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Ritva Koskela

MICROSCOPIC COLITIS: CLINICAL FEATURES AND GASTRODUODENAL AND IMMUNOGENETIC FINDINGS

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INSTITUTE OF CLINICAL MEDICINE,
DEPARTMENT OF INTERNAL MEDICINE,
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DEPARTMENT OF PATHOLOGY,
DEPARTMENT OF MEDICAL MICROBIOLOGY

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MICROSCOPIC COLITIS: CLINICAL FEATURES AND GASTRODUODENAL AND IMMUNOGENETIC FINDINGS

Academic dissertation to be presented with the assent of the Faculty of Medicine of the University of Oulu for public defence in Auditorium 7 of Oulu University Hospital, on 20 May 2011, at 12 noon

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Abstract

The aims of this study were to investigate the clinical features, the endoscopic and histological abnormalities of ileocolonic and gastroduodenal mucosa and immunogenetic background of microscopic colitis (MC) and its subtypes collagenous colitis (CC) and lymphocytic colitis (LC). 30 patients with CC and 54 with LC were examined with different control groups used according to the study.

The mean age at diagnosis was in the sixties in both CC and LC, with a female preponderance in both Autoimmune conditions such as celiac diseased (CD) were common in MC. Bronchial asthma associated with LC. Lactose intolerance associated with MC but colonic diverticulosis was rare.

Ileal histological changes were common in MC. Focal gastritis did not associate with MC. Lymphocytic gastritis was found only in LC. Gastric endoscopic erosions were more prevalent in CC than in LC. The age at diagnosis of MC was higher in *H. pylori* positive than negative patients. The patients with MC had shorter duodenal villi than controls even when patients with CD were excluded.

HLA-DR3-DQ2 haplotype and TNF2 allele carriage were more frequent in patients with MC compared to controls. The genotype GG of IL-6-174 was more prevalent in MC compared to the controls. IL-6 genotype did not associate with the serum IL-6 concentration. The concentration of IL-6 was higher in patients with CC than in LC.

In conclusion, in addition to colonic typical inflammation, histological abnormalities were detected also in gastric, duodenal and ileal mucosa. CD was common in MC, but there was no association with specific types of gastritis. HLA association was found in MC. Polymorphism in the proinflammatory IL-6-174 gene displayed a possible association with MC. Although CC and LC share many clinical features, the differences in the occurrence of immune conditions, gastric abnormalities and IL-6 response point to differences in their pathogenesis.

Keywords: celiac disease, collagenous colitis, cytokine gene polymorphism, focal gastritis, Helicobacter pylori, HLA-DR3-DQ2, interleukin-6, lymphocytic colitis, lymphocytic gastritis, microscopic colitis, TNF2

Koskela, Ritva, Mikroskooppisten koliittien kliiniset, histopatologiset ja immunogeneettiset piirteet.
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Tiivistelmä

Tutkimuksen tavoitteena oli tutkia mikroskooppisen koliitin sekä sen alaryhmien, kollageenikoliitin ja lymfosyyttisen koliitin kliinisiä piirteitä, mahalaukun ja ohutsuolen limakalvon muutoksia sekä immunogeneettistä taustaa. Tutkimukseen osallistui 30 kollageeni- ja 54 lymfosyyttikoliittipotilasta sekä verrokkeja.

Sekä kollageenikoliitti että lymfosyyttinen koliitti diagnosoitiin keskimäärin 50-60 v iässä, ja molemmissa tautiryhmissä naisia oli enemmän kuin miehiä. Autoimmuunisairaudet kuten keliakia olivat yleisiä liitännäissairauksia. Astmaa esiintyi lymfosyyttistä koliittia sairastavilla verrokkeja enemmän. Laktoosi-intoleranssi oli yleistä, mutta paksusuolen divertikuloosia oli harvoin mikroskooppista koliittia sairastavilla potilailla.

Ileumin muutokset olivat yleisiä. Mikroskooppinen koliitti ei assosioitunut fokaaliseen gastriittiin. Lymfosyyttigastriittia todettiin vain lymfosyyttisessä koliitissa. Mahalaukun eroosioita esiintyi enemmän kollageenikoliitissa kuin lymfosyyttisessa koliitissa. Mikroskooppinen koliitti ilmeni iäkkäämpänä niillä, joilla todettiin helikobakteeri. Pohjukaissuolen suolinukka oli keliakiasta riippumatta matalampaa kuin verrokeilla.

HLA-DR3-DQ2 haplotyyppiä, TNF-2 alleelia ja IL-6-174-GG genotyyppiä esiintyi enemmmän mikroskooppista koliittia sairastavilla potilailla kuin verrokeilla. IL-6 genotyyppi ei vaikuttanut seerumin IL-6-pitoisuuteen. IL-6 pitoisuus oli korkeampi kollageenikoliitissa kuin lymfosyyttisessä koliitissa.

Havainnot osoittavat, että mikroskooppisessa koliitissa limakalvomuutoksia on paksusuolen lisäksi myös muualla mahasuolikanavassa. Keliakia on tavallinen liitännäistauti. HLA-DR3-DQ2 on yleinen mikroskooppista koliittia sairastavilla myös ilman keliakiaa. IL-6-174-GG genotyypin yleisyys viittaa siihen, että tämä polymorfismi saattaa altistaa mikroskooppiselle koliitille. Vaikka kollageenikoliitti ja lymfosyyttinen koliitti ovat kliinisesti samankaltaisia sairauksia, erot tautiassosiaatioissa, mahan limakalvon muutoksissa ja seerumin IL-6-tasoissa viittaavat erilaisiin syntymekanismeihin.

Asiasanat: fokaalinen gastriitti, Helicobacter pylori, HLA-DR3-DQ2, IL-6, keliakia, kollageenikoliitti, lymfosyyttigastriitti, lymfosyyttinen koliitti, mikroskooppinen koliitti, sytokiinigeenipolymorfismi, TNF2

Lennä, lennä
hetken tulinen lintu
Tee pesä pilvien
väliin
Sitä se onni on
ettei hetkeen katso
taakseen
eikä eteen.

Tommy Tabermann

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Oulu, April 2011 Ritva Koskela

Abbreviations

A	Adenine
ANA	Anti-nuclear antibodies
ASA	Acetylsalicylic acid
BAM	Bile acid malabsorption
BMI	Body mass index
C	cytosine
CRP	C reactive protein
CD	Celiac disease
CC	Collagenous colitis
DD	Diverticular disease
DH	Dermatitis herpetiformis
ESPGAN	European Society of Paediatric Gastroenterology
ESR	Erythrocyte sedimentation rate
5-ASA	5-aminosalicylic acid
FG	Focal gastritis
G	Guanine
GFD	Gluten-free diet
GWA	Genome-wide association
H. pylori	Helicobacter pylori
HLA	Human leukocyte antigen
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IFN	Interferon
IEL	Intraepithelial lymphocyte
IL	Interleukin
IL-1-RA	Interleukin-1 receptor antagonist
LC	Lymphocytic colitis
LG	Lymphocytic gastritis
MC	Microscopic colitis
MHC	Major histocompatibility complex
NSAID	Non-steroidal anti-inflammatory drug
RA	Rheumatoid arthritis
RCD	Refractory celiac disease
SCL	Subepithelial collagen layer
SD	Standard deviation

SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SSRI	serotonin reuptake inhibitors
T	Thymidine
Th	T helper
TNF	Tumour necrosis factor
TTG	Tissue transglutaminase
UC	Ulcerative colitis

List of original articles

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals.

I Koskela RM, Niemela SE, Karttunen TJ & Lehtola JK (2004) Clinical characteristics of collagenous and lymphocytic colitis. Scand J Gastroenterol 39: 837–845.

II Koskela RM, Niemelä SE, Lehtola JK, Bloigu RS & Karttunen TJ (2011) Gastroduodenal mucosa in microscopic colitis. Scand J Gastroenterol 46: 567–576.

III Koskela RM, Karttunen TJ, Niemela SE, Lehtola JK, Ilonen J & Karttunen RA (2008) Human leucocyte antigen and TNFα polymorphism association in microscopic colitis. Eur J Gastroenterol Hepatol 20: 276–282.

IV Koskela RM, Karttunen TJ, Niemelä SE, Lehtola JK, Bloigu RS & Karttunen RA Cytokine gene polymorphisms in microscopic colitis. Association with IL-6-174 GG genotype. Eur J Gastroenterol Hepatol, in press.

Contents

1 Introduction

Microscopic colitis (MC) is a term used to describe those entities characterized by chronic watery diarrhoea, normal endoscopical appearance of the colon, and typical histological inflammatory changes in the colon (Read *et al.* 1980). MC is found in 9–10%, even up to 20% of the colonoscopies performed for non-bloody chronic diarrhoea (Fine *et al.* 2000b, Pardi *et al.* 2007).

Collagenous colitis (CC) and lymphocytic colitis (LC) are the main types of MC, and the differential diagnosis of these entities is based on typical histological appearance of colon mucosa (Lazenby *et al.* 1989). The typical feature in CC is a clear subepithelial collagen band in addition to microscopic inflammatory changes in the mucosa of the colon (Lindstrom 1976). In LC, the most characteristic feature is an increased number of colonic intraepithelial lymphocytes (IEL) which may be found also in some cases of CC (Lazenby *et al.* 1989). The relation of CC and LC is not clear. It is not known whether they are both manifestations of a single disease entity or whether they represent separate but related clinical conditions (Veress *et al.* 1995). These diseases resemble each other in many of their features and cannot be distinguished by the clinical presentation or disease course. There have been reports of a progression of CC to LC and vice versa (Nyhlin *et al.* 2006).

The pathogenesis of MC is not understood. (Pardi 2004.) The associations with various autoimmune diseases such as celiac disease (CD), thyroid dysfunction and rheumatoid diseases, female predominance and the connection with specific human leucocyte antigen (HLA) – type have given rise to proposals of an autoimmune reaction being involved in pathogenesis of MC. Some compounds such as non-steroidal anti-inflammatory drugs (NSAID) have also been connected to the onset of symptoms of MC. Bile acid malabsorption (BAM) and infective agents have also suspected to play a role in disease pathogenesis.

This study investigated the clinical characteristics of MC, its associations with other illnesses and medications. It was wished to determine if, in addition to CD, there are other characteristic abnormalities which can be detected in the upper gastrointestinal tract. In addition, the specific immunologic and genetic background of MC was investigated such as the occurrence of HLA-DQ-haplotypes associated with CD and polymorphisms of certain cytokine genes (Tumor necrosis factor (TNF)-α, Interleukin (IL)-6, IL-1 β, IL-1 receptor antagonist (IL-1RA), IL-10, and CD14).

2 Review of the literature

2.1 Microscopic colitis

2.1.1 History and terminology

In 1976, Lindström described microscopic inflammatory changes and the subepithelial collagen layer (SCL) in a macroscopically normal colon of a woman suffering from diarrhoea and called the condition collagenous colitis (CC) (Lindstrom 1976). Microscopic colitis (MC) is a term originally introduced by Read *et al.* (1980) to describe patients with idiopathic chronic diarrhoea, normal endoscopical findings and microscopic evidence of an inflammatory infiltrate in the colonic mucosa, the symptoms of which are distinct although somewhat similar to those seen in CC. Lazenby *et al.* (1989) later showed that an increased number of colonic IELs was the most characteristic feature of MC and suggested a more accurate term: lymphocytic colitis (LC). In the literature MC is often used as an umbrella term covering all cases of colitis with abnormal histology but a normal endoscope appearance, and thus LC and CC are considered as two basic subtypes of MC (Pardi 2004). Towards the end of the 1990´s and in the 2000´s also atypical forms of microscopic colitis have been described: paucicellular LC or MC not otherwise specified, MC with the presence of giant cells, pseudomembranous CC, MC with granulomatous inflammation, cryptal lymphocytic coloproctitis and clear cell colitis (Chang *et al.* 2005).

2.1.2 Epidemiology

The prevalence of chronic diarrhoea is 4–5% in the population and 7–14% in an elderly population, with the frequency depending on definitions of diarrhoea and geographical region (Thomas *et al.* 2003). MC is found in 8–10% of patients colonoscopied because of diarrhoea (Olesen *et al.* 2004b, Fernandez-Banares *et al.* 2010). There is published population-based epidemiologic data of MC from several different regions which is summarized in Table 1. The incidence increases gradually with age in MC (Olesen *et al.* 2004b). According to some epidemiological studies, the incidence of MC has increased during the past decades. This trend may partly be due to the increased awareness of the condition among clinicians and histopathologists, and this has led to improved diagnosis.

The geographical differences may represent true variation in the disease burden, but there may also be differences in the clinical routine for examining patients with diarrhoea.

Table 1. Incidence rates (new diagnosis per 100 000 person years) of collagenous colitis and lymphocytic colitis in epidemiological studies

Region	Study period	Collagenous colitis	Lymphocytic colitis	Study
Sweden	1984–1993	1.8	not available	Bohr 1995
	1993–1998	4.9	4.4	Olesen 2004
	1999–2004	5.2	5.5	Wickbom 2006
Spain	1993–1997	2.3	3.7	Fernandez-Banares 1999, 2010
	2004–2008	2.9	2.3	
Iceland	1995–1999	5.2	4.0	Agnarsdottir 2002
USA	1985–1997	1.6	2.7	Pardi 2007
	1998–2001	7.1	12.6	
Canada	2002–2004	4.6	5.4	Williams 2008

2.1.3 Demographic features

MC is usually considered as a disease of the aged. However, a wide age range has been reported, and the disease may even rarely occur in childhood (Pardi 2004). Demographic features are summarized in Table 2. The peak incidence of MC is in the 6th and 7th decades although the peak seems to have moved to older age groups over 80 years as observed in a recent Spanish study (Fernandez-Banares *et al.* 2010). Accordingly, in a Swedish study, MC was found in 20% of patients older than 70 years who had been referred for colonoscopy for non-bloody diarrhoea compared to 10% of diagnostic yield in average (Olesen *et al.* 2004b). A predominance of women has been repeatedly reported in MC.

Table 2. Demographic features in microscopic colitis.

Characteristics	Microscopic colitis N = 1859	Collagenous colitis N = 702	Lymphocytic colitis N = 1157
Gender (M:F)	1:1.6–1:6.2	1:2.7–1:7.5	1:1.1–1:4.5
Age (years) at diagnosis			
Mean[1]		55–70[1]	56–68[1]
(range)		(18–94)	(17–98)
Median[2]		63–64[2]	61–64[2]
Diagnostic delay[3] (months)			
Median		4–25	2–5
(range)		(0–600)	(1–240)

[1]mean (Fernandez-Banares *et al.* 1999, Baert *et al.* 1999, Kao *et al.* 2009, Fernandez-Banares *et al 2010.*

[2]median (Bohr *et al.* 1996a, Pardi *et al.* 2002, Olesen *et al.* 2004b, Pardi *et al.* 2007).

