Oral Findings in Dermatitis Herpetiformis and Coeliac Disease

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To my family

CONTENTS

1. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-IV)

I. **Patinen P**, Björksten F, Malmström M, Savilahti E, Reunala T (1995). Salivary and serum IgA antigliadin antibodies in dermatitis herpetiformis. Eur J Oral Sci 103: 280-284.

II. **Patinen P**, Savilahti E, Hietanen J, Malmström M, Mäki M, Reunala T (1997). Intraepithelial lymphocytes bearing the gamma/delta receptor in the oral and jejunal mucosa in patients with dermatitis herpetiformis. Eur J Oral Sci 105: 130-135.

III. **Patinen P**, Hietanen J, Malmström M, Reunala T, Savilahti E (2002). Iodine and gliadin challenge on oral mucosa in dermatitis herpetiformis. Acta Derm Venereol 82: 86-89.

IV. **Patinen P**, Aine L, Collin P, Hietanen J, Korpela M, Enckell G, Kautiainen H, Konttinen YT, Reunala T (2004). Oral findings in coeliac disease and Sjögren's syndrome. Oral Dis, in press

2. ABBREVIATIONS

AGA	antigliadin antibody
CD	coeliac disease
CD3+	CD3 positive lymphocytes
CD4+	helper T lymphocytes
CD8+	suppressor T lymphocytes
DH	dermatitis herpetiformis
DMFT	decayed, missing, filled teeth
ELISA	enzyme-linked immunosorbent assay
EmA	endomysium antibody
GFD	gluten-free diet
α/β TcR+	α/β T cell receptor positive lymphocytes
γ/δ TcR+	γ/δ T cell receptor positive lymphocytes
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
KI	potassium iodine
PMI	panoramic mandibular index
RAU	recurrent aphthous ulcer
TGA	transglutaminase antibody
TG2	tissue type transglutaminase
SS	Sjögren's syndrome
TcR	T cell receptor

3. ABSTRACT

Patinen P. Oral findings in coeliac disease and dermatitis herpetiformis.
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Hospital for Skin and Allergic Diseases, Hospital for Children and Adolescents, Huslab / Oral Pathology Unit, and Department of Oral and Maxillofacial Surgery, Helsinki University Central Hospital and Departments of Otorhinolaryngology and Maxillofacial Surgery, Internal Medicine and Dermatology, Tampere University Hospital, Tampere, Finland

Coeliac disease (CD) is an autoimmune disorder with small-intestinal mucosal inflammation caused by wheat gluten and related cereal peptides in genetically predisposed individuals. Dermatitis herpetiformis (DH) is a blistering skin disease in which the patients have mostly subclinical gluten sensitive enteropathy, i.e. CD, and pathognomonic IgA deposits in the skin and oral mucosa. Sjögren's syndrome (SS), which occurs in about 3% of the patients with CD and DH, also has an autoimmune background and is characterized by an inflammation of the salivary glands resulting in dryness of mouth.

In the first study (I) the occurrence of IgA antigliadin antibodies (AGA) in the saliva and serum of ten untreated and ten gluten-free diet (GFD) treated patients with DH was examined by a solid-phase enzyme immunometric assay (Gluten IgA EIA; Pharmacia, Uppsala, Sweden). The salivary IgA AGA levels, however, were only about 10% of the corresponding levels in the serum and similar to those of healthy controls. In addition, salivary IgA AGA levels did not decrease during GFD treatment unlike the serum IgA AGA levels. Therefore, it was concluded that salivary IgA AGA cannot be used for screening the enteropathy of the patients with DH or monitoring the GFD treatment. In the second study (II), immunohistochemical stainings detected only very low numbers of intraepithelial γ/δ T cell receptor (TcR) bearing lymphocytes in the buccal mucosa of 13 untreated and 13 GFD treated patients with DH. This was in sharp contrast to the jejunal epithelium, where the numbers of γ/δ TcR+ lymphocytes were high in line with their indicator role for gluten-sensitive enteropathy.

In the third study (III) wheat gliadin, but not potassium iodine, challenged buccal epithelium showed an influx of CD4+ lymphocytes ($p=0.06$) at 12 h in all five patients with DH but no marked changes in the numbers of CD8+ cells. The numbers of α/β TcR+ lymphocytes were increased ($p=0.03$) in the lamina propria but the numbers of γ/δ TcR+ cells remained at a very low level both in the epithelium and lamina propria.. It was concluded that the oral mucosa in DH are capable of reacting to locally applied gliadin by CD4+ and α/β TcR+ lymphocyte response. However, the oral mucosa were resistant to producing any macroscopic or microscopic DH lesions when challenged by potassium iodine which is in sharp contrast to what happens in the skin.

Patients with CD are known to have an increased frequency of recurrent aphthous ulcers and coeliac-type dental enamel defects whereas patients with SS suffer from dryness of mouth and have an increased risk for dental caries. The fourth study (IV) examined whether patients suffering concomitantly from CD and SS have an even higher frequency of oral mucosal, dental and salivary abnormalities than patients with either disorder alone. Twenty patients with CD+SS and 20 age and sex matched controls with CD and 20 with SS were studied. Oral mucosal changes were most common in SS (80%) followed by CD+SS (65%) and CD (40%). Coeliac-type dental enamel defects were found in 89% in CD+SS and in 88% in CD compared to only 25% in SS ($p<0.001$). The median number of teeth was 6 in the CD+SS, 24 in the CD and 22 in the SS group. The DMF index was higher ($p<0.005$) in the CD+SS than in the CD group. Interestingly, the CD+SS group was characterized by a higher salivary flow rate ($p<0.001$) and lower inflammarory focus score in the salivary glands ($p<0.01$) than the SS group. These differences suggest that GFD treatment may alleviate autoimmune inflammation in SS when the patient has concomitant CD. Overall, the co-occurrence of CD and SS should be recognized due to its effects on dental and oral mucosal health.

4. INTRODUCTION

Coeliac disease (CD) is an autoimmune disorder with small intestinal mucosal inflammation caused by wheat gluten and related cereal peptides in genetically predisposed individuals (Trier 1991, Mäki&Collin 1997). In the past few years, knowledge about the pathogenesis of CD has increased greatly. Gliadin triggers a cascade of reactions in which activated T cells induce an inflammatory process in the jejunal mucosa with subsequent formation of autoantibodies to transglutaminase enzyme (Dieterich et al 1997). The end point of these events is jejunal mucosal damage, i.e. atrophy of the villi, leading to various gastro-intestinal symptoms and signs of malabsorption (Sollid et al 1989). Gluten-sensitive enteropathy is frequently also subclinical and recent serological screening studies have shown that the prevalence of CD may be as high as 1/100 (Mäki et al 2003). Dermatitis herpetiformis (DH) is a cutaneous phenotype of CD showing pathognomonic IgA deposits in the skin and oral mucosa (Reunala 2001). The blistering rash and the mostly subclinical enteropathy respond well to treatment by a gluten-free diet (GFD) (Garioch 1994).

In the early 1980s tests for the measurement of antigliadin antibodies (AGA) in the serum were developed (Unsworth et al 1981, Vainio et al 1983). The results showed that screening of IgA class AGA was useful in search for gluten-sensitive enteropathy in CD and DH (Vainio et al 1985). At the same time an increased number of intraepithelial γ/δ TcR+ lymphocytes in the jejunal mucosa was found to be a constant marker for gluten-sensitive enteropathy in CD and DH (Halstensen et al 1989, Savilahti et al 1992). The first aim of the present study in patients with DH was to examine whether IgA AGA are secreted into the saliva and these salivary antibodies could then be used as a marker for gluten-sensitive enteropathy. A further aim was to investigate whether γ/δ TcR+ lymphocytes occur in the oral mucosa in similar high numbers as in the jejunal mucosa and whether application of gliadin or potassium iodine causes increased influx of these cells into the mucosa.

Patients with CD and DH are known to have recurrent aphtous ulcers (RAU) and coeliac-type dental enamel defects, both of which might be the only presenting symptoms in CD (Fergusson et al 1976, Aine 1996). Earlier reports have described also glossitis and cheilitis as typical findings associated with CD (Cooke et al 1953, Barry et al 1974). CD and DH are

associated with Sjögren's syndrome (Collin et al 1994, Iltanen et al 1999), an autoimmune disorder not linked with gluten sensitivity but characterized by inflammation of the salivary glands. Dryness of the mouth and increased risk for caries are consequences of the decreased salivary secretion in SS (Konttinen et al 1997). CD patients with associated SS could have an even higher risk for oral mucosal and dental lesions. Therefore, one aim of the present study was to examine whether patients suffering concomitantly from CD and SS have higher frequencies of oral mucosal, dental and salivary abnormalities than patients with either disorder alone.

5. REVIEW OF LITERATURE

5.1 Coeliac disease and dermatitis herpetiformis

5.1.1 Coeliac disease

CD is an autoimmune disorder characterized by inflammation, villous atrophy and crypt hyperplasia of the small-intestinal mucosa (Mäki&Collin 1997). The mucosal lesion develops in genetically susceptible individuals after ingestion of dietary gluten and heals when gluten-containing cereals are withdrawn from the diet (Trier 1991). The prevalence in Western countries may be as high as 1/100 (Fasano et al 2003, Mäki et al 2003). CD can appear at any age and in adults it is more common in women than in men (Mäki&Collin 1997). Patients may present with typical minor symptoms, such as loose stools and abdominal discomfort, or with apparent symptoms, such as steatorrhoea or malabsorption, leading to iron and folic acid deficiency and hypocalcaemia. About 20% of the patients do not have apparent gastrointestinal symptoms and therefore present with a so-called silent or subclinical form of the disease (Collin et al 1997). Due to this CD easily remains underdiagnosed if no serological screening tests are performed (Corazza et al 1993, Mäki et al 2003). Current diagnostic criteria for CD comprise the finding of a typical mucosal lesion of subtotal or partial villous atrophy, after which induction of a GFD should result in histological recovery and disappearance of gastrointestinal symptoms (Trier 1991, Mäki&Collin 1997).

5.1.2 Dermatitis herpetiformis

Dermatitis herpetiformis (DH) is a life-long blistering skin disease with predilection sites on elbows, knees and buttocks. All patients have pathognomonic granular IgA deposits in the skin (van der Meer 1969) and also in the oral mucosa (Hietanen&Reunala 1984). A very close relation exits between DH and CD, all patients with DH have at least subclinical or latent CD in the small intestine, and the rash also responds to a GFD. Patients with DH may also present gastrointestinal symptoms typical of CD. Several findings indicate that DH is a cutaneous phenotype of CD (Hervonen et al 2000, Reunala 2001, Fry 2002).

The prevalence rate of DH in Finland has been reported 1:1500, which is a quarter of that of coeliac disease (Collin et al 1997). Men slightly predominate among Finnish patients with DH, unlike in CD (Collin 1996, Mäki&Collin 1997). The common age at the onset of DH is 30-40 years, but it can appear at any age (Reunala 2001).

The formation of clinically visible blisters in DH starts with the formation of neutrophil micro-abscesses at the summits of dermal papillae. These are quickly transformed by oedema into micro-vesicles which then coalescence to form a unilocular subepidermal bulla. The whole process takes about 24 hours. The split occurs in lamina lucida (Klein et al 1983). DH blister fluid contains increased collagenase-, gelatinase-, and elastase-like enzymatic activity (Oikarinen et al 1986). Oral KI aggravates DH, and KI applied to the uninvolved skin will produce clinical lesions similar to those of DH (Haffenden et al 1980). By using a KI application method, Airola et al (1997) showed that the initial event in blister formation is early actication of urokinase plasminogen activator with subsequent activation of various metalloproteases and disruption of the basement membrane. The actication of some of these enzymes occurred already before any influx of lymphocytes or polymorphonuclear leucocytes was seen in the dermis (Airola et al 1997).

5.1.3 Jejunal findings

In untreated CD the characteristic abnormalities in the small-intestinal mucosae are villous atrophy, crypt hyperplasia and an increased density of inflammatory cells in the epithelium and lamina propria (Trier 1991). The mucosal lesion heals with a gluten-free diet and deteriorates further if the patient resumes a gluten-containing diet (Mäki&Collin 1997).

Increased density of lymphocytes bearing the γ/δ T cell receptor (TcR) is present in the jejunal mucosa, and this seems to be a constant marker for untreated CD and DH (Halstensen et al 1989, Holm et al 1992, Savilahti et al 1992). In GFD treated CD patients a decrease occurs in the density of the γ/δ T cells, but the level, however, remains elevated although the mucosae are macroscopically recovered (Iltanen et al 1999, Järvinen et al 2003). The density of jejunal α/β TcR+ lymphocytes is also increased in untreated patients with CD, but their numbers decrease to normal levels after the initiation of a GFD (Järvinen 2003).

Lymphocytes bearing γ/δ TcR are found, particularly in the jejunal epithelium, whereas α/β TcR+ lymphocytes are found both in the jejunal epithelium and the lamina propria.

The term latent CD is applied to patients who have been shown to have a normal villous architecture on a normal gluten-containing diet, but show increased intraepithelial γ/δ T cell counts and/or IgA EmA or TGA levels in the serum, and who later on develop villous atrophy. Patients with subclinical CD present no symptoms. Increased γ/δ T cell counts together with intestinal fluid IgA AGA also seem to be markers for latent CD (Holm et al 1992).

It has been suggested that γ/δ TcR+ lymphocytes may have a special function in protecting mucosal surfaces by eliminating infected or otherwise damaged epithelial cells (Janeway et al 1988). These cells have the ability to recognize non-peptidic molecules commonly associated with micro-organisms and stressed cells (Boismenu&Havran 1997). The γ/δ TcR+ lymphocytes may also play a role in various inflammatory and autoimmune conditions. These cells can secrete lymphokines and express cytolytic activity in response to antigenic stimulation. A consistent feature of the γ/δ TcR+ lymphocytes is a rapid and transient release of bioactive polypeptides such as interferon-gamma (Ferrick et al 1995). In the normal human oral epithelia the γ/δ TcR+ lymphocytes are rare (Pepin et al 1993).

