted against TG2 (21,22). For double labelling, sections were stained for human IgA (green, as above, this paragraph), and for TG2 (red) using monoclonal mouse antibodies against TG2 (CUB7402, NeoMarkers, Fremont, CA), followed by rhodamine-conjugated anti-mouse immunoglobulin antibodies (Dako), both diluted 1:200 in phosphate-buffered saline. In our laboratory, intraobserver and interobserver agreement in the detection of present or absent TG2-specific IgA deposits has been 98% among 5 investigators. All of the evaluations were carried out blindly without knowledge of disease history or laboratory findings.

Serology

Serum IgA-class reticulin (ARA) or endomysial antibodies were determined by an indirect immunofluorescence method as described earlier (14). A positive staining pattern seen in a serum dilution of 1:5 or more was considered positive in both tests. During the study period, endomysial antibodies replaced ARA in clinical practice. If the endomysial antibodies result was not available, then the ARA result was used instead. These 2 coeliac autoantibody tests have proved in our laboratory to be almost identical (28), and both have been shown to be directed against TG2 (13). In this study, these antibodies are indicated as serum coeliac autoantibodies. In retrospect, it was impossible here to determine serum TG2-antibodies by enzyme-linked immunosorbent assay in all of the patients and controls.

HLA Typing

HLA typing was based on polymerase chain reaction with allele-specific primers identifying HLA DQ2 and DQ8, and performed with a Dynal DQ low-resolution SSp kit (Dynal, Oslo, Norway). In coeliac disease, 90% to 95% of patients carry the HLA DQ2-haplotype and most of the rest carry HLA DQ8 (29).

Statistics

Quantitative data are expressed as medians and ranges and qualitative data as percent of abnormal values. Statistical differences were evaluated using the Mann-Whitney *U*, Wilcoxon, and McNemar tests, as appropriate. All of the testing was 2-sided, and $P < 0.05$ were considered statistically significant. All of the calculations were performed with the Statistical Package for Social Sciences version 14.0 (SPSS, Chicago, IL).

RESULTS

Small bowel mucosal TG2-specific IgA deposits already were seen in the first biopsy in all but 1 of the patients with latent coeliac disease, when mucosal villous architecture was still intact (Table 2, Fig. 1). When the patients continued on a normal gluten-containing diet, small bowel mucosal villous atrophy and crypt hyperplasia developed; in parallel, the intensity of mucosal IgA deposits increased, and the deposits were present in all of the patients at the time of the diagnosis of coeliac disease (Figs. 1 and 2). The intensity of mucosal IgA deposits again decreased significantly when the patients were placed on a gluten-free diet, albeit minor depositions still were seen in many. A total of 7 patients in the latent coeliac disease group were initially serum autoantibody negative; 6 of them still had positive small bowel mucosa IgA deposits, 2 had family history for coeliac disease, and all 6 cases tested had coeliac-type HLA. Sixteen of the

TABLE 2. *Abnormal findings and HLA DQ2 or DQ8 haplotypes in different patient groups at the time of first biopsy*

	Latent coeliac disease	Potential coeliac disease	Overt coeliac disease	Noncoeliac controls
Small bowel mucosal TG2-specific IgA deposits present, n (%)	25/26 (96)	20/20 (100)*	13/13 (100)	1/22† (5)
Small bowel mucosal CD3+ IELs, density increased, n (%)	13/21 (62)	15/20 (75)	9/13 (69)	18/42 (43)
Small bowel mucosal γδ+ IELs density increased, n (%)	15/21 (71)	18/20 (90)	11/13 (85)	20/42 (48)
Serum IgA class coeliac autoantibodies (ARA or EmA) present, n (%)	19/26 (73)	14/19 (74)	11/13 (85)	4/41† (10)
HLA DQ2 or DQ8 haplotype present, n (%)	12/12 (100)	16/16 (100)	12/12 (100)	18/42 (43)

TG2 = transglutaminase-2; IgA = immunoglobulin A; IEL = intraepithelial lymphocyte; ARA = reticulin antibody; EmA = endomysial antibody.
* By definition in this study.
† All 4 antibody-positive (all ARA+, see methods) noncoeliac controls were HLA DQ2 and DQ8 negative; 1 of them had positive mucosal IgA deposits.

