KATRI KAUKINEN

Small Bowel Mucosa in Suspected and Confirmed Coeliac Disease

University of Tampere
Tampere 2000

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Acta Universitatis Tamperensis 770

ACADEMIC DISSERTATION
University of Tampere, Medical School
Tampere University Hospital,
Department of Internal Medicine
Finland

Supervised by
Docent Pekka Collin
University of Tampere

Reviewed by
Professor Juhani Lehtola
University of Oulu
Professor Erkki Savilahti
University of Helsinki

Distribution

University of Tampere
Sales Office
P.O. Box 617
33101 Tampere
Finland

Tel. +358 3 215 6055
Fax +358 3 215 7150
taju@uta.fi
http://granum.uta.fi

Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 770
ISBN 951-44-4927-4
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 62
ISBN 951-44-4928-2
ISSN 1456-954X
http://acta.uta.fi

Tampereen yliopistopaino Oy Juvenes Print
Tampere 2000

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ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the main auditorium of Building K,
Medical School of the University of Tampere,
Teiskontie 35, Tampere, on October 27th, 2000, at 12 o'clock.

University of Tampere
Tampere 2000

LIST OF ORIGINAL PUBLICATIONS

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

I 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. Scand J Gastroenterol 34:163-169.

II Kaukinen K, Collin P, Holm K, Karvonen A-L, Pikkarainen P and Mäki M (1998): Small-bowel mucosal inflammation in reticulin or gliadin antibody positive patients without villous atrophy. Scand J Gastroenterol 33: 944-949.

III Kaukinen K, Turjanmaa K, Mäki M, Partanen J, Venäläinen R, Reunala T and Collin P: Intolerance to cereals is not specific for coeliac disease. Scand J Gastroenterol. In press.

IV Kaukinen K, Collin P, Mykkänen A-H, Partanen J, Mäki M and Salmi J (1999): Celiac disease and autoimmune endocrinologic disorders. Dig Dis Sci 44: 1428-1433.

ABBREVIATIONS

AIT	autoimmune thyroiditis
AGA	gliadin antibody
APECED	autoimmune polyendocrinopathy-candidosis-ectodermal dystrophy
ARA	reticulin antibody
ATD	autoimmune thyroid disorder
BMI	body mass index
CI	confidence interval
CD	cluster design
ECH	enterocyte height
ELISA	enzyme-linked immunosorbent assay
EmA	endomycial antibody
ESPGAN	European Society of Paediatric Gastroenterology and Nutrition
EU	ELISA unit
HLA	human leukocyte antigen
IDDM	insulin-dependent diabetes mellitus
IEL	intraepithelial lymphocyte
IF	immunofluorescence
Ig	immunoglobulin
NT	not taken
PVA	partial villous atrophy
RAST	radioallergosorbent test
SD	standard deviation
SVA	subtotal villous atrophy
tTG	tissue transglutaminase antibody
VH/CrD	villous height and crypt depth ratio

INTRODUCTION

In coeliac disease ingestion of gluten results in T-cell-mediated small bowel mucosal damage, and a life-long gluten-free diet ensures clinical and histological recovery. The prevalence of coeliac disease is possibly as high as 1:100 in the general population (Johnston et al. 1997). The disease is strongly associated with HLA DQ2, less commonly with DQ8, haplotype; 96-100% of coeliac disease patients share either of these haplotypes, compared to 20-30% in the general population (Sollid et al. 1989, Polvi et al. 1996). Evidence suggests that the disease develops gradually from small bowel mucosal inflammation to crypt hyperplasia and subsequently to overt villous atrophy. Consequently, some subjects consuming gluten and evincing apparently normal small bowel mucosal villous architecture may in fact be gluten-sensitive; they have latent coeliac disease and develop villous atrophy later in their disease process (Ferguson et al. 1993). However, severe partial (PVA) or subtotal villous atrophy (SVA) with crypt hyperplasia is still required for diagnosis of the disease (Walker-Smith et al. 1990), and diagnostic difficulties frequently arise when biopsy specimens show borderline findings.

Apart from small bowel mucosal villous atrophy and crypt hyperplasia, a prominent feature in untreated coeliac disease is an increase in intraepithelial lymphocytes (IELs). Closer inspection of these inflammatory cells reveals that most IELs are $\alpha\beta$ T-cell receptor-bearing lymphocytes. In coeliac disease the density of $\gamma\delta^+$ IELs is also typically increased (Savilahti et al. 1990, Spencer et al. 1991), and according to case reports these $\gamma\delta^+$ cells have already been found in the latent stage of the disease (Mäki

et al. 1991a, Arranz and Ferguson 1993). This increase seems, however, not to be pathognomonic for coeliac disease.

Patients affected by coeliac disease may suffer from severe malabsorption syndrome with diarrhoea and weight loss, but the majority of subjects with untreated disease have nowadays only mild if any symptoms (Collin et al. 1990, Watson et al. 1992). In the diagnosis of atypical and asymptomatic cases determination of coeliac serology, i.e. antireticulin (ARA), antiendomysial (EmA), antigliadin (AGA) and tissue transglutaminase antibodies (tTG), is helpful (Mäki 1995a, Dieterich et al. 1998, Sulkanen et al. 1998a). It is known that patients with untreated coeliac disease are at risk of considerable complications such as small bowel lymphoma (Holmes et al. 1989) and osteoporosis (Molteni et al. 1990), and early detection and treatment of the condition is thus warranted.

The purpose of the present study was to investigate small bowel mucosal structural and inflammatory changes suggestive of coeliac disease in patients suspected of the condition. In addition, the frequency of such changes was investigated in a special group carrying an increased risk of coeliac disease. A particular objective was to detect minor small bowel mucosal changes indicative of coeliac disease in patients with apparently normal mucosal villous architecture, in other words excluded for coeliac disease on the basis of current diagnostic criteria. Such small bowel mucosal changes were compared to those observed in untreated and treated coeliac disease patients. In patients on gluten-free diet the focus was to investigate how small amounts of ingested gluten affect small bowel mucosal integrity.

REVIEW OF THE LITERATURE

1. Classical coeliac disease

1.1. Historical background

The typical features of coeliac disease were described by Samuel Gee in 1888. The signs of the condition were severe steatorrhoea and cachexia occurring mainly in young children, and without proper treatment the outcome was poor. Gee also suggested that if patients could be cured at all, it was by means of diet (Gee 1888). However, it was more than 60 years before the harmful effect of ingested wheat gluten was recognised by Dicke (1950). A few years later Paulley reported that small bowel mucosal villous atrophy with chronic inflammation was a uniform finding in coeliac disease (Paulley 1954), and soon the development of peroral intestinal biopsy equipment made specific diagnosis of coeliac disease possible (Shiner 1957).

1.2. Classical features and complications

In the 1970s the most prominent symptoms of coeliac disease included steatorrhoea, malabsorption syndrome, weight loss and diarrhoea in both children (Visakorpi et al. 1970, Young and Pringle 1971) and adults (Cooke and Holmes 1984). Related to malabsorption, almost half of the patients were found to have neurological complications (Morris et al. 1970, Banerji and Hurwitz 1971, Cooke and Holmes 1984). Bone disorders such as bone pain or osteomalacia were common (Cooke and Holmes 1984), and malignancies, especially small bowel lymphomas, were reported in 7-15% (Harris et al. 1967, Holmes et al. 1976). During the early 1980s it seemed that coeliac disease was disappearing (Challacombe and Bayliss 1980, Langman et al.

1985). However, this conception was probably due to referral bias based on declining hospital admissions. At the same time, it was shown that coeliac disease may also appear with milder symptoms and that due to better recognition of the disease even more cases were diagnosed (Table 1). Nowadays steatorrhoea and the malabsorption syndrome have become rare; isolated malabsorption of iron, calcium and folic acid are still common, but a deficiency of these nutrients does not invariably lead to clinical manifestations (Biemond et al. 1987, Ståhlberg et al. 1991, Bode and Gudmand-Hoyer 1996a, Hin et al. 1999). It is noteworthy that many patients feel well and can even be overweight (Corazza et al. 1994).

Table 1. Frequency (%) of clinical features in three series of adults with untreated coeliac disease in two different observation periods.

	Frequency (%) of clinical features					
	Logan et al. 1983		Pare et al. 1988		Corazza et al. 1993	
Symptoms	1960-64 (n=38)	1975-79 (n=102)	1966-75 (n=7)	1981-85 (n=29)	1972-77 (n=22)	1984-89 (n=141)
Malabsorption/ weight loss	63	22	100	63	91	56
Diarrhoea	18	27	71	55	91	55
Abdominal pain			100	52	68	45
Borborygmia			100	50	55	52
Anaemia	18	15	0	38	36	34
Bone pain			29	0	45	11
Dermatitis herpetiformis			0	3	0	16

Dermatitis herpetiformis, an itching and blistering skin disease, is one classical manifestation of coeliac disease. Granular immunoglobulin (Ig)A deposits in the papillary dermis of unaffected skin are pathognomonic for the disease (van der Meer

1969). Most patients with dermatitis herpetiformis have small bowel villous atrophy and crypt hyperplasia consistent with coeliac disease (Fry et al. 1972, Reunala et al. 1984). Both the skin and the small bowel mucosal lesions improve during a gluten-free diet, but also medication with dapsone is needed to control the skin symptoms especially at the beginning of dietary treatment (Reunala et al. 1977, Reunala et al. 1984).

1.3. Small bowel biopsy

In untreated coeliac disease the characteristic abnormalities in the small bowel mucosa are villous atrophy, crypt hyperplasia and an increased density of IELs (Roy-Choudhury et al. 1966, Ferguson and Murray 1971, Kuitunen et al. 1982). Increased numbers of chronic inflammatory cells are also found in the lamina propria (Lancaster-Smith et al. 1975), and enterocyte height (ECH) is reduced (Stewart et al. 1967, Chapman et al. 1974, Kuitunen et al. 1982). During a gluten-free diet these histological features improve concomitant with clinical recovery, but reappear if gluten is again introduced to the diet (McNicholl et al. 1976, McNicholl et al. 1979). For the diagnosis of coeliac disease it is crucial to show the gluten dependency of the small bowel mucosal lesion, since villous atrophy and intraepithelial lymphocytosis may be seen in other conditions such as cow's milk allergy, giardiasis and postenteritis syndrome (Kuitunen et al. 1975, Katz and Grand 1979, Kuitunen et al. 1982); on the other hand, especially in developed countries these disorders are rare in adults. In coeliac disease small bowel mucosal villous atrophy is more prominent in the proximal part of the intestine and the mucosa in the ileum may even be undamaged (MacDonald et al. 1964, Stewart et al. 1967). Based on data on only 11 adults with untreated coeliac disease, it has been suggested that the severity of

symptoms correlates with the length of abnormal small bowel mucosa and not with the degree of villous atrophy (MacDonald et al. 1964).

1.4. Diagnostic criteria

Until the late 1950s the diagnosis of coeliac disease was based solely on the presence of gastrointestinal symptoms and impaired intestinal absorption function (Cooke and Holmes 1984). The first criteria proper for coeliac disease were defined in 1969 by an expert board of the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) (Meeuwisse 1970). They were originally formulated for children and based on three small bowel biopsies: typical small bowel mucosal villous atrophy with crypt hyperplasia while taking a gluten-containing diet, a recovery of villous architecture on gluten-free diet, and a further deterioration of the mucosa upon gluten challenge. The permanence of gluten intolerance was introduced for the first time. These ESPGAN criteria were modified in 1990 (Walker-Smith et al. 1990), and are still employed in both children and adults (Walker-Smith 1997). The finding of characteristic small bowel mucosal atrophy remains the basis for the diagnosis, and the effect of a gluten-free diet must be shown by clinical and, especially in asymptomatic coeliac disease patients, also by histological recovery. Gluten challenge is no longer routinely required but is recommended if there are any doubts about the initial diagnosis. The presence of circulating antibodies (AGA, ARA and EmA) while on gluten-containing diet and their disappearance on a gluten-free diet support the diagnosis of coeliac disease (Walker-Smith et al. 1990).

2. New clinical aspects of coeliac disease

2.1. Screening tests for coeliac disease

Over the past years, reliable non-invasive screening tests have been developed to select patients for small bowel biopsy when coeliac disease is suspected. Nowadays intestinal absorption and permeability tests such as faecal fat excretion, d-xylose and ^{51}chromium-labelled EDTA absorption tests are no longer widely used due to their low specificity and low positive predictive value (Sanderson et al. 1975, Bode and Gudmand-Hoyer 1987). More recently the focus has been on serological screening tests. IgA-class AGA, ARA and EmA tests have been found to have a high degree of sensitivity and specificity for untreated coeliac disease, as shown in Table 2. The

Table 2. Recent studies in adults with untreated coeliac disease comparing sensitivity (%) and specificity (%) of IgA-class EmA, ARA and IgA- and IgG-class AGA tests.

Author	IgA-EmA	IgA-ARA	IgA-AGA	IgG-AGA
Sensitivity (%)				
Mäki et al. 1991b	92	92	31	46
Volta et al. 1991	85	44	68	
McMillan et al. 1991	89		100	57
Ferreira et al. 1992	100	91	91	76
Vogelsang et al. 1995	100		82	73
Sulkanen et al. 1998b	85	78	80	35
Specificity (%)				
Mäki et al. 1991b	95	95	87	89
Volta et al. 1991	100	100	100	
McMillan et al. 1991	100		100	87
Ferreira et al. 1992	99	99	85	88
Vogelsang et al. 1995	100		83	74
Sulkanen et al. 1998b	100	100	86	97

variation in percentages obtained in each test probably results from differing patient series. Determination of IgA- and IgG-class AGA by enzyme-linked immunosorbent assay (ELISA) is reliable in young children (Savilahti et al. 1983, Ascher et al. 1990, Troncone and Ferguson 1991), but in adults the specificity of the test has varied from 30-100% (Table 2). False-positive AGA have been found in other gastrointestinal disorders (Burgin-Wolff et al. 1983, Unsworth et al. 1983) and even in healthy subjects (Grodzinsky et al. 1990, Uibo et al. 1993).

The serum antibodies ARA and EmA are detected by immunofluorescent (IF) methods; in the ARA test rat tissues (kidney, liver and stomach) (Seah et al. 1971) and in the EmA test monkey oesophagus (Chorzelski et al. 1984) or human umbilical cord (Ladinser et al. 1994) serve as antigens. Altogether the EmA test seems to be less markedly observer-dependent and more sensitive than the ARA test (Table 2), but in experienced hands the correlation between these two tests is good (Mäki 1995a).