5.1.4 Serological findings

Antigliadin antibodies

The jejunal mucosa of patients with untreated CD is always heavily infiltrated with plasma cells containing and producing IgA (Rubin et al 1965), most of which seem to be IgA AGA (Falchuk&Strober 1974). The number of IgA-containing cells falls rapidly when a GFD is started and becomes normal within a few months both in CD and DH (Reunala 1978). At the same time IgA AGA levels decrease in the serum and then rise again when a gluten challenge is performed (Vainio et al 1985). Methods of measuring IgA AGA include immunofluorescence (Unsworth et al 1981), enzyme-linked immunosorbent assay (ELISA)

(Vainio et al 1983), diffusion-in-gel ELISA (Kilander et al 1983) and solid-phase radioimmunoassay (Ciclitira et al 1985).

The median sensitivity of IgA AGA in screening of CD is 87% and the specificity somewhat lower, 83% (Table 1). AGA and especially IgG AGA are also found in other gastrointestinal diseases and in disorders such as rheumatoid arthritis and atopic dermatitis (Collin et al 2002). They may also occur in healthy individuals not carrying the risk of HLA DQA/DQB alleles for CD (Mäki&Collin 1997). IgG-class AGA detects CD when the patient has selective IgA deficiency, the frequency of which is 2-3% in CD (Savilahti et al 1971, Kokkonen et al 1982, Korponay-Szabo et al 2003).

IgA AGA measured by in-house ELISA had a high negative predictive value (99%) in one study and it was suggested as a suitable first-line test to exclude CD (Bowron et al 2000). When IgA AGA is positive, a suitable confirming test is a some more specific test, i.e. measurement of IgA EmA or TGA. This step-wise approach to screening for CD was shown to yield cost savings in one recent study (Bowron et al 2000).

Table 1. Sensitivities and specificities of various serological tests in the screening of coeliac disease.

IgA antibody test	Sensitivity %		Specificity %	
	Median	Range	Median	Range
Antigliadin antibody ELISA/EIA	87	31-100	83	82-100
Endomysial antibody Indirect immunofluorescence	93	85-100	100	95-100
Transglutaminase antibody ELISA	95	92-98	95	94-99

Data from seven studies (Collin et al 2002)

Endomysium and transglutaminase antibodies

IgG-class reticulin antibodies were first described in sera of patients with CD using an indirect immunofluorescence method. Thereafter, IgA-class reticulin antibodies were shown to be more specific in the screening of CD (Hällström 1989). Due to a rather low sensitivity, the IgA-class reticulin antibody measurements are now considered to be of limited value in the diagnosis of CD (Collin et al 1994).

The IgA-class endomysial antibody test (EmA) was introduced by Chorzelski et al (1984). This indirect immunofluorescene test using monkey oesophagus and then human umbilical cord as substrate was shown to be more sensitive and specific than the previous reticulin antibody test in the screening and diagnosis of CD (Table 1). Recently, it was shown that the endomysial antigen binding IgA antibodies from the serum of patients with CD was tissue type transglutaminase which occurred also in human tissues (Dieterich et al 1997, Schuppan 2000). Thereafter, an ELISA test was developed to measure these antibodies in the sera of patients with CD and DH (Dieterich et al 1998, Sulkanen et al 1998, Dieterich et al 1999). The IgA TGA ELISA test using recombinant tissue transglutaminase is both sensitive and specific and is now widely used in the screening and diagnosis of CD (Johnston et al 2003, Table 1). In addition, the efficacy of GFD treatment can be monitored by measuring IgA TGA (Dieterich et al 1998, Sulkanen et al 1998, Dieterich et al 1999). The antibody levels decrease when the jejunal mucosa heals on a GFD, and continuously positive results often indicate faults on the dietary treatment.

5.1.5 Treatment

Gluten-free diet in coeliac disease

The treatment of CD is a life-long GFD (Trier 1991, Mäki&Collin 1997). Wheat gluten can be divided into alcohol-soluble fractions, prolamins, and non-soluble fractions. Prolamins in wheat are called gliadins**,** which can be further divided into alpha, beta, gamma and omega fractions according to electrophoretic mobility. Prolamins in rye are called secalins, in barley hordeins and in oats avenins. Oats, however, has been reported to be tolerated by patients with

CD without any harm to the small intestine and is now generally considered safe for these patients. It seems that only 60-80% of patients with CD maintain a strict GFD (Collin et al 1996). Occasional intake of gluten may not necessarily produce gastrointestinal symptoms, but continuously eating small amounts of gluten seems to be a reason for mucosal inflammation with increased numbers of intraepithelial lymphocytes and increased levels of IgA AGA, EmA and TGA (Mayer et al 1991, Collin et al 1996).

Gluten-free diet and dapsone in dermatitis herpetiformis

The rash in patients with DH can be controlled by Dapsone and a GFD (Fry et al 1973, Reunala et al 1977, Garioch et al 1994). It takes several weeks or months for the rash to respond to the diet (Garioch) and therefore the initial treatment suitable for most patients is to start with a combination of dapsone and GFD. Dapsone produces rapid relief of itching, and the rash subsides within a few days. In contrast to GFD, it has no effect on the enteropathy, nor on the cutanous IgA deposits**.** Most patients with DH require 50 to 100mg of dapsone daily to control the rash (Fry et al 1973, Reunala et al 1977, Garioch et al 1994). The majority of patients tolerate dapsone well, but there are risks for dose-related haemotological side-effects. Dapsone 100mg daily or higher doses may cause hemolysis leading to a fall in haemoglobin and most patients have a small degree of methaemoglobinaemia (Leonard&Fry 1992). Sensory or motor neuropathies may appear after high-dose, long-term dapsone treatment, and the most severe side-effect, although very rare, is agranulocytosis, which usually appears within the first few months of treatment (Reunala 2001).

GFD is the treatment of choice for all patients with DH because both the rash and enteropathy improve (Fry et al 1973, Reunala et al 1977, Garioch et al 1994). The rash responds to GFD regardless of whether the patient has flat or normal appearing jejunal mucosa (Reunala 1978). In a few patients with DH the rash is very sensitive to minor amounts of eaten gluten, and flare-up the rash is seen especially at the onset of GFD treatment. About 90% of the Finnish patients with DH adhere well to a GFD and half of them can stop using dapsone within 2-3 years (Reunala et al 1977, Collin et al 1996). Similarly, in British patients it takes a mean of 2 years for cessation of dapsone therapy (Gawrodger et al 1984). The rash in patients with DH

is not activated by eating oats, and this cereal is now considered safe for the patiens (Hardman et al 1997, Reunala 2001).

5.1.6 Pathomechanisms

Autoimmunity in coeliac disease

It is widely accepted that the mucosal damage in CD is caused by an immunological mechanism. Both mucosal cellular (Trier 1991) and humoral (Sollid et al 1997) immune systems are activated, and the gliadin fraction of ingested gluten seem to be the trigger. Serum IgA antibodies recognize enzyme tissue transglutaminase (TGA2), which can be found in activated endothelial, fibroblast and mononuclear cells (Dieterich et al 1997). Tissue transglutaminase has an important role in cell homeostasis and regulates cell proliferation, differentiation and apoptosis (Shan et al 2002). Gliadin acts as a substrate for tissue transglutaminase (Dieterich 1999, Ciccocioppo et al 2003). Gliadin-specific HLA DQ2 and DQ8 restricted T cells can be found in the small-intestinal mucosal lesions (Lundin et al 1993). Antigen-presenting cells in the lamina propria present gluten peptides to CD4+ T cells via their HLA DQ molecules. Tissue transglutaminase modifies gliadin peptides through deamidation of glutamine residues to negatively charged glutamic acid, which facilitates the binding of gliadin peptides to the peptic groove of HLA DQ2 and DQ8 molecules resulting in increased T cell reactivity (Molberg et al 1998). Activated T cells induce a local inflammatory response that may continue as long as gliadin is present. Stimulated T cells secrete Th 1 cytokines such as TNFα and γ- interferon, which can further damage the small-bowel mucosa and lead to enteropathy (Sollid et al 1997, Nielsen et al 1998). TNFα triggers intestinal fibroblasts to secrete matrix metalloproteinases (MMPs), leading to mucosal destruction by dissolution of connective tissue. IL-15 increases the number of intraepithelial γ/δ TcR+ and CD 94+ cells only in coeliacs. Th 2 response at the intestinal level results in the formation of autoantibodies (Picarelli et al 1996), which may also have a direct role in the pathogenesis of small-intestinal mucosal damage.

The coexistence of autoimmune diseases and CD might be the result of molecular mimicry, by which gliadin or TG2 activates T cells that are cross-reactive with various self antigens in genetically susceptible hosts, and lead to chronic organ-specific autoimmune disease via epitope spreading. Tissue transglutaminase might also generate neoantigens by cross-linking or deamidation of other external or self-antigens (Schuppan 2000).

IgA in the skin in dermatitis herpetiformis

Granular IgA deposits in dermal papillae are characteristic for DH (van der Meer 1969). They are deposited throughout the skin, but greater amounts seem to be present together with complement (C3) near the active lesions (Zone et al 1996). IgA deposits have been localized in close association with microfibrillar bundles of elastic fibres in the papillary dermis and in the dermoepithelial junction below the basal lamina. The exact site of interaction between IgA and specified structure in DH skin is unknown. No circulating IgA autoantibodies against any dermal components in the sera of patients with DH have been found, which is in sharp contrast to findings in linear IgA disease. Recently Sardy et al (2002) showed that IgA deposits colocalize with epidermal transglutaminase (TG3) in patients with DH and suggested that this might be the autoantigen in DH skin.

Efforts to isolate functional IgA from DH skin have failed. IgA1 is the dominant subclass and either minimal or no deposits of IgA2 are found (Hall&Lawley 1985). The findings suggest that the IgA in DH skin may not arise from a gastrointestinal source as previously thought. After several months on GFD treatment IgA and C3 deposits decrease and may disappear, and IgA has been shown to return with the start of gluten-containing diet (Reunala 1978, Leonard et al 1983). This has led to a hypothesis that circulating immune complexes originating from the gut and composed of IgA antibodies attached to antigen, possibly gluten, could be deposited into DH skin. Circulating IgA immune complexes can be detected in the sera of patients with DH, and increased levels have been found after wheat ingestion. IgA immune complexes, however, occur also in the sera of patients with coeliac disease who have neither skin disease nor cutaneous IgA deposits. It has been suggested, that excess IgA tissue transglutaminase antibodies could be deposited into the skin due to cross-reactivity with cutaneous transglutaminases. Tissue transglutaminase is also involved in the intermolecular

closs-linking of type VII collagen that occurs near the areas where IgA depostis in DH skin are found (Raghunath et al 1996, Sardy et al 2002). Whether there is a specific dermal autoantigen in DH skin related to tissue transglutaminase is not yet known.

5.1.7 Genetic background

The occurrence of CD and DH in first-degree relatives ranges between 5% and 15% (Holm et al 1992). The coexistence of CD in monozygotic twins has been reported to be 80% (Polanco et al 1991, Hervonen et al 2000). Approximately 95% of patients with CD and DH share the HLA DR3- DQ2 haplotype, coded by the DQA1*0501 and DQB1*0201 alleles in the short arm of chromosome six (Sollid et al 1989, Polvi et al 1996). Most of the remainder express the DR4-DQ8 haplotype encoded by DQA1*0301, DQB1*0302 alleles (Polvi et al 1996).

The prevalence of HLA DQ2 is 20-30% in the normal population (Sollid et al 1989, Polvi et al 1996) and, therefore, only a minority of people with this HLA phenotype will develope CD. This suggests that additional, probably non-HLA-linked genes are involved in the pathogenesis of CD. Genome-wide screening studies have resulted in a number of proposals for candidate non-HLA gene regions. So far, no gene or gene region has been found outside HLA DQ, and any such additional genes are likely to have a moderate or minor effect on the pathogenesis of CD (Liu et al 2002).

5.1.8 Associated diseases

A close association exists between coeliac disease and Sjögren's syndrome. In a Finnish study, 3.3% of the patients with CD had associated Sjögren's syndrome, whereas the frequency was 0.3% in the disease controls (Collin et al 1994). On the other hand, 14.7% of the patients with SS were found to have CD in a recent Finnish study (Iltanen et al 1999). Testing serum anti TGA has been shown to be a reliable screening method to find coexisting CD in patients with SS (Luft et al 2003).

CD occurs in 2-5% of patients with type I diabetes or autoimmune thyroid disease and *vice versa* (Collin et al 2002, Hervonen et al 2004). Patients with multiple endocrine disorders and Addison's disease may also have concomitant CD. CD, SS and type I diabetes are associated with HLA DR3 and this genentic link is likely to contribute to the concomitant occurrence of these autoimmune disorders. GFD may be beneficial in the treatment of the associated endocrinological diseases in CD (Collin et al 2002).

Patients with CD and DH are known to be at risk of contracting lymphoma (Collin et al 1996). The most established lymphoma associated with CD is enteropathy-type intestinal T cell lymphoma. This rare lymphoma is often complicated by perforation of the small intestine, and the outcome is poor. B-cell lymphoma may also occur in CD. The precise frequency of malignancy in CD is unknown, but it has been estimated that for all lymphoma the prevalence is 0-7% in patients with untreated CD. GFD treatment has been shown to protect against lymphoma both in CD and DH (Holmes et al 1989).