[3]Diagnostic delay refers to the time interval between the onset of symptoms and the diagnosis of MC (Fernandez-Banares *et al.* 1999, Baert *et al.* 1999, Fernandez-Banares *et al.* 2003, Olesen *et al.* 2004a, Olesen *et al.* 2004b, Fernandez-Banares *et al.* 2010)

2.1.4 Clinical features

The symptoms and clinical features of CC and LC are indistinguishable. (Fernandez-Banares *et al.* 2003.) Chronic or recurrent non-bloody watery diarrhoea is the most typical hallmark of MC, ranging from mild to severe and medically refractory. Nocturnal stools and faecal incontinence are common. Patients with MC without diarrhoea and even with constipation have also been reported (Mullhaupt *et al.* 1998, Olesen *et al.* 2004a). Abdominal pain, flatulence, abdominal distension and urgency and other symptoms common in irritable bowel syndrome (IBS) have often been reported in patients with MC (Limsui *et al.* 2007). Weight loss, fatigue and nausea are also frequently encountered. (Bohr *et al.* 1996a, Pardi *et al.* 2002.) Although some patients may suffer from severe diarrhoea, serious dehydration is uncommon. Laboratory tests are usually in the normal range, but mild anaemia and hypersedimentation may be present (Fernandez-Banares *et al.* 2003).

2.1.5 Disease course and prognosis

The onset of symptoms can be sudden or gradual. (Bohr *et al.* 1996a, Baert *et al.* 1999, Olesen *et al.* 2004a.) The disease course is often variable with remissions and relapses. Self-limited single attacks and spontaneous resolution, as well as a

course with chronic refractory symptoms and constant diarrhoea have been reported.

The natural history for MC is usually benign, although disruptions in the quality of life might be significant (Madisch *et al.* 2005). In follow-up studies which varied in length from 6 months to 6.4 years, the resolution of symptoms spontaneously or with medications was achieved in 18–74% of patients with CC and 60–93% of patients with LC (Goff *et al.* 1997, Mullhaupt *et al.* 1998, Baert *et al.* 1999, Fernandez-Banares *et al.* 2003, Olesen *et al.* 2004a, Sveinsson *et al.* 2008). However, diarrhoea was chronic in 7–44% of patients with MC and continuous or intermittent medical treatment was needed in 22–50% of patients. A histologic improvement in follow-up endoscopies in MC patients with or without treatment has been found in 36–82% of patients (Mullhaupt *et al.* 1998, Baert *et al.* 1999, Olesen *et al.* 2004a).

Deaths described in follow-up cohorts were considered to be unrelated to MC and would be expected on the basis of the advanced age of the patients. (Fernandez-Banares *et al.* 2003, Pardi *et al.* 2007.) The overall cancer risk and mortality seem to be similar to those expected though long-term studies are still lacking. Some cases of colorectal cancer have been reported, and in only few cases of colorectal cancer occurring after a diagnosis of MC have been reported (Williams *et al.* 2008, Kao *et al.* 2009). In the study of Williams *et al.* (2008), the risk of developing MC was associated with past history of malignancy in older women. In addition, an excess of lung cancers in patients with CC has been reported in women (Chan *et al.* 1999).

2.1.6 Associated diseases and extracolonic manifestations

The occurrence of associated diseases is summarized in Table 3. Many studies have observed increased rates of autoimmune and chronic inflammatory disease in patients with MC with no differences being detected between CC and LC. In particular, the association to CD is remarkable as seen in Table 4. This connection will be discussed later in section 2.2.1. Using the anti-depressive drugs as serotonin reuptake inhibitors (SSRIs), as a proxy, depression seems to be common in patients with MC but only in one study of LC has the frequency been estimated to be 14% (Olesen *et al.* 2004a). No remarkable differences were found in the associated diseases between the CC and LC in most studies.

Inflammatory changes may not be restricted to the colon mucosa in MC although no systematic information of extracolonic abnormalities in MC has been

published. Ileal histologic changes such as villous atrophy, intraepithelial lymphocytosis and abnormal SCL have been reported in MC (Marteau *et al.* 1997, Sapp *et al.* 2002).

There are mainly case reports of gastric and duodenal changes in MC patients. In addition, in gastric and duodenal mucosa, an increased number of IEL may occur. (Marteau *et al.* 1997, Fine *et al.* 2000a.) In the duodenum elevated numbers of IEL are often accompanied by villous atrophy and cryptal changes indicative of concomitant CD. Lymphocytic gastritis (LG) and enteritis in association with LC and collagenous gastritis, gastrobulbitis and enterocolitis in association with CC and LC will be discussed in the section 2.2.4 (Wu & Hamilton 1999, Leung *et al.* 2009).

Table 3. Associated diseases in microscopic colitis.

Associated disease	Microscopic colitis (%)	Collagenous colitis (%)	Lymphocytic colitis (%)
Celiac disease	0–17	0–11	0–27
Diabetes mellitus (all types)	7–14	3–16	3–16
Insulin dependent diabetes	2	2–4	3–9
Thyroid disorders	5–13	5–21	5–21
Rheumatoid arthritis	3–7	2–10	2–7
Other collagenoses	2–9	1–11	3–7
Fibromyalgia	3–4	3–6	2–5
Chronic artrhalgia	23	25	21
Bronchial asthma	7	6–7	4–5
Autoimmune diseases	18–45	18–58	18–47

References: (Armes *et al.* 1992, Bohr *et al.* 1996a, Baert *et al.* 1999, Gillett & Freeman 2000, Ayata *et al.* 2002, Pardi *et al.* 2002, Fernandez-Banares *et al.* 2003, Olesen *et al.* 2004a, Barta *et al.* 2005, Williams *et al.* 2008, Kao *et al.* 2009)

Table 4. Prevalence of celiac disease (CD) in microscopic colitis.

Region, country	Microscopic colitis (collagenous colitis ; lymphocytic colitis) N (N;N)	Prevalence of celiac disease			Study*
		microscopic colitis N (%)	collagenous colitis (N%)	lymphocytic colitis N (%)	
Australia	38 (38; 0)		4 (11) (40)[3]		Armes 1992
Belgium	176 (96; 80)	10 (5.7)	3 (3.1)	7 (8.8)	Baert 1999
Canada	23 (8; 15)	4 (17.4)	0	4 (27)	Gillett 2000
Canada	104 (69; 35)	2 (2)	0	2 (5.7)	Chande 2005
Canada	164 (75; 89)	12 (7.3)	5 (6.7)	7 (7.9)	Williams 2008
Spain	81 (37;44)	0	0	0	Fernandez-Banares 2003
Sweden	163 (163; 0)		13 (8)		Bohr 1996
Sweden	97 (51;46)	7 (13.7) (17)[1]	3 (6.5) (7.5)[1]		Olesen 2004b
Sweden	199 (0; 199)			17 (9)	Olesen 2004a
United Kingdom	53 (43; 10)	5 (9.4)	3 (6)	2 (2)	Fraser 2002
United States	170 (0; 170)			10 (6)	Pardi 2002
United States	130 (46; 84)	5 (3.1) (10)[2]	2 (4.3)	3 (3.6)	Pardi 2007
United States	547 (171; 376)	18 (3.3)	5 (2.9)	13 (3.5)	Kao 2009

*The first author and year of the publication.

[1]42 pts of CC tested for CD, 7 found (17%)
40LC patients tested for CD,3 found (7.5%)

[2]50 patients tested for CD, 5 found (10%)

[3]10 patients tested for CD, 4 found (40%)

2.1.7 Diagnosis of MC

A history of chronic watery diarrhoea may arouse a suspicion of MC. Several other conditions causing chronic diarrhoea must be excluded. (Thomas *et al.* 2003.) Laboratory tests should be taken to exclude other causes of diarrhoea and to find diseases which may be worsening the symptoms. Lactose intolerance, other dietary agents or drugs possibly causing diarrhoea need to be determined and ruled out. Hyperthyreosis, CD and other diseases which may cause abdominal

symptoms should be excluded. It is recommended to undertake stool culture to exclude infectious colitis.

The most relevant intestinal diseases to be considered in the differential diagnosis are ulcerative colitis (UC), Crohn`s disease, intestinal infections and IBS. (Fine *et al.* 2000b, Thomas *et al.* 2003.) The differential diagnosis is mostly based on the endoscopic view and evaluation of histological samples taken from the colon. However, it is notable that some histological features often associated with inflammatory bowel disease (IBD) or even ischemic or infectious colitis may be present in patients with clinically established MC (Ayata *et al.* 2002). The differential diagnosis and relationship of MC with CD, IBD and IBS are reviewed in sections 2.2.1, 2.2.2 and 2.2.3.

Endoscopy

In colonoscopy, the surface of the colon is typically normal-looking to the eye, but subtle non-specific abnormalities such as erythema, oedema or abnormal vessel-patterns have been described in 20–34% of cases, and in rare cases mucosal tearing has been observed (Bohr *et al.* 1996a, Cruz-Correa *et al.* 2002, Ayata *et al.* 2002, Olesen *et al.* 2004a). In chromoendoscopy with indicocarmine staining of the mucosa, an uneven and coarse staining pattern distinguishable from normal mucosa has been reported in CC (Sato *et al.* 2003). In addition, new techniques such as endoscopic ultrasonography and confocal endomicroscopy have been tested in MC although the data about the benefit of those advanced techniques is still rather limited (Hollerweger *et al.* 2003, Kiesslich *et al.* 2006).

Since the histological changes in colon may be distributed in a patchy manner, multiple biopsies are recommended. None of the colonic segments alone provides a 100% diagnostic yield, although the inflammatory changes and possible collagenous bands are often most prominent in the proximal part of the colon. It has been reported that a sigmoideoscopic examination may miss the diagnosis in 5–44% of cases. (Offner *et al.* 1999, Agnarsdottir *et al.* 2002, Pardi 2004.) Ileocolonoscopy is recommended in patients over 50 years of age in order to obtain samples from different colonic segments to maximize the likelihood of a correct diagnosis and also to exclude malignancies, adenomas and Crohn`s disease of proximal colon and ileum.

Histopathology

The diagnosis of MC is based on histological samples taken from the colon. The diagnosis of LC is based on an increased number of IEL (>20/100 epithelial cells) (Figure 1), and the diagnosis of CC is established by the presence of SCL beneath the basal membrane exceeding 10 µm in thickness (Figure 2). Both changes are seen as diffusely visible in more than one biopsy specimen (Lindstrom 1976, Lazenby *et al.* 1989). The collagen deposition of SCL consists mainly of type VI collagen but also type III collagen may be detectable. SCL contains also a significant amount of the glycoprotein, tenascin (Aigner *et al.* 1997).

An additional finding in both forms of MC is inflammation in lamina propria dominated by lymphocytes and plasma cells (Lazenby *et al.* 1989). In addition, eosinophils and mast cells may be found, but neutrophils are scanty. Surface epithelial degenerative changes are seen; these consist of flattening and vacuolization of the epithelial cells and detachment of the surface epithelium. In CC, there may be an excess of IEL, although often to a lesser degree than found in LC. A simultaneous increase in the numbers of IEL (more than 20%) and the SCL has been reported in up to 28–81% of patients with CC (Veress *et al.* 1995, Baert *et al.* 1999, Fernandez-Banares *et al.* 2003). The IELs are predominantly CD8+ T-lymphocytes and lymphocytes in lamina propria consist of CD4+ T helper (Th)-cells (Mosnier *et al.* 1996). MC shares some morphologic features in common with IBD e.g. active cryptitis and crypt abscesses and Paneth cell metaplasia (Ayata *et al.* 2002).

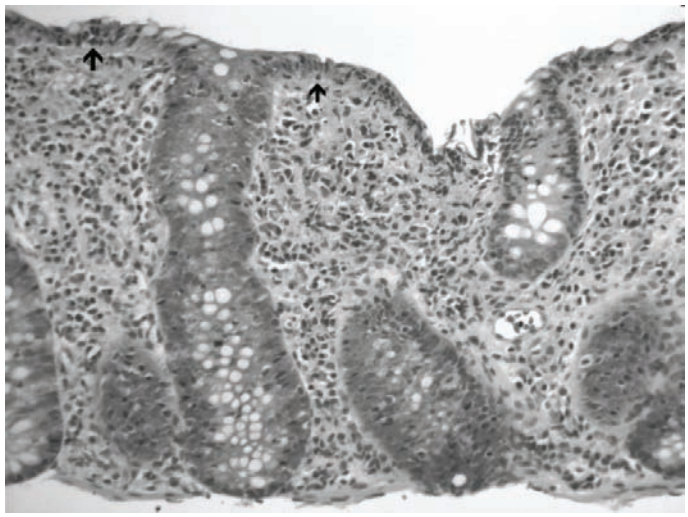

Fig. 1. Biopsy findings in lymphocytic colitis showing the characteristic increased number of intraepithelial lymphocytes (arrow) and an increased number of lamina propria mononuclear inflammatory cells. (Stained with hematoxylin and eosin), original magnification x 200).

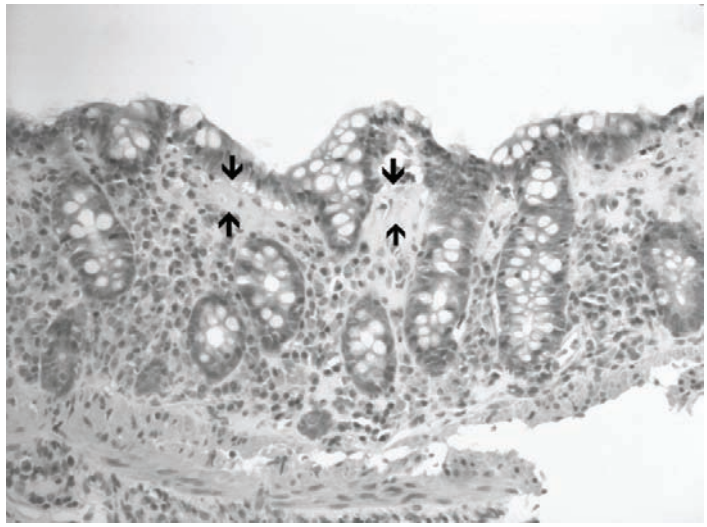

Fig. 2. Biopsy findings in collagenous colitis showing the characteristic thick subepithelial collagen layer (arrow). There is also an increased number of mononuclear inflammatory cells in the lamina propria and a mild increase of intraepithelial lymphocytes (Stained with hematoxylin and eosin, original magnification x 200).

2.1.8 The relationship of CC and LC

There has been discussion about whether CC and LC are the same disease at different stages of development or rather two different but related conditions. Usually those conditions are considered to be subtypes of MC with similar clinical expression and partly similar histopathological features except for the SCL in CC. The clinical course may differ as more chronic and severe symptoms have been described in CC compared to LC in some but not all reports (Baert *et al.* 1999, Fernandez-Banares *et al.* 2003, Sveinsson *et al.* 2008).

The female preponderance has been reported in both forms of MC, though in many reports it has been clearer in CC compared to LC (Table 2). Differences in HLA-associations have been reported though the results are conflicting as discussed in section 2.1.9. The conversion of LC to CC or the opposite has been reported (Nyhlin *et al.* 2006).

2.1.9 Etiopathogenesis of MC

The cause of MC is largely unknown and probably multifactorial. It has been postulated that an inflammatory reaction in the mucosa arises from an abnormal immune response to a luminal antigen in predisposed individuals. (Nyhlin *et al.* 2006.) The increased number of T lymphocytes in epithelial layer of mucosa may support this proposal. The role of luminal factors is supported by regression of colonic inflammation after diversion of the faecal stream (Jarnerot *et al.* 1995). No specific luminal antigens have been detected, although different infectious agents and bacterial degradation products such as toxins, or other luminal factor as gluten have been suggested as triggering factors of the disease. Factors increasing epithelial permeability, i.e. certain drugs (NSAIDs), may allow luminal antigens to enter the lamina propria and evoke an inflammatory response (Pardi 2004). The accumulation of MC cases in certain families and its association with CD suggest some degree of genetic susceptibility (Jarnerot *et al.* 2001).