Table 3. Recent studies about sensitivity (%) and specificity (%) of IgA-class tTG test in untreated coeliac disease.

Author	Sensitivity (%)	Specificity (%)
Dieterich et al. 1998	98	95
Sulkanen et al. 1998a	95	94
Troncone et al. 1999	92	98
Bazzigaluppi et al. 1999	97	96
Dieterich et al. 1999	89*	98
Biagi et al. 1999	95	90
Sblattero et al. 2000	92	99

*patients with dermatitis herpetiformis

The most recently developed tissue autoantibody test, tTG ELISA, has proved promising (Dieterich et al. 1998, Sulkanen et al. 1998a). Compared to the EmA test,

this ELISA is time-saving and easier to interpret; it shows sensitivity figures similar to the EmA, but its specificity has been somewhat lower (90-99%) (Table 3).

2.2. Atypical symptoms

Especially in the light of serological screening tests it became apparent that the clinical presentation in coeliac disease may be monosymptomatic or atypical; abdominal symptoms can be mild or absent, and only minor if any haematological or biochemical abnormalities may be evinced (Collin et al. 1990, Watson et al. 1992). Table 4 shows such atypical features; it is often impossible to distinguish whether some of these conditions are true gluten-dependent immunologically induced extra-intestinal manifestations or complications of malabsorption. Nevertheless, in many cases no clear evidence of such malabsorption is present.

2.2. Silent coeliac disease and associated disorders

A typical small bowel mucosal lesion can be found in apparently asymptomatic subjects (Ferguson et al. 1993). Large numbers of such silent cases have been detected by serological screening in at-risk groups such as first-degree relatives of coeliac patients (Auricchio et al. 1988, Mäki et al. 1991b), in patients having selective IgA deficiency (Meini et al. 1996) and in various autoimmune disorders shown in Table 5. Similarly, individuals with silent coeliac disease have been found in general populations enrolled in screening programmes (Table 6).

Earlier many disorders (including those listed in Table 5) have been taken to be connected with coeliac disease; for instance in 1984 Cooke and Holmes (1984) described in their thorough review almost 100 such disease associations. However, the prevalence of coeliac disease has increased with time due to better recognition

Table 4. Non-intestinal symptoms and complications in coeliac disease.

Symptoms or complications	References	Summary of results
Chronic oral mucosal aphthous ulceration	Ferguson et al. 1976, Ferguson et al. 1980, Jokinen et al. 1998	4-24% of patients with chronic oral mucosal disorders have coeliac disease
Dental enamel hypoplasia	Aine et al. 1990, Ballinger et al. 1994, Martelossi et al. 1996	10-69% of coeliac patients have dental enamel defects in permanent teeth
Infertility and unfavourable outcome of pregnancy	Ferguson et al. 1982, Sher and Mayberry 1994, Ciacci et al. 1996, Collin et al. 1996a, Kolho et al. 1999, Martinelli et al. 2000	Coeliac patients may have late menarche, early menopause, a higher frequency of infertility, recurrent abortions and stillbirths; offspring may have lower birthweights
Hypertransaminasaemia of unknown origin	Lindgren et al. 1994, Volta et al. 1998a, Bardella et al. 1999	1.5-9% of patients with chronic cryptogenic hypertransaminasaemia have coeliac disease
Neurological manifestations of unknown origin	Hadjivassiliou et al. 1996, Hadjivassiliou et al. 1998	At least 35% of patients with neurological dysfunction or ataxia of unknown origin have coeliac disease
Epilepsy and posterior cerebral calcifications	Gobbi et al. 1990	67% of patients with epilepsy and posterior cerebral calcifications have coeliac disease
Dementia and brain atrophy	Collin et al. 1991	5 cases with brain atrophy, dementia and coeliac disease at a relatively young age
Depression	Hallert and Derefeldt 1982, Ciacci et al. 1998	Depression occurs more often in coeliac disease than in controls
Non-specific mono- or polyarthritis	Bourne et al. 1985, Mäki et al. 1988, Collin et al. 1992	Non-specific arthritis may be the only feature of coeliac disease.
Osteoporosis	Molteni et al. 1990, Mazure et al. 1994, Valdimarsson et al. 1996a, Vazquez et al. 2000	Decreased bone mineral density and a high prevalence of bone fractures in untreated disease, bone mineral density improves after introduction of a gluten-free diet
Exocrine pancreatic insufficiency	Regan and DiMagno 1980, Carroccio et al. 1997	Coexistent exocrine pancreatic impairment may complicate recovery during gluten-free diet
Hyposplenism	O'Grady et al. 1984, Corazza et al. 1999	Signs of hyposplenism in 76-33% of patients with coeliac disease

of the disease, and the majority of these associations are therefore likely to be coincidental (Collin et al. 1994a). In diseases having similar genetic background and immunological alterations, on the other hand, the associations are probably significant (Mäki and Collin 1997).

Table 5. Frequency (%) of coeliac disease in adults with autoimmune disorders

Autoimmune disorders Author	No of patients with autoimmune disease	Frequency of coeliac disease (%)	Screening method
Type 1 diabetes mellitus			
Collin et al. 1989	195	4.1	ARA
Page et al. 1994	767	2.0	AGA
Rensch et al. 1996	47	6.1	EmA
Cronin et al. 1997	101	5.0	EmA
Autoimmune thyroid disease			
Collin et al. 1994b	83	4.8	ARA
Sategna-Guidetti et al. 1998	152	3.3	EmA
Berti et al. 2000	172	2.9	EmA
Alopecia areata			
Corazza et al. 1995	256	1.2	AGA, EmA
Volta et al. 1997	232	1.0	AGA, EmA
Primary biliary cirrhosis			
Logan et al. 1978		Case reports (n=4)	
Dickey et al. 1997	57	7.0	EmA
Kingham and Parker 1998	67	6.0	Medical records
Autoimmune hepatitis			
Volta et al. 1998b	181	2.8	AGA, EmA
Primary Sjögren's syndrome			
Pittman and Holub 1965		Case report (n=1)	
Iltanen et al. 1999a	34	14.7	Biopsy to all
Addison's disease			
Reunala et al. 1987		Case reports (n=3)	
Collin et al. 1994a		Case reports (n=2)	
Inflammatory bowel disease			
Salem and Truelove 1965		Case reports (n=14)	
Kumar et al. 1979		Case reports (n=2)	
Kitis et al. 1980		Case reports (n=4)	

2.4. Epidemiology

Ten years ago, the prevalence of clinically manifest coeliac disease was estimated to be 1 in 1000 in Europe, with a range from 1 in 250 observed in Sweden to 1 in 4000 in Denmark (Greco et al. 1992). However, recent screening studies in general populations have yielded higher prevalence figures, 1 in 300 or even 1 in 100 (Table 6).

Table 6. Recent studies on the prevalence of coeliac disease

Author	Population		Prevalence of coeliac disease per 100,000	Screening method
	Area	n		
Bode and Gudmand-Hoyer 1996b	Adults, Denmark	500,000	46	Clinical data
Gandolfi et al. 2000	Blood donors, Brazil	2045	143	AGA, EmA
Corazza et al. 1997	Adults, Italy	2237	180	EmA
Not et al. 1998	Blood donors, USA	2000	250	EmA, no biopsy
Collin et al. 1997	Adults, Finland	147,000	270	Screening at-risk groups
Hovdenak et al. 1999	Blood donors, Norway	2096	294	AGA, EmA
Rostami et al. 1999	Blood donors, Netherlands	1000	303	EmA
Catassi et al. 1994	Students, Italy	3351	328	AGA, EmA
Grodzinsky et al. 1992	Blood donors, Sweden	1866	375	AGA
Ivarsson et al. 1999	Adults, Sweden	1894	530	AGA, EmA
Kolho et al. 1998	Adults, hospital staff, Finland	1070	769	EmA
Johnston et al. 1997	Adults, Ireland	1823	820	ARA, EmA
Meloni et al. 1999	School children, Sardinia	1607	1058	AGA, EmA
Korponay-Szabo et al. 1999	Preschool children, Hungary	427	1171	EmA
Catassi et al. 1999	Saharawi children, Western Sahara	989	5600	EmA, no biopsy

The frequency of coeliac disease may indeed differ between countries, but it has been suggested that more patients with coeliac disease remain undetected in areas with a low prevalence (Ferguson 1997). For instance in the USA, the first epidemiological study showed a prevalence of 1 in 5000 (Talley et al. 1994), but subsequently 1 in 400 of blood donors were found to have IgA-class EmA (Not et al. 1998). Knowing the high specificity of this test, the latter study probably represents a closer estimate of the true prevalence of coeliac disease in the USA, even though the presence of enteropathy was not confirmed by small bowel biopsy. Particularly high figures have recently been reported; in a study by Catassi et al. (1999) 5.6% of Saharwi children from Algeria were EmA-positive (Table 6). In a smaller series studied by Lionetti et al. (1999) as many as 41% of 54 children from the same area had positive EmA. Apart from differences between geographical areas, the prevalence figures for coeliac disease may also be affected by study settings; the age and gender of subjects affect the results, since coeliac disease is more common in females than in males, and the highest prevalence figures occur in the age group 40-50 years (Hallert et al. 1981, Bode and Gudmand-Hoyer 1996a).

2.5. Genetic factors

Susceptibility to coeliac disease is determined to a significant extent by genetic factors. The disease was first described as occurring in patients with human leukocyte antigen (HLA) B8 (Falchuk et al. 1972, Stokes et al. 1972), but this HLA-association appeared to be secondary and in linkage disequilibrium with alleles in the HLA class II region, that is HLA DR3 and DR5/DR7 (Keuning et al. 1976, Mearin et al. 1983, Verkasalo et al. 1983). Subsequently, an even stronger association was shown with HLA DQ2 encoded by alleles DQA1*0501 and DQB1*0201 located either in *cis* (in

DR3-DQ2-positive individuals) or in *trans* position (in DR5/7-DQ2 heterozygous subjects) (Tosi et al. 1983, Sollid et al. 1989). Approximately 90% of coeliac disease patients share this HLA DQ2 configuration compared to 20-30% found in the population in general (Tosi et al. 1983, Sollid et al. 1989, Polvi et al. 1996). Most DQ2-negative coeliac disease patients express the DR4-DQ8 (DQA1*0301, DQB1*0302) haplotype (Spurkland et al. 1992, Michalski et al. 1996, Polvi et al. 1998). HLA DR3-DQ2 and DR4-DQ8 are also typical of a number of autoimmune diseases such as insulin-dependent diabetes mellitus, autoimmune thyroiditis and Sjögren's syndrome (Dalton and Bennet 1992).

Differences in the concordance of coeliac disease between monozygotic twins (70%) and HLA-identical siblings (30%) suggest that non-HLA genes are also involved in the disease process (Polanco et al. 1981, Mearin et al. 1983). Genome-wide screening studies have led to some proposals for candidate non-HLA gene regions; however, for the present no uniform gene or gene region has been found outside HLA DQ (Zhong et al. 1996, Houlston et al. 1997, Greco et al. 1998).

2.6. Small bowel mucosal morphology and immunology

2.6.1. Morphology

In coeliac disease small bowel mucosal damage develops gradually, as depicted in Figure 1. First, an increased density of IELs is observed in an otherwise normal mucosal architecture (infiltrative type); thereafter the crypts become elongated together with minor villous shortening (infiltrative-hyperplastic type). The lesion further progresses to severe partial (PVA) or subtotal villous atrophy (SVA) with crypt hyperplasia (flat destructive type), which finding is today considered diagnostic for coeliac disease (Marsh 1992). In crypt epithelial cells the frequency of mitosis is

increased, and the rate of production of these cells is two times greater in untreated coeliac patients than in control subjects (Wright et al. 1973). Moss and associates (1996) showed that an increased enterocyte apoptosis correlated with proliferating crypt cells, which might explain the occurrence of flat mucosa and hyperproliferation of epithelial cells.

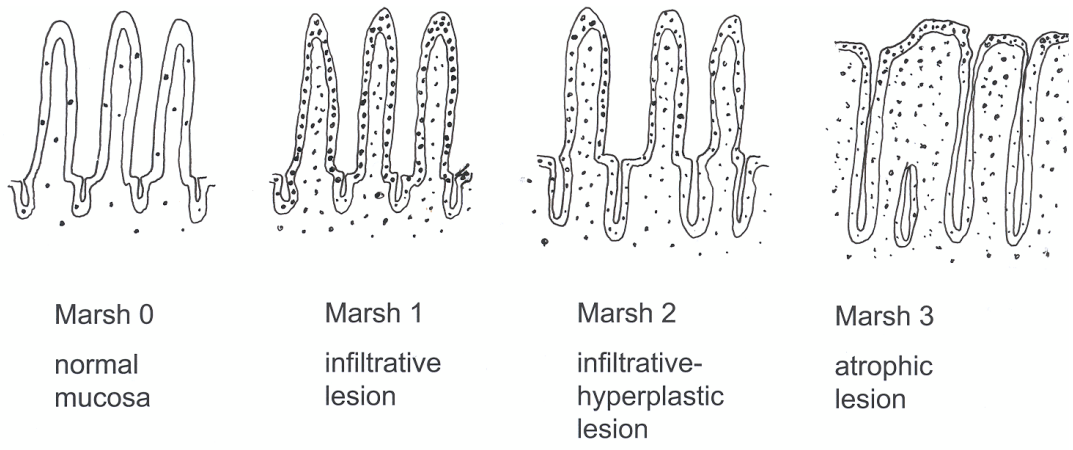

Figure 1. Classification of small bowel mucosal changes according to Marsh (Marsh 1992).

2.6.2. Intraepithelial lymphocytes

Both in coeliac and in healthy individuals almost all IELs are cluster-design (CD) 3^+ T-cells; B-cells are only very rarely found in the epithelium (Selby et al. 1983, Verkasalo et al. 1990). Distributions of different T-cell subsets in the epithelium and lamina propria of normal small bowel mucosa are shown in Table 7.

Table 7. Occurrence of T-cell subsets in epithelium and lamina propria in normal human small bowel mucosa.