Bone mass density has been shown to be significantly lower in patients with untreated CD compared to CD patients treated with GFD or controls (Corazza et al 1995). Osteopenia has also been reported in patients with DH (Di Stefano et al 1999). Untreated CD is characterized by low serum calcium and increased levels of 1,25 -vitamin D and secondary hyperparathyroidism which causes increased bone turnover (Corazza et al 1995,Valdimarsson et al 2000). Untreated patients with CD seem to be at higher risk for bone fractures than other people (Vasquez et al 2000). Prospective studies in GFD-treated patients with CD have shown that the dietary treatment improves bone mass densitity (Kemppainen et al 1999).

5.2 Oral findings in coeliac disease and dermatitis herpetiformis

5.2.1 Oral lesions

Recurrent aphthous ulceration (RAU) is one of the most common lesions of the oral mucosa, affecting about 20% of the general population at some time (Axell&Hendricsson 1985). Its cumulative prevalence varies from 5 to 66% of the population depending on the group studied

(Miller&Ship 1977). There are several reports in the medical literature of RAU being a common feature in patients with CD. Ferguson et al (1976) published a study dealing with jejunal mucosal abnormalities in patients with RAU, and the improvement in the oral lesions when treated with a GFD. Of the 33 patients with RAU, 24% were found to have CD. In other studies the prevalence of CD in patients with RAU has varied from 0 to 40% (O'Farelly et al 1991), but most authors consider the prevalence to be 2-5%. Interestingly, RAU without the intestinal lesion typical of CD might also respond to a GFD (Walker et al 1980, Wray 1981, O'Farelly et al 1991). In earlier reports glossitis has been reported in up to 90% of patients with untreated CD (Cooke et al 1953). Cheilitis and angular stomatitis are considered by most authors to support a diagnosis of malnutrition and deficiencies of iron, folic acid and/or vitamin B12 (Barry et al 1974, Wray et al 1978), all of which are also typical signs of CD. The prevalence of cheilitis and angular stomatitis in CD varies in different studies between 4 and 50% (Wray et al 1978). A proper dietary treatment with a GFD frequently leads to remission of the oral lesions (Barry et al 1974, Ferguson et al 1976).

There is a certain discrepancy in opinions as to the prevalence of oral lesions in DH. Oral mucosal involvement is considered rare by most authors (Rusotto&Ship 1971, Munston&Hodgson 1973, Economopoulou&Laskaris 1986) and is seen in less than 10% of patients with DH. In contrast, Frazer et al (1973) reported oral mucosal lesions in ten of 15 patients with DH. The oral lesions seen were of four main types: erythematous, vesicular, purpuric and erosive. The patient group was small and therefore the conclusion on the prevalence of oral lesions in two thirds of the patients with DH should be interpreted with caution. Both keratinized and non-keratinized oral mucosa can be affected in DH. Lesions have been detected on the lips, anterior fauces, hard and soft palate, upper alveolar ridge and buccal mucosa. Uvula and tonsillae rarely show lesions in DH (Fraser et al 1973, Economopoulou&Laskaris 1986). The precence of lesions in tonque also seems to be uncommon (Rusotto&Ship 1971, Munston&Hodgson 1973, Fraser et al 1973). Nowadays agreement exists that, if present, oral DH lesions are small vesicles which soon rupture, leaving fibrine-covered, superficial erosions resembling aphthae. Hyperkeratotic areas and lesions resembling oral lichen planus might also occur. DH is now distinguished from linear IgA disease, which is not linked with gluten-sensitive enteropathy. In this rare disease oral mucosal involvement is apparently more common and consists of often large mucosal

erosions (Chan et al 1990). In immunofluorescence examination the subepithelial IgA deposists are granular in DH, whereas in linear IgA disease they occur along the basement membrane zone in linear fashion (Rantala et al 1985).

5.2.2 Salivary findings

In addition to being important in speaking and swallowing, saliva is part of the defence system of the human body. Oral mucosae are in continuous contact with different micro-organisms and food. Mechanical rinsing of the oral mucosa by saliva moves foreign bodies towards strongly acidic stomach. A sufficient amount of saliva is necessary for the protection against oral diseases. Saliva has many antimicrobial properties. These can be classified as non-immunological (lysozyme, lactoferrin, histatins and salivary peroxidase systems) or immunological which consist e.g immunoglobulins (Tenovuo 1989).

Salivary flow rate

The average unstimulated flow rate over a waking period of 16 hours is about 0.3ml/min and the total volume will be about 300 ml of saliva. During sleep, the maximum flow will fall to less than 0.1 ml/min, thus producing less than 40 ml of saliva in 7 hours. Studies with various foods suggest that during eating the average stimulated flow rate is about 4 ml/min, which equal to about 200 ml of saliva per day produced during meals. The total daily flow of saliva amounts about 500-600 ml, which is much less than the 1500 ml/24 hours quoted in many textbooks (Edgar&O'Mullane 1996). Approximately 45% of this is produced by the parotid and 45% by submandibular glands, 5% by sublingual glands and another 5% by oral mucosal minor salivary glands (Mandel&Wotman 1976). Besides glandular secretions, human "whole saliva" or "mixed saliva" consists of gingival crevicular fluid, oral microbes and epithelial cells (Tenovuo 1989). Normal resting saliva and stimulated salivary secretion rates are shown in table 2. Several diseases affecting salivary glands, such as Sjögren's syndrome, are associated with diminished salivary flow rate (Syrjänen 1982, Atkinson 1990)

Table 2. Classification of salivary secretion rate (ml/min) according to the reference intervals of Ericsson and Hardwick (1978) for resting and paraffin-stimulated whole saliva

	Very low	Low	Normal
Resting saliva	<0.1	0.1 – 0.25	0.25 – 0.35 (mean: 0.30)
Stimulated saliva	<0.7	0.7 – 1	1 – 3 (mean 1.5)

Edgar&O'Mullane 1996

Salivary immunoglobulins

The immunoglobulins in the whole saliva are mostly IgA class, but also immunoglobulins G, M, D and E classes also are present in much lower concentrations (Brandtzaeg 1989). The most abundant immunoglobulin in saliva is dimeric secretory IgA (sIgA), which is produced by local plasma cells in the major and minor salivary glands (Mestecky et al 1986). In the blood, IgA exists primarily as monomer, whereas in secretions IgA is composed of a 300 kDA IgA dimer, a 70 kDA secretory component and a 15 kDA J -chain (Brandtzaeg 1989). Immunofluorescence studies (Korsrud&Brandtzaeg 1982, Moro et al 1984) have localized IgA containing plasma cells in salivary glands in the connective tissue around intercalated or intralobular ducts, as well as around acini. J chain is usually found within IgA secreting plasma cells as well (Moro et al 1984). The secretory component has been localized within epithelial cells of the intercalated and intralobular ducts (Korsrud&Brandtzaeg 1982, Moro et al 1984). Secretory IgA is remarkably stable and therefore well suited to function in protease-containing external secretions such as whole saliva (Brandtzaeg 1989).

Two IgA subclasses are present in saliva. IgA1 forms the major component, IgA2 a minor component though its concentration is higher in saliva than in other secretions (Tappuni&Challacombe 1994). Salivary IgA is absent at birth, but is readily detectable in infants at the age of one week (Cole et al 1998). The IgG is the only detectable immunoglobulin in the saliva of a newborn, but the concentration decreases to non-detectable levels after some months and appears again after tooth eruption (Brandtzaeg 1989). Low concentrations of IgG can be detected in the stimulated parotid saliva, but most of the IgG

detected in whole saliva enters the mouth from the gingival crevicular fluid, and to it from serum (Brandtzaeg 1989). Inversely, only 1.6% - 4% of the total salivary IgA is reported to leak from serum into saliva (Strober et al 1970, Delacroix et al 1982).

IgA1 subclass antibodies have been found to *Streptococcus mutans, S sanguis and S mitior*, all important pathogens of dental caries, but also to *Bacteroides* and *Capnocytophaga* species, which are involved in periodontal diseases. Parotid antibodies to *Bacteroides fragilis, B gingivalis* and *Eschericia coli* are mainly of the IgA2 subclass (Brown&Mestecky 1985).

McClelland et al and Wallington et al (1972) suggested that elevated salivary IgA levels observed in CD and DH derive from the influx of IgA secreting B cells from the intestine. Al-Bayaty et al (1989) and Hakeem et al (1992) found significantly increased salivary IgA AGA levels in untreated children and adults with CD. Due to this they suggested that salivary IgA AGA measurements could be used as a non-invasive screening test for CD. Kelly et al (1991) and O'Mahony et al (1991) also found salivary IgA AGA in their patients with CD, but the levels in saliva did not correlate with the serum levels. No studies on salivary IgA AGA in the patiens with DH were published.

5.2.3 Dental findings

In 1958, Lindemann described enamel defects in children with non-specific diarrhoea, and thereafter several case reports of enamel defects in patients with CD were published (Smith&Miller 1979, Rasmussen&Espelid 1980, Nikiforuk&Fraser 1981). Andersson-Wenckert et al (1984) reported severe enamel hypoplasia in children who suffered from CD. Aine (1986) presented a new classification for permanent-tooth enamel defects in CD. They consisted of four grades: Grade I, defect in colour of enamel; grade II, slight structural defects with typical horizontal grooves; grade III, evident structural defects with deep horizontal grooves and large vertical pits; and grade IV, severe structural defects where the shape of the tooth may be changed. As many as 96% of the children (Aine 1986) and 83% of adults with CD were found to have systematically occurring enamel defects (Aine 1996). The frequency was 80% in children with DH and 53% in adults with DH (Aine et al 1992, Aine 1996). This enamel finding in DH gave further evidence that subclinical CD has an important effect on

the pathophysiology of DH, as enamel is formed in early childhood and DH is often diagnosed at a later age. Dental enamel defects were shown to correlate with the small-intestinal mucosal damage and the presence of HLA-DR3 by Mäki et al (1991). Lower frequencies of coeliac-type enamel defects in CD than in Finland have been reported in Spain (53%) and Italy (24%) (Aguirre et al 1997, Rea et al 1997). Overall, coeliac-type dental enamel defects have led to a diagnosis of CD in otherwise asymptomatic patients (Aine 1996).

The frequency of dental caries when compared with that of a control group has been reported to be lower in the patients with CD (Andersson-Wenckert et al 1984) or equivalent (Fulstow 1979, McLoughlin et al 1980).

5.3 Sjögren's syndrome

5.3.1 Clinical features

Sjögren's syndrome (SS) has been defined as an autoimmune epithelitis characterised by lymphocytic infiltration of exocrine glands and epithelia in multiple sites. In addition to the involvement of the exocrine glands, it is also characterized by clinically important complications, general symptoms and visceral changes. The involvement of lacrimal and salivary glands results in the typical features of dry eyes and dry mouth (sialopenia and xerostomia), the so-called sicca complex. Diminished tear fluid secretion causes a sandy sensation in the eyes, burning, redness, photosensitivity, eye fatigue, itching and disturbances of vision. Parotid gland enlargement occurs in half of the SS patients. Important complications include caries and oral candidosis. Although keratoconjunctivitis sicca and stomatitis sicca are emphasized in the current diagnostic criteria, all other exocrine glands are also involved. This leads to rhinitis sicca (crust building and nose bleeding), pharyngitis sicca (dysphagia, hoarse voice), dry trachea (respiratory tract infections) and vaginitis sicca (dyspareunia). Most of the patients also have systemic extraglandular manifestations. These include general symptoms, the most common of which are fatigue, joint and muscle pain and Raynaud's phenomenon. Visceral symptoms often have an autoimmune character and include

Hashimoto's autoimmune thyreoiditis, various forms of interstitial pneumonias, chronic atrophic gastritis, primary biliary cirrhosis and autoimmune hepatitis, renal tubular acidosis, interstitial cystitis, vasculitic skin changes and neurological manifestations from the peripheral and central nervous system. Polyarthralgia is reported in 84% of the patients and synovitis up to 77%, which is why these patients are often taken care of by rheumatologists. In addition, these patients have an increased risk of developing a mucosal-associated lymphatic tissue (MALT) derived marginal zone lymphoma. Pregnancies in SS-A/Ro and/or SS-B/La positive mothers can be complicated by neonatal lupus and/or cardiac conduction abnormalities, which are caused by the transplacental passage of the autoantibodies from the mother to the child.

SS can be seen alone (primary SS) or in association with other autoimmune rheumatic disease (secondary SS) (Moutsopoulos 1994). The diagnostic approach to SS is rather complicated because it must include two different goals: firstly, assessment of the ocular and salivary components and demonstration of autoimmune features, and secondly, differentiation between the primary and secondary variants of the syndrome. Like most of the rheumatic diseases, SS lack a single distinguishing feature, however, and the disease is diagnosed based on the presence of a combination of clinical and laboratory manifestations.

Different classification criteria sets have been suggested for SS, both before and during the First International Symposium on Sjögren's Syndrome held in Copenhagen in 1986, but none of these had been validated and universally accepted. In addition to the Copenhagen criteria, also Greek, Californian and Japanese criteria were presented. The first validated criteria were created and tested in a European Union supported project (Vitali et al 1993). Later these EU criteria have been extended to create the American-European consensus criteria (Vitali et al 2002) which are compiled in Table 3. The American-European Group also reached a consensus on a list of exclusion criteria (Table 4). This list is quite similar to the so-called Californian criteria and the exclusions in the Californian criteria were also adopted by the European Study Group in its preliminary criteria set (Vitali et al 1993). Some modifications were made to the earlier list: (*a*) the category of "anticholinergic" drugs was used instead of "antidepressant, antihypertensive, parasympatholytic drugs and neuroleptic agents"; (*b*) "past head and neck radiation treatment" was added as an exclusion criterion; (*c*) sialoadenosis was

deleted. Hepatitis C virus (HCV) infection was added as an exclusion criterion, taking into account most of the data emerging from the current literature.