Drugs

Diarrhoea is a relatively frequent adverse event induced by some drugs, accounting for about 7% of all adverse effects of drug therapy. More than 700 drugs have been claimed to cause diarrhoea (Chassany *et al.* 2000). There are several reports suggesting that certain drugs have induced MC. Beaugerie and

Pardi (2005) have suggested a scoring system to classify drugs according their propensity for causality and 17 drugs were identified as having a high or intermediate causality to MC (Table 5). In the study by Fernandez-Banares et al (2007), drug-consumption seemed to increase the risk of MC. NSAIDs and SSRIs were often used by patients with MC and may have acted as a triggering factor for colonic inflammation or for worsening self-evolving MC. According to several clinical studies, 15–71% of patients with CC and 13–53% of patients with LC have used NSAIDs regularly and correspondingly 16–28% and 20–27% have been taking SSRIs at the time of onset of colitis (Goff *et al.* 1997, Baert *et al.* 1999, Fernandez-Banares *et al.* 2003).

Table 5. Drugs associated with microscopic colitis (modified from Beaugerie & Pardi 2005).

Causality inducing microscopic colitis	Drugs
Definite causality	Ranitidine, Acetylsalicylic acid, NSAIDs, Lansoprazole, Sertraline, Acarbose, Cyclo-3-Fort, Cirkan
Probable causality	Lisinopril,Paroxetine, Carbamazebine, Simvastatin, Flutamide, Modopar, Tardyferon, Vinburnine, Oxetorone
Low probability	Cimetidine,Gold salts, Daflon, Piascledine

Infection

A sudden onset of MC and a positive response to antibiotic treatment in some of these patients have given rise to the idea of a possible infectious cause. (Nyhlin *et al.* 2006.) In addition, the positive effect of cholestyramine treatment has been suggested to result from chelation of microbial cytotoxins. LC shares many features with Brainerd diarrhoea, an acute watery diarrhoea with a long duration associated with mucosal lymphocytosis. (Osterholm *et al.* 1986.) There is epidemiological evidence to suggest an infectious etiology for Brainerd diarrhoea, though no causative organism has been identified. Serological evidence for a *Yersinia enterocolitica* and other *Yersinia* species have been found in patients with CC (Makinen *et al.* 1998, Bohr *et al.* 2002b). There are also case reports for an association of MC with other bacteria as *Campylobacter jejuni* and *Clostridium difficile* (Nyhlin *et al.* 2006).

Autoimmunity

The reported association of MC and various autoimmune conditions, characteristic lymphocyte infiltration at the site of inflammation, HLA-DR expression in epithelial cells, female preponderance and the responsiveness to corticosteroids are features possibly reflecting the autoimmune background of MC. (Pardi 2004.) The putative association of MC and CD with autoimmunity is considered in detail in section 2.2.1. The inflammation primarily initiated by a foreign luminal agent may lead to immunologic cross reactivity with an endogenous antigen in certain predisposed individuals in the pathogenesis of an autoimmune condition. Autoimmune markers such as anti-nuclear antibodies have been detected repeatedly in MC, but the results for other antibodies such as perinuclear antineutrophil cytoplasmic antibodies and anti-*Saccharomyces cerevisiae* antibodies have been controversial (Bohr *et al.* 1996b, Freeman 1997, Holstein *et al.* 2006). No disease-specific markers for MC have so far been identified.

Genetic background and HLA associations

There are case reports of an accumulation of MC in families (Jarnerot *et al.* 2001). An HLA association could point to a genetic predisposition towards MC, but no other studies of possible genetic background of MC have been reported. An increased prevalence of HLA-A1 and a decreased prevalence of HLA-A3 in LC have been reported but no such an association was found in CC (Giardiello *et al.* 1992). The association of MC to HLA-DQ haplotypes similar to those found in CD have been reported (Fine *et al.* 2000a), but in a Spanish study, the association with HLA-DQ2 genes was observed only in LC (Fernandez-Banares *et al.* 2005).

Bile acid malabsorption

It has been speculated whether concomitant BAM could be the pathogenetic mechanism in MC, i.e. an increase of bile acids possibly triggering the mucosal inflammation in colon, but the role of BAM is contradictory. The prevalence of BAM has been detected in 27–44% of patients with CC and in 9–60% of those with LC. (Ung *et al.* 2000, Fernandez-Banares *et al.* 2001, Ung *et al.* 2002.) In uncontrolled studies, cholestyramine, a bile acid binding drug, has been reported to be effective (ad 80%) in both forms of MC where there is concomitant BAM,

even though no improvement in mucosal inflammation was detected (Ung *et al.* 2001). However, as shown in the same studies, the efficacy of cholestyramine in MC patients without BAM also seems to be good (20–67%).

Mechanism of diarrhoea in MC

The precise mechanism of diarrhoea in MC is not fully understood. Colonic perfusion studies have suggested that diarrhoea in these conditions is secretary in nature i.e. due either to increased secretion or reduced absorption (Rask-Madsen *et al.* 1983). However fasting seems to reduce diarrhoea, which would indicate that there is an osmotic component behind the diarrhoea in some patients as well (Bohr *et al.* 2002a) . In CC, the volume and weight of the diarrhoea stools seem to be related to the intensity of inflammation in the lamina propria but not to the extent or the thickness of the collagenous band (Wang *et al.* 1987, Lee *et al.* 1992). Thus the severity of chronic inflammation and surface epithelial damage of the colonic mucosa is believed to be the main determinant of diarrhoea in both CC and LC (Bo-Linn *et al.* 1985). Nitric oxide has been suggested to be involved in the pathophysiology of the diarrhoea in MC i.e. colonic nitric oxide production is elevated in MC and the levels of nitric oxide seem to correlate with the activity of MC (Lundberg *et al.* 1997).

2.1.10 Treatment of MC

There is no specific treatment for MC. Treatment has been based only in anecdotal evidence and treatment options have been mainly empirical. (Nyhlin *et al.* 2006.) It is recommended that drugs and agents possibly exacerbating diarrhoea such as NSAIDs, excess of caffeine, sweeteners or lactose should be discontinued. (Pardi 2004.) Concomitant CD should be treated by a gluten-free diet (GFD). Anti-diarrhoeal treatment with loperamide is often effective and safe to use when tolerated and recommended as a symptomatic first-line therapy. If symptomatic treatment does not help, the same drugs as used in IBD and cholestyramine have been prescribed. There are some published uncontrolled reports about treatment with some drugs normally used in MC (corticosteroids, 5-aminosalicylic acid (5-ASA) compounds, cholestyramine, bulking agents, and antibiotics) and in small series or in complicated cases of MC (azathioprine, methotrexate, cyclosporine, octreotide, pentoxifylline) but placebo-controlled and randomized studies are few with limited patient series. Those studies are

summerized in Table 6. At present, only treatment with oral budesonide has resulted with both a symptomatic and histological response, however the symptoms did reappear after discontinuation of the drug. Surgery is seldom necessary, but case reports of ileostomy with or without colectomy, subtotal colectomy or colectomy with an ileal pouch anal anastomosis have been published (Jarnerot *et al.* 1995, Nyhlin *et al.* 2006).

Table 6. Randomized placebo-controlled treatment studies in microscopic colitis (MC), collagenous colitis (CC) and lymphocytic colitis (LC).

Treatment	Dosage/day Duration of trial	type of MC N	Clinical response/remission	Histologic response/remission	Study[1]
Bismuth subsalicylate	2358 mg 8 weeks	CC 9 LC 5	MC: 7/7 vs.0/7 p = 0.03 CC 4/4 vs 0/5 p = 0.03 LC 3/3 vs. 0/2 p = ns	CC 4/4 vs. 0/0 p = 0.03 LC 2/3 vs. 1/2 p = ns	Fine 1999
Boswellia serrata	1200 mg 6 weeks	CC 31	20/23 vs. 3/22 p < 0.001	p = NS	Madisch 2007
Prednisolone	50 mg 2 weeks	CC 11	5/8 vs 0/3 p = 0.15	no data	Munck 2003
LA-5 and BB-12[2]	2 capsules 12 weeks	CC 29	6/21 vs 1/8 p = 0.38	no effect	Wildt 2006
Budesonide	9 mg 8 weeks	CC 28	8/14 vs 3/14 p = 0.05	10/11 vs 4/12 p = 0.01	Baert 2002
	9 mg 8 weeks	CC 45	20/23 vs. 3/22 p < 0.001	14/26 vs, 1/25 p = 0.002	Mielhke 2002
	9 mg 8 weeks	CC 20	10/10 vs. 2/10 p < 0.001	10/10 vs. 3/10 p = 0.02	Bonderup 2003
	6 mg 6 months[3]	CC 80	33/40 vs. 11/40 p = 0.0002	19/25 vs. 6/19 p = 0.002	Bonderup 2009 Mielhke 2008
	9 mg 6 weeks	LC 42	18/21 vs. 10/21 p = 0.01	11/15 vs. 4/13 p = 0.030	Miehlke 2009

[1]First author and year of publication.

[2]Lactobacillus acidophilus LA-5 and Bifidobacterium animalis supsp. lactis.

[3]Maintenance of response

2.2 Other chronic inflammatory states in the gastrointestinal mucosa

2.2.1 Celiac disease

CD is defined as an immune-mediated condition with abnormal proximal small intestinal mucosa triggered and maintained by the ingestion of gluten-containing grains and improved with a GFD affecting genetically susceptible persons (Abadie *et al.* 2011). CD is a common disease in the Western world, with prevalences increasing being around 0.5–1% according to serologic screening studies (Mustalahti *et al.* 2010). In Finland, the biopsy proven prevalence has been assessed at between 0.45%–1%, with the frequency in the elderly as high as 2.34%, and when also positive serology was included then prevalences of 1% in adolescents, 2% in adults and 2.74% in elderly people have been estimated (Collin *et al.* 1997, Maki *et al.* 2003, Lohi *et al.* 2007, Vilppula *et al.* 2009, Mustalahti *et al.* 2010).

The golden standard for the diagnosis of CD is the duodenal biopsy exhibiting the typical histology with increased IEL count, crypt hyperplasia and villous atrophy (Green & Cellier 2007). However, there is a range of histological changes from normal villous architecture with an mere intraepithelial lymphocytosis, through partial villous atrophy to total villous atrophy with crypt hyperplasia (Marsh 1992). The first diagnostic criteria for CD were produced in 1969 and modified later by an expert board of the European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) (Walker-Smith JA *et al.* 1990). The latest criteria revised in 2001 are also known as the Amsterdam criteria (United European Gastroenterology 2001). Serological tests, such as endomycial and tissue transglutaminase (TTG) antibodies, have been used in screening of CD (Collin *et al.* 2007).

The classical symptoms of CD are weight loss, diarrhoea, steatorrhea, but often gastrointestinal symptoms are mild and may resemble symptoms of IBS. (Green & Cellier 2007, Collin *et al.* 2007.) The silent form of the disease comprises the majority of patients with screening-detected CD. Latent CD refers to patients with positive serologic tests for CD, a high density of IEL (with γ/δ T cell receptors) without villous atrophy in duodenal mucosa and carriage of susceptibility genes for CD (Kaukinen *et al.* 2007). CD is frequently found in conjunction with other autoimmune diseases (Green & Cellier 2007).

The effect of a GFD, which is the treatment of CD, is monitored by taking duodenal biopsies after approximately one year after starting the diet (United European Gastroenterology 2001). The most common cause of a poor response is continued gluten contamination in food intentionally or by accident. (Green & Cellier 2007.) In refractory celiac disease (RCD), villous atrophy does not improve in spite of adhering to a strict GFD.

The mucosa damage develops gradually and is most prominent in the proximal small intestine with the severity of the damage, gradually decreasing towards the distal small intestine, and rarely does the lesion extend to the ileum (Murray *et al.* 2008). Abnormalities of the gastric and colorectal mucosa may also be observed in some cases. CD, especially RCD may be associated with diffuse epithelial T cell infiltration at all levels of the gastrointestinal tract (Fine *et al.* 1998, Verkarre *et al.* 2003). The IEL count in gastric mucosa, especially in antrum, has been reported to be higher in patients with CD, especially in those not with GFD, compared to controls (Karttunen & Niemela 1990, Diamanti *et al.* 1999). The association of LG to CD will be discussed in 2.2.4. In addition collagenous gastritis and collagenous sprue have been described in patients with CD (Leung *et al.* 2009).

There is a strong genetic influence on the susceptibility of CD. (Karell *et al.* 2002, Abadie *et al.* 2011.) The reported concordance rate among monozygotic twins is about 75%, and 10–15% of first degree relatives are affected. Almost all, 90–95% of CD patients possess the HLA-DR3 or -DR5/7 combination-associated DQ2 molecule with most of the rest having HLA-DR4-DQ8. On the other hand, since HLA-DR3-DQ2 is present in 10–30% of Caucasians without CD, less than 10% of HLA-DR3-DQ2-positive individuals actually develop CD. As the contribution of HLA to the familial risk of CD has been estimated to be up to 35%, the susceptibility conferred by the HLA-DR3-DQ2 may be influenced by other either inside or outside the MHC region. The so-called genome-wide association (GWA) studies had identified a large number of other genes implicated in CD (Dubois *et al.* 2010). In studies about cytokine polymorphisms the results have been controversial, depending partly on the study population and partly on the geographical region those results will be interpreted in section 2.3.3.

The relationship of CD and MC

The inflammatory characteristics of MC, i.e. mononuclear inflammatory cell infiltration of the lamina propria and intraepithelial lymphocytosis, are strikingly

similar to those observed in the small bowel in CD. In untreated CD, mild colonic lymphocytosis has been reported in 19% of patients, but it differs from LC by the lack of surface epithelial abnormalities (Fine *et al.* 1998).

In several MC studies, the association with CD has been documented (Table 4). In CD, the prevalence of MC is higher than in the normal population, with frequencies varying upon the timing of the colonoscopy to the diagnostic gastroscopy, use and effect of GFD and also the diagnostic criteria. The overall frequency of MC in CD varies from 2.7% up to 36%, frequencies such as 22–67% being reported in nonresponsive CD, and in untreated CD values as high as 75% detected in small series (Fine *et al.* 1998, Fine *et al.* 2000b, Verkarre *et al.* 2003, Hopper *et al.* 2005). An association of HLA-DR3-DQ2 with MC, especially with LC, has been described. (Fine *et al.* 2000a, Fernandez-Banares *et al.* 2005.) In those series, the relatively low prevalence of CD did not explain this association.