Characteristics of $CD3^+$ T-cells	Epithelium	Lamina propria
$CD8^+$	80-90%	20%
$CD4^+$	6-12%	80%
$CD8^-$ $CD4^-$	6%	
$\alpha\beta^+$	90%	>99%
$\gamma\delta^+$	2-10%	<1%

Data from Brandtzaeg et al. 1989, Spencer et al. 1989a, MacDonald and Spencer 1991, Cerf-Bensussan et al. 1997

In untreated coeliac disease the density of $\alpha\beta^+$ IELs decreases to normal with a gluten-free diet and again increases during gluten challenge (Savilahti et al. 1990, Savilahti et al. 1992, Kutlu et al. 1993). It is thus suggested that $\alpha\beta^+$ IELs are activated by gluten; however, there is no evidence that these cells would recognise gluten peptides directly. The role of $\alpha\beta^+$ IELs in the pathogenesis of coeliac disease is unknown; 80% of $\alpha\beta^+$ IELs are $CD8^+$ and thus suggestive of a cytotoxic-suppressor cell function (Tredjosiewics and Howdle 1995). The observation that an increase in the density of IELs ($\alpha\beta^+$) parallels the increase in IELs with cytolytic granules containing granzyme B, a protein characteristic of activated lymphocytes, would further point to the cytotoxity of these $\alpha\beta^+$ IELs (Oberhuber et al. 1996).

An increased density of $\gamma\delta^+$ IELs is considered to be a typical and constant finding in coeliac disease; the number of these cells appears to be elevated in untreated disease, as well as during a gluten-free diet (Halstensen et al. 1989, Spencer et al. 1989b, Savilahti et al. 1990, Spencer et al. 1991, Savilahti et al. 1992). According to case reports, they can be detected even before the development of villous atrophy (Mäki et al. 1991a, Arranz and Ferguson 1993). In a family study by Holm and colleagues

(1992) a significant association between an increased density of $\gamma\delta^+$ IELs and coeliac-type HLA (DQ2) was found. However, an increased density of these cells in the small bowel mucosal epithelium is not specific for coeliac disease; they have also been found in children with conditions such as cow's milk allergy or postenteritis syndrome (Spencer et al. 1991, Chan et al. 1993, Pesce et al. 1996).

The role of $\gamma\delta^+$ IELs in the gut epithelium remains unknown. These cells have been held to be cytotoxic for epithelial cells and to produce proinflammatory mediators (Brenner et al. 1987, Viney et al. 1990, McMenamin et al. 1994). However, the assumed constant increase in these $\gamma\delta^+$ IELs in coeliac disease would not support this hypothesis. It has, moreover, also been suggested that their role is protective; $\gamma\delta^+$ T-cells are known to express keratocyte growth factor, which may be important in the surveillance and in repair of damaged epithelial cells (Boismenu and Havran 1994). They may also downregulate the inflammatory response of $\alpha\beta^+$ IELs (Mukasa et al. 1995). In $\gamma\delta^+$ deficient mice more severe epithelial damage has been observed in intestinal infection by Eimeria vermiformis than in controls (Roberts et al. 1996). Animal studies suggest that $\gamma\delta^+$ cells may play a role in regulating oral tolerance and in mediating autoimmune phenomena (Barrett et al. 1993, Ke et al. 1997).

2.6.3. Lamina propria cells

T-cells comprise about 25-40% of the total leukocytes in the lamina propria; in addition, large numbers of B-lymphocytes, plasma cells, granulocytes, macrophages and fibroblasts are present. In untreated coeliac disease the densities of all of these cell subsets are increased (Dhesi et al. 1984). Most of the lamina propria T-cells, unlike IELs, are $CD4^+$ phenotype, and virtually all T-cells express $\alpha\beta^+$ T-cell receptor

(MacDonald and Spencer 1991). There is evidence that in the coeliac mucosal lesion there are class II HLA-restricted gliadin-specific $CD4^+$ cells, which respond to antigen stimulation by secreting cytokines such as interleukin 2, interferon-γ and tumour necrosis factor-α; this enhanced cytokine production may be involved in the development of mucosal damage (Lundin et al. 1993, Tredjosiewics and Howdle 1995, Molberg et al. 1997). In untreated coeliac patients there is also an increased density of plasma cells producing IgA, IgG and IgM in the lamina propria (Savilahti 1972). It has been shown that AGA and EmA can be produced in *in vitro* culture of small bowel mucosal biopsy samples from coeliac patients (Picarelli et al. 1996a), and furthermore, IgA- and IgM-class antibodies against gliadin have been detected in the jejunal fluid of coeliac disease patients (O'Mahony et al. 1991, Arranz and Ferguson 1993). These findings suggest that coeliac antibodies are of intestinal origin and may be produced by lamina propria plasma cells.

2.6.4. Mucosal HLA DR expression

HLA DR antigens are present in activated T-lymphocytes, B-cells, and macrophages and also on the surface of epithelial cells in the human intestine. In the normal small bowel mucosa, HLA DR expression is seen in villous enterocytes, being most prominent on the top and decreasing towards the base of the villi; in the crypts the expression is usually absent (Arnaud-Battandier et al. 1986, Arato et al. 1987, Scott et al. 1987). In untreated coeliac disease, enterocytes on the surface epithelium and cells in the lamina propria of small bowel mucosa express HLA DR more heavily, and these antigens are also found along the whole length of the crypts; the presence of this enhanced mucosal HLA DR expression has been associated with local mucosal lymphocytic infiltration, and it has been suggested that enhanced DR expression is a

sign of cell-mediated immune response (Arnaud-Battandier et al. 1986, Arato et al. 1987). It has, moreover, been shown that this DR expression is gluten-dependent, and in treated coeliac patients the DR pattern reverts to normal (Ciclitira et al. 1986, Marley et al. 1987).

2.7. Latency and early changes in coeliac disease

Small bowel mucosal atrophy develops gradually from mucosal inflammation to mild and finally to severe villous atrophy with crypt hyperplasia (Marsh 1992). Thus, individuals who consume normal amounts of gluten and have normal small bowel villous architecture may still be gluten-sensitive; they may have latent coeliac disease and develop typical villous atrophy with crypt hyperplasia in the course of time (Ferguson et al. 1993). Dermatitis herpetiformis is a good model of coeliac disease latency; in most patients with dermatitis herpetiformis typical coeliac-like small bowel mucosal damage is present, but in about 10% of the patients the lesion is limited to minor villous atrophy or to mucosal inflammation only (Reunala et al. 1984). In patients with only minor mucosal abnormalities, typical coeliac-like mucosal damage can develop consumption of extra amounts of gluten (Weinstein 1974, Ferguson et al. 1987). Furthermore, virtually all dermatitis herpetiformis patients have increased densities of $\gamma\delta^+$ IELs (Savilahti et al. 1992, Vecchi et al. 1992, Sturgess et al. 1993). Although latent coeliac disease is a well-known entity, only a small number of proven cases have so far been documented (Table 7), and there are no data on the prevalence of this condition. As shown in Table 7, many patients already suffer from abdominal symptoms during the latent phase, even though they yield no evidence of small bowel mucosal damage.

Table 7. Clinical, serological and histological data on earlier cases with proven latent coeliac disease without additional gluten challenge.

Author	Patients with proven latent coeliac disease	At the time of the first small bowel biopsy			Follow-up time before villous atrophy
		Symptoms	Serology	Small bowel mucosal alterations	
Egan-Mitchell et al. 1981	1 child	Diarrhoea, iron deficiency, impaired growth	Not done	None	14 months
Marsh 1989	2 adults	2 malabsorption, anaemia	Not done	2 increased density of IELs, 1 enlarged crypts	3 years
Mäki et al. 1990	3 children, 1 adult	1 poor weight gain, 2 loose stools, 1 rash	1 IgG-AGA positive	None	2½-9 years
Mäki et al. 1991a	1 adult	No (family study)	IgA-ARA positive	$CD3^+$ and $\gamma\delta^+$ IELs increased	2 years
Mäki et al. 1991b	3 patients	No (family study)	3 IgA-ARA positive	None	3 years
Collin et al. 1993	7 adults	Abdominal symptoms	3 IgA-ARA, AGA positive 2 IgA-ARA positive 2 IgA-AGA positive	3 mild villous alterations or increased density of IELs	1-5 years
Mäki et al. 1995b	1 child	No (diabetes mellitus)	IgA-ARA positive	None	1 year
Troncone 1995a	14 children	4 impaired growth, 3 diarrhoea, 2 anaemia, 1 arthralgia, 1 rash, 3 no symptoms (diabetes mellitus)	4 IgA-AGA positive 2 IgA-EmA positive	Increased density of IELs in 1	8 months – 10 years
Corazza et al. 1996a	3 adults	1 epigastric pain, 1 iron deficiency anaemia, 1 loose stools	2 IgA-EmA positive	None	2 months – 13 years

By definition, the diagnosis of latent coeliac disease is retrospective. The concept of potential coeliac disease has been applied to patients who are with great likelihood at risk of developing coeliac enteropathy later in life (Ferguson et al. 1993). For instance, in patients having normal villous architecture, an increased density of IELs, and especially $\gamma\delta^+$ cells, positive serum ARA or EmA or a coeliac-like antibody pattern in the jejunal fluid, are taken to be markers of forthcoming coeliac disease (Arranz and Ferguson 1993, Arranz et al. 1994, Troncone et al. 1996). The natural history of patients with potential coeliac disease is unclear. It is not known when, if ever, individual patients develop enteropathy, since only a small number have so far been followed up (Mäki et al. 1991a,b, Collin et al. 1993, Troncone 1995). Apart from increased gluten intake (Doherty and Barry 1981, Ferguson et al. 1987), the factors responsible for deterioration of the small bowel mucosa are still poorly understood. In any case, coeliac-type genetics, that is HLA DR3-DQ2 or DR4-DQ8, should be found in patients with latent coeliac disease (Mäki et al. 1990, Corazza et al. 1996a, Mäki and Collin 1997).

2. 8. Pathogenetic aspects

It is nowadays widely accepted that immunological mechanisms are implicated in the development of the mucosal damage in coeliac disease. In untreated coeliac patients there are signs of activation of both mucosal cellular and humoral immune systems (Tredjosiewics and Howdle 1995, Sollid et al. 1997). The major environmental trigger is ingested gluten (gliadin), but it has been suggested that enteric adenovirus infection may also play a part in the causation of the disease (Kagnoff et al. 1987).

Gluten-specific HLA DQ2 and DQ8 restricted T-cells are present in the coeliac disease lesion in the small bowel mucosa (Lundin et al. 1993). Recently Dietrich and

associates (1997) established that serum EmA, a specific indicator of active coeliac disease, recognises tissue transglutaminase. This enzyme is now considered to be the predominant, if not the sole, autoantigen for coeliac disease. In active coeliac disease the expression of tissue transglutaminase is increased (Bruce et al. 1985), and the enzyme enhances the binding of gliadin peptides to HLA DQ2 and DQ8 molecules through deamination of glutamine residues. This peptic groove further results in better binding affinity and increased T-cell reactivity (Molberg et al. 1998). The secretion of interferon-γ and other inflammatory cytokines by activated lamina propria T-cells can damage the small bowel mucosa (Sollid et al. 1997). Moreover, it has been suggested that antibodies against tissue transglutaminase can play a direct role in the pathogenesis of coeliac disease. In an *in vitro* model tTG has been seen to inhibit epithelial differentiation on the crypt-villous axis (Halttunen and Mäki 1999); whether tTG contributes to coeliac-type small bowel mucosal damage *in vivo* is not known.

2.9. Treatment of coeliac disease

Patients with coeliac disease and dermatitis herpetiformis have to withdraw gluten permanently from the diet. Rye (secalins) and barley (hordeins) prolamins bear a close taxonomic relationship to wheat prolamin (gliadin), and are also toxic in coeliac disease (Anand et al. 1978). Controversy has prevailed as to the toxicity of oat prolamin (avenin) (van de Kamer et al. 1953, Dissanayake et al. 1974a, Baker and Read 1976); however, recent studies suggest that adults with coeliac disease or dermatitis herpetiformis tolerate oats (Janatuinen et al. 1995, Srinivasan et al. 1996, Hardman et al. 1997, Reunala et al. 1998). With a strict gluten-free diet most coeliac disease patients show a rapid clinical recovery; in some, however, chronic diarrhoea has continued, even if the diet has been adequate. According to one series, other

conditions, for exemple collagenous or microscopic colitis or exocrine pancreatic insufficiency, can give rise to these continuing symptoms (Fine et al. 1997).

Small bowel mucosal histological improvement is often slower than clinical response, and complete recovery of the mucosa can take more than a year (Grefte et al. 1988). Interestingly, many patients observing a long-term strict gluten-free diet are still reported to evince marked small bowel mucosal abnormalities (Table 8). The most common explanation here is continued, intentional or inadvertent, ingestion of gluten (Baker et al. 1975). In clinical practice immunosuppressive medication is needed only rarely. In unresponsive cases the presence of refractory coeliac disease, ulcerative jejunoileitis and even small bowel lymphoma must be considered (O'Mahony et al. 1996).

A strict gluten-free diet is recommended for the prevention of complications of coeliac disease such as lymphoma and osteoporosis (Holmes et al. 1989, Molteni et al. 1990, Mazure et al. 1994). Nonetheless, in clinical practice compliance is poor, only 17-65% of patients adhering strictly to the diet (Kluge et al. 1982, Kumar et al. 1988, Mayer et al. 1991, Bardella et al. 1994). There are few and contradictory data regarding lax dieting in the treatment of coeliac disease (Table 8). According to the authors of some studies, regular consumption of 5g of gluten daily was well-tolerated and did not cause significant small bowel mucosal damage (Kumar et al. 1988, Montgomery et al. 1988), whereas in others a daily intake of 1g gluten has resulted in mucosal inflammation (Mayer et al. 1991, Catassi et al. 1993, Troncone et al. 1995b). Wheat-starch based gluten-free flour products, meeting today’s Codex Alimentarius Standards (1981), may contain residual gluten (40-60 mg gluten or 20-30 mg gliadin/ 100g flours), and theoretically these trace amounts of gluten can be harmful. On the other hand, such products have been widely used in the UK and Northern Europe for

over 30 years and if the diet has been otherwise strict, no excess mortality or morbidity has been noted (Holmes et al. 1989, Collin et al. 1994a, Collin et al. 1996b). Similarly, according to three small series these products have not been found to have harmful effects on the small bowel mucosa (Table 8). In contrast, in one open challenge study without small bowel mucosal biopsies, wheat starch-based gluten-free products evoked more abdominal symptoms than a naturally gluten-free diet, i.e. a strict diet based on rice, maize and buckwheat (Chartrand et al. 1997).