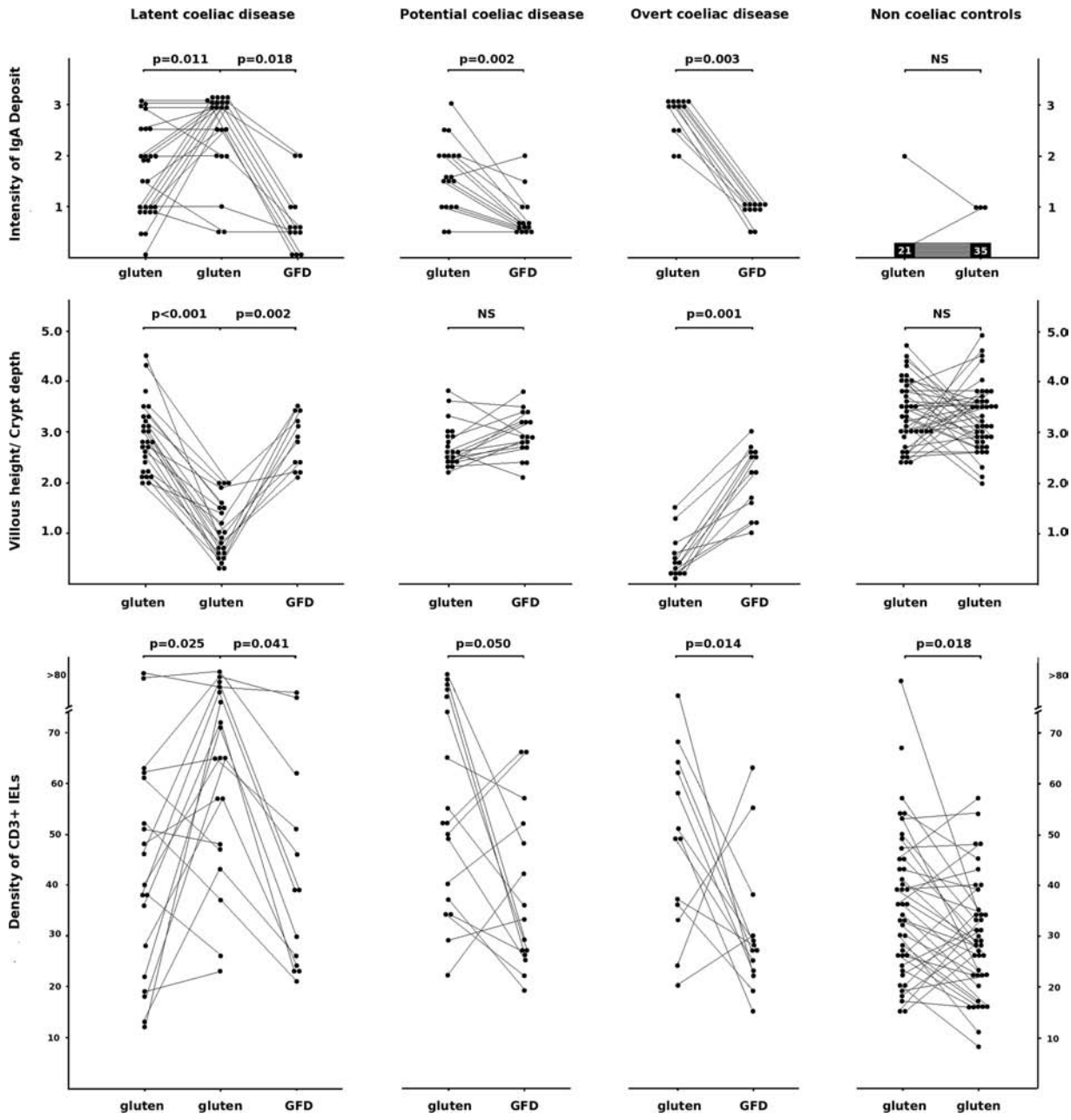

FIG. 1. Changes in small bowel mucosal tissue transglutaminase-2 (TG2)–specific immunoglobulin (Ig)A deposits, villous height–crypt depth ratios, and densities of CD3+ intraepithelial lymphocytes in patients having latent, potential, and overt coeliac disease and in noncoeliac controls at different time points. Gluten = normal gluten-containing diet; GFD = gluten-free diet.

20 patients having TG2-specific small bowel mucosal IgA deposits in intact villi (potential coeliac disease) agreed to a follow-up biopsy after 1 year on a gluten-free diet. The intensity of the deposits decreased in 15 (94%) of the 16 on a gluten-free diet (Figs. 1 and 2). Parallel to these changes, abdominal symptoms and signs of malabsorption resolved in 13 (65%) and improved in 5 (25%) of the patients whilst on the gluten-free diet (Table 3). TG2-specific small bowel mucosal IgA deposits were present in all of the patients with untreated overt coeliac disease, and the intensity of the deposits decreased with the diet. In noncoeliac controls the deposits were seen initially in 1 of 22 (5%) and in 3 of 38 (8%) patients on a gluten-containing diet in the follow-up biopsies (Fig. 1); these deposit-positive cases did not present with any particular clinical sign and had no family history of coeliac disease.