2.2.2 Inflammatory bowel disease

UC and Crohn`s disease are the two main types of IBD. In Finland, the prevalence of UC was 291/100 000 and Crohn`s disease 124/100 000 inhabitants, 1999 the annual incidences were estimated 19.6 and 9.4/100 000 inhabitants, respectively. (Manninen *et al.* 2010.) Both diseases manifest typically in young adolescents, the peak onset in 15 to 30 years of age with a male preponderance limited in UC. Both diseases are characterized by their intermittent courses. (Baumgart & Sandborn 2007.) In the active stage, bloody diarrhoea, abdominal cramping are usual and some patients suffer fever and lose weight. Diagnosis and differential diagnosis between the different forms of IBD are based on clinical presentation and endoscopic, histologic, radiologic and laboratory studies. In UC, biopsies of colon show diffuse mucosal inflammatory changes, basal plasmacytosis, often diffuse cryptitis, crypt abscesses and marked crypt irregularities. In Crohn`s disease, mucosal histologic changes similar to UC may be focal but marked, but submucosal inflammation and granulomas are typical for Crohn`s disease.

IBD often clusters in families, with first-degree relatives having a 4- to 20-fold higher risk of developing the disease. Both genetic and environmental factors seem to contribute to the incidence and prevalence of IBD. (Baumgart & Carding 2007.) IBD has been suggested to arise from a disruption of mucosal immune homeostasis in genetically susceptible individuals, resulting in altered processing

of enteric antigens, and chronic inflammation. GWA studies have identified several IBD susceptibility loci which have been renamed as IBD 1–9 respectively (Gaya *et al.* 2006). Mutations of the CARD15/NOD2 have been associated with Crohn`s disease, which is also demonstrated in Finnish population (Helio *et al.* 2003). A stronger association exists between the genes of HLA region in UC than in Crohn`s disease (Cho & Weaver 2007). Cytokines have been implicated in the pathogenesis of chronic inflammatory diseases and have a regulatory and effector's role in the mucosal immune and inflammatory response in IBD. The studies of cytokine polymorphism in IBD (interpreted in section 2.3.3) have been conflicting and a possible connection found is often associated with variations in IBD phenotype, the samples studied have been small and the results not reproducible in different populations. Polymorphism of IL-23 receptor gene locus has been revealed to be associated to susceptibility of Crohn`s disease and the IL-23 pathway with Th-17 cells expressing IL-23 receptor is suggested to play a major role in the pathogenesis of IBD, at least in Crohn`s disease (Cho & Weaver 2007, Baumgart & Carding 2007).

Relationship of IBD with MC

A few cases of MC have been reported to develop into Crohn`s disease or UC (Bohr *et al.* 1996a, Fernandez-Banares *et al.* 2003, Nyhlin *et al.* 2006). A family history of UC or Crohn`s disease has been previously reported in at least 2–7% of patients of LC (Pardi *et al.* 2002, Olesen *et al.* 2004a). The relationship of MC and IBD is unclear whether these two disorders occur only incidentally sometimes in the same individual or whether they share genetic predisposition or immunological pathways.

Patients affected by IBD are often younger than patients with MC in IBD, but the onset of IBD may sometimes occur also at older ages. The female preponderance found in MC does not exist in IBD. Diarrhoea with relapses and remissions is the leading symptom also in MC as in IBD, but bloody diarrhoea is not as usual in MC as in IBD. (Baumgart & Sandborn 2007.) The same drugs including 5-ASA oral formulations and corticosteroids, especially budesonide, are effective in both MC and IBD. The ultimate differential diagnosis is based on the findings in endoscopy and histology. Colonoscopic views in UC and Crohn`s disease present with distinct abnormalities differing from normal endoscopic view or very minor abnormalities in MC (Nikolaus & Schreiber 2007). Histopathology reveals occasionally neutrophilic cryptitis or crypt abscesses, surface erosions,

Paneth cell metaplasia and even crypt architectural irregularities in MC and be confused with IBD, but the degree of architectural irregularity is usually milder in MC as compared to IBD (Ayata *et al.* 2002).

2.2.3 Irritable bowel syndrome

IBS is a symptom complex referring to functional bowel disorder diagnosis based on a number of the characteristic symptoms in the absence of any detectable abnormalities. (Drossman *et al.* 2002.) The symptoms consist of abdominal discomfort associated with altered bowel habits accompanied by changes in stool form and frequency, a sensation of incomplete evacuation, bloating, with abdominal distension, and the passage of mucus per rectum. In order to specify the diagnosis of IBS, symptom-based diagnostic criteria have been elaborated, the most recent being the Rome III consensus (Longstreth *et al.* 2006). IBS is a common disorder with a prevalence varying from 3.5 to 25% depending on the population and the diagnostic criteria used. According to a Finnish study, the prevalence of IBS was 5.1% in Finnish population when Rome II criteria were used (Hillila & Farkkila 2004). There is a female preponderance, and the prevalence is highest in 20–50 year olds usually decreasing with age (Drossman *et al.* 2002). According to the pattern of bowel habits or stool consistency, IBS can be divided into subgroups such as constipation or diarrhoea prominent, mixed or unspecified form of IBS (Longstreth *et al.* 2006). IBS symptoms typically fluctuate, and some patients lose the symptoms during follow-up. About 90% of patients with IBS suffer also from other somatic symptoms and/or psychiatric symptoms (Drossman *et al.* 2002).

A careful interview of the patient is the basis of the diagnosis. Additional testing depends on the patient's age, duration, severity and type of symptoms. Laboratory tests to exclude anaemia, thyroid dysfunction, lactose intolerance, CD, parasitic infection and occult blood in stools are often worthwhile performing. (Drossman *et al.* 2002.) Colonoscopy is often indicated, especially in older patients with diarrhoea prominent IBS.

The pathophysiology of IBS is multifactorial with altered visceral sensitivity, altered gastrointestinal motility, psychosocial factors and genetic predisposition influencing the symptom generation (Drossman *et al.* 2002, Longstreth *et al.* 2006). Increased cellularity of the colonic mucosa and lamina propria and lymphocytic infiltrates of the myenteric plexus have been described in IBS patients (Chadwick *et al.* 2002). Increased levels of proinflammatory cytokines

such as TNFα, IL-1β, IL-6 in peripheral blood mononuclear cells have been analysed in IBS patients (Liebregts *et al.* 2007). Studies about the cytokine gene polymorphisms including IL-10 and TNFα gene polymorphisms suggest that IBS is associated with genetic regulation of cytokine secretion, indicative of a proinflammatory state in IBS (Gonsalkorale *et al.* 2003, van der Veek *et al.* 2005). These studies may be evidence that an abnormal mucosal immune response has a role in the pathogenesis of IBS.

Relationship of IBS with MC

Diarrhoea prominent IBS and MC share chronic recurring symptoms of altered bowel habits, diarrhoea associated with abdominal pain or discomfort, although the onset of symptoms of IBS is usually at younger ages than in MC. In a study by Limsui et al (2007), up to 56% of patients with MC (73 of 131 patients) met Rome II criteria of IBS. In the retrospective study of Kao et al (2009), 17% (30/171) patients with CC and 11.4% (43/376) patients with LC had a prior diagnosis of IBS. In a recent prospective study, MC was diagnosed in 1.5% of all the patients (7/466) and in 2.3% of patients over 45 years of age (4/171) with non-constipated IBS (Chey *et al.* 2010). An association with CD has also been described in IBS (Leeds *et al.* 2007).

The inflammatory changes in colon with increased number of IEL and increased cellularity in lamina propria in some of the IBS patients resembles but does not fulfil the histological criteria of MC (Chadwick *et al.* 2002). It has been speculated that there is a subset of patients in whom IBS may form a continuum with MC, especially LC.

2.2.4 Gastritis

Gastritis displays a broad variety histopathological and topographical features and types (Sipponen 2001.) After *Helicobacter pylori* (*H. pylori*) was discovered in 1983, the role of this bacterium in the etiology of most cases of chronic gastritis became rapidly apparent (Marshall & Warren 1984). Even when *H. pylori* in gastritis has been identified, it must not be assumed that it is the sole etiologic factor, it is possible that there are other agents causing damage to gastric mucosa, i.e. drugs, alcohol, dietary factors and infectious agents. (Dixon *et al.* 1996.) Gastritis diagnosis is mostly based on histological findings, and pathologists emphasize the importance of combining topographical, morphological and

etiological information into the classification of types of gastritis since in this way, one can generate clinically useful diagnosis. Gastritides are divided into three main categories: acute, chronic, and special forms; chronic gastritis is further separated in subcategories according to the presence or absence and distribution of atrophy. The revised Sydney system is recommended in the classification and grading of gastritis (Dixon *et al.* 1996).

The morphological variables in chronic gastritis

Important information in grading the gastritis is whether *H. pylori* is present. (Sipponen 2001.) The activity of chronic gastritis is characterised by the presence of neutrophil polymorphs in a background of chronic inflammation, those being in the lamina propria, within the epithelium and within the foveolar lumen. Neutrophil activity is an almost universal phenomenon in *H. pylori* gastritis.

A few mononuclear leukocytes i.e. lymphocytes, plasma cells and macrophages are always present in the lamina propria of gastric mucosa, normally at maximum from 2 to 5 cells per one high power (×40 objective) microscopic field. (Dixon *et al.* 1996.) Any increase in the numbers of mononuclear cells in the lamina propria is indicative of chronic gastritis. IELs may occasionally be detected in up to 5 per 100 epithelial nuclei. Lymphoid follicles (lymphoid aggregates with germinal centres) are characteristic of chronic *H. pylori* gastritis and can be considered as a hallmark diagnosis.

Atrophy of the gastric mucosa is defined as a loss of glandular tissue, which leads to thinning of the mucosa. (Sipponen 2001.) Atrophy in the oxyntic mucosa causes loss of acid secretion and to the development of intestinal or pyloric metaplasia. Atrophy and intestinal metaplasia can be either diffuse or multifocal. The pattern of inflammation and atrophy may be an indicator of its etiology (Dixon *et al.* 1996).

Foveolar hyperplasia, which may be seen in all forms of gastritis, the foveolae are increased in length and tortuosity. (Dixon *et al.* 1996.) This may develop either as a compensatory response to increased cell exfoliation from the surface or as a response to stimulation by cytokines or other inflammatory mediators.

H. pylori gastritis

The prevalence of *H. pylori* infection varies depending on the age of the subject, the socioeconomic status, the method used, the geographical region and the ethnicity and it is decreasing in developed countries such as in Finland. The reported seroprevalence rates have declined from 56 to 31% from 1973 to 1994 in 15–74 year old individuals studied in the Vammala region in Finland (Kosunen *et al.* 1997). The seroprevalence rates varied according to age from 7.3% in 15 year olds up to 68.1% in 75-year-old subjects in a 1994 patient cohort. In a study of 561 outpatients undergoing gastroscopy in 1994–2002 in the same region, *H. pylori* was detected in 32.3% of patients, the frequency of infection ranging according to age from 13.4% to 41.5% (Salomaa-Rasanen *et al.* 2004). In this study patients with *H. pylori* eradication treatment were excluded, but the possible treatment of *H. pylori* gastritis with antimicrobials had decreased the prevalence rates further, but population studies in Finland at present are lacking.

H. pylori gastritis is usually prominent in the antral mucosa. (Sipponen 2001.) Clinical outcomes of *H. pylori* gastritis range from simple asymptomatic gastritis to some more serious conditions since it has been associated with atrophic gastritis, gastric erosions, duodenal and gastric ulcer disease, gastric carcinoma and in gastric lymphoma emerging from the mucosa-associated lymphoid tissue lymphoma. (Dixon *et al.* 1996, Toljamo *et al.* 2005.) Antrum predominate *H. pylori* gastritis is associated with a peptic ulcer risk and gastric erosion risk whereas body predominant carries a low peptic ulcer risk. Multifocal or body predominant atrophy of *H. pylori* gastritis is associated with the risk of gastric cancer. The severity and distribution of *H. pylori* gastritis the host genetic factors in influencing the disease risk and *H. pylori* strain affect whether the *H. pylori* gastritis remains as simple asymptomatic gastritis or progresses to more serious conditions.

Other forms of gastritis

Focal gastritis (FG) is an idiopathic form of chronic gastritis affecting antral mucosa when *H. pylori* is not present. (Dixon *et al.* 1996.) The characteristic appearance of FG is a focal, patchy inflammation with polymorphonuclear, eosinophilic and mononuclear cells destroying parts of the crypts. FG is known to associate with IBD (Halme *et al.* 1996a, Sharif *et al.* 2002, Kundhal *et al.* 2003,

Danelius *et al.* 2009.) In Crohn`s disease, the prevalence rates of FG vary 34%-65%, in UC, the prevalence is lower 8–21%.

Lymphocytic gastritis (*LG*) is a chronic inflammatory process of gastric mucosa with unknown etiology and clinical significance. (Dixon *et al.* 1996.) LG is characterised by a marked increase in the number of lymphocytes, mainly T-lymphocytes, in the surface and foveolar epithelium of the gastric mucosa, the diagnostic threshold used for LG being greater than 25 IELs per 100 cells. The increased number of IEL is sometimes associated with marked chronic inflammatory cell infiltration of the lamina propria. The histological features of LG are more often detected in the gastric body mucosa. In follow–up studies, inflammatory changes in LG seem to be persistent and progressive. (Niemela *et al.* 1995, Hayat *et al.* 1999a.) The endoscopic appearance in LG may be normal, but gastric erosions, hypertrophic gastropathy or varioliform gastritis have also been described. The reported prevalence of LG has varied from 0.83% to 2.5% in unselected gastric biopsy material, in 4.5% of patients with chronic active gastritis, 0.83–9% in patients with dyspepsia, and at 3.7% in children and adolescents. LG is more common among females and the mean age of LG is 45–49 years (Hayat *et al.* 1999b).

H. pylori infection has been detected histologically or serologically in 40–100% of cases with LG. (Niemela *et al.* 1995, Hayat *et al.* 1999a.) Though in LG, the characteristic corpus predominating gastritis may eventually lead to the disappearance of *H. pylori* in the gastric mucosa, serologic testing may well be more sensitive in detecting *H. pylori* infection. *H. pylori* eradication has been shown to lead to a significant improvement in the mucosal changes present in LG. An association has been reported between CD and LG (Karttunen & Niemela 1990). The probability of LG seems to be higher in patients with untreated (6–61%) or refractory disease (33–64%) as compared to patients responding to a GFD (0–30%) (Wu & Hamilton 1999, Diamanti *et al.* 1999, Verkarre *et al.* 2003). Patients with both LC and CD have also been reported to suffer from LG (Verkarre *et al.* 2003).

Granulomas may be detected as a reaction to other endogenous or foreign materials, but so called *granulomatous gastritis* may be an isolated finding or a mark of various granulomatous diseases such as Crohn`s disease, sarcoidosis or tuberculosis (Dixon *et al.* 1996). *Collagenous gastritis* and collagenous gastro-duodenitis are rare disorders characterized by a deposition of a collagen band beneath the surface epithelium of gastric mucosa, in the latter case in duodenum, and they are often associated with MC, CD and LG (Leung *et al.* 2009)

2.3 Immunologic aspects of intestinal inflammation

2.3.1 Gut related immunology

The intestinal microbiota refers to the set of micro-organisms inhabiting the gut. The microbiome is acquired at birth, changes rapidly in infancy but remains rather stable in adulthood though fluctuations occur due environmental factors and diseases. (Baumgart & Carding 2007, Abraham & Cho 2009.) Close contact with high concentrations of luminal bacteria consisting of more than 500 species is a unique feature of the intestinal immune system and it is crucial for the development of normal mucosal immune function and oral tolerance.