The etiology of SS is unknown, but recent years have brought a clearer understanding of its pathogenesis (Pertovaara 2001). In an appropriate genetic background, ie. HLA DR3-DQ2, an unknown trigger factor, possibly viral, leads to an immunologically mediated inflammatory mechanisms which harms exocrinic glands and their function. Viruses can indeed lead to very similar conditions, which is also the reason for the fact that the currently valid American-European criteria include human immunodeficiency virus (HIV) and HCV among the exclusion criteria. T-cell mediated autoimmune responses in the glandular tissue are considered of major importance in the pathogenesis of SS (Pertovaara 2001). The role of Sjögren specific circulatory anti-SS-A and anti-SS-B antibodies in the inflammatory or autoimmune process is not at present solved. The current speculations on the pathogenesis do not yet explain the female dominance (90 %), the age of onset (usually 40-50 years) and targeting of the exocrine glands.

Table 3. American-European Consensus Group classification criteria for Sjögren's syndrome (Vitali et al 2002)

I. Ocular symptoms: a positive response to at least one of the following questions:
1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?
2. Do you have a recurrent sensation of sand or gravel in the eyes?
3. Do you use tear substitutes more than 3 times a day?

II. Oral symptoms: a positive response to at least one of the following questions:
1. Have you had daily feeling of dry mouth for more than 3 months?
2. Have you had recurrent or persistently swollen salivary glands as an adult?
3. Do you frequently drink liquids to aid in swallowing dry food?

III. Ocular signs – that is, objective evidence of ocular involvement defined as positive result for at least one of the following two tests:

1. Schirmer's I test, performed without anaesthesia ($\leq$ 5mm in 5 minutes)
2. Rose bengal score or other ocular dye score ($\geq$ 4 according to van Bijsterveld's scoring system)

IV. Histopathology: In minor salivary glands (obtained through normal-appearing mucosa) focal lymphocytic sialoadenitis evaluated by an expert histopathologist, with a focus score $\geq$ 1, defined as lymphocytic foci (which are adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes) per 4mm^2 of glandular tissue.

V. Salivary gland involvement: objective evidence of salivary gland involvement defined as positive result for at least one of the following diagnostic tests:

1. Unstimulated salivary flow ($\leq$ 1.5ml in 15 minutes)
2. Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitary or destructive pattern), without evidence of obstruction in the major ducts.
3. Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer.

VI. Autoantibodies: presence in the serum of the following antibodies:
1. Antibodies to Ro(SSA) or La(SSB) antigens, or both.

Table 4. American-European rules for classification and exclusion criteria of Sjögren's syndrome (Vitali et al 2002)

For primary SS
In patients without any potentially associated disease, primary SS may be defined as follows:

1. The presence of any 4 of the 6 items as indicative of primary SS, as long as either item IV (Histopathology) or VI (Serology) is positive.
2. The presence of any 3 of the 4 objective criteria items (that is, items III, IV, V and VI)
3. The classification tree procedure represents a valid alternative method for classification , although it should be more properly used in clinical-epidemiological surveys.

For secondary SS
In patients with a potentially associated disease (for instance, another well defined connective tissue disease), the presence of item I or item II plus any 2 among items III, IV and V may be considered as indicative of secondary SS.

Exclusion criteria:

Past head and neck radiation treatment
Hepatitis C infection
Acquired immunodeficiency disease (AIDS)
Pre-existing lymphoma
Sarcoidosis
Graft versus host disease
Use of anticolinergic drugs (since a time shorter than 4 –fold the half life of the drug)

5.3.2 Oral findings

Salivary insufficiency may cause difficulty in chewing, swallowing and phonation, adherence of food to buccal surfaces, abnormalities in taste or smell, loss of filiform papillae, fissures on tongue, buccal membranes and lips, need for frequent ingestion of liquids and worsening of dental caries, but also a chronic *Candida* infection (Najera et al 1997, Konttinen et al 1997, Pertovaara et al 2001). The patients with SS have shown a higher frequency of oral mucosal changes, higher decayed, missing and filled surface (DMFS) scores, increased alveolar bone loss and higher risk of having adult periodontitis than healthy controls (Najera et al 1997, Pedersen et al 1999).

6. AIMS OF THE PRESENT STUDY

1. To study the occurrence of antigliadin antibodies in the saliva in patients with DH and to examine whether these antibodies could be used for screening of gluten sensitive enteropathy.
2. To examine whether γ/δ T cell receptor bearing lymphocytes occur in the oral mucosa in patients with DH in similar high numbers as in the jejunal mucosa.
3. To investigate the inflammatory reaction caused by potassium iodine and gliadin application on the oral mucosa in patients with DH.
4. To study whether patients suffering concomitantly from CD and SS have a higher frequency of oral mucosal, dental and salivary abnormalities than patients with either disorder alone.

7. MATERIALS AND METHODS

7.1 Patients and controls

A total of 32 patients with DH (19 untreated, 13 GFD treated) and 20 CD patients with associated SS, together with 20 case controls with CD and 20 with SS, were examined in the four different study protocols.

Table 5. Patients with dermatitis herpetiformis and coeliac disease in the present studies (I-IV)

Study design	Patients	N Sex (m/f)	Mean age (range) years	Controls	N Sex (m/f)	Mean age (range) years
I Measurement of salivary and serum IgA-AGA	1. Untreated patients with DH 2. GFD treated patients with DH	10 (7/3) 10 (8/2)	53 (24-75) 41 (23-75)	Non-disease controls (hospital staff)	20 (3/17)	44 (29-58)
II Occurrence of intraepithelial γ/ δ TcR+ lymphocytes in oral mucosa	1. Untreated patients with DH 2. GFD treated patients with DH	13 (9/4) 13 (9/4)	48 (24-75) 40 (23-61)	Non-disease controls (hospital staff)	9 (3/6)	41 (25-52)
III Potassium iodine and gliadin challenge of oral mucosa	Dapsone treated patients with DH on a normal diet	6 (5/1)	49 (35-75)	Non-disease controls (hospital staff)	2 1/1	39 (37-42)
IV Occurrence of oral findings in CD patients associated with Sjögren's syndrome	Patients with CD*+SS	20 (1/19)	61 (48-78)	1. Case controls with CD* 2. Case controls with SS	20 (1/19) 20 (1/19)	61 (50-80) 62 (47-79)

* three patients with concomitant dermatitis herpetiformis

7.1.1 Dermatitis herpetiformis (I-III)

All patients with DH were from a special out-patient clinic in the Hospital for Skin and Allergic diseases, Helsinki University Central Hospital. The diagnosis of DH was based on showing IgA deposits in the skin by direct immunofluorescence as previously described (Hietanen&Reunala 1984). After the diagnosis the patients received dapsone (25-50mg/day) if the rash was active, and stared GFD treatment as previously described (Reunala et al 1977).

Study I. Salivary and serum IgA AGA were measured in 10 newly diagnosed DH patients at the onset of GFD treatment and after the patients had been three months on a GFD. Samples were also obtained from 10 patients with DH, who had been on long-term GFD treatment (mean 33 months, range 3-156 months). One of these patients had failed to follow a strict GFD and was excluded from the study. Twenty healthy members of hospital staff served as controls.

Study II. Oral mucosal biopsies were taken from 13 consecutive, newly diagnosed patients with DH and from 13 patients with DH treated with GFD for a mean of 4.5 years (range 0.5 – 15 yr). Nine healthy members of hospital staff on a normal gluten-containing diet volunteered as controls. Control jejunal specimens with normal villous architecture were obtained from 11 patients with gastrointestinal symptoms on a normal gluten containing diet.

Study III. Six patients with DH volunteered for the potassium iodine and gliadin challenge study. All patients used dapsone (25-75mg/day) to control the rash. Three newly diagnosed patients were on a normal gluten-containing diet, and three had tried GFD, but at the time of the study had eaten some gluten every week. Five of the patients had increased IgA AGA and four showed IgA EmA (titer 10-1600) in the serum. Small-intestinal biopsy showed subtotal or partial villous atrophy in five and inflammatory changes in one patient. Dapsone was withdrawn 48 h before the study. Two healthy members of hospital staff served as controls.

7.1.2 Coeliac disease and Sjögren's syndrome (IV)

Study IV. The twenty patients with CD+SS, and the age and sex matched case controls with either CD or SS, were from the Department of Internal Medicine, Tampere University Hospital. The diagnosis of CD was based on the demonstration of subtotal or partial villous atrophy with crypt hyperplasia in small-intestinal mucosal biopsy. SS was diagnosed according to the American-European consensus group criteria (Vitali et al 2002). The mean duration of CD was 9.3 years and that of SS 8.2 years. Fourteen patients were on a strict GFD and six had occasional faults in the diet. Four patients used prednisone daily (daily dose below 10mg) for SS.

7. 2. Clinical investigations (IV)

7.2.1 Oral lesions

Oral mucosal abnormalities were recorded according to the WHO ICD-DA classification (WHO 1978) and were grouped topographically (Roed-Pedersen&Renstrup 1969). A thorough dental examination was performed and caries status recorded using the DMF index (WHO 1997).

7.2.2 Dental enamel defects

Coeliac-type dental enamel defects in the incisors were graded by a classification method described by Aine (1986).

7.3 Collection of saliva and IgA antigliadin antibody assay

7.3.1 Collection of saliva (I, IV)

Whole saliva samples were collected according to a method described by Heintze et al (1983). After at least 5 min rest, unstimulated whole saliva was collected for 5 min (study I). Then the salivary flow was stimulated by chewing paraffin (2g, melting point 42-44^{o}C, Orion Diagnostica, Espoo, Finland), and the stimulated whole saliva was collected for 5 min.

The salivary samples were incubated immediately at 56^{o}C for 30 min in order to inactivate proteolytic enzymes and complement. The samples were then centrifuged for 15 min at 3000 x g at room temperature. The supernatants were collected and stored at -40^{o}C. Serum samples were collected simultaneously and they were also stored at -40^{o}C.

7.3.2 Antigliadin antibody assay (I)

Serum IgA AGA was assayed using a solid phase enzyme immunometric assay obtained in the form of a commercial ELISA kit (Gluten IgA EIA; Pharmacia, Uppsala, Sweden). IgA AGA in the serum (diluted 1/200) is allowed to react with gliadin coated on the surface of a microplate well. The absorbance at 420 nm was measured using a microplate photometer and the value for each sample was compared with that of a reference serum (pooled patient sera).

Salivary IgA AGA was assayed similarly, except that a 10-fold volume of saliva (diluted 1/20) was used per assay, as the immunoglobulin concentration in saliva was generally lower than in serum. For every patient, the saliva and serum IgA AGA measurements were performed in the same ELISA runs. Salivary and serum IgG AGA were measured with a gluten IgG EIA assay kit (Pharmacia).

Salivary total IgA was measured using an immunoturbidometric method with an EPOS 5060 analyzer (Eppendorff, Hamburg, Germany). The anti-IgA antiserum was from DAKO (Glostrup, Denmark).

7.4 Potassium iodine and gliadin challenge on oral mucosa (III)

Fifty per cent potassium iodine in petrolatum and gliadin powder (30mg; Sigma Chemicals, St. Louis, MO, USA) moistened with physiological saline was applied in 8 mm diameter aluminium patch test chambers (Finnchamber, Epicon Ltd., Tuusula, Finland). These were fixed with occlusive bandage on buccal mucosa of the molar area just below linea alba. The chambers were removed after 12h and 4 mm punch biopsies were taken from the challenged and non-challenged (control) mucosa under 2% lidocaine with epinephrine.

7.5 Biopsies and immunohistochemical stainings (II-IV)

7.5.1 Oral and jejunal biopsies

Punch biopsies of 3 mm (II) and 4 mm (III) were taken under 0.5% lidocaine with epinephrine from the buccal mucosa of the molar region just below linea alba. A jejunal biopsy was taken under gastroscopy (Savilahti 1992). The biopsy specimens were snap-frozen in liquid nitrogen, embedded in a mounting media (OCT, Tissue Tek®; Miles, Elkhart, IN, USA) and stored at -70^{o}C before use.

7.5.2 Immunohistochemical stainings and cell counting

An avidin-biotin peroxidase method was used for staining of the cells as previously described by Savilahti (1992). Serial cryostat sections (4-5 μm) were cut, fixed in acetone for 20 min at 4^{o}C, then in chloroform for 20 min at 20^{o}C and finally washed three times in phosphate-buffered saline (PBS), pH 7.2. The buffer was removed, and the sections covered with a dilution of monoclonal antibodies in PBS for 1 h. After washing, endogenous peroxidase was blocked by incubation with 0.5% hydrogen peroxidase for 30 min. A Vectastain Elite ABC kit (PK-6102, Vector Laboratories, Burlington, CA, USA) was used for 30 min to show the binding of the monoclonal antibodies in accordance with the manufacturer's instructions. Counterstaining was performed with haematoxylin.

The monoclonal antibodies used were anti-Leu4 (dilution 1:400; Becton Dickinson, Mountain View, CA, USA) for CD3+ cells, OKT8 (dilution 1:20; Becton Dickinson) for CD8+ cells, T4 (dilution 1:20; Coulter Immunology, Hialeah, FL, USA), TcRδ1 (dilution 1:200; T cell Sciences, Cambridge, MA, USA) for γ/δ TcR+ cells and βF1 (dilution 1:100, T cell Sciences) for α/β TcR+ cells.

Positively stained cells were counted in the epithelium and lamina propria with a light microscope through a calibrated graticule at 1000 x magnification. At least 30 fields (0.09 x 0.16mm) were counted and the results were given as cells/mm^2. In the jejunal epithelium the cell numbers were given as cells/mm (Savilahti 1992).

7.5.3 Salivary gland biopsies and inflammatory focus score

An incision biopsy was taken from minor salivary glands in the lower lip of 20 patients with CD+SS, 20 case controls with CD and 20 case controls with SS (study IV) under local anaesthesia (lidocaine with 2% epinephrine). At least five minor salivary glands were harvested. An inflammatory focus score was calculated by counting from 5μm sections the number of mononuclear cell infiltrates containing at least 50 inflammatory cells in a $4mm^2$ glandular section under a light microscope using a graticule and 40x magnification (Segerberg-Konttinen et al 1986).