Whether patients with clinically silent coeliac disease should be treated with a gluten-free diet is a matter of debate. These patients may simply be accustomed to their gastrointestinal symptoms and may eventually benefit from treatment. In symptomatic coeliac patients a gluten-free diet has reduced the long-term risk of lymphoma (Holmes et al. 1989). However, it is not known whether clinically silent subjects run a similar risk of developing malignancies without dietary treatment (Catassi 1997). On the other hand, the decreased bone mineral density observed in some patients with silent coeliac disease favours dietary treatment (Corazza et al. 1996b, Mustalahti et al. 1999, Cellier et al. 2000).

Table 8. Small bowel biopsy findings in coeliac patients on a strict gluten-free diet or taking small amounts of gluten.

Author	n	Age group	Consumption of gluten	Duration of gluten-free diet	Symptoms	Abnormal findings in small bowell biopsy
A *Strict diet*						
Dissanayake et al. 1974b	18	Adults	No	6-72 months	Asymptomatic	SVA in 1, PVA in 1
Baker et al. 1975	18	Adults	No	4-132 months	-	SVA in 4, PVA in 9
Ciclitira et al. 1985	10	Adults	No	> 1 year	Asymptomatic	No
Colaco et al. 1987	16	Adolescents	No	8-28 years	-	Slightly abnormal mucosa in 6
Kumar et al. 1988	24	Adolescents	No	Several years	Asymptomatic	PVA or SVA in 5
Montgomery et al. 1988	10	Adults	No	6-27 months	-	Median villous height lower than in controls
Mayer et al. 1991	6	Adolescents	No	9-16 years	Different symptoms in 34%	Considerable villous shortening in 3
Gawkrodger et al. 1991	31	Adults*	No	14 months-11 years	Need for dapsone/ sulfapyridine in 10	SVA or PVA in 7; increase of IELs in 8
Catassi et al. 1993	20	Children	No	14 ± 3 months	Asymptomatic	Mean VH/CrD 1.6, mean of IELs normal
Troncone et al. 1995b	4	Adolescents	No	≥10 years	Asymptomatic	No
B *Small amounts of gluten*						
Dissanayake et al. 1974b	13	Adults	< 0.5 g gluten daily	6-72 months	Abdominal symptoms in 1	PVA in 10
Baker et al. 1975	24	Adults	< 2 g gluten daily	4-132 months	-	PVA in 16
Ejderhamn et al. 1988	11	Adolescents	4-14 mg gliadin daily†	8-14 years	Asymptomatic	No
Kumar et al. 1988	15	Adolescents	Occasional gluten (2.5-10 g)	Several years	Asymptomatic	PVA or SVA in 7

Author	n	Age group	Consumption of gluten	Duration of gluten-free diet	Symptoms	Abnormal findings in small bowell biopsy
B *Small amounts of gluten*						
Mayer et al. 1991	14	Adolescents	0.06-2 g gluten daily	9-16 years	Symptoms in 29%	Flat mucosa in 4, villous shortening in 7, increase in mean crypt epithelial volume and IELs in crypts
Troncone et al. 1995b	6	Adolescents	< 0.5 g gluten daily	≥10 years	Asymptomatic	PVA in 1; increased epithelial volume and IELs in crypts in 2
C *Challenge studies*						
Ciclitira et al. 1984a	7	Adults	1.2-2.4 mg gliadin daily, 1 week†	> 1 year	-	No significant changes in VH/CrD, in IELs and in enterocyte height
Ciclitira et al. 1985	10	Adults	1.2-2.4 mg gliadin daily, 6 weeks†	> 1 year	Diarrhoea in 4	No significant changes in VH/CrD, in IELs and in enterocyte height
Montgomery et al. 1988	8	Adults	2.5-5 g gluten daily, median 6 months	6-27 months	-	Increase in mean density of IELs
Catassi et al. 1993	1)10 2)10	Children Children	1) 100 mg 2) 500 mg gliadin daily, 4 weeks	14 ± 3 months	1) Asymptomatic 2) Anorexia and pale stools in 3	1) Mean VH/CrD 1.5‡ and 1.3§, mean IELs 11/100‡ and 19/100§ enterocytes 2) Mean VH/CrD 1.6‡ and 1.1§, mean IELs 10/100‡ and 25/100§ enterocytes

VH/CrD = villous height and crypt depth ratio

* patients with dermatitis herpetiformis; † gliadin from the wheat starch-based gluten-free products; ‡ values before challenge study; § values after challenge study

PURPOSE OF THE PRESENT STUDY

The aims of the present study were:

1. to study small bowel mucosal structure and inflammation in untreated (**I-IV**) and treated coeliac disease (**I**);

2. to study small bowel mucosal structure and inflammation indicating coeliac disease in patients positive for IgA-class ARA and AGA (**II**), and in subjects with a history of intolerance to cereals (**III**);

3. to assess the frequency of coeliac disease, and the occurrence of minor small bowel mucosal changes indicating coeliac disease in patients with multiple endocrine disorders (**IV**).

4. to assess the value of small bowel mucosal morphometrical and immunohistochemical methods in the diagnosis and treatment of coeliac disease (**I-IV**).

SUBJECTS AND METHODS

1. Patients and controls

1.1. Newly-diagnosed and treated coeliac disease patients (**I**)

Small bowel biopsy specimens from 21 newly diagnosed coeliac disease and 14 dermatitis herpetiformis patients were analysed at the Department of Medicine, Tampere University Hospital, during the period 1997-98 (Table 9). A follow-up biopsy was taken from all 21 coeliac disease patients according to current clinical practice after adherence to our standard gluten-free diet for a median of 10 months (range 6-24 months). In addition, a control biopsy was taken from five out of 14 patients with dermatitis herpetiformis.

The findings in the newly detected patients were compared with long-term treated coeliac patients; this group comprised 41 patients with coeliac disease and 11 with dermatitis herpetiformis, who had been on a gluten-free diet for a median of 10 years (range 2-16 years) and were willing to undergo a follow-up small bowel biopsy (Table 8). Current gastrointestinal and cutaneous symptoms were recorded, and body mass index (BMI) was calculated by the formula weight/height^2 (kg/m^2).

All patients with coeliac disease fulfilled the ESPGAN diagnostic criteria (Walker-Smith et al. 1990). The diagnosis of dermatitis herpetiformis was based on the typical rash, and on the finding of granular IgA deposits in uninvolved skin (van der Meer 1969).

Table 9. Patients and control subjects in studies **I-IV**

	Study group			Control group		
Study number	Subjects	n (female)	Median age (range), years	Subjects	n (female)	Median age (range), years
I	Newly diagnosed coeliac disease and dermatitis herpetiformis patients before and 6-24 months after introduction of gluten-free diet	35 (25)	39 (22-84)	Non-coeliac subjects with dyspepsia	27 (11)	45 (22-81)
	Long-term treated patients with coeliac disease and dermatitis herpetiformis	52 (36)	44 (7-67)			
II	IgA-class ARA and AGA positive patients suspected of coeliac disease	96 (72)	42 (15-75)	Non-coeliac subjects with dyspepsia	27 (11)	45 (22-81)
III	Patients having abdominal complaints after ingestion of cereals	93 (70)	39 (17-73)	Non-coeliac subjects with dyspepsia	30 (13)	45 (22-81)
IV	Patients having multiple autoimmune endocrinological disorders	62 (41)	33 (16-72)	Non-coeliac subjects with dyspepsia	27 (11)	45 (22-81)

1.2. Coeliac disease antibody-positive patients (**II**)

The study involved 96 consecutive adult patients (Table 9) found upon screening for coeliac disease positive for IgA-class ARA or AGA at the Department of Medicine, Tampere University Hospital, during the period 1995-96. The screening had been carried out because these patients had relatively mild symptoms, which did not warrant direct small bowel biopsy. The main symptoms or signs leading to this screening and the duration of symptoms were recorded. All patients were on a normal diet containing at least 10 g gluten per day. Patients with dermatitis herpetiformis and previously diagnosed coeliac disease were excluded from this study.

Twelve patients whose initial small bowel biopsy showed normal VH/CrD (≥2) volunteered for a second biopsy after 4-18 months, mainly by reason of the symptoms. They continued to take a gluten-containing diet all the time.

1.3. Patients reporting abdominal symptoms after ingestion of cereals (**III**)

The study group comprised 93 consecutive adults (Table 9) from health centres spontaneously reporting abdominal symptoms after consumption of wheat, rye or barley. The examinations were carried out at the Department of Medicine, Tampere University Hospital during the years 1995-97. Local general practitioners were responsible for the enrolment. Any prior serological, endoscopical or histological investigations were discouraged, and patients were told to continue with normal gluten-containing diet. Suspicion or presence of malabsorption was not an inclusion criterion. Family history of coeliac disease, gastrointestinal symptoms after ingestion of cereals and other symptoms were evaluated. Patients were further asked whether they had sometimes tried to adopt a gluten-free diet on their own account.

1.4. Patients having multiple autoimmune endocrinological disorders (**IV**)

This study included all 62 adult patients with more than one autoimmune endocrinological disorder treated at the endocrinological outpatient clinic of Tampere University Hospital during the years 1994-96 (Table 9). Insulin-dependent diabetes mellitus (IDDM) was diagnosed in patients in need of exogenous insulin to prevent ketoacidosis and to maintain adequate metabolic stability. Graves´ disease and autoimmune thyroiditis (AIT) were classified as autoimmune thyroid disorders (ATD). The diagnosis of Graves´ disease was based on clinical evidence of hyperthyroidism and typical eye symptoms. AIT was diagnosed in patients having primary hypothyroidism with an atrophic thyroid gland, or typical goiter and lymphocytic infiltration established by needle aspiration, and positive microsomal antithyroid antibodies. The diagnosis of Addison´s disease was based on the detection of hypocortisolism and the presence of antibodies to adrenal cortical cells. Autoimmune polyendocrinopathy-candidosis-ectodermal dystrophy (APECED), one form of the autoimmune polyglandular syndrome, was defined on the basis of at least two out of three component disorders, namely chronic mucocutaneus moniliasis, hypoparathyroidism and Addison´s disease. Alopecia areata was also considered eligible in the present study, since it often appears concomitantly with autoimmune endocrinological disorders and has a distinct autoimmune background (Hoffmann 1999).

Data on previously detected coeliac disease patients were scrutinised; in these cases the diagnosis of coeliac disease had been based on ESPGAN criteria (Walker-Smith et al. 1990). All non-coeliac subjects with multiple autoimmune endocronological disorders were requested to undergo upper gastrointestinal endoscopy and small bowel biopsy; 28 consented to endoscopy, 23 refused, three could not be traced and two had

died. Patients who underwent endoscopy were asked about possible gastrointestinal symptoms.

1.4. Non-coeliac control subjects (**I-IV**)

Consecutive adult patients undergoing upper gastrointestinal endoscopy due to dyspepsia, upper abdominal pain or heartburn at the Department of Medicine, Tampere University Hospital, served as controls for small bowel biopsy findings (Table 9). All control subjects were consuming gluten, were negative for ARA and AGA and had no autoimmune diseases or relatives with coeliac disease.

2. Dietary assessment

In study **I**, a detailed history of occasional or regular consumption of gluten-containing products was assessed by a dietitian, and by a four-day record of food intake. A wheat starch-based gluten-free diet was considered strict when no additional gluten intake was shown. The diet was considered naturally gluten-free when a strict diet did not contain even wheat starch-based gluten-free products. The daily consumption of wheat starch-containing gluten-free flour was evaluated in grams. This amount was converted to milligrams of gluten per day as follows: a nitrogen content of 0.05g/100g flour equals 0.3% protein. Measurements made by Skerritt and Hill (1992) and Hekkens (1991) showed that 0.3% protein corresponds to 40-60 mg gluten. It was assumed that 100 g of gluten-free wheat starch-based flours contains the maximum amount of gluten allowed by the Codex Alimentarius standard (1981), that is 50 mg gluten/100 g flour.

3. Small bowel biopsy

3.1. Morphometrical studies

In adults seven forceps biopsy specimens were taken from the distal part of the duodenum upon upper gastrointestinal endoscopy; five for morphological examination and two for immunohistochemical staining. In children specimens were obtained with the Watson capsule from the proximal jejunum at the ligamentum of Treitz. The specimens for morphologic examination were stained by hematoxylin-eosin and studied under light microscopy. Morphometrical studies including VH/CrD (**I-III**) and the ECH (μm) (**I**) were made from well-oriented specimens as described elsewhere (Kuitunen et al. 1982, Ciclitira et al. 1985). In study **IV** small bowel biopsy findings were histologically classified into normal, mild PVA, severe PVA and SVA. It was considered that in morphometrical analysis VH/CrD < 2.0 (**I-III**) and detection of SVA or severe PVA with crypt hyperplasia (**I-IV**) in adequately oriented specimens indicated coeliac disease (Walker-Smith et al. 1990, Holm 1993). ECH < 34 μm, indicating mean – 2 SD for controls, was considered abnormal.

3.2. Immunohistochemical studies

Two small bowel biopsy specimens from adults (**I-IV**) and one piece of the jejunal biopsy specimen from children (**I**) were freshly embedded in optimal cutting-temperature compound (OTC, Tissue-Tec, Miles Inc, Elkhart, IN, USA) and stored at -70^{o}C. Immunohistochemical studies were carried out on 5-μm-thick frozen sections. $CD3^{+}$ IELs were stained with Leu-4 monoclonal antibody (Becton Dickinson, San Jose, CA, USA), and $\alpha\beta^{+}$ and $\gamma\delta^{+}$ IELs were detected by monoclonal βF1 antibody (T

Cell Diagnostics, Woburn, MA, USA) and TCR γ (T Cell Diagnostics), respectively. Monoclonal antibody Leu-4 was diluted 1:15, antibody βF1 in a dilution of 1:80, and TCR γ in a dilution of 1:140. Positive IELs were counted with a x100 flat field light microscope objective. Surface epithelium comprising at least 30 fields, 1.6 mm, was counted and the density of IELs expressed as cells per millimetre of epithelium, as reported elsewhere (Savilahti et al. 1997). Reference values for $CD3^+$ IELs were ≤ 54 cells/mm (cut-off values indicating mean + 2 SD for control subjects), for $\alpha\beta^+$ IELs 39 cells/mm and for $\gamma\delta^+$ IELs 4.3 cells/mm.

HLA DR expression (**I-IV**) was examined by staining biopsy specimens with monoclonal antibody HLA DR (Becton Dickinson) in a dilution of 1:1500 (Holm et al. 1994). DR expression was considered enhanced when strong in villous epithelium or present in crypts. Negative crypt together with only slight to moderate villous epithelium expression was considered normal.