The intestinal epithelium forms the interface between the intestinal microbiome and the mucosal lymphoid tissue and serves as a physical barrier against the entry of microbes and other antigens from the intestinal lumen into the circulation. (Abraham & Cho 2009.) The mucosal barrier is composed of enterocytes, goblet and Paneth cells, all with intercellular junctions sealing the paracellular space. Intact mucosal barrier with mucus produced by goblet cells and antimicrobial peptides secreted by epithelial cells and Paneth cells are parts of the defence to combat against microbial invasion. A large part of mucosal lymphoid tissue is in the gut, and most of the antibodies in human system are produced in the intestine. The mechanism that establishes and maintains oral tolerance with immunological non-responsiveness to the microbiota and food-derived antigens involves complex and close collaboration between anatomical, cellular and humoral factors (Baumgart & Carding 2007).

Under normal conditions, the intestinal mucosa is in a state of controlled inflammation regulated by the balance of proinflammatory and anti-inflammatory cytokines (Baumgart & Carding 2007). *The innate immunity* is the first line non-specific inborn defence against pathogens and is provided by the epithelial cells, macrophages, dendritic cell, natural killer cells, neutrophils and the complement system. (Abraham & Cho 2009.) Without prior immune learning after detecting a pathogen, innate immunity responds rapidly by recruiting immune cells, activating the complement system, identifying and removing foreign substances and stimulating the adaptive immune system. *The adaptive immune* system helps clear the infection and builds specific immunity with a memory component. Activation of the adaptive response occurs through cytokine secretion, antigenic processing and presentation and differentiation of effector cells (Hornef *et al.* 2002). T– and B-cells are the main cells in the adaptive immune system. B-cells

are antibody producers, while T cells are divided into cytotoxic T cells which possess the cell-surface molecule CD8 and Th cells which have CD4 on their surface. Activated cytotoxic T-cells can produce interferon (IFN)- γ and kill their effector cells by apoptosis. Th cells regulate the specific immune response and are classified on the basis of function as well as according to their ability to elaborate specific cytokines. Th1 cells induce the cell to mediate immune responses through cytokines such as IL-2, IL-12, IFN γ and TNFβ. Th2 cells mediates humoral responses and produces cytokines as IL-4, IL-5, IL-6, IL-10 and IL-13. IFNγ suppresses the development of Th2, whereas IL-4, IL-10 and IL-13 inhibit Th1 responses. (Brand 2009.) Proinflammatory cytokines activate macrophages and induce those cells to secrete large amounts of cytokines such as TNFα, IL-1 and IL-6. Regulatory T cells and Th17 cells producing IL-17 and other cytokines such as IL-6, IL-21, IL-22 and TNFα, are a novel subset of CD4+T cells which partly explains discrepancies in the classic Th1/Th2 model. These cells play a major role in autoimmunity and tolerance toward intestinal antigens and Th17 cells which possess IL-23 receptor as well as IL-23 play a major role in the inflammation of gut. Similar to Th2 cells, Th1 cells negatively crossregulate the differentiation of Th17 cells, and proinflammatory cytokines such as IL-1, IL-6 induce the differentiation of Th17 cells (Brand 2009). During active inflammation, effector T-cells predominate over regulatory T-cells. A defect in mucosal barrier for different causes or abnormalities in innate and adaptive immune responses to the indigenous flora and other luminal antigens may lead to abnormal mucosal inflammation in the gastrointestinal tract (Baumgart & Carding, Abraham & Cho 2009).

2.3.2 Genetic variation behind the immune responses

There is an extensive natural occurring inter-individual variation in DNA sequences with the presence of tens of thousands of genes with three billion of DNA base-pairs in human genome and approximately ten million positions in their genetic code are polymorphic at a significant frequency. (Rotimi *et al.* 2007.) Inheritance of susceptibility to many diseases is multifactorial, and the strongest disease associations are with combination of genes of alleles at multiple loci rather than with individual alleles. In addition, because of the linkage equilibrium of single nucleotide polymorphisms (SNP), the true disease susceptibility gene may be difficult to identify. Specific polymorphisms in cytokine genes and HLA genes are known to modulate the immune response and thus the inflammatory

response (Cho & Weaver 2007). In general, the combination of genes and environmental factors may predispose an individual to a certain disease, protect against disease or be neutral depending on the context of the interaction (Martinez 2007). Diseases with a polygenic pattern of heredity may be manifested when a threshold of susceptibility burden is overwhelmed by an accumulation of risk factors.

Genetic variation related to susceptibility to complex disease is generally investigated by association studies-method, using population-based or case-control subjects in comparison with the patients (Burgner *et al.* 2006.) One common method is to use the candidate gene approach. This means that one select certain genes as the study object. Recently genetic susceptibility studies have started using a GWA approach where several million SNPs throughout the whole genome are examined with modern techniques (Xavier & Rioux 2008, Dubois *et al.* 2010).

2.3.3 Cytokines and cytokine gene polymorphisms

Cytokines are biologically active molecules secreted by one cell to alter the behaviour of itself or another cell. (Bidwell *et al.* 1999.) Cytokines act on their target cells by binding to specific receptors, initiating signal transduction and triggering second messenger pathways resulting in phenotypic changes in the cell via altered gene regulation. The cytokines take part in a highly complex coordinated network in which they induce or repress their own synthesis as well as that of other cytokines and cytokine receptors. The inflammatory reaction in infectious and autoimmune diseases is regulated by the fine balance between the pro- and anti-inflammatory cytokines. (Howell *et al.* 2002.) A proinflammatory cytokine will be induced during the course of an inflammatory response and will be associated with its onset and/or progression. Functionally IL-1, IL-6 and TNFα are classified as proinflammatory cytokines, whereas IL-1-receptor antagonist (IL-1RA) and IL-10 molecules function as anti-inflammatory cytokines.

There is significant evidence to indicate that an individual´s inflammatory response can be modulated by specific polymorphisms in the promoter regions of many cytokine genes, which may influence the level or expression of these genes and thus influence cytokine production. (Bidwell *et al.* 1999.) There are several different types of polymorphisms in the genome including SNPs, deletions, insertions and repeat polymorphism. In the cytokine genes, SNPs are the most common form of polymorphisms, and they represent nucleotide replacement by

one of the four others bases - adenine (A), thymine (T), cytosine (C), guanine (G). (Wilson *et al.* 1992, Kinane & Hart 2003.) SNPs can modify cytokine production and in this way polymorphism may expose an individual to certain diseases, lead to variations in phenotypic expression and influence the disease course. In many diseases, there does not appear to be any clear-cut correlation between alleles or genotypes that are associated with higher pro-inflammatory cytokine levels and disease occurrence. The disconcordances of associations of gene polymorphism to certain diseases in different studies may be attributable to differences in the ethnicities of populations, patient and control cohort selection or small sample size, disease classification or status or method of statistical analysis (Burgner *et al.* 2006).

TNFα and gene polymorphism

TNFα is a proinflammatory cytokine produced by activated monocytes, macrophages and T cells. (Papadakis & Targan 2000.) It has an important role in the initiation, regulation and perpetuation of the inflammatory response in host defence, but inappropriately high TNFα production has been associated with several pathological conditions. In the treatment of IBD, the positive effects of anti-TNFα- monoclonal antibodies support the assumption that TNFα is important in the pathogenesis of IBD (Baumgart & Sandborn 2007).

The TNFα gene is located within the class III region of the major histocompatibility complex (MHC) on chromosome 6 (6p21) between the HLA class II and class I loci and it is in strong linkage disequilibrium with the HLA-B8 and DR3 alleles (Price *et al.* 1999). One of the polymorphisms identified in the promoter region of TNFα gene is a SNP at position -308, which involves the substitution of guanine by adenine (G→A) in the uncommon allele TNFA, also called TNF2 (Wilson *et al.* 1992). The presence of TNF2 is usually correlated with enhanced TNF production (Wilson *et al.* 1997). Polymorphisms in TNF genes have been associated with susceptibility to several autoimmune diseases, such as juvenile rheumatoid arthritis (Ozen *et al.* 2002), systemic lupus erythematosus (SLE) (Wilson *et al.* 1994), dermatitis herpetiformis (DH) (Messer *et al.* 1994) and CD (McManus *et al.* 1996). Most studies have concluded that the association between TNF2 and autoimmune disease may be explained by linkage to an extended HLA-B8-DR3 haplotype. In CD, the presence of the TNF2 allele could also independently be associated with an increased risk for CD (Polvi *et al.* 1998, Garrote *et al.* 2002, Woolley *et al.* 2005). The significance of TNFα-

polymorphisms in the risk of IBD is controversial, most of them indicating a tendency towards lower or to the same carriage rate for TNF2 allele as seen in controls (Koss *et al.* 2000,Vatay *et al.* 2003, Balding *et al.* 2004). Patients with IBS have displayed significantly higher baseline TNFα levels, and also TNF2 has been found more frequently in these subjects than in controls (O'Mahony *et al.* 2005, Liebregts *et al.* 2007)

IL-6 and gene polymorphism

Since IL-6 has both pro- and anti-inflammatory functions, it has a range of pleiotropic activities, involved in the regulation of the acute phase response controlling local or systemic acute inflammatory response and the stimulation of T cells and B cells, their growth and differentiation. (Hirano 1992.) IL-6 is produced by various types of cells including T and B cells, monocytes and fibroblasts. IL-6 synthesis and release are stimulated by IL-1β and TNFα, but IL-6 remains substantially longer in the plasma, thus serving as a good marker of inflammation (Song & Kellum 2005).

IL-6 reflects of the activation of the inflammatory processes in many autoimmune diseases and in acute and in chronic inflammatory states. High serum levels of IL-6 have been measured in periodontal disease (Raunio *et al.* 2007), in primary Sjögren´s syndrome (Hulkkonen *et al.* 2001b), in rheumatoid arthritis (RA) (Pawlik *et al.* 2005) and in Crohn`s disease both in serum and in the mucosa of inflamed intestine (Gross *et al.* 1992).

IL-6 gene is situated on chromosome 7 (7p21) and several SNPs at different sites in the IL-6 gene have been described, which are relevant to regulate the final cytokine levels. The most widely studied of these is a SNP situated at position -174 which is caused by a G to C transition (Fishman *et al.* 1998). Most of the studies indicate that the allele G may partly determine the level of IL-6 in basal and stimulated conditions but effects of this SNP on IL-6 expression may depend on the specific cell or tissue type and stimulus involved (Fishman *et al.* 1998, Hulkkonen *et al.* 2001b, Raunio *et al.* 2007). IL-6-174 polymorphism is associated with susceptibility and outcome of several acute and chronic diseases and inflammatory states. The IL-6-174-GG-genotype is associated with severe chronic periodontal disease (Raunio *et al.* 2007) and the IL-6-174-G allele has been associated to systemic-onset juvenile RA (Fishman *et al.* 1998) and to RA (Marinou *et al.* 2007). On the other hand, the IL-6-174-GG-genotype may also confer beneficial effects by protecting against atherosclerosis (Hulkkonen *et al.*

2009) and improving survival in cases of sepsis (Schluter *et al.* 2002). In CD, one study has been published into the association of IL-6-G allele in DQ2 negative patients with CD (Garrote *et al.* 2005), but no such an association was observed in Finnish material (Woolley *et al.* 2005). The studies into IBD have detected no significant differences in IL-6-polymorphisms between patients and healthy subjects (Klein *et al.* 2001, Balding *et al.* 2004).

Interleukin-1 family and their gene polymorphisms

IL-1 is produced by monocytes and macrophages, being stimulated by many proinflammatory cytokines such as TNFα, IFNα, IFNβ and IL-1β, and inhibited by anti-inflammatory cytokines such as IL-4 and IL-10 (Barksby *et al.* 2007). The IL-1 gene complex codes for three proteins, IL-1α, IL-1β and IL-1RA, of which the first two are strong inducers of inflammation, while IL-1RA is an anti-inflammatory cytokine and a physiologically effective antagonist to the other IL-1 cytokines. (Dinarello 1996). IL-1β binds to cell surface receptors and activates B – and T-cell functions, regulates fever reaction, and participates in the initiation of the synthesis of acute-phase protein.

IL-1RA is produced in macrophages, monocytes and neutrophils in response to the same stimulus as IL-1. (Dinarello 1996.) It binds to the IL-1 receptors with high affinity without activating the cell, thus inhibiting the IL-1 activity. The balance between IL-1 and IL-1RA seems to be important in the normal physiology of various organs and tissues and in the regulation of inflammatory responses. In several diseases, IL-1RA levels are elevated and it seems plausible that IL-1RA serves as a natural compensating mechanism for the IL-1 induced by the disease process and thus an imbalance between IL-1 and IL-1RA can lead to exaggerated chronic inflammation and may have an effect on the pathogenesis of many inflammatory diseases such as RA, kidney diseases, diabetes, leukaemia, graft-versus-host disease and arterial diseases (Arend & Guthridge 2000). Both evidence from animal models and studies in diseased tissues have emphasized to the importance of IL-1RA in IBD and it has been claimed that an imbalance in the IL-1RA/IL-1 ratio in the mucosa of colon may contribute to the chronic inflammative response (Mansfield *et al.* 1994, Carter *et al.* 2004).

IL-1 genes are arranged in a cluster on the long arm of chromosome 2 (2q13). In the IL-1 β gene, several polymorphisms have been reported: one of them is a SNP at position -511 (Di Giovine *et al.* 1992) and another one at position +3953 at the 5th exon site (Pociot *et al.* 1992). Both of these SNPs are caused by C to T

transitions, called also allele 2. In intron 2 of the IL-1RA gene, there are variable numbers of an 86 bp repeat sequence; the most common allele 1 contains four repeats but allele 2 contains two repeats (IL-1RA-2) (Tarlow *et al.* 1993). In linkage studies, the more rare IL-1 β (-511) allele T is associated with the presence of IL-1RA allele 2, while in case of IL-1 β (+3953) it associates with C allele. (Hurme & Santtila 1998).

Polymorphisms of the IL-1 gene cluster, inducing an imbalance between IL-1 and IL-1RA, have been associated with both susceptibility to and severity of a number of chronic inflammatory and autoimmune conditions (Hurme *et al.* 1998, Arend & Guthridge 2000). In normal healthy individuals, but apparently in many diseases and infections, the plasma IL-1RA levels are influenced by alleles of both the IL-1RA and IL-1β genes (Hurme & Santtila 1998). The IL-1 gene family has been extensively studied in IBD, including the relationship between gene polymorphisms, and both disease susceptibility and disease progression, but the results have been conflicting. Accordingly, the IL-1RA-allele 2 has been connected to UC but studies examining the allelic distribution of IL-1β-511, -3953-polymorphism have produced controversial results in UC and Crohn's disease (Mansfield *et al.* 1994, Nemetz *et al.* 1999, Carter *et al.* 2004).

IL-10 and gene polymorphism

IL-10 is an anti-inflammatory multifunctional cytokine produced by Th2 cells, B cells, monocytes and keratinocytes. (Moore *et al.* 2001.) It has an important role in the regulation of immune responses and affects many cell types. IL-10 inhibits activation, proliferation and cytokine production of T cells responding to antigens and IFN-γ production by natural killer cells. IL-10 downregulates HLA II expression and antigen presentation of special cells. Additionally IL-10 is a potent inhibitor of the production proinflammatory cytokines through its action on monocyte or macrophage (IL-1β, IL-8, IL-6, IL-12, TNFα,) and Th1 cells (IL2, IFNδ). IL-10 also promotes B cell activation and differentiation and induces immunoglobulin synthesis and autoantibody production. This cytokine has been studied in infections, systemic inflammations and autoimmune diseases (Hurme *et al.* 1998).