7.6 Radiological investigations (IV)

A panoramic radiograph was taken from all 60 patients and the state of alveolar bone was evaluated by using a panoramic mandibular index (PMI, Benson et al 1991). This was obtained by dividing the height of the inferior cortex on the right side of the mandible by the distance from the lower border of the mandible to the inferior edge of the mental foramen. The alveolar bone resorption index (Packota et al 1988) was calculated for 45 patients with teeth by dividing the distance from the inferior border of the mandible to the alveolar crest by the distance from the inferior border of the mandible to the lower edge of mental foramen.

7.7 Statistical methods

In Study I, the Mann-Whitney U test was used to compare the salivary and serum IgA AGA levels in the untreated and GFD treated DH patiets and the control subjects. Rank correlation analysis (Spearman test) was used in calculating the correlations between IgA AGA concentrations in serum and saliva. The Wilcoxon-Pratt paired rank sum test was used for comparison of IgA AGA levels before and after GFD treatment.

In Study II, the Mann-Whitney U test was used to compare the numbers of CD3, γ/δ TcR+ cells and α/β TcR+ cells in the patients with DH and the control subjects.

In Study III, the permutation test for paired replicates was used to compare the cell counts in the challenged and control mucosa.

In Study IV, the results for the patient groups of CD+SS, CD and SS were expressed as means and standard deviations, or for skewed parameters as medians and interquartile ranges (IQR). Statistical comparison between the groups was made using the Kruskal-Wallis test, Chi-square, Fisher's exact test or Fisher-Freeman-Halton test as appropriate. Post hoc testing of several univariate comparisons was made with Hommel adjustments or Dwass-Steel-Chritchlow-Flinger method at significance level 0.05.

7.8 Ethics

The study protocols for the research studies were approved by the Ethical Committees of the University Hospital for the Skin and Allergic Diseases, Helsinki (I-III) and the University Hospital of Tampere (IV). All patients in the four studies signed confirmation to participate.

8. RESULTS

8.1 Antigliadin antibodies in patients with dermatitis herpetiformis (I)

8.1.1 Salivary and serum IgA and IgG antigliadin antibodies

The median salivary IgA AGA concentration was not significantly different in the ten untreated patients with DH compared to the nine patients with DH on a long-term GFD or to the 20 control subjects (I, table 2). In contrast to salivary IgA AGA, the untreated patients with DH had significantly higher serum IgA AGA levels than the patients with DH on a long-term GFD ($p=0.043$) or the healthy controls ($p=0.006$). No correlation was found between salivary and serum IgA AGA levels in the DH patient groups or the control subjects. Measurement of IgG AGA in three patients with DH did not disclose these antibodies in the saliva although one of the patients had a markedly increased level in the serum (I, table 1).

8.1.2 Effect of gluten-free diet treatment on the IgA antigliadin antibodies

The three-month GFD treatment did not alter the median salivary IgA AGA levels in the ten patients with DH, and individual changes were small as well (I, table 3). In contrast, the median serum IgA-AGA level decreased from 10.5% to 5.5% ($p=0.0078$).

8.2. γ/δ T cell receptor positive lymphocytes in oral and jejunal mucosa in patients with dermatitis herpetiformis (II)

The median number of γ/δ TcR+ cells was very low in the buccal epithelium of 13 untreated (0.4 cells/mm^2; II, table 1) and 13 GFD-treated patients with DH (0.3 cells/mm^2). In contrast, much higher numbers of α/β TcR+ cells were found, but the densities did not differ between the untreated (154 cells/mm^2) and GFD-treated (250 cells/mm^2) patients with DH. The median numbers of CD3 positive lymphocytes were also similar in the two DH patient groups and healthy control subjects.

In contrast to buccal epithelium, the jejunal specimens (II, table 2) showed significantly increased ($p<0.001$) densities of intraepithelial γ/δ TcR+ cells in the untreated and GFD-treated patients with DH compared to specimens from the control patients. The median numbers of intraepithelial γ/δ TcR+ lymphocytes were somewhat lower than the numbers of α/β TcR+ lymphocytes both in the untreated and GFD-treated patients with DH (II, table 2).

8.3 Potassium iodine and gliadin challenge on oral mucosa in dermatitis herpetiformis (III)

8.3.1 Clinical and histological findings

Though all six patients with DH had active lesions in the skin at the end of the 12h oral mucosal challenge experiment, none of them showed blisters or clinically visible inflammatory changes on the potassium-iodine or gliadin-challenge site of the buccal mucosa. In agreement with this, none of the biopsy specimens from the challenge sites showed subepithelial vesicles or accumulation of polymorphonuclear leukocytes typical of DH lesions.

8.3.2 T lymphocyte response

In the buccal epithelium all five patients with representative biopsy specimens showed increased numbers of CD4+ T lymphocytes (median 114 cells/mm^2) in the gliadin challenge sites compared to the control sites (50 cells/mm^2, $p = 0.06$, Fig.1). Potassium iodine did not cause any marked influx of CD4+ lymphocytes. Similarly, no significant change were found in the numbers of CD8+ cells in the gliadin or potassium iodine challenge sites. The α/β TcR+ cells did not show any statistically significant change either in the gliadin or potassium iodine challenge sites.

In the lamina propria the numbers of CD4+ cells in the gliadin or potassium iodine challenge sites did not show any marked increase compared to the control sites. However, the numbers of α/β TcR+ cells were increased ($p=0.03$) in the gliadin challenge site (median 152 cells/

mm^2) compared to the control site (57 cells/ mm^2), whereas the potassium iodine challenge site did not show any increase.

In contrast to α/β TcR+ cells, only very few γ/δ TcR+ cells (maximum 5 cells/mm^2) were seen intraepithelially or in the lamina propria in the gliadin or potassium iodine challenged sites. Similarly, the oral blister specimen from one patient (II, p.133) did not show any γ/δ TcR+ cells in contrast to marked amounts of α/β TcR+ cells.

8.4 Oral findings in coeliac disease patients with associated Sjögren's syndrome (IV)

8.4.1 Oral mucosal findings

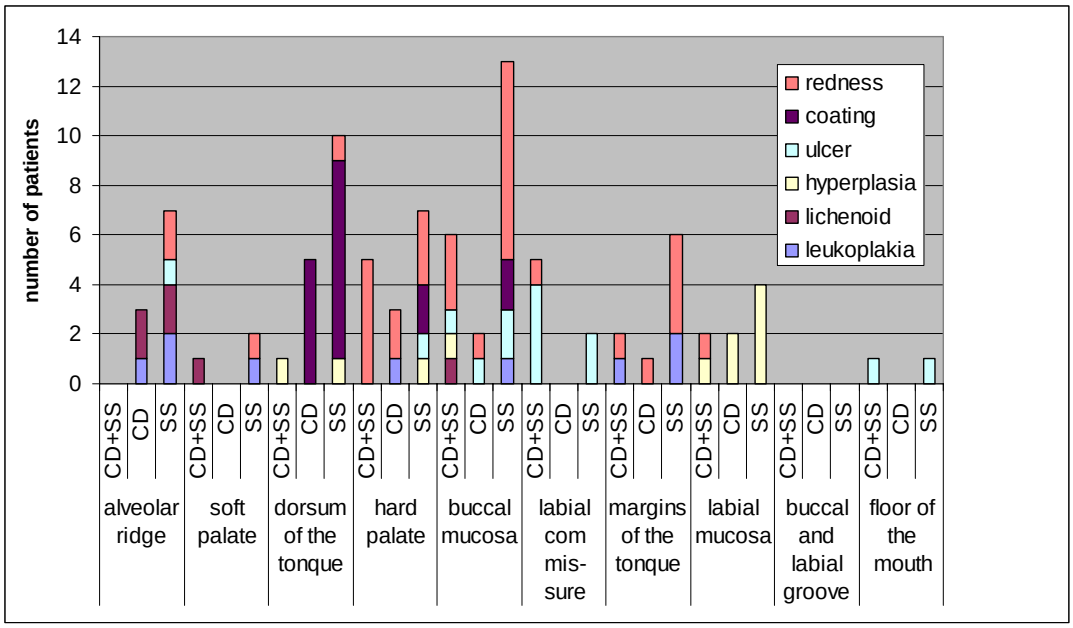
The patients with CD+SS (90%) and with SS (95%) more often ($p<0.001$) gave a history of mouth dryness than the patients with CD (40%, IV: Table 2). The frequency of macroscopic mucosal lesions was high in the SS (80%), lower in the CD+SS (65%) and lowest in the CD (40%, $p=0.043$) group (Table 6). Six patients with CD+SS, six with SS and one with CD had mucosal ulcers. The mucosal and dental findings in the three patients with DH in each study group were in the line with those observed in the patients with CD.

Table 6. Oral mucosal findings in the patients with coeliac disease (CD) and associated Sjögren's syndrome (SS), and in the case controls of the patients with either coeliac disease or Sjögren's syndrome alone.

	CD+SS group (n=20)	CD group (n=20)	SS group (n=20)
Mucosal Abnormalities	13 (65%)	8 (40%)	16 (80%)
Patchy redness	8	3	13
Ulcers	6	1	6
Lichenoid/ Hyperplastic changes	3	4	7
Leukoplakia	1	2	5

The mean number of affected mucosal locations was 1.7 (range 1-4) in the CD+SS, 2.0 (range 1-3) in the CD and 3.1 (range 1-5) in the SS group (table 5.).

Figure 1. Distribution of oral mucosal lesions in the patients with coeliac disease associated with Sjögren's syndrome and in the patients with coeliac disease and Sjögren's syndrome alone.



8.4.2 Dental findings

Twelve patients with CD+SS had teeth compared to 17 with CD and 16 with SS (p=0.25, Table 2). The median number of teeth was 6 in the CD+SS, 24 in the CD (p=0.072) and 22 in the SS group. The DMF index was similar in the CD+SS (28) and SS (27) groups, but significantly lower (22, p=0.005) in the CD group. The frequency of coeliac-type enamel defects was 89% in the CD+SS and 88% in the CD group compared to 25% in the SS group (p<0.001, Table 2).

8.4.3 Radiological findings

The mean PMI index was about the same in the CD+SS (0.37), CD (0.38) and SS (0.40) group. Similarly, no differences were found in the mean mandibular alveolar bone resorption index in the CD+SS (2.82), CD (2.83) and SS (2.94) groups.

8.4.4 Salivary flow rates and salivary gland inflammatory focus score

The stimulated salivary flow rate was within the normal range (>0.7ml/min) in the patients with CD+SS and CD, but significantly low in the patients with SS (0.3ml/min, $p<0.001$). The lacrimal excretion value in the Shirmer test was normal (8.2 mm/5min) in the patients with CD, but decreased in patients with CD+SS (3.7 mm/5min) and SS (1.5 mm/5min) ($p=0.003$). The salivary gland inflammatory focus score was highest (median 5.5) in the patients with SS, lower in the CD+SS (3.7) and lowest ($p=0.002$) in the CD (2.1) group (table 2).

9. DISCUSSION

9.1 Salivary IgA antigliadin antibodies in the patients with dermatitis herpetiformis. (I)

Earlier studies had shown that patients with CD can have salivary IgA AGA antibodies in the saliva (Al-Bayaty et al 1989, Hakeem et al 1992). The salivary IgA AGA levels in the patients with DH, however, were only about 10% of the corresponding levels in the serum and similar to those of healthy controls. In addition, the salivary IgA AGA levels did not decrease during GFD treatment unlike the serum IgA AGA levels. These results are in agreement with the findings of the studies in children (Rujner et al 1996) and adults with CD (Kelly et al 1991, O'Mahony et al 1991, Lähteenoja et al 1999). As salivary IgA is mostly of secretory type, separate control mechanisms for serum and salivary IgA AGA responses could explain the present results. In contrast to IgA antibodies, salivary IgG is mostly derived from gingival crevicular fluid. In agreement with this, we found IgG AGA only in only a third of the present patients with DH. Similarly Lähteenoja et al (1999) found salivary IgG AGA in only 20% of patients with CD.

9.2 Inflammatory changes in oral mucosa of the patients with dermatis herpetiformis (II-III)

Increased density of γ/δ TcR+ lymphocytes in the jejunal mucosa is a constant marker of gluten-sensitive enteropathy in CD and DH (Holm et al 1992, Savilahti et al 1997). Therefore, we examined whether increased numbers of γ/δ TcR+ lymphocytes might also occur in the oral mucosa of patients with DH (II). We found, however, only a few γ/δ TcR+ cells in the buccal epithelium or lamina propria, but rather high densities of α/β TcR+ cells. These findings were confirmed in a similar study performed by Lähteenoja et al (2000) in the patients with CD. In contrast to DH and CD, increased amounts of γ/δ TcR+ lymphocytes were found in the lesions of recurrent aphthous stomatitis (Natah et al 2000).

We also challenged buccal mucosa in the patients with DH by potassium iodine and gliadin to study the macroscopic and microscopic inflammation and especially whether gliadin causes an influx of γ/δ TcR+ cells into the mucosa (III). The gliadin challenge caused a marked

influx of CD4+ lymphocytes in the epithelium and increase of α/β TcR+ lymphocytes in the lamina propria, whereas the numbers of CD8+ lymphocytes and γ/δ TcR+ cells remained unaltered. These findings are in general similar to those observed in the patients with CD by Lähteenoja et al (2000a, 2000b). In contrast to the findings in the oral mucosa and small intestine, a gluten challenge did not cause any influx of T lymphocytes into the DH skin and the few T lymphocytes observed seem not to be gluten-specific (Baker et al 1995, Garioch et al 1995). Oral mucosal challenge with potassium iodine did not cause macroscopic lesions like in the skin.