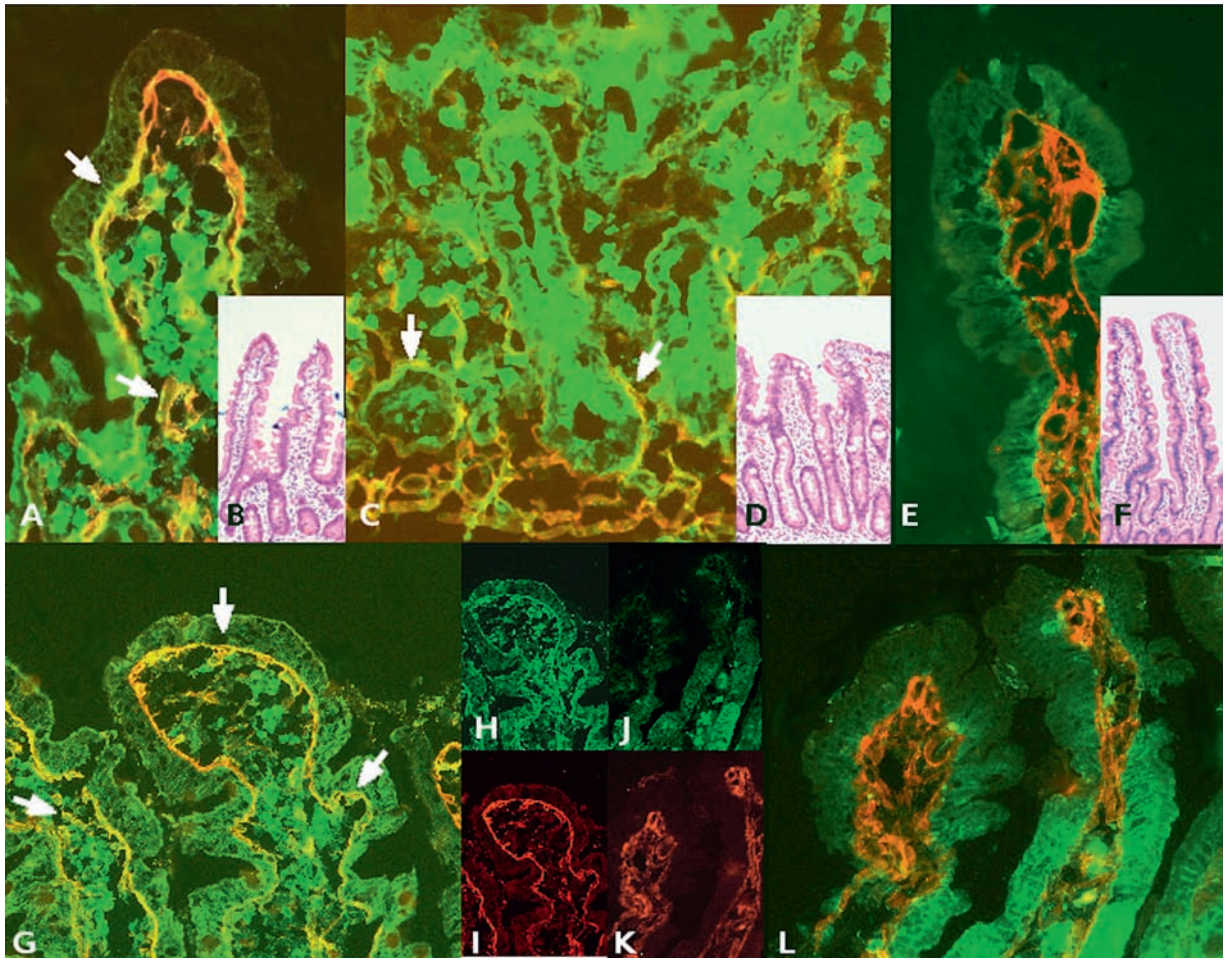

FIG. 2. A–F, Small bowel mucosal immunoglobulin (Ig)A deposits in a 48-year-old woman having latent coeliac disease. The first small bowel mucosal biopsy (B) showed normal villous morphology, but (D) 2 years later when she continued on a normal gluten-containing diet villous atrophy with crypt hyperplasia developed. Villous morphology (F) recovered on a gluten-free diet. Serum celiac autoantibodies were negative throughout, but (A) strong subepithelial tissue transglutaminase (TG2)-targeted IgA autoantibody deposits (yellow, arrow) already were present in the small bowel mucosa in the first biopsy, and (C) also when villous atrophy was detected. The deposits disappeared (E) with a gluten-free diet. G-L, Mucosal IgA deposits are shown from a 21-year-old woman with potential coeliac disease having (G) normal small bowel mucosal architecture and strong subepithelial tissue TG2-specific IgA deposits. After 1 year on a gluten-free diet her symptoms recovered and IgA deposits disappeared (L). IgA is stained with green (H, J), TG2 with red (I,K), and colocalization of IgA and TG2 is shown in yellow (A,C,G).

The histological, serological, and HLA DQ findings are set out in Table 2 and Figure 1. At the first biopsy in the noncoeliac control group, densities of CD3+ IELs were increased in 41% and γδ+ IELs in 48%, and during the study the cell densities decreased significantly with time without any dietary intervention (Table 3, Fig. 1). At the outset, 4 (10%) of the noncoeliac controls were coeliac autoantibody positive, but all of them seroconverted negatively during the study (Table 2).

DISCUSSION

The present study confirms earlier data indicating that the borders of coeliac disease clearly extend beyond small bowel mucosal villous atrophy (30). The current diagnostic criteria have been applied for many years and small intestinal mucosal atrophy has been sine qua non for the diagnosis (1). To revise the criteria of coeliac disease beyond villous atrophy requires the demonstration of gluten dependency of the symptoms and histology in genetically susceptible individuals. We have shown in this article that by careful examination we are able to find coeliac disease in patients with normal villous structure. Once the criteria have been revised, the inevitable consequence is that these patients should be treated with a gluten-free diet. In this study, mucosal TG2-specific IgA deposits were accurate markers for gluten sensitivity; they were detected early on in the