All specimens were evaluated by the same investigator and without prior knowledge of disease history, laboratory findings or diet. The correlation coefficients for intraobserver variation for $CD3^+$, for $\alpha\beta^+$ and for $\gamma\delta^+$ IELs were 0.95, 0.85 and 0.98, and the coefficients for interobserver variations were 0.92, 0.82 and 0.98, respectively. The intraobserver estimations of enhanced upregulation of small-bowel HLA DR expression were similar in 86%, interobserver in 91%.

4. Serological tests

Serum IgA-class EmA (**I, III, IV**) (Ladinser et al. 1994) and ARA (**I-II**) (Hällström 1989) were determined by IF, using human umbilical cord and a composite block of

rat tissues (rat kidney, liver, stomach and heart) as antigens, respectively. A screening dilution of 1:≥5 was considered positive. Serum AGA were investigated by ELISA; the lower limit of positivity for IgA-class AGA (**I-IV**) was 0.2 ELISA units per millilitre (EU/ml) and for IgG-class ≥10.0 EU/ml (**I, III, IV**) (Vainio et al. 1983). In study **I** two coeliac patients had selective IgA deficiency, and in these cases serum IgG-class EmA and ARA were also determined (reference values 1:≥10 for IgG-class EmA and ARA) (Sulkanen et al. 1998b). Serum IgA-class tTG analysis was performed by ELISA (Inova Diagnostics, San Diego, CA, USA) a unit value (U) ≥ 20 was considered positive (Sulkanen et al. 1998a) (**III**).

5. Genetic markers

HLA DQ alleles encoding HLA DQ2 and DQ8 were determined at the Tissue Typing Laboratory of the Finnish Red Cross Blood Transfusion Service in Helsinki. In study **IV** the HLA A1 alleles were analysed using the polymerase chain reaction/restriction fragment length polymorphism method of Ota and associates (1991) and the DQ B1 alleles typed using the Inno-Lipa DQ B reverse dot blot kit (Immunogenetics, NV, Zwijndrecht, Belgium). In study **III**, HLA-typing was performed using the Dynal SSP low resolution DQ typing kit (Dynal AS, Oslo, Norway).

6. Differential diagnosis: studies on cereal allergy

For differential diagnosis, investigations were made of cereal allergy in study **III**. Serum total IgE and specific IgE antibodies against wheat with commercial

radioallergosorbent test (RAST) (CapRAST, Pharmacia, Uppsala, Sweden; positive value > 0.35 kU/l) were measured. Skin prick and patch tests for wheat, rye and barley were applied with self-made allergen preparations from the respective flours and the results determined as previously described (Majamaa et al. 1999). Three patients were subjected to an open wheat challenge, prior to which they had observed a wheat elimination diet and recorded any skin and gastrointestinal symptoms. The first challenge took place in hospital. Thereafter they consumed 4-5 slices of bread daily; the challenge was discontinued if clear adverse effects occurred.

7. Statistical analysis

The data on coeliac disease patients, on patients excluded for coeliac disease and on controls were compared with each other by Student's *t* test (**I-III**), Mann-Whitney U test (**IV**), chi-square test and Fisher's exact test (**I-IV**) when appropriate. In addition, the small bowel biopsy results were given as mean with 95% confidence intervals (CI) (**I-III**).

8. Ethics

The study protocols were approved by the ethical committee of Tampere University Hospital. All subjects gave informed consent.

RESULTS

1. Untreated coeliac disease

Villous atrophy and crypt hyperplasia compatible with coeliac disease (VH/CrD <2) was found in 29 out of 96 (30%) IgA-class ARA- or AGA-positive patients with a suspicion of coeliac disease (**II**), in 8 out of 93 (9%) patients suffering from abdominal symptoms after ingestion of cereals (**III**) and in one out of 28 (4%) newly biopsied patients with multiple autoimmune endocrinological disorders (**IV**). Of these 38 coeliac patients altogether 27 suffered from abdominal complaints such as loose stools, flatulence and abdominal discomfort; eight had oral mucosal or cutaneous symptoms, two had mild anaemia and iron deficiency, and one was asymptomatic. The median duration of symptoms was two years (range from 0 to 10 years).

The VH/CrD and ECH were significantly lower and the densities of IELs higher in untreated coeliac disease and dermatitis herpetiformis patients than in non-coeliac control subjects (Table 10, Figure 2) (**I-IV**). Enhanced small bowel mucosal HLA DR expression was seen in 81 % of coeliac patients and in 10% of controls; this difference was statistically significant ($p<0.0001$).

Altogether 92% (35/38) of untreated coeliac disease patients were ARA- or EmA-positive and 72% (26/36) IgA-AGA-positive (**II-IV**); IgG-AGA was found in only 14% of coeliac patients (**II-IV**). In study **III** all eight coeliac patients had positive tTG. The genetic susceptibility marker for coeliac disease, HLA DQ2, was detected in all seven patients tested (**III-IV**).

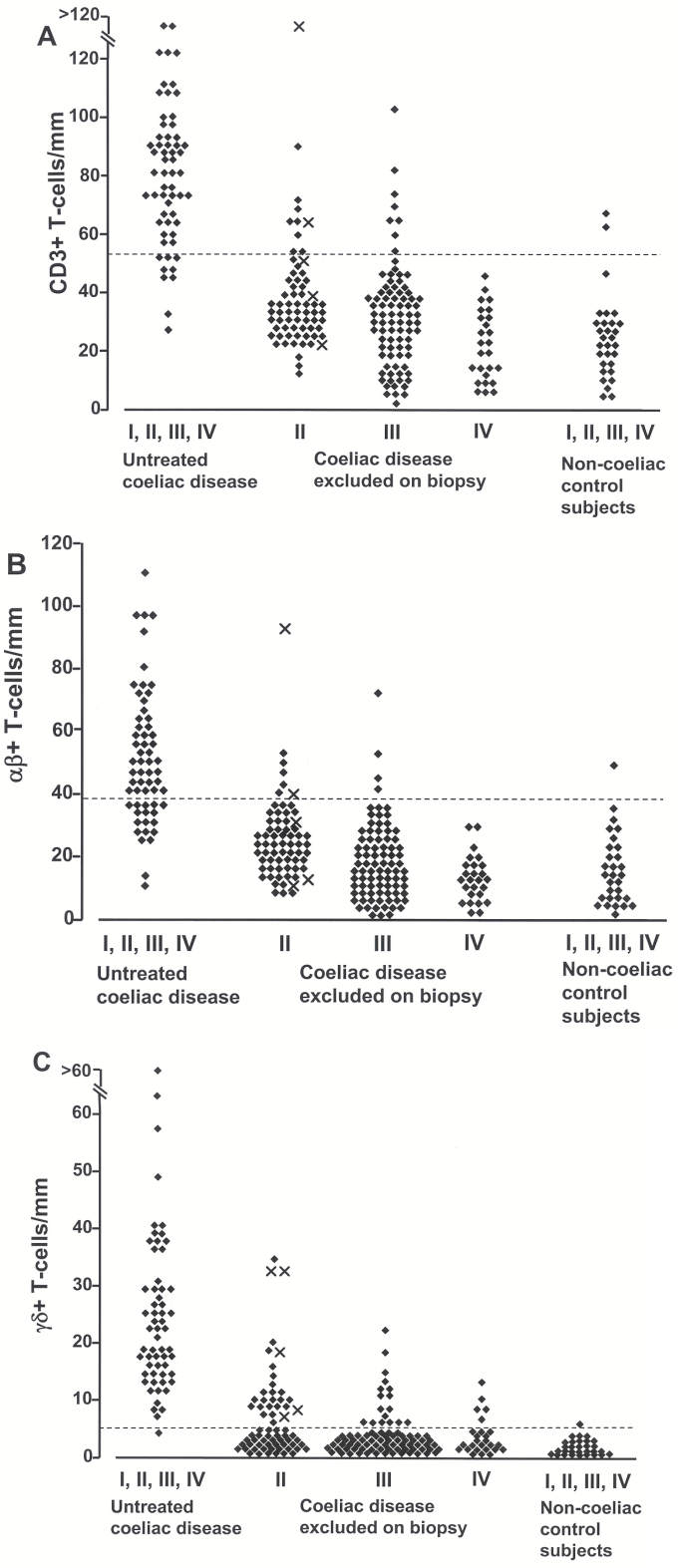

Figure 2. Densities of $CD3^+$ (A), $\alpha\beta^+$ (B) and $\gamma\delta^+$ IELs in patients having coeliac disease, patients excluded for coeliac disease on biopsy and in non-coeliac control subjects (**I-IV**). Mean + 2 SD of control subjects are shown by dotted horizontal lines. Patients with proven latent coeliac disease are marked separately (×)

Table 10. The mean, 95% CI and number of abnormal results (%) of small bowel mucosal morphological and immunohistochemical findings in untreated coeliac disease and dermatitis herpetiformis patients (**I-IV**) and in control subjects (**I-IV**).

		Untreated coeliac disease (n=45) and dermatitis herpetiformis patients (n=14)	Non-coeliac control subjects (n=30)
VH/CrD	mean	0.6*	2.9
	95% CI	0.4-0.8	2.7-3.1
	abnormal findings (%)	55/59 (93%)‡	0/30 (0%)
ECH; μm	mean	31.2†	38.2
	95% CI	30.0-32.4	37.5-38.9
	abnormal findings (%)	28/35 (80%)‡	0/30 (0%)
$CD3^+$ IELs; cells/mm	mean	82	23
	95% CI	74-90	17-29
	abnormal findings (%)	50/59 (85%)‡	2/30 (7%)
$\alpha\beta^+$ IELs; cell/mm	mean	50	15
	95% CI	45-55	11-19
	abnormal findings (%)	41/59 (69%)‡	1/30 (3%)
$\gamma\delta^+$ IELs; cell/mm	mean	25.0	1.3
	95% CI	20.3-29.7	0.8-1.8
	abnormal findings (%)	58/59 (98%)‡	1/30 (3%)
Enhanced HLA DR expression		48/59 (81%)‡	3/30 (10%)

* VH/CrD was ≥2 in four patients with dermatitis herpetiformis.

† includes 35 coeliac disease and dermatitis herpetiformis patients from study **I**.

‡ $p<0.0001$ compared with non-coeliac controls.

2. Treated coeliac disease (**I**)

In the long-term treated group in study **I**, 40 (76%) patients were found to be on a strict wheat starch-based gluten-free diet. Their mean daily intake of gluten from wheat starch-containing flour products was calculated to be 34 mg (range 5-150 mg). In addition, six (12%) on a wheat starch-based diet admitted occasional consumption

of gluten-containing food, e.g. normal bread or cake (containing about 1-2 g gluten) every week or at least once a month. Six (12%) patients were on a strict naturally gluten-free diet.

Four (8%) of the 52 patients with long-term treated coeliac disease admitted upon inquiry to mild gastrointestinal symptoms; none of them had dietary lapses. The 11 patients with dermatitis herpetiformis were asymptomatic, and did not take dapsone. None of the 16 children with coeliac disease had growth disturbances, and the mean BMI in adults was normal, 25 kg/m2 (SD 4.4, range 17-35).

In the 46 patients on a strict diet (either wheat starch-based or naturally gluten-free), the VH/CrD and ECH were similar to those in non-coeliac subjects. (Table 11). One of the six patients admitting occasional gluten intake had SVA and one severe PVA. In newly diagnosed patients, mucosal recovery was not complete after they had been on a gluten-free diet for 10 months on average (Table 11).

The mean density of $CD3^+$ IELs was higher in long-term treated patients than in non-coeliac control subjects. However, looking at $CD3^+$ subgroups, the densities of $\alpha\beta^+$ cells in the long-term treated were similar to, but those of $\gamma\delta^+$ cells higher than in controls. Even the densities of $\gamma\delta^+$ cells seemed to decrease with time during the gluten-free diet (Table 11). The densities of IELs were not dependent on the trace amounts of gluten coming from daily consumption of wheat starch (in study **I**: Figure 3).

Enhanced mucosal HLA DR expression was found more often in treated coeliac disease patients than in non-coeliac controls. However, the expression was not significantly more common in patients on a strict wheat starch-based diet than in those using a naturally gluten-free diet (p=0.24) (Table 11).

Table 11. The mean, 95% CI and number of abnormal results (%) of small bowel mucosal morphological and immunohistochemical findings in long-term treated coeliac disease and dermatitis herpetiformis patients compared to newly detected patients before and after short-term gluten-free diet and to non-coeliac control subjects (**I**).

		Newly diagnosed patients with coeliac disease and dermatitis herpetiformis		Long-term treated patients with coeliac disease and dermatitis herpetiformis			
		Untreated disease n=35	Short-term gluten-free diet n=26	Naturally gluten-free diet n=6	Wheat starch based gluten-free diet n=40	Occasional dietary lapses n=6	Non-coeliac controls n=27
VH/CrD	mean	0.7*	1.9	3.0	3.3	1.9	2.8
	95% CI	0.4-1.0	1.6-2.2	2.7-3.3	3.1-3.5	1.1-2.7	2.6-3.0
	abnormal findings (%)	31/35 (89%)†	14/26 (53%)†	0/6 (0%)	1/40 (3%)	2/6 (33%)	0/27 (0%)
ECH; µm	mean	31.2	37.3	38.5	38.0	35.8	38.2
	95% CI	30.1-32.3	36.5-38.1	37.4-39.5	37.5-38.5	32.7-38.1	37.5-38.9
	abnormal findings (%)	28/35 (80%)†	1/26 (4%)	0/6 (0%)	1/40 (3%)	2/6 (33%)‡	0/27 (0%)
$CD3^+$IELs; cells/mm	mean	82	52	43	39	38	25
	95% CI	70-94	42-62	28-58	35-44	30-46	19-31
	abnormal findings (%)	27/35 (77%)†	12/26 (46%)§	2/6 (33%)	9/40 (23%)	0/6 (0%)	2/27 (7%)
$\alpha\beta^+$ IELs; cell/mm	mean	51	25	15	19	23	16
	95% CI	43-59	19-31	10-20	17-21	14-32	12-20
	abnormal findings (%)	25/35 (71%)†	4/26 (15%)	0/6 (0%)	0/40 (0%)	0/6 (0%)	1/27 (4%)
$\gamma\delta^+$ IELs; cell/mm	mean	25.9	16.3	10.1	11.6	9.5	1.4
	95% CI	18.7-33.1	11.1-21.5	4.8-15.4	9.5-13.7	5.8-13.2	0.9-1.9
	abnormal findings (%)	35/35 (100%)†	23/26 (88%)†	5/6 (83%)†	36/40 (90%)†	5/6 (83%)†	1/27 (4%)
Enhanced HLA DR expression		27/35 (77%)†	14/26 (54%)‡	1/6 (17%)	15/40 (37%)†	5/6 (80%)†	3/27 (11%)

*VH/CrD was ≥2 in four patients with dermatitis herpetiformis; † $p<0.0001$ compared with non-coeliac controls;

‡$p<0.05$ compared with non-coeliac controls; §$p=0.001$ compared with non-coeliac controls.