9.3 Oral findings in coeliac disease patients with associated Sjögren's syndrome (IV)

More than a half of the patients with CD have been reported to present with various oral mucosal abnormalities (Lähteenoja et al 1998), and coeliac-type dental enamel defects are typical features both for CD and DH (Aine 1996, Aquirre et al 1997). Patients with SS frequently have dental caries due to decreased salivary flow (Najera et al 1997, Konttinen et al 1997, Pertovaara 2001). CD and SS are frequently associated (Collin et al 1994) and patients suffering concomitantly from both disorders could be at even higher risk for mucosal and dental abnormalities. In agreement with this, we found that eight of the 20 patients with CD+SS had lost their teeth, and the median number of teeth in the CD+SS group was only 6 compared to 24 in the CD and 22 in the SS group. This difference did not reach statistical significance ($p=0.072$). However, in the patients with CD+SS the DMF index was significantly higher than in those with CD and as high as in the patients with SS. This suggests that the increased caries index is in part linked to impaired salivary gland function typical of SS (Pedersen et al 1999). The stimulated salivary flow rate was, however significantly less impaired in the patients with CD+SS than in those with SS only. It is therefore, possible that the coeliac-type dental enamel defects, the prevalence of which was as high as 89% in the CD+SS group, could have an additive effect on the caries risk.

In agreement with Lähteenoja et al (1998) we found macroscopic mucosal changes in as many as 40% of the present patients with CD. The prevalence was even higher in the patients with CD+SS (65%) and highest (80%) in those with SS. This suggests that the low salivary flow rate and concomitant dryness in the mouth in SS affect mucosal health more than the mucosal

inflammation typical for CD. In line with the lower frequency of mucosal changes and higher salivary flow rates, the patients with CD+SS had a lower salivary gland inflammatory focus score than the patients with SS only, though this was not significant. The reason could be that the patients with CD+SS had adhered to a GFD for several years. Diagnosis of CD at an early age has been shown to prevent the later development of associated autoimmune disorders (Ventura et al 1999) and the same seems true in adult patients adhered to a GFD for many years (Collin et al 1997).

Interestingly, the median focus score of the 20 patients with CD was 2.1 and the interquartile range values of 1.0 and 3.1. This implies that many of the patients with CD could have a subclinical autoimmune focal sialoadenitis characteristic of SS. This finding also suggests that the overlap between CD and SS may be more extensive than is apparent at first sight. It is also a matter of discussion whether SS in the patients with CD should be classified as secondary or primary SS.

10. CONCLUSIONS AND FUTURE ASPECTS

DH, a life-long blistering skin disease with pathognomonic IgA deposits in the skin and oral mucosa, is at present considered to be a cutaneous phenotype of CD (Reunala 2001, Fry 2002, Oxentenko&Murray 2003, Karpati 2004). CD is a common autoimmune disorder in which the small-intestinal mucosa are damaged by wheat gluten and related cereal peptides by a HLA DQ2 restricted, CD4+ T cell driven inflammation (Mäki&Collin 1997, Shan et al 2002, Mowat 2003). The formation of IgA class autoantibodies to tissue type transglutaminase (TG2) and their deposition in the intestinal mucosa seems to be of importance in the pathomechanism of small intestinal lesion (Dieterich et al 2003, Korponay-Szabo et al 2004, Reif&Lerner 2004).

A recent serological screening study by Mäki et al (2003) in Finland showed that the prevalence of CD may be as high as 1/100. Many CD cases remain subclinical due to which there has been an obvious need to develop sensitive and specific serological screening tests (Mäki&Collin 1997). At first, screening was performed by measuring IgA and IgG class AGA with ELISA (Unsworth et al 1981, Vainio et al 1983). When IgA AGA was found also in saliva, it was suggested that salivary IgA AGA measurements could be used as a non-invasive screening test for gluten-sensitive enteropathy in CD (Al-Bayaty et al 1989, Hakeem et al 1992). *The first aim of the present study (1) was to examine whether IgA AGA also occurs in the saliva of patients with DH* and, if so, whether measurement of salivary IgA AGA could be used to monitor the progress of GFD treatment as well as measurement of serum IgA AGA (Savilahti et al 1983, Vainio et al 1985). The salivary IgA AGA levels in the patients with DH, however, were only about 10% of the corresponding levels in the serum and similar to those of healthy controls (I). Though IgA class EmA is more specific than AGA in serological screening of gluten-sensitive enteropathy (Reunala et al 1987), measurement of IgA EmA from saliva in patients with CD also gave unsatisfactory results (Rujner et al 1995, Lähteenoja et al 1999). The same was true when IgA TGA was measured with ELISA (Baldas et al 2004). However, another recent study using radioimmunoassay found IgA TGA in the saliva of almost every patient with CD, but in none of the controls (Bonamico et al 2004). One reason for the high sensitivity and specificity seems to be the use of genetically produced TG2 antigen and a sensitive fluid-phase RIA method. In the same study it was found that the saliva volume to be used in the TGA RIA had to be 15 times that

of the serum volume (Bonamico et al 2004), a finding which is in line with the present IgA AGA ELISA results from saliva and serum. Due to the much lower IgA antibody concentration in saliva compared to serum and the need for radioactive reagents, it seems that saliva TGA RIA cannot at present replace serum TGA ELISA in the routine screening of gluten-sensitive enteropathy in CD or DH.

The second aim of the present study (2) was to examine whether gamma/delta T cell receptor bearing lymphocytes occur in the oral mucosa of patients with DH in similar high numbers as in the jejunal mucosa. Previously it has been shown that an increase of the number of γ/δ TcR+ lymphocytes in the jejunal mucosa is a constant marker for untreated patients with CD and DH (Halstensen et al 1989, Holm et al 1992, Savilahti et al 1992). γ/δ TcR+ lymphocytes are found particularly in the epithelium, whereas α/β TcR+ lymphocytes occur both in the epithelium and lamina propria. In the present study in the patients with DH (II) we found only single γ/δ TcR+ cells in the buccal epithelium and lamina propria, but rather high densities of α/β TcR+ cells. These findings were confirmed in a similar study performed by Lähteenoja et al (2000) in the patients with CD.

The third aim of the present study (3) was to investigate the inflammatory reaction caused by potassium iodine and gliadin application on the oral mucosa in patients with DH. Gliadin challenge caused an influx of CD4+ lymphocytes in the epithelium and a significant increase of α/β TcR+ lymphocytes in the lamina propria, whereas the numbers of CD8+ lymphocytes and γ/δ TcR+ cells remained unaltered (III). These findings are in general similar to those observed in patients with CD by Lähteenoja et al (2000a) although their patients were GFD treated and the present patients untreated. Lähteenoja et al (2000b) also injected a synthetic gliadin-like peptide submucosally and found an influx of α/β TcR+ cells and increased expression of IL-2 receptor, an activation marker, on the T lymphocytes. Knowledge about the homing receptors expressed by orally invading T cells would also be of interest. To be active in the gut, gliadin has to be digested by trypsin and pepsin, and then modified by tissue transglutaminase in order to obtain the immunodominant, 33-dimer peptide, which then causes T lymphocyte activation and cytokine secretion (Sollid et al 1997, Shan et al 2002, Dieterich et al 2003). Whether this peptide causes a similar influx of CD4 + and α/β TcR+ cells in the oral buccal mucosa as that observed in the present study or that by Lähteenoja et al (2000b) needs to be examined.

The fourth aim of the present study (4) was to examine whether patients suffering concomitantly from CD and SS have a higher frequency of oral mucosal, dental and salivary abnormalities than patients with either disorder alone. Previously patients with CD and DH have been reported to present with recurrent aphthous ulcers and coeliac-type dental enamel defects (Fergusson et al 1976, Aine 1996). In agreement with a previous study by Lähteenoja et al (1998), 40% of the present patients with CD had macroscopic mucosal changes (IV). Like the patients with Lähteenoja et al (1998), the present patients had been on a GFD several years suggesting that the observed mucosal changes were not related to malabsorption-related deficiency of iron, vitamins or trace elements (Barry et al 1974, Ferguson et al 1976). A prospective study on the occurrence of macroscopic mucosal changes at diagnosis and after adherence to a GFD with serial determinations of calcium, iron and various vitamins would confirm or exclude the possible malabsorption-related background of the observed mucosal changes.

CD and DH are also associated with other autoimmune diseases such as type I diabetes and SS (Collin et al 2002, Hervonen et al 2004). SS, which occurs in about 3% of the patients with CD, also has an autoimmune background and is characterized by inflammation of the salivary glands (Syrjänen 1982). Decreased salivary flow causes dryness of the mouth, which increases risk for caries in SS (Konttinen et al 1997). Whether patients with CD+SS might have a higher prevalence of oral mucosal and dental lesions than those with either disease alone has not been previously studied. In the present study (IV) eight (40%) of the 20 patients with CD+SS had completely lost their teeth and the mean number of teeth was only 6 in the CD+SS group compared to 24 in the CD and 22 in the SS group. The DMF index was high (28 and 27, respectively) in the CD+SS and SS groups, but significantly lower in the CD group. The high DMF in the CD+SS group could be linked to the impaired salivary function typical of SS and also to coeliac-type dental enamel defects seen in 89% of these patients. These coeliac-type enamel defects can lead to the diagnosis of otherwise asymptomatic patients (Aine 1996). The diagnosis and treatment of both CD and SS as early as possible is a general goal and a further prospective study is needed to show whether active treatment of both these diseases and careful follow-up would prevent loss of teeth and improve oral health.

Interestingly, the median inflammatory focus score in the minor salivary glands of the 20 patients with CD was 2.1, whereas the normal score is < 1. This implies that many of the patients could have subclinical autoimmune focal sialoadenitis characteristic for SS. This

finding should be studied in a larger patient series consisting of both untreated and GFD treated patients with CD and DH. If confirmed, the increased salivary gland inflammation in patients with CD and those with CD+SS could be caused by an autoimmune inflammation specific for CD. Recently, Korponay-Szabo et al (2004) showed IgA TGA deposition in addition to jejunum also to extraintestinal tissues such as liver and lymph nodes. This is true also for DH skin (Sardy et al 2002). TG2 is a ubiquitous enzyme, which is present in many tissues including epithelial cells of salivary glands (Lee et al 1996, Lorand&Graham2002). A further study is, therefore, needed to examine whether the salivary glands could also be a target for IgA TGA deposition and further harmful inflammation in CD and DH.

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12. REFERENCES

Aquirre JM, Rodriguez R, Orib D, Vitoria JC. Dental enamel defects in celiac patients. Oral Surg Oral med Oral Pathol Oral Radiol Endod 1997;84:646-650.

Aine L. Coeliac-type permanent-tooth enamel defects. Ann Med 1996;28:9-12.

Aine L. Dental enamel defects and dental maturity in children and adolescents with coeliac disease. Proc Finn Dent Soc 1986;82(suppl 3):1-71.

Aine L, Mäki M, Reunala T. Coeliac-type dental enamel defects in patients with dermatitis herpetiformis. Acta Derm Venereol (Stockh) 1992;72:25-27.

Airola K, Reunala T, Salo S, Saarialho-Kere UK.Urokinase plasminogen activator is expressed by basal keratinocytes before interstitial collagenase, stromelysin-1, and laminin-5 in experimentally induced dermatitis herpetiformis lesions. J Invest Dermatol, 1997;108:7-11.

Al-Bayaty HF, Alfred MJ, Walker DM, Newcombe RG, Swift G, Smith PM, Ciclitira PJ. Salivary and serum antibodies to gliadin in the diagnosis of celiac disease. J Oral Pathol Med 1989;18:578-581.

Andersson-Wenckert I, Blomquist HK, Fredrikzon B. Oral health in coeliac disease and cow's milk protein intolerance. Swed Dent J 1984;8: 9-14.

Atkinsson JC, Travis WD, Pillemer SR, Bermudez D, Wolff A, Fox PC. Major salivary gland function in primary Sjögren's syndrome andits relationship to clinical features. J Rheumatol 1990;17:318-322.

Axell T, Henricsson V. The occurrence of recurrent aphthous ulcers in an adult Swedish population. Acta Odontol Scand 1985;43:121-125.

Baker BS, Garioch JJ, Bokth S, Leonard JN, Fry L. Absence of gluten-specific T lymphocytes in the skin of patients with dermatitis herpetiformis. J Autoimmunity 1995;8:75-82.

Baldas V, Tommassini A, Santon D, Not T, Gerarduzzi T, Clarich G, Sblattero D, Marzari R, Florian F, Martellossi S, Ventura A. Testing for anti-human transglutaminase antibodies in saliva is not useful for diagnosis of celiac disease. Clin Chem 2004;50:216-219.

Barry RE, Baker P, Read AE. Coeliac disease: The clinical presentation. Clin Gastroenterol 1974;3:55-69.

Benson BW, Prohoda TJ, Glass BJ. Variations in adult cortical bone mass measured by a panoramic mandibular index. Oral Surg Oral Med Oral Pathol. 1991;71:349-356.

Boismenu R, Havran W. An innate view of γδ T cells. Curr Opin Immunol 1997;9:57-63.

Bowron A, Moorghen M, Morgan JE, Osborne JR, Stansbie D, Stone JE. Cost-effective strategy for the serological investigation of coeliac disease. Ann Clin Biochem, 2000;3:467-470.

Brandtzaeg P. Salivary immunoglobulins. In: Human saliva: clinical chemistry and microbiology. Vol II. Tenovuo J, editor. Boca Raton, FL, CRC Press, 1989.

Brown TA, Mestecky J. Immunoglobulin A subclass distribution of naturally occurring salivary antibodies to microbial antigens. Infect Immun 1985;49:459-462.

Chan SL, Regezi JA Cooper KD. Oral manifestations of linear IgA disease. J Am Acad Dermatol 1990;22:362-365.