TABLE 3. *Findings and subjective symptoms in 20 patients having potential coeliac disease before and after GFD*

Sex, age (y)	HLA DQ2 or DQ8	Family history of coeliac disease	Positive serum autoantibodies		Symptoms and signs	
			Before GFD	After GFD	Before GFD	After GFD
F46	+/−	−	+	+[†]	Abdominal pain	Resolved
M54	+/+	−	+	−	Abdominal pain	Resolved
F47	+/+	−	+	−	Loose stools, anaemia	Resolved
F43	+/−	+	+	−	Abdominal pain	Resolved
F48	+/−	−	+	−	Diarrhoea, flatulence, anaemia	Resolved
F72	+/−	−	−	−	Diarrhoea	Resolved
F28	+/−	+	−	−	Abdominal pain, flatulence	Resolved
F37	+/−	−	+	−	Loose stools, flatulence[*]	Resolved
F59	ND	−	+	−	Abdominal pain[*]	Resolved
F59	+/−	−	+	−	Loose stools, flatulence	Resolved
F58	+/−	−	+	−	Abdominal pain, flatulence,	Resolved
F33	+/−	−	−	−	Diarrhoea	Resolved
M52	+/−	+	+	−	Weight loss, anaemia[*]	Resolved
F44	+/−	−	+	−	Abdominal pain[*]	Improved
F21	+/−	−	+	−	Dyspepsia	Improved
F59	+/−	−	−	−	Diarrhoea, arthralgia	Improved
F41	ND	−	ND	ND	Abdominal pain	Improved
F39	ND	−	+	ND	Diarrhoea	Improved
M36	+/−	+	−	−	Arthritis	No change
M51	+/−	−	+	−	Refractory epilepsy	No change

+, positive finding; −, negative finding; GFD, gluten-free diet; ND, no data.
[*] At the baseline Marsh 0-type small bowel mucosal lesion; all of the rest had Marsh 1 lesion.
[†] Autoantibody titre decreased.

disease process, their intensity increased as enteropathy progressed on a gluten-containing diet, and their intensity decreased along with mucosal recovery on a gluten-free diet. These intestinal coeliac autoantibody deposits proved to be better initial markers for gluten sensitivity than small bowel mucosal IEL densities, and they also were able to detect serum coeliac autoantibody-negative cases having mild enteropathy coeliac disease (Table 2). This was demonstrated in patients with latent coeliac disease for whom the development of villous atrophy had been confirmed by follow-up on a gluten-containing diet. Furthermore, in patients with TG2-specific IgA deposits but no evidence of progression to overt coeliac disease, the deposits were shown to be gluten dependent (Fig. 1). The process was akin to that of overt coeliac disease and latent coeliac disease. In this study, the majority of patients with latent coeliac disease had experienced gluten-dependent symptoms before subsequent diagnostic enteropathy had developed (Table 1).

Furthermore, 18 (90%) of the 20 patients found to have small bowel mucosal TG2-specific IgA deposits, but considered in view of normal small bowel histology not to have celiac disease, benefited from gluten-free dietary treatment (Table 3). Similar cases have been reported in the literature (6,31,32); some subjects have even been diagnosed as having osteopenia or osteoporosis (4,7,33). Of note, in this study group the nonresponsive symptoms were refractory epilepsy and arthritis, indicating that these extraintestinal manifestations were not gluten dependent. Assuming that the diagnostic criteria for the disease should indeed be widened to cover mild enteropathy coeliac disease, some issues should be stressed to avoid overdiagnosis of the disorder. Marsh 1-type small bowel mucosal lymphocytosis is an unspecific finding (8–10), as also seen in the present study; the densities of CD3+ and γδ+ IELs also decreased with time in noncoeliac controls without any dietary intervention (Fig. 1). Some noncoeliac patients evinced negative seroconversion during the follow-up; these cases were ARA positive without HLA DQ2 or DQ8.