Of the long-term treated patients ARA or EMA was positive in only two patients admitting to occasional gluten intake; villous atrophy was evident in both. In addition, despite normal small bowel mucosal architecture, two patients had positive IgA-class AGA and two patients with selective IgA deficiency IgG-class AGA.

3. Findings when serology suggests coeliac disease (**II**)

In patients suspected to have coeliac disease and found to have IgA-ARA or -AGA abdominal complaints were the main symptom leading to antibody screening in 49

Table 12. The means, 95% CI and number of abnormal findings (%) of IELs and enhanced mucosal HLA DR expression (%) in small bowel biopsy specimens in serum IgA-class ARA- and AGA-positive patients found to have coeliac disease, in patients excluded for coeliac disease on biopsy, and in control subjects (**II**).

		Coeliac disease excluded on biopsy n=67	Coeliac disease n=29	Non-coeliac controls n=27
$CD3^+$ IELs; cells/mm	mean	38	87	23
	95% CI	32-44	77-97	17-29
	abnormal findings (%)	10/67 (15%)*	27/29 (93%)‡	2/27 (7%)
$\alpha\beta^+$ IELs; cell/mm	mean	25	52	15
	95% CI	22-28	44-60	11-19
	abnormal findings (%)	7/67 (10%)*	21/29 (72%)‡	1/27 (4%)
$\gamma\delta^+$ IELs; cell/mm	mean	6.1	28.9	1.3
	95% CI	4.3-7.9	23.8-34.0	0.8-1.8
	abnormal findings (%)	27/67 (40%)*†	28/29 (97%)‡	1/27 (4%)
Enhanced HLA DR expression		11/67 (16%)*	24/29 (83%)‡	3/27 (11%)

* $p<0.0001$ compared with patients with coeliac disease.
† $p=0.0002$ compared with non-coeliac controls
‡ $p<0.0001$ compared with non-coeliac controls.

patients, signs of malabsorption in 13 and oral mucosal and cutaneous symptoms (excluding dermatitis herpetiformis) in 34. The median duration of symptoms was 1.5 years (range one month to 20 years). Twenty-one out of the 96 antibody-positive patients had both IgA-class ARA and AGA, 15 were positive for ARA only, and 60 for IgA-AGA only. Twenty-nine (30%) were found to have villous atrophy and crypt hyperplasia compatible with coeliac disease (VH/CrD <2), and 6-12 months after the introduction of a gluten-free diet clinical and serological or histological improvement was evident in all. In the remaining 70% (67/96) villous morphology did not differ from that in non-coeliac control subjects and coeliac disease was thus excluded; these patients continued on a gluten-containing diet.

Table 13. Distribution of small bowel mucosal IELs and enhanced HLA DR expression according to serum ARA or AGA positivity in 67 patients excluded for coeliac disease, and in non-coeliac controls (**II**).

		Patients excluded for coeliac disease		Non-coeliac controls n=27
		ARA-positive n=9	only AGA-positive n=58	
$CD3^+$ IELs; cells/mm	mean	58	34	23
	95% CI	26-90	30-38	17-29
	abnormal findings (%)	3/9 (33%)	5/58 (9%)	2/27 (7%)
$\alpha\beta^+$ IELs; cell/mm	mean	37	23	15
	95% CI	23-51	20-26	11-19
	abnormal findings (%)	2/9 (22%)	5/58 (9%)	1/27 (4%)
$\gamma\delta^+$ IELs; cell/mm	mean	13.4	5.0	1.3
	95% CI	5.4-21.4	3.5-6.5	0.8-1.8
	abnormal findings (%)	6/9 (67%)*	21/58 (36%)†	1/27 (4%)
Enhanced HLA DR expression		3/9 (33%)	8/58 (14%)	3/27 (11%)

* p=0.0003 compared to non-coeliac controls
† p=0.0007 compared to non-coeliac controls

In the 67 seropositive patients excluded for coeliac disease the densities of IELs were significantly higher than those in non-coeliac controls, but still significantly lower than those in coeliac patients (Figure 2, Table 12). In these patients excluded for coeliac disease enhanced mucosal HLA DR expression was as uncommon as in non-coeliac controls (Table 12). The distributions of small bowel mucosal findings according to serum IgA-class ARA or AGA positivity are shown in Table 13. Altogether, mucosal inflammation was slightly more prominent in ARA-positive patients than in patients having IgA-AGA only.

A second follow-up biopsy was carried out in 12 patients having normal mucosal villous morphology at the first biopsy and taking a gluten-containing diet; 10 of them had initially increased and two had normal densities of $\gamma\delta^+$ IELs. At the time of rebiopsy five of these 12 patients (Figure 2, and in study **II**; Table III, patients no. 1 to 5) had villous atrophy compatible with coeliac disease (VH/CrD <2). These patients started a gluten-free diet and six months later clinical and serological or considerable histological improvement was found in four; one patient declined follow-up examinations.

4. Findings when symptoms suggest coeliac disease (III)

Of the 93 patients reporting gastrointestinal symptoms after ingestion of cereals, 83% (77/93) suffered from belching or bloating, 63% (59/93) from diarrhoea, 34% (32/93) from abdominal discomfort and 2% from constipation. In addition, two patients complained of mouth ulcers. None had clinical signs of steatorrhoea. The median duration of the symptoms was 10 years (range 3 months - 30 years). Ingestion of wheat evoked symptoms in 82 (88%) patients, rye in 81 (87%) and barley in 64

(69%). Four patients had ATD, four vitiligo, two alopecia areata and one ulcerative colitis, and a family history of coeliac disease was noted in 14 (15%) patients. Forty-three (46%) patients had previously maintained a gluten-free diet on their own account and also experienced an alleviation of abdominal complaints. At the time of the present study all patients were again consuming gluten; six were not able to maintain this diet for longer than 2-3 weeks, all others managed for at least one month.

Table 14. The mean, 95% CI and number of abnormal findings (%) of IELs and mucosal HLA DR expression (%) in small bowel biopsy specimens from patients having abdominal symptoms after ingestion cereals and found to have coeliac disease, patients excluded for coeliac disease on biopsy, and control subjects (**III**).

		Coeliac disease excluded on biopsy n=85	Coeliac disease n=8	Non-coeliac controls n=30
$CD3^+$ IELs; cells/mm	mean	29	82	23
	95% CI	25-33	71-93	17-29
	abnormal findings (%)	8/85 (9%)*	7/8 (88%)‡	2/30 (7%)
$\alpha\beta^+$ IELs; cell/mm	mean	18	50	15
	95% CI	16-20	36-64	11-19
	abnormal findings (%)	4/85 (5%)*	5/8 (62%)‡	1/30 (3%)
$\gamma\delta^+$ IELs; cell/mm	mean	3.0	17.5	1.3
	95% CI	2.2-3.8	13.0-22.4	0.8-1.8
	abnormal findings (%)	17/85 (20%)†	8/8 (100%)‡	1/30 (3%)
Enhanced HLA DR expression		16/85 (19%)*	7/8 (88%)‡	3/30 (10%)

* $p<0.001$ compared with patients having coeliac disease.
† $p=0.01$ compared with patients having coeliac disease.
‡ $p<0.0001$ compared with non-coeliac controls.

Villous atrophy and crypt hyperplasia compatible with coeliac disease (VH/CrD <2) was found in 8 (9%) out of 93 patients and in these patients symptoms and small bowel mucosal morphology improved during the gluten-free diet. In the remaining 85 patients, villous architecture was comparable to that in non-coeliac controls; only one patient had patchy villous shortening and crypt elongation, albeit not fulfilling the criteria for coeliac disease (VH/CrD range 0.5-2.6). HLA DQ2 was present in all six coeliac disease patients tested. In addition, 35 (47%) out of 75 patients excluded for coeliac disease had HLA DQ2 or DQ8, or both; the one with patchy mucosal lesion had DQ8. HLA-typing was for technical reasons unsuccessful in two coeliac and 10 non-coeliac subjects.

The densities of $CD3^+$ and $\alpha\beta^+$ IELs were higher and enhanced mucosal HLA DR expression more common in patients with coeliac disease than in symptomatic patients excluded for coeliac disease, or in non-coeliac controls. Compared to the control group the densities of $\gamma\delta^+$ IELs were also increased in patients excluded for coeliac disease (Table 14). When individual patients excluded for coeliac disease were evaluated separately, seven evinced an increased density of $CD3^+$ IELs, four $\alpha\beta^+$ IELs and 17 $\gamma\delta^+$ IELs, and 16 enhanced mucosal DR expression (Figure 2); none of these findings, however, was restricted to coeliac-type HLA, (Table 15).

Serum tTG tests were positive in all coeliac patients and EmA in all except one. None of the 85 patients excluded for coeliac disease was positive for EmA or tTG, but 39 (46%) were positive for IgA-class AGA and 10 for IgG-class AGA. In these patients excluded for coeliac disease HLA DQ2 or DQ8 was found in 45% of IgA-AGA positive, and in 9% of IgG-AGA positive patients.

Table 15. Small bowel mucosal IELs and enhanced mucosal HLA DR expression in patients excluded for coeliac disease but having abdominal symptoms after ingestion of cereals. Results are divided according to the presence of coeliac-type HLA, DQ2 and DQ8 (**III**).

		Coeliac disease excluded		
		HLA DQ2 or DQ 8 positive n=35	HLA DQ 2 and DQ8 negative n=40	HLA unknown n=10
$CD3^+$ IELs; cells/mm	mean	29	28	32
	95% CI	23-35	22-34	22-42
	abnormal findings (%)	4/35 (11%)*	2/40 (5%)	1/10 (10%)
$\alpha\beta^+$ IELs; cell/mm	mean	18	18	19
	95% CI	14-22	15-21	14-24
	abnormal findings (%)	1/35 (3%)*	3/40 (8%)	0/10 (0%)
$\gamma\delta^+$ IELs; cell/mm	mean	2.7	3	3.8
	95% CI	1.3-4.1	1.8-4.2	1.1-6.5
	abnormal findings (%)	7/35 (20%)*	8/40 (20%)	2/10 (20%)
Enhanced HLA DR expression		7/35 (20%)*	8/40 (20%)	1/10 (10%)

* the differences between HLA DQ2- or DQ8-positive patients and patients without HLA DQ2 and DQ8 were not statistically significant.

Of the 93 patients reporting abdominal symptoms after ingestion of cereals 56 (60%) had a history of atopic symptoms (13 asthma, 35 allergic rhinitis, 38 atopic dermatitis). Serum total IgE was increased in 15. Seventy-four of these patients underwent a serum radioallergosorbent test (RAST) test against wheat, and skin prick and patch tests against cereals. At least one of the tests was positive in 19 patients; six of them had two and in one all three tests were positive. Nine out of 19 patients started an elimination diet and an alleviation of symptoms was achieved in all. Three of these patients were willing to participate in an open oral challenge with cereals, and a recurrence of symptoms was seen in two.

5. Application of morphological and immunohistochemical studies to a coeliac disease risk group (IV)

Coeliac disease had previously been detected in six (10%) out of 62 patients with multiple autoimmune endocrinological disorders (Table 15); they all fulfilled the ESPGAN criteria. Two were diagnosed on the basis of abdominal symptoms, and one because of growth disturbance; three were asymptomatic and had been found by serological tests outside the current study protocol.

Of the 56 non-coeliac patients with multiple endocrinological disorders 28 consented to upper gastrointestinal endoscopy; upon inquiry 15 reported suffering from subtle abdominal symptoms. In one asymptomatic patient (4%) SVA with crypt hyperplasia compatible with coeliac disease was found, and on gluten-free diet mucosal recovery was evident. Thus the overall frequency of coeliac disease in patients with multiple autoimmune endocrinological disorders is at least 11% (7/62). In addition, two patients had mild PVA and crypt hyperplasia, albeit not fulfilling the diagnostic criteria for coeliac disease.

An increased density of $CD3^+$ and $\alpha\beta^+$ IELs was found only in the newly detected patient with coeliac disease; in the rest the densities of these cells were within normal limits (Figure 2). However, the density of $\gamma\delta^+$ IELs was also increased in two patients with mild PVA and in three with normal villous structure (Table 16). Enhanced mucosal HLA DR expression was seen in the newly detected coeliac patient, as well as in one of the two with mild PVA and in two of the three with an increased density of $\gamma\delta^+$ IELs only. In all patients evincing small bowel mucosal abnormalities

suggestive of coeliac disease HLA DQ2 or DQ8 was present. These HLA-types were also found in 16 (57%) patients with normal mucosal architecture.

Of the newly biopsied patients with multiple autoimmune disorders, EmA was positive only in the one patient with coeliac disease (4%). Moreover, seven (25%) patients had IgA-class AGA and one (4%) IgG-class AGA. AGA-positivity was not restricted to minor mucosal findings or coeliac-type HLA.

Table 16. Patients with multiple autoimmune disorders and small bowel mucosal villous atrophy or increased density of $\gamma\delta^+$ IELs (**IV**).

Sex, age at diagnosis, (years)	HLA DQ2/DQ8	Autoimmune endocrinological disorders
Coeliac disease		
M24†	NT*	IDDM + ATD + Addison's disease
M49†	+	IDDM + ATD
F10†	NT	IDDM + ATD
F13†	NT	IDDM + ATD
F10†	+	IDDM + alopecia areata
F25†	+	ATD + Addison's disease
F33	+	ATD + alopecia areata
Mild partial villous atrophy and increased $\gamma\delta^+$ IELs		
F17	+	IDDM + ATD
F50	+	IDDM + Addison's disease + APECED
Increased $\gamma\delta^+$ IELs without villous atrophy		
M49	+	IDDM + ATD
F32	+	IDDM + ATD
M18	+	Addison's disease + APECED

NT = not taken
*HLA DR3, B8 positive; †previously detected patients with coeliac disease.