Ciccocioppo R, Di Sabatino A, Ara C, Biagi F, Perilli M, Amicosante G, Cifone MG, Corazza GR. Gliadin and tissue transglutaminase complexes in normal and coeliac duodenal mucosa. Clin Exp Immunol 2003;134:516-524.

Ciclitira PJ, Ellis HJ, Evans TJ, Lennox ES. A radioimmunoassay for wheat gliadin to assess the suitability of gluten free food for patients with coeliac disease. Clin Exp Immunol 1985;59:703-708.

Chorzelski TP, Beutner EH, Sulej J, Tchorzewska H, Jablonska S, Kumar V, Kapuscinska A. IgA antiendomysium antibody. A new immunological marker of dermatitis herpetiformis and coeliac disease. Br J Dermatol 1984;111:395-402.

Cole MF, Bryan S, Evans MK, Pearce CL, Sheridan MJ, Sura PA, Wientzen R, Bowden GH. Humoral immunity to commensal oral bacteria in human infants: salivary antibodies reactive with Actinomyces naeslundii genospecies 1 and 2 during colonization. Infect Immun 1998;66:4283-4289.

Collin P, Kaukinen K, Välimaki M, Salmi J. Endocrinological disorders and celiac disease. Endocr Rev 2002;23:464-483.

Collin P, Pukkala E, Reunala T. Malignacy and survival in dermatitis herpetiformis: A comparison to coeliac disease. Gut 1996;38:528-530.

Collin P, Reunala T, Pukkala E, Laippala P, Keyriläinen O, Pasternack A. Coeliac disease – associated diseases and survival. Gut 1994;35:1215-1218.

Collin P, Reunala T, Rasmussen M, Kyrönpalo S, Pehkonen E, Laippala P, Mäki M. High incidence and prevalence of adult coeliac disease. Augmented diagnostic approach. Scand J Gastroenterol 1997;32:1129-1133.

Cooke WT, Peeney ACP, Hawkins CF. Syptoms, signs and diagnostic features of idiopathic steatorrhoea. Quart Med J 1953;22:59-77.

Corazza GR, Di Sario A, Cecchetti L, Tarozzi C, Corrao G, Bernardi M, Gasbarrini G. Bone mass and metabolism in patients with celiac disease. Gastroenterology 1995;109:122-128.

Corazza GR, Frisoni M, Treggiari EA, Valentini RA, Filipponi C, Volta U, Gasbarrini G. Subclinical celiac sprue. Increasing occurrence and clues to its diagnosis. J Clin Gastroenterol 1993;16:16-21.

Delacroix DL, Dive C, Rambaud JC, Vaerman JP. IgA subclasses in various secretions and in serum. Immunology 1982;47:383-385.

Di Stefano M, Jorizzo RA, Veneto G, Cecchetti L, Gasbarrini G, Corazza GR.Bone mass and metabolism in dermatitis herpetiformis. Dig Dis Sci 1999;44:2139-2143.

Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997;3:797-801.

Dietrich W, Esslinger B, Schuppan D. Pathomechanisms in celiac disease. Int Arch Allergy Immunol 2003;132:98-108.

Dieterich W, Laag E, Bruckner-Tuderman L, Reunala T, Karpati S, Zagoni T, Riecken EO, Schuppan D.Antibodies to tissue transglutaminase as serologic markers in patients with dermatitis herpetiformis. J Invest Dermatol 1999;113:133-136.

Dieterich W, Laag E, Schopper H, Volta U, Ferguson A, Gilltet H, Riecken EO, Schuppan D. Autoantibodies to tissue transglutaminase as predictors of celiac disease. Gastroenterology 1998;115:1317-1321.

Economopoulou P, Laskaris G. Dermatitis herpetiformis: Oral lesions as an early manifestation. Oral Surg Oral Med Oral Pathol 1986;62:77-80.

Edgar WM, O'Mullane DMO. Saliva and Oral Health. Thanet Press Limited, London, 2 ed., 1996.

Ericsson Y, Hardwick L. Individual diagnosis, prognosis and counselling for caries prevention. Caries Res 1978;12(Suppl 1),94.

Falchuk ZM, Strober W. Gluten-sensitive enteropathy: synthesis of antigliadin antibody in vitro. Gut 1974;15:947-952.

Fasano A, Berti I, Geraduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PH, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wasserman SS, Murray JA, Horvath K. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med 2003;163:286-292.

Ferrick DA, Schrenzel MD, Mulvania T, Hsieh B, Ferlin WG, Lepper H. Differential production of interferon-γ and interleukin-4 in response to Th1- and Th2-stimulating pathogens by γ/δ T cells in vivo. Nature 1995;1973:255-257.

Frazer NG, Kerr NW, Donald D. Oral lesions in dermatitis herpetiformis. Br J Dermatol 1973;89:439-450.

Ferguson R, Basu MK, Asquith P, Cooke WT. Jejunal mucosal abnormalities in patients with recurrent oral ulceration. BMJ 1976;1:11-13.

Fry L, Seah PP, Riches DJ. Clearance of skin lesions in dermatitis herpetiformis after gluten withdrawal. Lancet 1973;1:288-291.

Fry L. Dermatitis herpetiformis: problems, progress and prospects.Eur J Dermatol 2002;12:523-531.

Fulstow ED. Incidence of dental caries in coeliac children. Arch Dis Child 1979;54:166.

Garioch JJ, Lewis HM, Sargent SA, Leonard JN, Fry L. 25 years' experience of a gluten-free diet in the treatment of dermatitis herpetiformis. Br J Dermatol 1994;9:403-408.

Garioch JJ, Unsworth DJ, Baker BS, Leonard JN, Fry L. Failure of intradermal skin testing with gluten to produce delayed hypersensitivity reactions in patients with dermatitis herpetiformis. Br J Dermatol 1995;132:698-702.

Gawrodger JDC, Blackwell JN, Gilmour HK, Rifkind EA, Heading RC, Barnetson RS. Dermatitis herpetiformis, diagnosis, diet and demography. Gut 1984;25:151-157.

Hakeem V, Fifield R, Al-Bayaty HF, Alfred MJ, Walker DM, Williams J, Jenkins HR. Salivary IgA antigliadin antibody as a marker for coeliac disease. Arch Dis Child 1992;67: 724-727.

Haffenden GP, Blenkinsopp WK, Ring NP, Wojnarowska F, Fry L. The potassium iodide patch test in dermatitis herpetiformis in relation to treatment with a gluten-free diet and dapsone. Br J Dermatol 1980;102:313-317.

Hall RP, Lawley TJ. Characterization of circulating and cutaneous IgA immune complexes in patients with dermatitis herpetiformis. J Immunol 1985;135:1760-1765.

Halstensen TS, Scott H, Brandtzaeg P. Intraepithelial T cells of the TCRγδ+ CD8- and Vδ1,Jδ1(+) phenotype are increased in coeliac disease. Scand J Immunol 1989;30:665-672.

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

Heinze U, Birkhed D, Björn H. Secretion rate and buffer effect of resting and stimulated whole saliva as a function of age and sex. Swed Dent J 1983;18:578-581.

Hervonen K, Karell K, Holopainen P, Collin P, Partanen J, Reunala T. Concordance of dermatitis herpetiformis and coeliac disease in monozygous twins. J Invest Dermatol 2000;115:990-993.

Hervonen K, Viljamaa M, Collin P, Knip M, Reunala T.The occurrence of type 1 diabetes in patients with dermatitis herpetiformis and their first-degree relatives. Br J Dermatol 2004;150:136-138.

Hietanen J, Reunala T. IgA deposits in the oral mucosa of patients with dermatitis herpetiformis. Scand J Dent Res 1984;92:230-234.

Holm K, Mäki M, Savilahti E, Lipsanen V, Laippala P, Koskimies S. Intraepithelial γ/δ T-cell receptor lymphocytes and genetic susceptibility to coeliac disease. Lancet 1992;339:1500-1503.

Holmes GKT, Prior P, Lane MR, Pope D, Allan RN. Malignancy in coelic disease – effect of a gluten free diet. Gut 1989;30:333-338.

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

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

Janeway CA jr, Jones B, Hayday A. Specificity and function of T cells bearing γ/δ receptors. Immunol Today 1988;9:73-76.

Johnston SD, McMillan SA, Collins JS, Tham TC, McDougall NI, Murphy P. A comparison of antibodies to tissue transglutaminase with conventional serological tests in the diagnosis of coeliac disease. Eur J Gastroenterol Hepatol 2003;15:1001-1004.

Järvinen TT, Kaukinen K, Laurila K, Kyrönpalo S, Rasmussen M, Mäki M, Korhonen H, Reunala T, Collin P. Intraepithelial lymphocytes in celiac disease. Am J Gastroenterol 2003;98:1332-1337.

Karpati S. Dermatitis herpetiformis: close to unravelling a disease. J Dermatol Sci 2004;34:83-90.

Kelly CP, Feighery CF, Gallagher RB, Gibney MJ, Weir DG. Mucosal and systemic IgA anti-gliadin antibody in celiac disease. Dig Dis Sci 1991;36:743-751.

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

Kilander AF, Dotevall G, Fallstrom SP, Gillberg RE, Nilsson LA, Tarkowski A. Evaluation of gliadin antibodies for detection of coeliac disease. Scand J Gastroenterol 1983;18:377-383.

Klein GF, Hintner H, Schuler G, Fritsch P. Junctional blisters in acquired bullous disorders of the dermal-epidermal junction zone; role of lamina lucida as the mechanical locus minoris resistentiae. Br J Dermatol 1983;109:499-508.

Kokkonen J, Similä S, Vuolukka P. The incidence of coeliac disease and pyloric stenosis in children in Northern Finland. Ann Clin Res 1982;14:123-128.

Konttinen YT, Platts LA, Tuominen S, Eklund KK, Santavirta N, Tornwall J, Sorsa T, Hukkanen M, Polak JM. Role of nitric oxide in Sjogren's syndrome. Arthritis Rheum 1997;40: 875-883.

Korponay-Szabo IR, Dahlbom I, Laurila K, Koskinen S, Woolley N, Partanen J, Kovacs JB, Mäki M, Hansson T. Elevation of IgG antibodies against tissue transglutaminase as a diagnostic tool for coeliac disease in selective IgA deficiency. Gut 2003;52:1567-71.

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

Korsrud FR, Brandzaeg P. Characterization of epithelial elements in human major salivary glands by functional markers: localization of amylase, lactoferrin, lysozyme, secretory component and secretory immunoglobulins by paired immunofluorescence staining. J Histochem Cytochem 1982;30:657-666.

Lee CH, Lee SK, Chi JG, Park SC, Chung SI, Saitoh M, Shrestha P, Mori M. Immunohistochemical evaluation of transglutaminase C in tumours of salivary glands. Eur J Cancer B Oral Oncol 1996;201:401-406.

Leonard JN, Fry L. Treatment and management of dermatitis herpetiformis. Clin Dermatol 1992;135:363-367.

Leonard JN, Haffenden G, Tucker W, Unsworth J, Swain F, McMinn R, Holborow J, Fry L. Gluten challenge in dermatitis herpetiformis. NEJM 1983;308:816-819.

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

Luft LM, Barr SG, Martin LO, Chan EK, Fritzler MJ. Autoantibodies to tissuen transglutaminase in Sjogren's syndrome and related rheumatic diseases. J Rheumatol 2003;30:2613-1619.

Lundin KEA, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O, Thorsby E, Sollid LM. Gliadin specific, HLA-DQ (alpha 1*0501, beta 1*0201) restricted T cells isolated from the small intestinal mucosa of coeliac patients. J Exp Med 1993;178:187-196.

Lundqvist C, Baranov V, Teglund S, Hammarström S, Hammarström ML. Cytokine profile and ultrastructure of intraepithelial γ/δ T cells in chronically inflamed human gingiva suggest a sytotoxic effector function. J Immunol 1994;153:2302-2312.

Lähteenoja H, Toivanen A, Viander M, Mäki M, Irjala K, Räihä I, Syrjänen S. Oral mucosal changes in coeliac patients on a gluten-free diet. Eur J Oral Sci 1998a;105:899-906.

Lähteenoja H, Irjala K, Viander M, Vainio E, Toivanen A, Syrjänen S. Oral mucosa is frequently affected in patients with dermatitis herpetiformis. Arch Dermatol 1998b;134:756-758.

Lähteenoja H, Toivanen A, Räihä I, Syrjänen S, Viander M. Salivary antigliadin and antiendomysium antibodies in coeliac disease. Scand J Immunol 1999;50:528-535.

Lähteenoja H, Toivanen A, Viander M, Räihä I, Syrjänen S, Mäki M. Increase of T cell subsets of oral mucosa: a late response in patients with coeliac disease? Scand J Immunol, 2000:52:602-608.

Lähteenoja H, Mäki M, Viander M, Toivanen A, Syrjänen S. Local challenge of oral mucosa with gliadin in patients with coeliac disease. Clin Exp Immunol 2000a:120:38-45

Lähteenoja H, Mäki M, Viander M, Räihä I, Vilja P, Rantala I, Toivanen A, Syrjänen S. Local challenge on oral mucosa with an alpha-gliadin related synthetic peptide in patients with celiac disease. Am J Gastroenterol 2000b;95:2880-2887.

Mandel ID, Wotman S. The salivary secretions in health and disease. Oral Sci Rev 1976;8:25-27.

Mayer M, Greco L, Troncone R, Auricchio S, Marsh MN. Compliance of adolescents with coeliac disease with a gluten-free diet. Gut 1991;32:881-885.

McClelland DB, Parkin DM, Heading RC, Barnetson RS, Warwick RR, Sherman DJ. Small-intestinal immunoglobulin levels in dermatitis herpetiformis. Lancet 1972;2:1108-1110.

McLoughlin MJ, McNeill S, Egan-Mitchell B, McNicholl B. Incidence of dental caries in coeliac children. Arch Dis Child 1980;55:80.

van der Meer JB. Granular deposits of immunoglobulins in the skin of patients with dermatitis herpetiformis. Br J Dermatol 1969;81:493-503.