Furthermore, 8% of these controls had minor small bowel mucosal IgA deposits without any signs of gluten sensitivity in the follow-up biopsy. Long-term follow-up is needed to show whether these subjects will eventually develop overt coeliac disease (7,34,35). To conclude, no single test alone can reliably detect early-stage coeliac disease without villous atrophy, but gluten-dependent TG2-specific IgA deposits offer a good diagnostic tool. Based on the findings in this study, we suggest that the deposits should be investigated when coeliac disease is suspected but the small bowel mucosal villous morphology is equivocal. In symptomatic patients (as was the case in the present series) having signs of minor enteropathy coeliac disease without villous atrophy, gluten-free dietary treatment should be considered. In asymptomatic cases, the benefits of early treatment are more ambiguous and subject to further studies. In the meantime, follow-up with a normal gluten-containing diet is recommended. In coeliac disease, however, gluten-induced symptoms may occur outside the

intestine (30), and during the follow-up the wide clinical spectrum of the disease should be kept in mind.

REFERENCES

1. Walker-Smith JA, Guandalini S, Schmitz J, et al. Revised criteria for diagnosis of coeliac disease. *Arch Dis Child* 1990; 65:909–11.
2. Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity (''celiac sprue''). *Gastroenterology* 1992;102:330–54.
3. Mäki M, Holm K, Koskimies S, et al. Normal small bowel biopsy followed by coeliac disease. *Arch Dis Child* 1990;65:1137–41.
4. Kaukinen K, Mäki M, Partanen J, et al. Celiac disease without villous atrophy. Revision of criteria called for. *Dig Dis Sci* 2001; 46:879–87.
5. Kaukinen K, Peräaho M, Collin P, et al. Small bowel mucosal transglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: a prospective and randomized study. *Scand J Gastroenterol* 2005;40:564–72.
6. Troncone R. Latent coeliac disease in Italy. *Acta Paediatr* 1995; 84:1252–7.
7. Matysiak-Budnik T, Malamut G, de Serre NP, et al. Long-term follow-up of 61 patients diagnosed in childhood: evolution towards latency is possible on a normal diet. *Gut* 2007;56:1379–86.
8. Kakar S, Nehra V, Murray JA, et al. Significance of intraepithelial lymphocytosis in small bowel biopsy samples with normal mucosal architecture. *Am J Gastroenterol* 2003;98:2027–33.
9. Salmi TT, Collin P, Järvinen O, et al. Immunoglobin A autoantibodies against transglutaminase 2 in the small intestinal mucosa predict forthcoming coeliac disease. *Aliment Pharmacol Ther* 2006; 24:541–52.
10. Lähdeaho ML, Kaukinen K, Collin P, et al. Celiac disease: from inflammation to atrophy. A longterm follow-up study. *J Pediatr Gastroenterol Nutr* 2005;41:44–8.
11. Paparo F, Petrone E, Tosco A, et al. Clinical, HLA, and small bowel immunohistochemical features of children with positive serum antiendomysium antibodies and architecturally normal small intestinal mucosa. *Am J Gastroenterol* 2005;100:2294–8.
12. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* 1997;3:797–801.
13. Korponay-Szabo IR, Laurila K, Szondy Z, et al. Missing endomysial and reticulin binding of coeliac antibodies in transglutaminase 2 knockout tissues. *Gut* 2003;52:199–204.
14. Sulkanen S, Halttunen T, Laurila K, et al. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. *Gastroenterology* 1998;115:1322–8.
15. Rostom A, Dube C, Cranney A, et al. The diagnostic accuracy of serologic tests for celiac disease: a systematic review. *Gastroenterology* 2005;128:S38–46.