DISCUSSION

1. Difficulties in interpretation of small bowel biopsy specimens

The current diagnostic criteria for coeliac disease are based on the finding of small bowel mucosal villous atrophy with crypt hyperplasia (Walker-Smith et al. 1990), but it is widely accepted that gluten intolerance is not restricted to such a lesion only. Evidence suggests that small bowel mucosal damage in coeliac disease develops gradually from mucosal inflammation to crypt hyperplasia and finally to partial and subtotal villous atrophy (Marsh 1992). In keeping with this, the present study shows that patients excluded for coeliac disease on the grounds of apparently normal villous architecture still may have minor mucosal abnormalities suggestive of coeliac disease, and five such patients developed unequivocal villous atrophy compatible with coeliac disease during the follow-up (**II**).

However, it is not always easy to interpret whether these minor changes are due to gluten intolerance, and diagnostic difficulties may arise. First, a proper orientation of small bowel biopsy specimens is essential in the assessment of biopsy samples. Secondly, the results can be influenced by inter- and intraobserver variation. Thirdly, the possible patchiness of atrophic changes in small bowel biopsy specimens may cause problems (Scott and Losowsky 1976, Scott and Losowsky 1977, Picarelli et al. 1996b). To overcome these pitfalls, as many as seven small bowel biopsy specimens were taken in adults; in addition, orientation of the specimens and changes in intra- and interobserver variation were regularly scrutinised. As a result, intra- and interobserver variation was found acceptable. Moreover, in the present series

patchiness seemed to be rare and in only one patient (**III**) was one specimen found to be slightly patchy.

2. Untreated coeliac disease with unequivocal villous atrophy

In the present study VH/CrD measurements were applied to quantitate more exactly severe PVA or SVA and crypt hyperplasia. Altogether this ratio, as well as ECH, was definitely lower in untreated coeliac disease than that in non-coeliac controls. In accord withearlier studies (Ferguson and Murray 1971, Kuitunen et al. 1982, Savilahti et al. 1990, Savilahti et al. 1992), the feature common to these untreated coeliac disease patients was an increase in IELs (Table 10). An increased density of $\gamma\delta^+$ cells was found in all but one patient with untreated disease, and determination of $\gamma\delta^+$ IELs can thus clearly be helpful in the diagnosis of coeliac disease, especially if the small bowel biopsy finding is equivocal. By contrast, there was a clear overlap in the densities of $\alpha\beta^+$ IELs between untreated coeliac patients and control subjects and in a third of untreated patients these cells were normal (Figure 2). It would thus appear that determination of $\alpha\beta^+$ IELs adds little to the diagnostic work-up of coeliac disease. HLA DQ2 was found in all seven new coeliac patients tested. The number of samples investigated was small, but it has recently been shown in a larger series that most Finnish coeliac disease patients share this HLA haplotype, and in a HLA DQ2-negative minority mostly HLA DQ8 haplotype was present (Polvi et al. 1998). This is in line with findings elsewhere in Europe (Tosi et al. 1983, Sollid et al. 1989, Ploski et al. 1993), and there is much evidence to indicate that coeliac disease is unlikely in Caucasian patients negative for both HLA DQ2 and DQ8. The significance of the

genetic background was supported in the present study; 69% of patients affected by two or more autoimmune endocrinological diseases had the HLA DQ2 or DQ8 haplotype. Altogether 11% of these patients had overt coeliac disease and their risk of coeliac disease was even higher than that in patients having one such disorder (Table 5).

In patients presenting with only subtle if any symptoms, serology is helpful in the detection of coeliac disease. In the present series, sensitivity figures of 90-100% for IgA-class ARA, EmA and tTG tests and 70-80% for IgA-AGA test are in agreement with those in earlier studies (Mäki et al. 1991b, Volta et al. 1991, Vogelsang et al. 1995, Volta et al. 1995, Dieterich et al. 1998, Sulkanen et al. 1998a). However, the present results are in contrast to studies showing that IgA-class ARA, EmA and AGA tests are almost 100% specific for small bowel mucosal villous atrophy and coeliac disease (Hällström 1989, McMillan et al. 1991, Ferreira et al. 1992, Valdimarsson et al. 1996b). As seen in study **II,** 86% of IgA-class ARA- and AGA-positive patients, 60% of patients with IgA-ARA only, and 3% of patients with only IgA-AGA had villous atrophy. Similarly, several authors have reported normal-appearing mucosa in many patients with positive serology (Grodzinsky et al. 1992, Ladinser et al. 1994, Vogelsang et al. 1995). One putative explanation for the discrepancy is the difference in patient series. Screening tests have been commonly applied in patients with a high suspicion of coeliac disease (McMillan et al. 1991, Valdimarsson et al. 1996b). In the present study the patients had relatively mild symptoms, and in such cases antibodies seem to be less specific. Thus the determination of coeliac disease antibodies is not alone sufficient for a diagnosis of the disease.

3. Small bowel mucosal changes during the gluten-free diet

Small bowel mucosal villous morphology recovered fully in all patients adhering to long-term strict gluten-free diet. The recovery was not complete and VH/CrD was lower than that in non-coeliac controls when the duration of the diet was less than 24 months or when patients had occasional dietary lapses. The ECH measurement was less sensitive than the VH/CrD ratio to show this incomplete mucosal healing (Table 11). The most sensitive indicator in small bowel mucosal response to gluten has been considered to be an increase in IELs (Ciclitira et al. 1984a, Leigh et al. 1985, Mayer et al. 1991, Catassi et al. 1993). A decrease in $\alpha\beta^+$ IELs is held to reflect the effect of a gluten-free diet (Savilahti et al. 1990, Savilahti et al. 1992, Kutlu et al. 1993). In the present series (**I**), the density of $\alpha\beta^+$ IELs decreased along with the gluten-free diet and was within normal limits in all long-term treated patients. However, the density of these cells was also normal in short-term treated patients having low VH/CrD and even in cases of dietary lapses.

Interestingly, also $\gamma\delta^+$ IELs decreased with the diet, even though this is in conflict with earlier reports (Halstensen et al. 1989, Savilahti et al. 1990). Nonetheless, in most patients $\gamma\delta^+$ IEL density remained high over the long period on a gluten-free diet. If a patient suspected of coeliac disease has started a gluten-free diet before the diagnostic procedures and small bowel biopsy and is reluctant to revert again to normal diet, the small bowel mucosal damage may have undergone full recovery. When a gluten-free diet has not been maintained for too long a time, a normal density of $\gamma\delta^+$ IELs suggests that the individual in question does not suffer from coeliac disease.

Enhanced small bowel mucosal HLA DR expression in the crypt epithelium was evident in some treated coeliac patients, which is in line with earlier observations (Ciclitira et al. 1986, Arato et al. 1987, Scott et al. 1987). According to the present study in the follow-up of dietary response, determination of mucosal DR expression contributed little information in individual cases. Coeliac serology might be an easy means of assessing the dietary response. However, as shown in the present and earlier studies (Mayer et al. 1991, Troncone et al. 1995b), they often fail to detect occasional slight dietary transgressions and incomplete mucosal healing. Altogether, in the present series measurement of VH/CrD from small bowel mucosal specimens was the best indicator of incomplete dietary response (Table 11).

Mucosal integrity was not dependent on the daily intake of trace amounts of residual gluten from wheat starch in patients on a strict diet. In the present study as many as 88% (46/52) of the long-term treated patients adopted a strict gluten-free diet, compared with 17-65% published elsewhere (Kluge et al. 1982, Kumar et al. 1988, Mayer et al. 1991, Bardella et al. 1994). It is too early to say whether wheat starch-based gluten-free products help to maintain a strict diet, but nevertheless these products were widely adopted and they seemed to be safe and well-tolerated provided the diet was otherwise strict.

4. Coeliac disease without villous atrophy

4.1. Abdominal symptoms suggesting coeliac disease

Results in study **III** indicate that since a positive history of abdominal symptoms after ingestion of cereals is a poor predictor of coeliac disease, all experimental dietary interventions before proper diagnosis of the disease are to be discouraged. Only nine

per cent of such symptomatic patients were found to have overt coeliac disease, and latent-potential coeliac disease also turned out to be rare; 17 (18%) patients with normal villous architecture had an increased density of $\gamma\delta^+$ IELs suggestive of latent coeliac disease, but nine of these were HLA DQ2- and DQ8-negative. This most likely excludes coeliac disease latency, since virtually all Finnish coeliac disease patients carry one or other of these DQ alleles (Polvi et al. 1998).

In study **III**, 60% of the adults suffering from abdominal symptoms after ingestion of cereals were affected by atopy and one fourth proved positive in allergy skin tests or RAST. By comparison, in Western European countries about 20% of adolescents suffer from atopic disorders (ISAAC 1998). In contrast to children and adolescents (Räsänen et al. 1994, Sampson and Ho 1997a, Majamaa et al. 1999), specificity of cereal allergy skin tests and RAST in atopic adults is not known because of the lack of double-blind, placebo-controlled oral challenge studies (Sampson 1997b). However, it seems that also in adults cereal allergy should be considered (Bengtsson et al. 1996), even though the evidence from the present study is not firm. On the other hand, not all symptoms evoked by cereals are due to gluten. Wheat starch is resistant to digestive enzymes and excessive fermentation by gastrointestinal bacteria may result in abdominal symptoms such as bloating and diarrhoea (Anderson et al. 1981).

4.2. Mucosal morphology suggestive of coeliac disease

Small bowel mucosal morphological examination from hematoxylin-eosin-stained samples should include determination of the density of total IELs (Kuitunen et al. 1982). In the present study immunohistochemical staining of $CD3^+$ cells was chosen instead, but it has previously been shown that both of these methods correlate well to each other (Arranz et al. 1994). Altogether 17 (9%) out of 179 patients excluded for

coeliac disease in studies **II-IV** were found to have minor small bowel mucosal abnormalities compatible with infiltrative or hyperplastic lesions according to Marsh's classification (Marsh 1-2 type lesions) (Marsh 1992). Interestingly, hyperplastic crypts and minor villous alterations were also seen without mucosal inflammatory cell infiltration (**IV**).

It is not easy to interpret without long surveillance whether these minor Marsh 1-2-type small bowel mucosal morphological changes are indicative of coeliac disease. As seen in study **III**, even in patients having abdominal symptoms after ingestion of cereals, an increased density of IELs was not always restricted to coeliac-type genetics, HLA DQ2 or DQ8 (Table 14). Similarly, in the study by Cooper et al (1980) nine patients with gluten-sensitive diarrhoea had an infiltrative-type small bowel mucosal lesion. In only three out of these nine patients was a coeliac-type HLA B8 haplotype present, and the authors concluded that there was no evidence of coeliac disease. Thus, an increased density of total IELs in the presence of normal villi does not inevitably indicate coeliac disease.

4.3. Immunohistochemical findings suggestive of coeliac disease

As many as a third of the patients excluded for coeliac disease according to currents diagnostic criteria had an increased density of $\gamma\delta^+$ IELs (**II-IV)**; in a majority of these patients the small bowel mucosal morphology was otherwise normal and in fact compatible with Marsh 0 type histology, since total counts of IELs were normal. A high density of $\gamma\delta^+$ IELs is considered to be especially characteristic (Halstensen et al. 1989, Spencer et al. 1989b, Savilahti et al. 1990), even though not pathognomonic, for coeliac disease (Spencer et al. 1991, Pesce et al. 1996). Increased densities of these cells have been detected in some patients suspected of coeliac disease but showing

normal small bowel mucosal villous architecture (Holm et al. 1992, Troncone 1995a); in two such cases coeliac-type villous atrophy has been reported to develop during follow-up (Mäki et al. 1991a, Arranz and Ferguson 1993). Thus, a high count of $\gamma\delta^+$ IELs in normal villi is considered to be a marker of latent coeliac disease; accordingly, in the present series five of the ten patients having initially an increased density of $\gamma\delta^+$ IELs and normal small bowel mucosal architecture were subsequently found to have mucosal atrophy compatible to coeliac disease in a follow-up biopsy. Recently, Iltanen and assiciates (1999b, c) have reported seven similar cases of latent coeliac disease. In an earlier family study it was shown that all individuals with a high density of γδ+ IELs had HLA DQ2 (Holm et al. 1992). This was also the case in the present series in patients suffering from multiple autoimmune endocrinological disorders (**IV**). By contrast, in study **III** in patients suffering abdominal complaints after ingestion of cereals only 47% of patients with an increase in γδ+ IELs and normal villous architecture had coeliac-type genetics (HLA DQ2 or DQ8). Similarly, Iltanen and colleagues (1999c) reported that only 54% of children with a high count of these cells and normal mucosa were HLA DQ2 positive. It is noteworthy that a close association between the increase in γδ+ IELs and coeliac-type HLA is seen in patient series where the frequency of HLA DQ2 or DQ8 is altogether 60-70% both in first degree family members of coeliac patients (Auricchio et al. 1988, Farre et al. 1999) and in patients with multiple autoimmune endocrinological disorders (**IV**). By comparison, the frequency of these HLA-types is only 20-30% in the general population (Tosi et al. 1983, Sollid et al. 1989, Ploski et al. 1993). It is important to note that an increase in γδ+ IELs does not necessarily indicate HLA DQ2 or DQ8 haplotype or coeliac disease in genetically unselected populations.

Enhanced HLA DR expression in the small bowel mucosa is another sign of inflammation, which implicates the activation of mucosal cell-mediated immunity. In agreement with earlier studies (Arnaud-Battandier et al. 1986, Arato et al. 1987), this was mainly seen here in patients with coeliac disease. In addition, enhanced DR expression was found in some subjects with apparently normal villous architecture; in many of them the density of $\gamma\delta^+$ IELs was also increased. Again enhanced HLA DR expression was not specific for coeliac disease since it was also seen in HLA DQ2-and DQ8-negative subjects (Table 14); it would thus appear that determination of mucosal HLA DR expression is of limited value in the diagnostics of latent coeliac disease.