The gene encoding IL-10 is located on chromosome 1q31-32. (Turner *et al.* 1997.) It is a relatively highly polymorphic gene and its promoter contains various SNPs and varying numbers of dinucleotide repeats (microsatellites) in its proximal and distal regions. Three SNPs have been described in the proximal part

of this gene at positions -1082 (A/G), -819 (T/C) and -592 (A/C). Four haplotypes have been described in the Caucasian population (GCC, ACC, ATA, and GTA). It has been postulated that the A-allele at the site -1082 is associated with low and the G-allele with high production of IL-10. However, when studying haplotypes, there was no concordance about which haplotype associated with elevated plasma IL-10 levels (Hulkkonen *et al.* 2001a, Kilpinen *et al.* 2002).

The allelic variation in the IL-10 promoter region has been associated with a variety of diseases in which immune dysregulation contributes to the pathogenesis. Polymorphisms in the IL-10-1082 gene have been associated with some inflammatory conditions including RA (Hajeer *et al.* 1998), SLE (Lazarus *et al.* 1997) and Sjögren´s syndrome (Hulkkonen *et al.* 2001a). The results of studies on IL-10 gene polymorphism in CD and IBD have been controversial (Tagore *et al*. 1999). There is some evidence for IL-10 gene polymorphism in IBS (Gonsalkorale *et al.* 2003).

CD14 and gene polymorphism

CD14 protein is present as a receptor on the surface of monocytes/macrophages, and neutrophils (membrane CD14) as well as in a soluble form. (Bas *et al.* 2004.) It is a receptor to bacterial lipopolysaccharide, peptidoglycan and other bacterial molecules, and it mediates monocyte/macrophage function, modulates lipopolysaccharide-triggered apoptosis, and regulates T- and B-lymphocyte activation and function (Wright *et al.* 1990). Thus, CD14 is an important innate immunity receptor, which participates in initiating the innate immune responses. In addition to its proinflammatory action, CD14 may also control the activation of the immune system by acting as a downregulatory molecule (Bas *et al.* 2004, Kitchens & Thompson 2005). CD14 has also an important role in the recognition and clearance of apoptotic cells by macrophages and may interact directly with T and B cells and thus modulate cellular and humoral immune responses i.e. it can possess a role in directing adaptive immunity reactions (Arias *et al.* 2000). Soluble CD14 is an acute phase protein and its levels are elevated in many systemic inflammatory, immunological and infectious diseases (Bas *et al.* 2004).

The CD14 gene is located within a cytokine gene cluster in the chromosomal region 5q31. A SNP in the promoter of the CD14 gene (T/C at position -159 or -260, which refer to the same polymorphism the discrepancy being due to a difference in the numbering system) has been described (Hubacek *et al.* 1999, Baldini *et al.* 1999.) Carriage of the T allele is associated with higher activation of

the immune response to lipopolysaccharides, and individuals who carry the TT-genotype seem to be higher producers of the soluble form of CD14 and they have been observed to express a higher density or membrane-bound CD14 receptors on their monocytes. The CD14 T-containing phenotype is associated with inflammatory and systemic states such as chronic periodontitis, UC and Crohn`s disease (Obana *et al.* 2002, Klein *et al.* 2002, Tervonen *et al.* 2007).

2.3.4 Major histocompatibility complex genes and human leukocyte antigens

MHC gene complex encodes HLA molecules. They are membrane-bound glycoproteins expressed as a heterodimer of α and β chains. (Beck & Trowsdale 2000.) The HLA molecules function to present processed antigenic peptide to T-cell receptor molecules expressed on the surface of T-cells. HLA class I molecules include the A, B and C series of molecules and are expressed on the surface of most of the cells and they are recognized by CD8+ T-cells, the majority of which have a cytotoxic phenotype. The HLA class II encompass the DR, DQ and DP series of molecules and genes of class II A and B loci encode the α and β chains, respectively. Class II molecules are expressed on the surface of specialized antigen-presenting cells, and present processed antigens to CD4+ T-cells, which are mainly of the helper phenotype. The class III region contains genes encoding some complement factors (C2, C4, and Bf), steroid 20-hydroxylase (CYP21), heat shock protein 70, and TNFα and β. The MHC genes on chromosome 6p21.3 are the most polymorphic genes found in the human genome, with multiple polymorphic sites within a single gene, with clusters of hypervariable sequence motifs resulting in multiple expressed alleles. (Howell *et al.* 2002.) This means that there is a huge genetic polymorphism in the HLA-genes modulating the immune response to multiple antigens, and it also means that there are major inter-individual differences in immune responses. Since the genes in HLA molecule are situated closely, they are often inherited linked together as certain allele combinations without recombination and thus some genes occur more often together on haplotypes than would be expected according to their gene frequencies. This phenomenon is named linkage disequilibrium. (Beck & Trowsdale 2000.) The strong linkage disequilibrium makes difficult to determine whether a particular gene is involved directly in disease susceptibility or whether it reflects the effect of linked genes. One example of the strong linkage disequilibrium is the extended ancestral haplotype HLA-8.1. including HLA class

I molecules HLA-A1, -B8 and DR-3 (HLA-A1, Cw7, B8, TNF AB*a2b3, TNFN*S, C2*C, Bf*s, C4A*Q0, C4B*1, DRB1*0301, DRB3*0101, DQA1*0501,DQB1*0201) (Price *et al.* 1999).

Particular HLA polymorphisms have been linked to susceptibility to a large number of immunologically-mediated diseases, including skin, gut, endocrine and joint diseases. HLA class II expression is often observed in inflammatory and autoimmune diseases. The HLA-association linked to CD is well known, 90–95% of all CD patients possess the HLA-DR3 or -DR5/7 combination-associated DQ2 molecule encoded by DQA1*0501 and DQB1*02 alleles, with most of the other patients having HLA-DR4-DQ8 (encoded by DQA1*03 and DQB1*0302) (Karell *et al.* 2003). In IBD studies, associations have repeatedly been found between the HLA-DRB1*1502 related haplotype and UC and the HLA-DRB1*0103 related haplotype and UC and colonic Crohn`s disease (Cho & Weaver 2007). In addition in Crohn`s disease, positive associations have been observed with HLA DRB3*0301-DRB1*1302 and HLA-DR1-DQw5 and HLA-DRB1*07 haplotypes and a negative association with HLA-DRB1*03 (Hirv *et al.* 1999).

3 Purpose of the present study

The clinical aspects of microscopic colitis and the characteristics of upper gastrointestinal endoscopical and histological abnormalities were investigated and specific immunogenetical aspects determined in MC. The relationship of MC with CD was investigated.

The specific aims of the study are as follows:

1. To characterize the essential clinical features of MC and its subtypes CC and LC the following aspects of these conditions were investigated: symptoms, disease course, associations to other chronic illnesses, occurrence of autoimmune diseases, and association with the use of drugs (I).
2. To explore endoscopical and histological findings of MC and its subtypes the data of endoscopy (both colonoscopy and gastroscopy) was collected, the histology of colon mucosa was re-evaluated to ensure the diagnosis of MC, and also the histology of ileal, gastric and duodenal mucosa was studied and reviewed (I, II). An attempt was made to study how widespread the inflammation in gastrointestinal channel would be in MC and if there was any characteristic pattern to the gastroduodenal abnormalities in MC.
3. The occurrence of HLA –DQ2-hapolotypes associated to CD in MC was examined and polymorphisms in TNF-α, IL-6, IL-1 β, IL-1RA, IL-10, and CD14 genes in MC were determined to define the specified immunologic and genetic background of MC (III, IV).

4 Subjects and methods

4.1 Study subjects

4.1.1 Patients

The flowchart of the patients is seen in Figure 3. Patients were identified by searching through the files of the Department of Pathology at Oulu University Hospital for all the patients with a diagnosis of MC, CC or LC in the years 1990 – 1996. Twentyone patients of the 48 patients with MC were excluded because they were old, in poor general condition, declined, or could not be contacted, but a further 23 patients with MC were found by searching the pathological databases of Oulu Health Center and Raahe Regional Hospital. Five additional patients of the retrospectively studied group were excluded because the diagnosis of MC could not be confirmed in a review of the specimens. A total of 39 patients with new diagnosis of MC in the years 1997–1999 were collected prospectively and included in the study. The patients were contacted by telephone and an informed consent letter was sent to them before their inclusion in the study. The patients who agreed to participate in the study were interviewed by the author. Thus, 84 patients, 54 with LC and 30 with CC, were included and served as the basic patient group, which was included as a whole in study I. The retrospective series was composed of 45 patients (24 LC, 21 CC) and the prospective series of 39 patients (30 LC, 9 CC). The number of patients included in the other studies varied depending on whether the blood or histology samples were available (Table 7).

Table 7. Number of patients and controls participating in Studies I-IV. Male female ratio marked in parenthesis

Group	Study I	Study II	Study III	Study IV
Microscopic colitis	84 (18:66)	75 (15:60)	80 (16:64)	81 (16:65)
Collagenous colitis	30 (10:20)	27 (8:19)	29 (9:20)	29 (9:20)
Lymphocytic colitis	54 (8:46)	48 (7:41)	51 (7:44)	52 (7:45)
Controls	84 (18:66)	60 (13:47)	Group 1: 3627	178 (56:122)
			Group 2: 178 (56:122)	

4.1.2 Controls

Control groups included in different studies are described in Table 7. For clinical studies, a control group of 84 subjects was prospectively collected at Oulu University Hospital, Kainuu Central Hospital and Oulu Health Center Hospital, aiming to obtain an age- and sex-matched series. All the control patients had endoscopically normal mucosa, with the exception of benign polyps in 36 subjects, and showed no inflammatory conditions or malignancies in large bowel biopsy specimens. This group served as a control group in study I. Histological samples from gastroduodenal mucosa were available from 60 of 84 controls, and they served as a control group in study II.

The control group for HLA-DQ analysis in study III (group 1) consisted of a series of 3654 schoolchildren (male 1826, female 1828; median age 12 years, range 7-16 years) from northern parts of Finland, of whom 3527 were tested for HLA haplotypes. (Maki et al. 2003.) Biopsy proved prevalence of CD in that series was estimated to be 1%. For controls in the cytokine polymorphism studies (III, group 2 and IV) a series of university staff and students (N = 178) from the northern Finnish population with a mean age 39.4(± 13.4 SD) years was used.

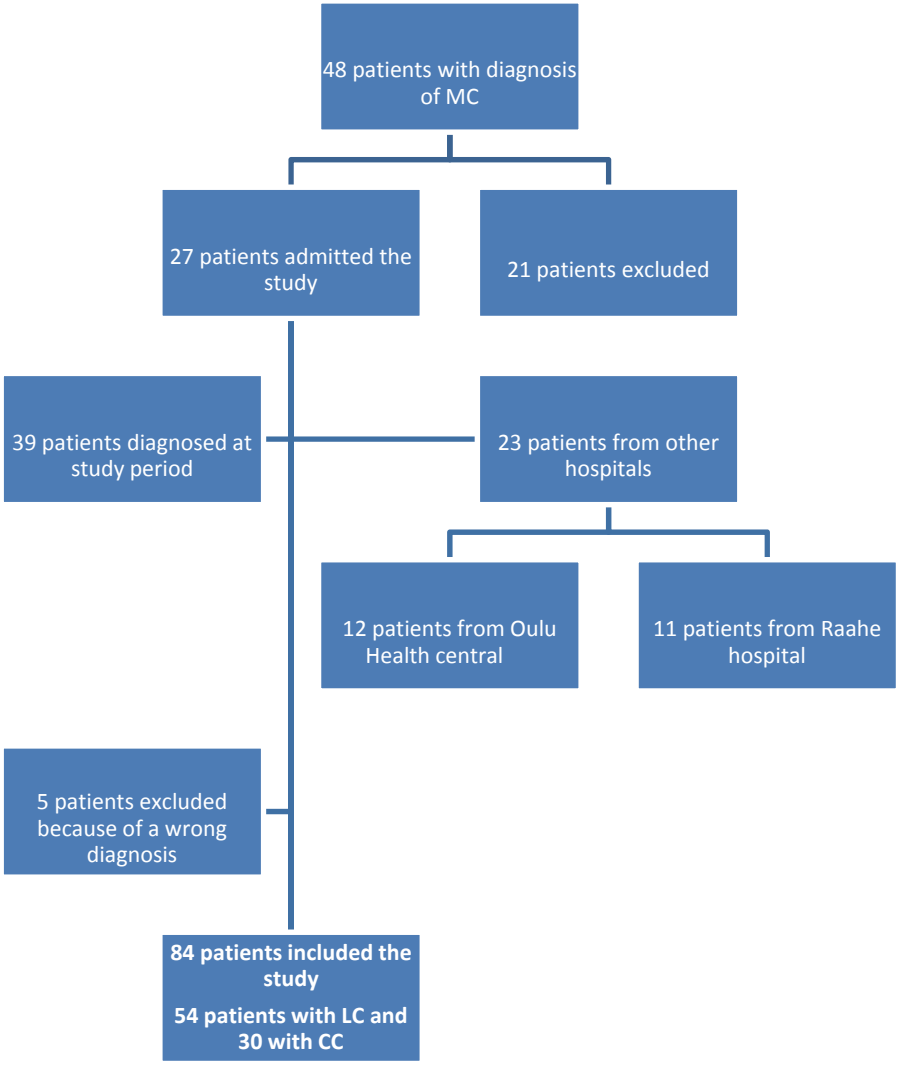

Fig. 3. Flowchart of the study patients.

4.2 Clinical data

Clinical data were obtained by reviewing the clinical records and interviewing patients personally using a structured questionnaire. Age at the time of onset of the symptoms and age at the time of histopathological diagnosis, the clinical picture of MC with gastrointestinal and general symptoms were registered and the

long-term course was evaluated from patients in a retrospective manner. Diarrhoea, or change in frequency and form of stool consistency, with other symptoms such as abdominal distention, flatulence and abdominal pain were considered as IBS-like symptoms. Other chronic diseases and regular use of medication were inquired.

4.3 Laboratory data

The results of blood tests made within 12 months of the date of the diagnosis of MC, and additionally, if the patient was still symptomatic at this time point were collected. The following results were recorded: haemoglobin concentration, C-reactive protein (CRP; normal < 11 mg/L), erythrocyte sedimentation rate (ESR; normal < 20 mm/h), serum creatinine, potassium, sodium, calcium, albumin (normal > 35 g/L), alanine transaminase, alkaline phosphatase, thyroid-stimulating hormone, and serum concentrations of IgG, IgA, IgM and IgE. The results of celiac serology and lactose intolerance test, if performed, were taken from the clinical data. The patients with HLA-DR3-DQ2 haplotype who had no duodenal biopsies available were further tested for the presence of TTG-antibodies (significant positive greater than 30 μmol/l).