16. Collin P, Helin H, Mäki M, et al. Follow-up of patients positive in reticulin and gliadin antibody tests with normal small bowel biopsy findings. *Scand J Gastroenterol* 1993;28:595–8.
17. Corazza GR, Andreani ML, Biagi F, et al. Clinical, pathological, and antibody pattern of latent celiac disease: report of three adult cases. *Am J Gastroenterol* 1996;91:2203–7.
18. Dickey W, Hughes DF, McMillan SA. Patients with serum IgA endomysial antibodies and intact duodenal villi: clinical characteristics and management options. *Scand J Gastroenterol* 2005; 40:1240–3.
19. Marzari R, Sblattero D, Florian F, et al. Molecular dissection of tissue transglutaminase autoantibody response in celiac disease. *J Immunol* 2001;166:4170–6.
20. Picarelli A, Maiuri L, Frate A, et al. Production of antiendomysial antibodies after in-vitro gliadin challenge of small intestine biopsy samples from patients with coeliac disease. *Lancet* 1996; 348:1065–7.
21. Korponay-Szabo IR, Halttunen T, Szalai Z, et al. In vivo targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. *Gut* 2004;53:641–8.
22. Salmi TT, Collin P, Korponay-Szabo I, et al. Endomysial antibody–negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. *Gut* 2006;55:1746–53.
23. Ziberna F, Baldas V, Martelossi S, et al. Intestinal mucosa anti-transglutaminase antibodies in early stage of gluten intolerance. *J Pediatr Gastroenterol Nutr* 2006;42:E19–20.
24. Sblattero D, Ventura A, Tomassini A, et al. Cryptic gluten intolerance in type 1 diabetes: identifying suitable candidates for gluten free diet. *Gut* 2006;55:133–4.
25. Maglio M, Paparo F, Franzese A, et al. Anti-tissue transglutaminase antibodies are deposited in the small intestinal mucosa of patients with type 1 diabetes. *Gastroenterology* 2006;130 (Suppl 2):A70.
26. Kuitunen P, Kosnai I, Savilahti E. Morphometric study of the jejunal mucosa in various childhood enteropathies with special reference to intraepithelial lymphocytes. *J Pediatr Gastroenterol Nutr* 1982;1:525–31.
27. Järvinen TT, Kaukinen K, Laurila K, et al. Intraepithelial lymphocytes in celiac disease. *Am J Gastroenterol* 2003;98:1332–7.
28. Mäki M. The humoral immune system in coeliac disease. *Baillieres Clin Gastroenterol* 1995;9:231–49.
29. Karell K, Louka AS, Moodie SJ, et al. HLA types in celiac disease patients not carrying the DQA1∗05-DQB1∗02 (DQ2) heterodimer: results from the European genetics cluster on celiac disease. *Hum Immunol* 2003;64:469–77.
30. Kaukinen K, Collin P, Mäki M. Latent coeliac disease or coeliac disease beyond villous atrophy? *Gut* 2007;56:1339–40.
31. Wahab PJ, Crusius JBA, Meijer JWR, et al. Gluten challenge in borderline gluten-sensitive enteropathy. *Am J Gastroenterol* 2001;96:1464–9.
32. Tursi A, Brandimarte G. The symptomatic and histologic response to a gluten-free diet in patients with borderline enteropathy. *J Clin Gastroenterol* 2003;36:13–7.
33. Esteve M, Rosinach M, Fernandez-Banares F, et al. Spectrum of gluten-sensitive enteropathy in first-degree relatives of patients with coeliac disease: clinical relevance of lymphocytic enteritis. *Gut* 2006;55:1739–45.
34. Hogberg L, Stenhammar L, Falth-Magnusson K, et al. Anti-endomysium and anti-gliadin antibodies as serological markers for a very late mucosal relapse in a coeliac girl. *Acta Paediatr* 1997;86:335–6.
35. Simell S, Hoppu S, Hekkala A, et al. Fate of five celiac disease–associated antibodies during normal diet in genetically at-risk children from birth in a natural history study. *Am J Gastroenterol* 2007;102:2026–35.