4.4. Serology suggesting coeliac disease

Seventy per cent of the patients found to have normal small bowel mucosal architecture and by definition excluded for coeliac disease were IgA-class ARA-or AGA-positive in study **II**, and 40% were positive for IgA-AGA in study **III**. The significance of these "false-positive" serum antibodies has so far remained obscure, although some preliminary data suggest that they might indicate gluten sensitivity (Collin et al. 1993, Picarelli et al. 1996b). It can thus be hypothetised that overt villous atrophy has not yet developed in all, and that IgA-class ARA, EmA and AGA tests detect gluten-sensitive subjects with still seemingly normal mucosal villous architecture. There was indeed evidence of this in the present study, as minor small bowel mucosal changes were also observed. In study **II**, apart from patients having overt coeliac disease, the densities of $CD3^+$, $\alpha\beta^+$ and $\gamma\delta^+$ IELs were also increased in antibody-positive patients without villous atrophy (Table 12). Five out of 12 such antibody-positive patients with initially normal small bowel mucosal villous architecture were later found to have mucosal damage compatible to coeliac disease

(VH/CrD <2); in all five an increased density of $\gamma\delta^+$ IELs was already present in the first small bowel biopsy. Interestingly, the increase in IELs was transient in some patients (in study **II**: Table III); the significance of such transient inflammatory changes remains to be elucidated.

It has previously been reported that 83% of IgA-ARA-positive patients with normal villous architecture have developed coeliac disease during follow-up (Collin et al. 1993), and that patients positive for ARA (or EmA) share coeliac-type HLA DQA1*0501 and HLA DQB1*0201 alleles (Mäki et al. 1991b). Hence, it has been suggested that ARA (or EmA)-positive patients having normal villous architecture may have latent coeliac disease (Ferguson et al. 1993). Our present findings are in line with these observations. However, in a study by Picarelli and assiciates (1996b), eight of 10 adults with positive EmA and normal small bowel mucosal villi were found to have HLA types different from HLA DQ2 or DQ8. The absence of HLA-types DQ2 and DQ8 strongly suggests that these patients were not suffering from coeliac disease, even though their diarrhoea was alleviated on gluten withdrawal. In other words, EmA-positivity does not invariably mean the presence of coeliac disease.

The significance of AGA in patients with normal small bowel mucosal morphology is obscure. There are findings, which imply that positive AGA is a sign of gluten sensitivity, even when villous atrophy is not present. O´Farrelly and colleagues (1991) found increased numbers of IELs in IgG-class AGA-positive subjects who had normal small bowel morphology. Corazza and group (1992) measured a mean surface to volume ratio of small bowel biopsy specimens in IgG-class AGA-positive first-degree relatives of coeliac disease patients. This ratio was, as expected, lowest in subjects found to have coeliac disease, but it was also significantly lower in IgG-class AGA-positive relatives without villous atrophy, when compared to relatives negative for

AGA, or to healthy control subjects. However, in a study by Collin and associates (1993), IgA-AGA positivity predicted forthcoming coeliac disease in only 10% of cases compared to 83% in ARA positivity. In addition, as seen in the present study (**III**), AGA positivity was not associated with coeliac-type genetics, which is in line with earlier studies (Mäki et al. 1991b, Pettersson et al. 1993). Further, in study **II** it was shown for the first time that many AGA-positive but ARA-negative subjects with normal villi had increased densities of $\gamma\delta^+$ IELs, and moreover, three such subjects subsequently had villous atrophy in the second biopsy. By contrast, in studies by Pittschiler and Ladinser (1996) and Johnston and colleagues (1999) IgA-class AGA-positive (but EmA-negative) subjects with normal villous morphology had normal densities of $CD3^+$, $\alpha\beta^+$ and $\gamma\delta^+$ IELs, and thus had no features of latent coeliac disease. However, they examined healthy individuals, whereas all patients in the present study (**II**) were suspected to be suffering from coeliac disease.

It is also interesting that in study **III** many HLA DQ2- and DQ8-negative patients experiencing abdominal symptoms after ingestion of cereals were positive for IgA-AGA; whether this "false" AGA positivity is a sign of an altered immune system or unspecific mucosal damage resulting in gluten permeability (Bonamico et al. 1997) is a subject for further studies.

4.5. New aspects for the diagnosis and treatment of coeliac disease

Based on earlier and the present data, the current diagnostic criteria for coeliac disease requiring definite villous atrophy (Walker-Smith et al. 1990) seem to be too strict. In widening the criteria to apply also to minor small bowel mucosal abnormalities, difficulties in differential diagnostics will emerge. However, in addition to detailed

small bowel mucosal morphological measurements, determination of total and $\gamma\delta^+$ IELs, together with serum ARA, EmA and tTG, helps to detect individuals who might be gluten-intolerant despite the absence of overt villous atrophy. Furthermore, determination of HLA DQ alleles can be used in the exclusion of coeliac disease; it is unlikely that Caucasian individuals negative for both DQ2 and DQ8 suffer from coeliac disease.

The increased risk of small bowel lymphoma and osteoporosis in untreated coeliac patients warrants early diagnosis and treatment of the condition (Holmes et al. 1989, Valdimarsson et al. 1996a). However, there is little evidence that patients with minor small bowel mucosal changes compatible with potential coeliac disease would benefit from a gluten-free diet before the appearance of villous atrophy. No studies have been carried out on bone mineral density or quality of life in these patients. There is only one case report of malignant lymphoma in the latent stage of coeliac disease (Freeman and Chiu 1986). Arranz and Ferguson (1993) found a resolution of diarrhoea in five out of eight patients with a coeliac-like intestinal antibody pattern during a gluten-free diet. Simultaneously a decrease in IELs in the small bowel mucosa was seen. Similarly a gluten-free diet alleviated diarrhoea in 10 EmA-positive patients without small bowel mucosal villous atrophy in a series by Picarelli and colleagues (1996b). It must be noted, however, that in the firstmentioned of these two studies no HLA-typing was carried out and in the latter eight of the 10 patients were negative for both HLA DQ2 and DQ8. To date, there are no data on the natural history or requirements for treatment of such HLA DQ2- and DQ8-negative patients suspected of coeliac disease.

As a rule, patients with minor small bowel mucosal changes suggestive of coeliac disease are nowadays still left on a normal gluten-containing diet and followed up to

detect possible development of villous atrophy at a later stage. However, based on the present and earlier data the diagnostic criteria for coeliac disease should be widened. Further studies are needed to show whether there are individuals who would benefit from a gluten-free diet before the manifestation of small bowel mucosal villous atrophy.

SUMMARY AND CONCLUSIONS

Small bowel mucosal villous atrophy with crypt hyperplasia and minor mucosal abnormalities indicative of an early stage of coeliac disease were examined in 96 consecutive patients suspected of coeliac disease and found to have IgA-class ARA or AGA, and in 93 adults with a history of cereal intolerance. In addition, the prevalence of coeliac disease and minor small bowel mucosal abnormalities was studied in a risk group comprising 62 adults with two or more autoimmune endocrinological disorders. For comparison, biopsy specimens were evaluated in 52 long-term treated coeliac and dermatitis herpetiformis patients and in 34 newly detected coeliac patients before and after adopting a gluten-free diet for an average of 10 months. Thirty adults complaining of indigestion served as non-coeliac biopsy controls.

Twenty-nine (30%) out of 96 patients having a suspicion of coeliac disease and positive IgA-ARA or AGA, eight (9%) out of 93 patients with a history of cereal intolerance and seven (11%) out of 62 adults with multiple autoimmune endocrinological disorders were found to have villous atrophy and crypt hyperplasia compatible with coeliac disease. VH/CrD was clearly lower and the densities of IELs, especially γδ+ cells, were increased in untreated coeliac patients when compared to non-coeliac controls. Altogether, $\gamma\delta^+$ IELs seemed to be a sensitive indicator of untreated coeliac disease, whereas in one third the density of $\alpha\beta^+$ IELs was normal. HLA DQ2 was found in all new coeliac disease patients tested. Ten per cent of untreated coeliac disease patients had negative serum IgA-ARA or EmA, and 20-30% negative IgA-AGA; in addition, IgA-class ARA or AGA were found in 40-70% of patients with apparently normal small bowel mucosa. Thus the determination of coeliac disease antibodies is not alone sufficient for a diagnosis of coeliac disease.

The small bowel mucosa recovered fully in all patients on a long-term strict gluten-free diet. The mucosal recovery was not complete and VH/CrD was lower than in non-coeliac controls when the duration of the gluten-free diet was less than 24 months or when patients had dietary lapses. Measurements of small bowel mucosal ECH, IELs or enhanced HLA DR expression or serum coeliac antibodies were not as sensitive indicators of mucosal damage as the VH/CrD ratio during the treatment. Even the density of $\gamma\delta^+$ IELs decreased, but in most patients these cells remained high over the long period on a gluten-free diet. This can be utilised in cases where the diagnostic small bowel biopsy is taken when the patient is already on a gluten-free diet and biopsy specimens show normal villous architecture, and if the gluten-free diet has not lasted too long. Altogether, in the present series the compliance with the diet was good, and small bowel mucosal integrity was not dependent on trace amounts of gluten coming from daily ingestion of gluten-free wheat starch.

In all, 179 patients were excluded for coeliac disease on the grounds of apparently normal small bowel mucosal villous architecture. Seventeen (9%) were found to have minor mucosal structural abnormalities compatible with infiltrative-hyperplastic lesion according to the Marsh classification, and in 50 (28%) patients the density of $\gamma\delta^+$ IELs was increased. Five out of ten patients having initially normal small bowel mucosal villous architecture but an increased density of $\gamma\delta^+$ IELs were found in the second follow-up biopsy to have villous atrophy compatible with coeliac disease. Two of these five were IgA-ARA-positive and three had only IgA-AGA present at the onset of the study. Even though an increased density of $\gamma\delta^+$ IELs seemed to predict forthcoming villous atrophy, the increase in $\gamma\delta^+$ cells was not restricted to coeliac-type HLA, that is HLA DQ2 or DQ8. Similarly, these HLA-types were not detected in all

patients yielding a high count of total IELs. Enhanced mucosal HLA DR expression was found in some patients excluded for coeliac disease; many of them also had an increased density of $\gamma\delta^+$, yet some of them were nonetheless negative for HLA DQ2 and DQ8. In the present study, positive serology did not necessarily indicate overt or latent coeliac disease; 64% of patients with AGA only had normal small bowel mucosa, and AGA positivity was not correlated to coeliac-type HLA. The specificity of ARA and EmA was confirmed in this study. A history of abdominal symptoms related to ingestion of cereals turned out to be an unspecific sign for overt or even potential coeliac disease.

Patients with multiple endocrinological autoimmune disorders appeared to carry an increased risk of coeliac disease; 18 (69%) of the 26 patients tested were found to have coeliac-type HLA and seven (11%) had villous atrophy compatible with coeliac disease. Even minor small bowel mucosal abnormalities were detected in an additional five patients (18% of those biopsied); in all of these five HLA DQ2 or DQ8 was also present.

In conclusion, the present diagnostic criteria for coeliac disease may fail to detect symptomatic gluten-sensitive individuals. However, if the criteria are widened to concern also minor small bowel mucosal abnormalities, “false-positive” cases may be found. A detailed small bowel mucosal morphological examination and determination of total and $\gamma\delta^+$ IELs, serum ARA, EmA or possible tTG, and HLA DQ alleles are not alone sufficient in the diagnosis of coeliac disease. With a combination of these tests gluten-intolerant patients may be detected even before the development of overt small bowel mucosal villous atrophy.

ACKNOWLEDGEMENTS

This study was carried out at the Department of Medicine, Tampere University Hospital and at the Medical School, University of Tampere.

I wish to express my deep gratitude to Professor Amos Pasternack, M.D., Head of the Department of Medicine, Tampere University Hospital, for his helpful advice and for placing the facilities of the Department at my disposal.

Above all, I am deeply indebted to my supervisor, Docent Pekka Collin, M.D., who aroused my interest in coeliac disease, and introduced me into the world of scientific research. He has always had time to advise and encourage me, and his optimism and excellent sense of humour has supported me to carry on.

I am very grateful to Professor Markku Mäki, M.D., for providing working facilities in his laboratory. His enthusiastic guidance and exceptional skills as a teacher and researcher has contributed markedly to this study.

I wish to express my deep appreciation to Emeritus Professor Jarmo Visakorpi, M.D., for all his work. I admire his fundamental coeliac disease research in Tampere. His close relationship with foreign researchers has helped us very much to become acquainted with international members of 'Coeliac Circus'.

I am indebted to Professor Erkki Savilahti, M.D., and Professor Juhani Lehtola, M.D., for their prompt and constructive criticism of the manuscript.

I am grateful to Professor Timo Reunala, M.D., for stimulating discussions and valuable advice during this work.

I want to express my sincere gratitude to my co-authors Kati Holm, M.D., Docent Anna-Liisa Karvonen, M.D., Anna-Helena Mykkänen, M.Sc., Docent Jukka Partanen, Ph.D., Docent Pekka Pikkarainen, M.D., Docent Immo Rantala, Ph.D., Docent Jorma Salmi, M.D., Docent Kristiina Turjanmaa, M.D., Rosa Venäläinen, M.Sc., and Dietitian Nanna Vuolteenaho, for their valuable help. I have been fortunate to have collaborators who posses great expertise in their fields and who have willingly shared their knowledge with me.

I wish to thank Mervi Hakanen M.S., Tuula Halttunen, M.Sc., Mrs. Anne Heimonen, Mrs. Mervi Himanka, Ms. Paula Huotari, Sari Iltanen, M.D., Ms. Jaana Inkinen, Kati Juuti, M.Sc., Docent Heikki Kainulainen, Ph.D, Mr. Jorma Kulmala, Kaija Laurila, M.Sc., Katri Lindfors, M.Sc., Ms. Katja Matinaho, Kirsi Mustalahti, M.D., Markku Peräaho, M.D., Satu Sulkanen, M.S., and all other members of Coeliac Disease Study Group, for their supportive attitude and co-operation. My sincere thanks are expressed to Kaija Kaskela, M.Sc, secretary of Coeliac Disease Study Group, for her great help during recent years.

I wish to thank Mr. Robert MacGilleon, M.A., for revising the language of this thesis. The personnel at the Medical Library of Tampere University Hospital is also acknowledged.

My warm thanks are expressed to my colleagues and nursing staff at the Departments of Medicine and Gastroenterology in Tampere University Hospital, for their positive attitude towards me and my work. Especially, my heart-felt thanks are due to my colleagues and friends in our football team; their encouragement and ‘constructive criticism’ during the games has been one souse of my inspiration.

And finally, I owe my deepest thanks to my close friends and my sister Outi Kaukinen-Vesa and her family for caring and support during these years.

This work was financially supported by the Emil Aaltonen Foundation, the Medical Research Fund of Tampere University Hospital, the Finnish Foundation for Gastroenterological Research and the Finnish Coeliac Society.

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Tampere, September 18, 2000

Katri Kaukinen

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