Blood samples for DNA and cytokine gene polymorphisms testing were available from 81 patients (29 with CC, 52 with LC) the number of samples examined varied in different series since there was not sufficient DNA in some samples to conduct all of the assays. The methods used for HLA-DR3-DQ2 and HLA-DR4-DQ8 typing are described in more detail in study III. The primers and methods used for polymorphisms in cytokine-coding genes i.e. TNFα-308, IL-6-174, IL-1β -511, IL-1β+3953, IL-1RA, IL-10-1082 and CD14-159 are described in studies III and IV.

Serum samples for IL-6 concentration determination were available for 72 patients (27 with CC, 45 for LC). Serum IL-6 levels were measured with sensitive ELISA quantikine HS Immunoassay kits (R&D) Systems GmbH, Wiesbaden-Nordenstadt, Germany) according to the manufacturer's instructions.

4.4 Endoscopy

The data of diagnostic procedure (colonoscopy or sigmoideoscopy) and gastroscopy were collected from the clinical records. If duodenal biopsies had not been analyzed previously, gastroscopy with biopsies from antral, gastric body and

duodenal biopsies was performed. Gastroscopy had been performed on 82 patients and 80 controls.

4.5 Histopathology

The biopsy specimens were fixed in neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin. All biopsy material (diagnostic colonoscopy and gastroscopy) were reviewed blindly without clinical information by an experienced pathologist.

The diagnosis of LC was based on an increased number of IEL (> 20/100 epithelial cells) the presence of CC on a SCL exceeding 10 μm in thickness, this being associated with an increase in the number of inflammatory cells in lamina propria. Cases with both an excess of IEL and a SCL were classified as CC.

Ileal biopsies were assessed for the presence and grading of villous atrophy on a four point scale from normal (0, no atrophy) to severe atrophy corresponding to flat mucosa (3). The diagnosis of ileal inflammation was based on the presence of increased chronic inflammatory cells, with or without neutrophilic leukocytes. An increase of IELs was diagnosed if the count of IELs was more than 25 /100 epithelial cells.

The histologic classification of gastric and duodenal mucosa is described in detail in study II. Histologic samples taken during gastroscopy were available from 75 of patients and 60 of controls for re-evaluation. In gastroduodenoscopy, 70 of patients (93.3%) and 51 of the controls (85%) a complete series of histological samples including all three anatomical areas had been taken. CD was diagnosed according to the revised ESPGAN diagnostic criteria for CD (Walker-Smith JA *et al.* 1990).

4.6 Statistical analysis

Results are expressed as percentages, means and standard deviation (SD) when appropriate. In addition, median and (interquartile) ranges were used (IV). The significances of the differences between the groups were tested by the independent samples T-test or by the Mann-Whitney test depending on the type and distribution of variables. Chi-Square statistics, and Fisher`s exact test were used to assess significant associations between categorical variables. Odds ratios and the corresponding 95% confidence intervals were estimated by cross-tabulation when appropriate (when p value was statistically significant) as a

measure of the magnitude of the association (III, IV). Differences were assumed as significant at p values of less than 0.05. Adjustment for multiple comparisons was made if needed (Rothman 1990, Hochberg & Benjamini 1990) and when $p < 0.05$, adjusted p is marked in parenthesis in studies of cytokine polymorphism (III, IV). The effect of confounding factors such as age, gender and body mass index on IL-6 concentration was tested by stratified analysis (IV). The statistical analysis was performed by using the different versions of SPSS software (v.10.0; 12.0; 16.0; SPSS Inc, Chicago, Illinois, USA).

5 Results

5.1 Clinical aspects (I)

The demographic features of 84 patients with MC (54 patients with LC and 30 with CC) are shown in Table 8. After re-evaluating the histology, the primary diagnosis of MC was specified to CC in 5 cases and to LC in 18 patients; in two patients the original diagnosis of CC was changed to LC and further more in two patients with the original diagnosis of LC was changed to CC. The trend for LC dominating in this material was more obvious in patients with a later diagnosis of MC. There was a female preponderance in MC, and a trend towards a higher female: male ratio in LC compared to CC ($p = 0.057$). The age distribution at the time of the diagnosis among both CC and LC patients seems to peak around the 6^{th} decade with no differences in the age pattern being between female and male patients (Figure 4). Three (10%) of the patients with CC and six (11%) of the patients with LC had been diagnosed before the age of 40 years. The age both at the onset of the symptoms of MC and at the diagnosis of MC was lower in CD patients (onset 42.7; diagnosis 47.9 years) as compared to patients without CD (onset 54.3, diagnosis 56.2 years; $p = 0.010$: $p = 0.027$)

The delay from the appearance of symptoms to diagnosis of MC displayed a trend to be longer in CC when compared to LC (median 19.5 months vs. 5 months). Patients with CD had a longer diagnostic delay than other patients (48 months, range 2–264 vs. 4.5 months, range 0.5–216 months; $p = 0.031$). The diagnostic delay was shorter in the prospective group compared with the retrospective group (12 months, range 0.5–264 vs. 4 months, range 1–216, $p = 0.025$). A familial occurrence was seen in two families: two sisters with CC and LC and a mother and a daughter both having LC.

The main indication for primary endoscopy was diarrhoea in 79 of 84 patients and other or additional reasons were anaemia, positive faecal blood, abdominal pain, changed bowel habits and constipation, haemorrhoids, follow-up of colon adenomas or suspicion of diverticular disease (DD). The most common symptom was watery diarrhoea associated with nocturnal diarrhoea and incontinence. The stool frequency estimated at the time of diagnosis was an average of 4 stools per day (median, range 1–30). About two-thirds of the patients had lost weight. The body mass index (BMI) of the patients was lower than controls ($p = 0.009$). About 80% of patients were estimated to have IBS like symptoms. About three out of

four (74%) of patients with a longer history of MC have had chronic intermittent disease course, 20% of patients had symptoms continuously and 6% had experienced only a single episode. The symptoms and disease course were similar in CC and LC. The symptoms were assessed as being relatively mild at the time of the study in 80% of patients, with the remainder having severe symptoms, although 90% of the patients did have symptoms and about 10% of patients were symptomless at the time of the interview.

Table 8. Clinical characteristics of patients with MC and controls (Study I).

Group	N (%)	Female: Male	Mean age (yr) at diagnosis ± SD (median) [1]	Mean age (yr) at the study± SD (median) [2]	BMI mean ± SD
Microscopic colitis	84	3.67:1	54.8 ± 13.4 (54)	57.0±12.8 (57)	24.4 ± 4.6**
Collagenous colitis	30 (36)	2:1	53 ± 12,7 (50.5)	56.5 ± 12.7 (53.5)	24.0 ± 3.6
Lymphocytic colitis	54 (64)	5.75:1	55.4 ± 13.2 (56)	57.2 ± 13.0 (58.5)	24.6 ± 5.1
Patients with CD	15 (18)	14:1	47.9 ± 16.1 (43)*	51.5 ± 16.8 (52)	23.3 ± 2.8
Patients without CD	69 (82)	3.06:1	56.2 ± 12.0 (56)*	58.3 ± 11.8 (57)	24.4 ± 4.8
Controls	84	3.67:1	-	57.7 ± 12.5 (58.5)	26.1 ± 3.9**

*p=0.027 Age at diagnosis less in MC patients with CD compared with others

**p=0.009 BMI lower in MC patients compared with controls

[1] Mean age at diagnosis means the age when MC was diagnosed and the age when colonoscopy for controls performed

[2] Mean age at the study means the age when patients were included in the study and interviewed and when colonoscopy was performed for the most of the controls. The difference between the age at colonoscopy of controls and diagnostic colonoscopy for patients was not statistically significant.

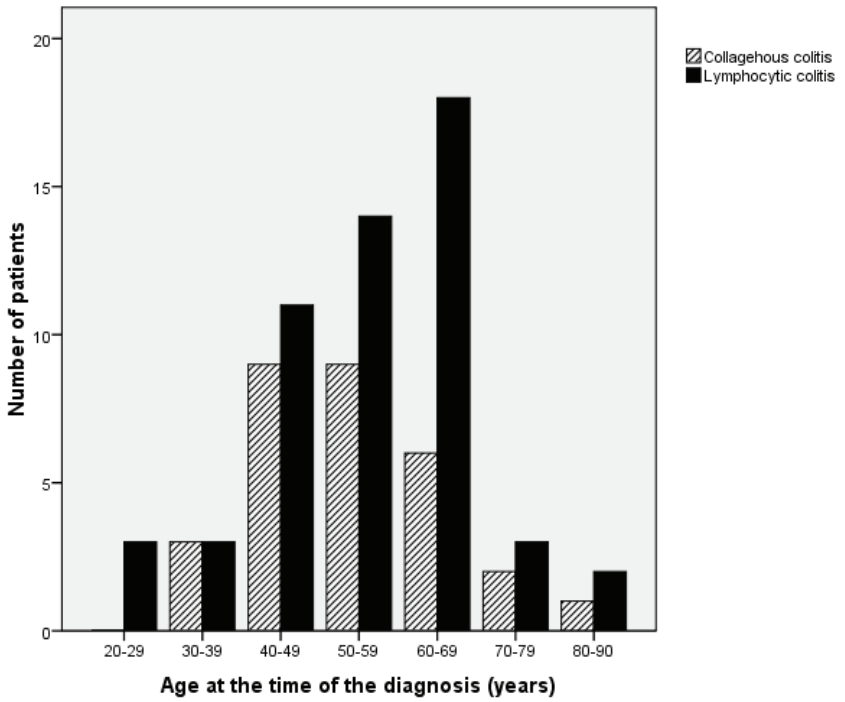

Fig. 4. Age distribution at the time of the diagnostic colonoscopy in male and female patients with MC.

5.1.1 Associated diseases and the use of medication

The diseases associated with LC and CC are shown in Table 9. Autoimmune conditions were more common in MC than in the controls, and were more prevalent in CC as compared to LC. Three (20%) patients with CD had an IgA deficiency compared to none of the patients without CD, the difference being statistically significant ($p = 0.01$). Two of the patients with CD had DH (1 with CC, 1 with LC). The subjects with LC had more often bronchial asthma than patients with CC, but no significant difference was seen as compared to controls. Six patients have had at least one malignancy: two had suffered breast cancer, one basalioma, one malignant brain tumour and two colon cancers, the other of those had had also prostate cancer. The colon cancer had been operated years before the diagnosis of MC in one patient, and the diagnosis of MC was made from the colon resecate in one patient. In controls, 4 malignancies had been treated: one

patient with breast and ovarian cancer, two endometrial cancers, one malignant lymphoma, and also one benign rectal carcinoid tumour which had been resected.

Hypolactasia was diagnosed at almost similar frequencies, around 45%, in both MC patients and controls, but lactose-related abdominal symptoms were more common in patients than controls ($p = 0.004$), and tended to be more common in patients with CC than with LC ($p = 0.06$).

Seven patients with LC had started a new drug shortly before they experienced symptoms related to MC (entacapone, ticlodipine, carbamazebine, NSAID, low dose of acetylsalicylic acid (ASA), ferrous sulphate, mianserin or fluoxetine) as had two patients with CC (NSAID, simvastatin). The temporal association of the introduction of a new drug and the onset of symptoms was more evident in LC than in CC (13% vs. 6.7%, $p = 0.047$). The clearest association to the beginning of symptoms to the started drug therapy was observed in two patients with LC, one of those started ticlodipine and the other entacapone.

There were no differences in the use of regular medication between patients and controls (78.5% vs. 64%). In all, 19% of patients with MC and 11% of controls were regularly using NSAIDs and accordingly no significant difference was found either in the use of NSAIDs or of low dose of ASA (38.1% vs. 33.3%). There was a trend that the consumption of antidepressive drugs was more common in patients than controls (14.3 vs. 4.8%; $p = 0.063$). Patients with LC used more often asthma drugs compared to patients with CC (20.4% vs. 0; $p = 0.006$) and cortisone inhalators were the most common drugs being used to treat asthma (16.7% vs. 0%, $p = 0.023$).

Table 9. Concomitant diseases and conditions with microscopic colitis and in controls.

Diseases or states	Microscopic colitis N = 84 (%)	Collagenous colitis N = 30 (%)	Lymphocytic colitis N = 54 (%)	Controls N = 84 (%)	P < 0.05
Celiac disease	15 (17.9)*	6 (20)	9 (16.7)	1 (1.2)*	* < 0.001
Diabetes mellitus [1]	8 (9.5)	2 (6.7)	6 (11.1)	7 (8.3)	
Thyroid disorders	12 (14.3)	5 (16.7)	7 (13)	7 (8.3)	
Bronchial asthma	16 (19)	2 (6.7)**	14 (25.9)**	19 (22.6)	**0.042
Rheumatic diseases	3 (3.6)	2 (6.8)	1 (1.9)	4 (4.8)	
Fibromyalgia	6 (7.2)	2 (6.7)	4 (7.4)	4 (4.8)	
Arthralgia, musculoskeletal pain etc	37 (44)	9 (30)	28 (51.9)	47 (56)	
Autoimmune disorders[3]	31 (36.9)†	16 (53.3)‡	15 (27.8)‡	11 (13.1)†	†0.001 ‡ 0.033
Lactose intolerance[2] n = 81	50/81 (61.7)º	22/29 (76)ºº	28/52 (54)ºº	28/74 (37.8)º	º0.004 ºº0.06
Pathologic lactose test n = 58	26/58 (44.8)	11/18 (61)	15/40 (38)	16/34 (47.1)	

[1] Adult-onset non insulin-dependent diabetes

[2] Either symptoms or a lactose test exhibit intolerance to food containing lactose

[3] Autoimmune states included here were celiac disease, autoimmune thyroid disease (9 in MC, 6 in controls), autoimmune hepatitis (1 in MC), pernicious anaemia (3 in MC), ankylosing spondylitis (2 in MC), rheumatoid arthritis (6 in MC, 4 in controls), Raynaud's disease (1 in MC), Sjögren syndrome (1 in both MC and controls) and autoimmune haemolytic anaemia (1 in both MC and in controls).

5.1.2 CD

The prevalence of CD was significantly higher in MC (18%; 15/84) than in the control group (1%; 1/84; p = 0.001). The mean age for CD diagnosis was 44 years (median 37.5 years, range 22–76 years). Nine patients had experienced CD for several years before the diagnosis of MC and had been on a gluten-free diet when MC was diagnosed with a mean length of treatment of 8 ± 3.4 years. Two patients had been on a GFD for less than one year. In three patients, CD was diagnosed during the next year following the diagnosis of MC. In addition in one patient, CD was diagnosed five years after the diagnosis of MC and this patient was not included in CD group until studies II-IV, what declares the different number of celiac patients in study I (14 patients). Subsequent this patient was found to have normal duodenal mucosa but a clearly positive TTG-antibody test during the time

that MC was diagnosed. Two patients without duodenal biopsy were also tested for TTG-antibodies, with one of them showing a clearly positive test result (66 µmol/l). These two patients were included as non-celiac patients in study I, but they were not included in either subgroups of patients with or without CD in further studies II-IV, because the presence or absence of CD could not be assessed due to the lack of duodenal histology.