Mestecky J, Russell MW, Jackson S, Brown TA. The human IgA system. A reassessment. Clinical Immunol Immunopathol 1986;40:105-114.

Miller MF, Ship II. A retrospective study of the prevalence and incidence of recurrent aphthous ulcers in a professional population, 1958-1971. Oral Surg Oral Med Oral Pathol 1977;43:532-537.

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

Moro I, Umemura S, Crago SS, Mestecky J. Immunohistochemical distribution of immunoglobulins, lactoferrin and lysozyme in human minor salivary glands. J Oral Pathol 1984;13:97-104.

Moutsopoulos HM. Autoimmune epithelitis. Clin Immunol Immunopathol 1994;72:162–165.

Mowat AM Coeliac disease – a meeting point for genetics, immunology, and protein chemistry. Lancet 2003;361:1290-1292.

Munston GC, Hodgson GA. Dermatitis herpetiformis with tonque lesions. Br J Dermatol 1973;89 (suppl 9):85-88.

Mäki M, Aine L, Lipsanen V, Koskimies S. Dental enamel defects in first-degree relatives of coeliac disease patients. Lancet 1991;337:763-764.

Mäki M, Collin P. Coeliac disease. Lancet 1997;349:1755-1759.

Mäki M, Mustalahti K, Kokkonen J, Kulmala P, Haapalahti M, Karttunen T, Ilonen J, Laurila K, Dahlbom H, Hansson T, Hopfl P, Knip M. Prevalence of celiac disease among children in Finland. NEJM 2003;348:2568-2570.

Mäki M, Sulkanen S, Collin P. Antibodies in relation to gluten intake. Dig Dis 1998;16:330-332.

Najera MP, Al-Hashimi I, Plemons JM, Rivera-Hidalgo F, Rees TD, Haghighat N, Wright J. Prevalence of periodontal disease in patients with Sjögren's syndrome. Oral Surg Oral Med Oral Pathol Radiol Endod 1997;83:453-457.

Natah SS, Häyrinen-Immonen R, Patinen P, Hietanen J, Malmström M, Savilahti E, Konttinen YT. Increased density of lymphocytes bearing the γ/δ T-cell receptors in recurrent aphthous ulceration (RAU). Int J Oral Maxillofac Surg 2000;29:375-380.

Nielsen EM, Jahnsen FL, Lundin KEA, Johansen F-E, Fausa O, Sollid LM, Jahnsen J, Scott H, Brandtzaeg P. Gluten induces an intestinal cytokine response strongly dominated by interferon γ in patients with celiac disease. Gastroenterology 1998;115:551-563.

Nikiforuk G, Fraser D. The etiology of dental hypoplasia: A unifying concept. J Paediatr 1981;98:888-893.

O'Farelly C, Kelly J, Hekkens W, Bradley B, Thopson A, Feighery C, Weir DG. α-Gliadin antibody levels: a serological test for coeliac disease. Br Med J 1983;286:2007-2010.

O'Farelly C, O'Mahony C, Graeme-Cook F, Feighery C, McCartan B, Weir D. Gliadin antibodies identify gluten sensitive ROU in the absence of villous atrophy. J Oral Pathol Med 1991;20:476-478.

Oikarinen AI, Reunala T, Zone JJ, Kiistala U, Uitto J. Proteolytic enzymes in blister fluids from patients with dermatitis herpetiformis. Br J Dermatol 1986;114:295-302.

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

Oxentenko AS, Murray JA. Celiac disease and dermatitis herpetiformis: the spectrum of gluten-sensitive enteropathy. Int J Dermatol 2003;42:585-587.

Packota GV, Hoover JN, Neufeld BD. A study of the height of intact alveolar bone on panoramic radiographs of adult patients. J Prosthet Dent 1988;60:504-509.

Pedersen AM, Reibel J, Nordgarden H, Bergen HO, Jensen JL, Nauntofte B. Primary Sjogren's sundrome: salivary gland function and clinical oral findings. Oral Dis 1999;5:128-138.

Pepin LF, Roger T, Morisset J, Seman M. Preferential V delta 1 expression among TcR gamma/delta –bearing T cells in human oral epithelium. Scand J Immunol 1993;37:289-294.

Pertovaara M. Clinical features and outcome of primary Sjögren's syndrome. Thesis, University of Tampere 2001.

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

Polanco I, Prieto G, Lama R, Carrasco S, Codoceo R, Larrauri J. Associated diseases in children with coeliac disease. In: Coeliac disease. 40 years gluten-free, 1991. Eds. ML Mearin and CJJ Mulder, Kluwer Academic Publishers, Dordrecht.

Polvi A, Eland C, Koskimies S, Mäki M, Partanen J. HLA DQ and DP in Finnish families with coeliac disease. Eur J Immonogen 1996;23:221-234.

Raghunath M, Höpfner B, Aeschlimann D, Luthi U, Meuli M, Altermatt S, Gobet R, Bruckner-Tuderman L, Steinmann B. Cross-linking og the dermo-epidermal junction of skin regenerating from keratinocyte autografts. J Clin Invest 1996;98:1174-1184.

Rantala I, Hietanen J, Soidinmaki H. Reunala T. Immunoelectron microscopic findings in oral mucosa of patients with dermatitis herpetiformis and linear IgA disease. Scand J Dent Res 1985;93:243-248.

Rasmussen P, Espelid I. Coeliac disease and dental malformation. J Dent Child 1980;47:190-192.

Rea F, Serpico R, Pluvio R, Busciolano M, Iovene A, Femiano F, Sessa G, Belnome G. Dental enamel hypoplasia in a group of celiac disease patients. Clinico-epidemiologic correlations. Minerva Stomatol 1997;46:517-524.

Reif S, Lerner A. Tissue transglutaminase – the key player in celiac disease: a review. Autoimmun Rev 2004;3:40-45.

Reunala T. Gluten-free diet in dermatitis herpetiformis II. Morphological and immunological findings in the skin and small intestine of 12 patients and matched controls. Br J Dermatol 1978;98:69-78.

Reunala T. Dermatitis herpetiformis. Clin Dermatol 2001;19:728-736.

Reunala T, Blomqvist K, Tarpila S, Halme H, Kangas K. Gluten-free diet in dermatitis herpetiformis. I. Clinical response of skin lesions in 81 patients. Br J Dermatol 1977;97: 473-480.

Reunala T, Chorzelski TP, Viander M, Sulej J, Vainio E, Kumar V, Beutner EH. IgA anti-endomysial antibodies in dermatitis herpetiformis: correlation with jejunal morphology, gluten-free diet and anti-gliadin antibodies. Br J Dermatol 1987;17:185-191.

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

Roed-Petersen B, Renstrup G. A classification of the oral mucosa suitable for electronic data processing its application to 560 leukoplakias. Acta Odontol Scand 1969;27:681-695.

Rubin W, Fauci SA, Sleisenger MH, Jeffries GH. Immunofluorescent studies in adult celiac disease. J Clin Invest 1965;44:475-485.

Rujner J, Socha J, Barra E, Gregorek H, Madalinski K, Wozniewicz B, Giera B. Seruma and salivary antigliadin antibodies and serum IgA anti-endomycium antibodies as a screening test for coeliac disease. Acta Paediatr 1996;85:814-817.

Rusotto SB, Shipp II. Oral manifestations of dermatitis herpetiformis. Oral Surg Oral Med Oral Pathol 1971;31:42-48.

Sardy M, Karpati S, Merkl B, Paulsson M, Smyth N. Epidermal transglutaminase (TGase3) is the autoantigen of dermatitis herpetiformis. J Exp Med 2002;195:747-757.

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

Savilahti E, Ormala T, Arato A, Hacsek G, Holm K, Klemola T, Nemeth A, Maki M, Reunala T. Density of gamma/delta+ T cells in the jejunal epithelium of patients with coeliac disease and dermatitis herpetiformis is increased with age. Clin Exp Immunol 1997;109:464-467.

Savilahti E, Pelkonen P, Visakorpi JK. IgA deficiency in children. A clinical study with special reference to intestinal findings. Arch Dis Child 1971;46:665-670.

Savilahti E, Reunala T, Mäki M. Increase of lymphocytes bearing the γ/δ T cell receptor in the jejunum of patients with dermatitis herpetiformis. Gut 1992;33:206-211.

Savilahti E, Viander M, Perkkio M, Vainio E, Kalimo K, Reunala T. IgA antigliadin antibodies: a marker of mucosal damage in childhood coeliac disease. Lancet 1983;1:320-322.

Schuppan D. Current concepts of celiac disease pathogenesis. Gastroenterology 2000;199:234-242.

Segerberg-Konttinen M, Konttinen YT, Bergroth V. Focus score in the diagnosis of Sjögren's syndrome. Scand J Rheumatol 1986;61(suppl):47-51.

Shan L, Molberg O, Parrot I, Hausch F, Filiz F, Gray GM, Sollid LM, Khosla C. Structural basis for gluten intolerance in celiac sprue. Science 2002;297:2275-2279.

Shmerling DH, Sacher M, Widmer B, Ben Zur ED. Incidence of dental caries in coeliac children. Arch Dis Child 1980;55:80-81.

Smith DMH, Miller J. Gastro-enteritis, coeliac disease and enamel hypoplasia. Br Dent J, 1979;147:91-95.

Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. Evidence for a primary association of celiac disease to a particular HLA-DQ α/β heterodimer. J Exp Med 1989;169:345-350.

Sollid LM, Molberg O, McAdam S, Lundin KE. Autoantibodies in coeliac disease: tissue transglutaminase – guilt by association? Gut 1997;41:851-852.

Strober W, Blaese RM, Waldmann TA. The origin of salivary IgA. J Lab Clin Med 1970;75:856-862.

Sulkanen S, Halttunen T, Laurila K, Kolho K-L, Korponay-Szabo I, Sarnesto A, Savilahti E, Collin P, Mäki M. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology 1998;115:1322-1328.

Syrjänen S. Salivary glands in rheumatoid arthritis. Thesis, University of Kuopio, Finland 1982.

Tappuni AR, Challacombe SJ. A comparison of salivary immunoglobulin A (IgA) and IgA subclass concentrations in predentate and dentate children and adults. Oral Microbiol Immunol 1994;9:142-145.

Tenovuo J. Nonimmunoglobulin defence factors in human saliva. In: Tenovuo JO, editor. Human saliva: clinical chemistry and microbiology, volume II. Boca Raton: CRC Press, 1989.

Tiberti C, Bao F, Bonamico M, Verrienti A, Picarelli A, Di Tola M, Ferri M, Vecci E, Dotta F, Eisenbarth GS, Di Mario U. Celiac disease-associated transglutaminase autoantibody target domains at diagnosis are age and sex dependent. Clin Immunol 2003;109:318-324.

Trier JS. Celiac sprue. NEJM 1991;325:1709-1719.

Unsworth DJ, Manuel PD, Walker-Smith JA, Cambell CA, Johnson GD, Holborow EJ. New immunofluorescent blood test for gluten sensitivity. Arch Dis Child 1981;56:864-868.

Vainio E, Kalimo K, Reunala T, Viander M, Palosuo T. Circulating IgA- and IgG class antigliadin antibodies in dermatitis herpetiformis detected by enzyme-linked immunosorbent assay. Arch Dermatol Res 1983;275:15-18.

Vainio E, Kalimo K, Viander M, Reunala T.Antigliadin antibodies and gluten-free diet in dermatitis herpetiformis. Acta Derm Venereol 1985;65:291-297.

Valdimarsson T, Toss G, Lofman O, Strom M.Three years' follow-up of bone density in adult coeliac disease: significance of secondary hyperparathyroidism. Scand J Gastroenterol 2000;35:274-280.

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

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

Ventura A, Neri E, Ughi C, Leopaldi A, Citta A, Not T. Gluten-dependent diabetes-related and thyroid-related autoantibodies in patients with celiac disease. J Pediatr 2000;137:263-265.

Vitali C, Bombardieri S, Moutsopoulos HM, Balestrieri G, Bencivelli W, Bernstein RM, Bjerrum KB, Braga S, Coll J, De Vita S, Drosos AA, Ehrenfeld M, Hatron PY, Hay EM, Isenberg DA, Janin A, Kalden JR, Kater L, Konttinen YT, Maddison PJ, Maini RN, Manthorpe R, Meyer O, Ostuni P, Pennec Y, Prause JU, Richards A, Sauvezie B, Schiødt M, Sciuto M, Scully C, Shoenfeld Y, Skopouli FN, Smolen JS, Snaith ML, Tishler M, Todesco S, Valesini G, Venables PJ W, Wattiaux MJ, Youinou P. Preliminary criteria for the classification of Sjögren's syndrome. Results of a prospective concerted action supported by the European Community. Arthritis Rheum 36:340-347, 1993.

Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, Daniels TE, Fox PC, Fox RI, Kassan SS, Pillemer SR, Talal N, Weisman MH and the European Study Group on Classification Criteria for Sjögren's Syndrome. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61:554-558.

Walker DM, Dolby AE, Mead J, Llewellyn, Rhodes J. Effect of gluten-free diet on recurrent aphthous ulceration. Br J Dermatol 1980;103:111.

Wallington T, Ajdukiewicz AB, Read AE, Jones JV. Levels of salivary IgA in treated and untreated coeliac disease. Gut 1972;13:718-720.

Wray D. Gluten-sensitive recurrent aphthous stomatitis. Dig Dis Sci 1981;26:737-740.

Wray D, Fergusson MM, Hutchinsson AW, Dagg JH. Nutritional deficienses in recurrent aphthae. J Oral Pathol 1978;7:418-423.

Zone JJ, Meyer LJ, Petersen MJ. Deposition of granular IgA relative to clinical lesions in dermatitis herpetiformis. Arch Dermatol 1996;132:912-918.