5.1.3 Clinical laboratory parameters

The blood tests were within the normal ranges in both CC and LC patients and controls. The median time interval from the diagnostic colonoscopy to the laboratory tests was 2 months (mean 4, range 0–69 months) for the patients and for the controls 0 months (mean 4, range 0–8 months). ESR was more often above normal in patients with MC than in controls (16/63, 25.4% vs. 5/72, 6.9%; $p = 0.004$). Elevated CRP values were more often detected in patients with MC compared to controls (8/46, 17.4% vs. 2/62, 3.2%; $p = 0.017$). Hypoalbuminemia was more common in patients with MC than in controls (13/58, 22.4% vs. 2/68, 2.9%; $p = 0.002$). IgA deficiency was found in three patients with MC all with concomitant CD (3.6% vs. 0%; $p = 0.01$).

5.2 Endoscopy and histology (I,II)

5.2.1 Colonoscopy and histology of colon and ileum mucosa (I)

The diagnostic endoscopy was sigmoideoscopy in 7 patients and colonoscopy in 76 patients. In one case, the diagnosis of MC was made from the right colon resected because of caecal carcinoma. Carcinoma was found by colonoscopy performed two weeks before the operation, at that time, biopsies had not been taken elsewhere from the colon.

Minor abnormalities of the mucosal vascular pattern, slight erythema, or patchy mild mucosal oedema without any ulcerations or friability were registered in 18 (21.7%) cases compared to 0 in controls ($p < 0.001$). Colonic diverticulosis was found more often in controls (39.3%) compared to patients with MC (15.5%; $p = 0.001$).

About half of the patients with CC (16/30) exhibited a mixed form of MC, where both a SCL and IEL infiltration were present. This subgroup did not differ

significantly for clinical features of the other patients with CC or LC, and comparisons were made between CC (including cases with intraepithelial lymphocytosis) and LC.

Ileum was reached in 70 of patients with MC in diagnostic colonoscopy (28 of CC, 42 of LC) and in 70 of 80 controls (90% of both groups) and no macroscopical lesions were registered. Ileal samples were available for revaluation from 56 patients with MC (67%), 21 patients with CC (70%) and 35 patients with LC (64.8%), and 70 controls (83%). Ileal histologic changes were detected in 10 patients with MC and none of controls (17.9% vs. 0; $p < 0.001$). Ileal histology was abnormal in 2 patients with CC and in 8 patients with LC (9.5% vs.22.9%; p = NS). Two patients with CC and 4 with LC had mild villous atrophy, 1 patient with LC displayed moderate villous atrophy and 3 patients with LC had ileitis in the ileum mucosa. Ileal histologic changes were found in 4/11 (36.4%) of patients with CD, three of them having mild villous atrophy and the other had ileitis.

5.2.2 Gastroscopy and gastric mucosa (II)

Study II evaluated 75 patients (27 with CC, 48 with LC) and 60 controls, whose histologic samples had been obtained by gastroscopy. Gastroscopy has been performed within one year of the diagnostic colonoscopy in 82% of MC patients and in 88% of controls. The mean age at the time of gastroscopy was 55.6 (± 13.3) years in patients and 55.6 (± 12.4) years in controls. The special forms of gastritis are described in table 10 and only the gastric histologic findings with significant differences in the comparisons are summarized in Table 10.

Table 10. Helicobacter pylori frequency and specific forms of gastritis in microscopic colitis (MC)

Type of gastritis	Microscopic colitis	Collagenous Colitis	Lymphocytic colitis	MC with celiac disease	MC without celiac disease	Controls
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
N (all)	72	25	47	14	58	60
H. pylori	11 (15.3)	5 (20.0)	6 (12.8)	1 (7.1)	10 (17.2)	17 (28.3)
Focal antral gastritis[1]	4 (5.6)	2 (8)	2 (4.3)	0	4 (6.9)	4/58 (6.9)
Lymphocytic gastritis[2]	4 (5.6)	0	4 (8.5)	3 (21.4) *	1 (1.7) *	6 (10)

*p = 0.021; Lymphocytic gastritis was more often found in MC patients with CD compared to MC patients without CD

[1] one patient with MC was *H. pylori* positive, 3 patients with MC and all the controls were *H. pylori* negative

[2]one patient with MC and three controls were *H. pylori* positive

Specific forms of gastritis

Presence of *H. pylori* infection could be studied in 72 patients and in 60 controls (Table 10). The age at the diagnosis of MC was higher in *H. pylori* positive (mean/median ± SD, 63.4/65.5 ± 9.6 years) than in *H. pylori* negative patients (54.4/54.1 ± 13.1 years; p = 0.034). *H. pylori* gastritis (excluding *H. pylori* associated cases of specialgastritides) was present in 14.1% (9/64) of MC and in 28% (14/50; p = 0.099) of the controls. Focal antral gastritis was detected in four patients with MC (Table 10). When *H. pylori* infection was excluded, the actual frequency of FG was still similar to the frequency of FG in controls (4.9 vs. 6.9%; p = NS). LG was similarly found in patients with MC and in controls (Table 10). All of the MC patients with LG had LC and three had concomitant CD. The frequency of LG was clearly elevated in MC patients with CD compared to those without CD, and LG was only found in untreated CD (3/5 of MC; 60%). The frequency of *H. pylori* in LG patients was similar in MC and controls (25%, vs. 50%, p = NS).

Other endoscopical and histological findings in gastric mucosa

Endoscopical findings during gastroscopy were normal in 77.3% of patients (58/75) and 73.3% of controls (45/60). The overall occurrence of *gastric erosions*

did not differ between MC and controls (10/75 13% vs. 3/60 5%), but among the *H. pylori* positive subjects, patients with MC exhibited more often erosions than the controls (5/11 45.5% vs. 1/17 5.9%; p = 0.022). MC patients using NSAIDs daily had more often gastric erosions than those not using these drugs (7/29 vs. 3/46; p = 0.039). No histologic differences in the gastric mucosa were found between patients and controls with endoscopic erosions. In the MC group, patients with erosions (n = 10) had higher antral glands (341/330 ± 71 vs. 277/270 ± 63 μm; p = 0.024), higher activity of antral gastritis (0.6/0 ± 0.84 vs. 0.1/0 ± 0.3; p = 0.01) than those without erosions (n = 65). In the body mucosa, MC patients with erosions had higher glands (563/575 ± 46 vs. 460/500 ± 121 μm; p = 0.001), but shorter foveolae (144/140 ± 33 vs. 195/150 ± 98 μm; p = 0.007), and a lower number of IEL in the mucosa than those without erosions (2.8/2 ± 1.6 vs. 7.1/3 ± 7.5; p < 0.0001). In the controls, no histological differences were detected between subjects with and without erosions.

Polypoid lesions of stomach were present only in *H. pylori* negative subjects. They were more common in the controls than in the patients with MC (9/60 15.0% vs. 2/75 2.7%; p = 0.012). The histology of polypoid lesions found in the patients with MC and four of the nine lesions in the controls were interpreted as being normal. The remaining five polypoid lesions in the controls were all cystic fundic gland polyps. Accordingly, the frequency of fundic gland polyps was higher in controls than in patients with MC (8.3% vs. 0%; p = 0.016). The presence of fundic gland polyps was not associated with the use of antisecretory drugs.

In gastric antral mucosa, the number of lymphatic follicles was lower in the patients with MC than in the controls (Table 11) and the same difference was seen in *H. pylori* positive subjects (1.8/2 ± 0.89 vs. 2.4/3 ± 0.80; p = 0.038). Similarly, the severity of gastritis (score of mononuclear cells) in the *H. pylori* positive population was less in the patients than in the controls (1.8/2 ± 0.9 vs. 2.4/3 ± 0.8; p = 0.048). Fibrosis of lamina propria was more abundant in patients with MC compared to controls (Table 11).

In the gastric body mucosa, the foveolae were shorter in patients with MC than in the controls (Table 11). This difference was also seen in the comparisons of *H. pylori* negative patients and controls (176/150 ± 79 vs. 240/195 ± 134 μm; p = 0.012). The difference in foveolar height was not related with the use of antisecretory drugs.

Table 11. Histological features of the antral and body mucosa in microscopic colitis and in the subgroups (collagenous colitis and lymphocytic colitis) in comparison with the controls.

Histology of gastric mucosa	Microscopic colitis N = 72 Mean /Median (SD)	*Collagenous* colitis N = 25 Mean/Median (SD)	*Lymphocytic* Colitis N = 47 Mean/Median (SD)	Controls N = 59 Mean/Median (SD)	P value < 0.05
Antrum					
Intestinal metaplasia	2.0/0 (7.4)	0*	3.0/0 (9.0)*	2.9/0 (13.0)	*0.026
Intraepithelial lymphocytes	4.8 /3 (6.5)	3.0/3 (1.7)*	5.8/3 (7.8)*	4.3/2 (6.2)	*0.018
Lymphatic follicles/biopsy	0.1/0 (0.2)**	0.02/0 (0.1)	0.07/0 (0.2)	0.2/0 (0.4)**	**0.035
Fibrosis in lamina propria	0.9/1 (0.7)**	1.0/1 (0.7)	0.8/1 (0.6)	0.6/1 (1.0)**	**0.018
Corpus					
Glandular height (μm)	476/500 (117)	516/550 (97)*	458/500 (122)*	479/550 (174)	*0.045
Foveolar height (μm)	187/150 (93)**	167/150 (58)	197/150 (105)	251/200 (131)**	**0.004

intestinal metaplasia as a percentage of mucosa, lymphatic follicles as a count/biopsy 0–5, and intraepithelial lymphocytes as a count/100 epithelial cells.

* Comparisons between patients with collagenous colitis and those with lymphocytic colitis

** Comparisons between patients with microscopic colitis and controls

Comparison of gastroduodenal findings in CC and LC

Endoscopic erosions were more common in CC than in LC (7/27 25.9% vs. 3/48 6.2%; $p = 0.030$). In CC, patients with *H. pylori* had more often erosions than those without *H. pylori* (4/5 80% vs. 3/20 15%; $p = 0.012$), but in LC, the erosion risk was not related to the occurrence of *H. pylori* (1/6 16.7% vs. 2/41 4.9%; p = NS). The use of NSAIDs contributed significantly to the higher erosion risk in CC patients compared to LC patients (6/13 vs. 2/19; $p = 0.038$). Endoscopical duodenitis or bulbitis tended to be more common in CC compared to LC (4/27 14.8% vs. 1/48 2.1%; $p = 0.053$), and among *H. pylori* negative patients, this difference was statistically significant (4/20 20% vs. 1/41 2.4%; $p = 0.036$). In CC, the presence of endoscopical erosions was associated with the higher activity of gastritis in the antrum ($0.9/1 \pm 0.9$ vs. $0.06/0 \pm 0.25$, $p = 0.009$), higher glands

(383/390 ± 70 vs. 266/265 ± 56 µm; p = 0.007) and deeper foveolae (455/460 ± 124 vs. 297/285 ± 73 µm; p = 0.003) in the antral mucosa, and to a lower IEL count in the body mucosa (2.0/2 ± 0.7 vs. 6.6/3 ± 6.4; p = 0.010).

Antral foveolae were higher in *H. pylori positive* patients with CC than in patients with LC (435/420 ± 125µm vs. 303/310 ± 52 µm; p = 0.047). In antral mucosa, the IEL count was higher in LC compared to CC (Table 11). In gastric body mucosa, the glandular height was increased in patients with CC compared to LC (Table 11), and this difference was more evident in *H. pylori* positive patients (585/595 ± 24 vs. 322/300 ± 68 µm; P = 0.008). In the *H. pylori* positive patients, the patients with CC displayed no atrophic changes, unlike patients with LC (0 vs. 1.5/2 ± 0.8; p = 0.029). The foveolae of body mucosa were higher in LC than in CC (330/350 ± 133.1 vs.148/140 ± 38.6 µm; p = 0.019) in *H. pylori* positive patients. In addition, in this population, the gastritis was also more active in LC compared to CC (1.0/0 ± 0 vs. 0.25/0 ± 0.5; p = 0.033) and tended to be more severe in LC compared to CC (2.7/3 ± 0.5 vs. 1.3/1 ± 1.3; p = 0.071).

Duodenum and duodenal mucosa

Eight patients with MC (of 15 patients with CD) had a diagnostic villous atrophy with increased IEL count. Three of those patients had been on a GFD for years and had still mild villous atrophy. When comparing patients with CD to the other patients with MC, they had shorter duodenal villi (420/440 ± 103 vs. 445/450 ± 74 µm; p = 0.004), deeper crypts (249/230 ± 77.8 vs. 191/180 ± 61 µm; p = 0.014) and a higher IEL count (39.5/38.5 ± 26.4 vs. 9.5/8 ± 6.7; p = 001) and higher mononuclear inflammatory cell score (4/4 ± 0.8 vs. 3.2/3 ± 0.8; p = 0.001). MC patients without CD had shorter duodenal villi (445/450 ± 74 vs. 480/490 ± 57 µm; p = 0.007), but lower IEL counts (9.5/8 ± 6.7 vs. 14.1/11 ± 10.4; p = 0.006) and lower mononuclear inflammatory cell score in the duodenal lamina propria (3.2/3 ± 0.8 vs. 3.5/4 ± 0.7; p = 0.028) compared to controls. Due to the high carriage rate of the HLA-DR3-DQ2 genotype also in patients without CD, the duodenal changes were compared with association of this genotype. The IEL count was lower in non-CD patients carrying the HLA-DR3DQ2 allele as compared to those without HLA-DR3DQ2 (6.41/6.5 ± 2.2 vs. 10.9/8 ± 7.7; p = 0.002).

Relationship of ileal abnormalities and gastroduodenal features

In MC patients with ileal changes, the duodenal IEL count was higher than in patients without ileal changes (37/19 ± 32.9 vs. 13.74/8.5 ± 14.3; p = 0.023). This difference between patients with or without ileal changes was also noted in patients without CD (31/9 ± 17 vs. 9.1/7.5 ± 5; p = 0.017) and patients with LC (29.0/19 ± 12.5 vs.13.0/8 ± 13.0; p = 0.025). In non-CD patients and in LC patients the antral IEL count was higher if the patients had also ileal changes (non-CD: 9.5/5 ± 5.0 vs. 3.3/3 ± 2.8; p = 0.006; LC: 8.9/6 ± 3.7 vs. 4.0/3 ± 3.6, p = 0.021). In CC patients with ileal changes, the IEL count in body mucosa was higher as compared to patients without ileal changes (17.5/17.5 ± 9.2 vs. 3.22 ± 2.5; p = 0.019).

5.3 Immunological aspects (III, IV)

Table 12. HLA-DR3-DQ2 and HLA-DR4-DQ8 frequencies in patients with microscopic colitis (MC) and subtypes of MC: collagenous colitis (CC), lymphocytic colitis (LC) and MC with or without celiac disease (CD)

HLA-type	MC, whole group N = 80 (%)	MC with CC N = 29 (%)	MC with LC N = 51 (%)	MC with CD N = 15[2] (%)	MC without CD N = 63[2] (%)	Control group N = 3627 (%)
HLA-DR3-DQ2[1]	35 (43.8)	13 (44.8)	22 (43.1)	13 (86.7)	21 (33.3)	655 (18.1)
p value	< 0.001	0.021	< 0.001	p < 0.001	0.003	
HLA-DR3-DQ2 and/or HLA-DR4-DQ8 [1]	43 (53.8)	16 (55.2)	27 (52.9)	14 (93.3)	28(44.4)	1411 (38.9)
p value	0.010	0.086	0.044	< 0.001		

[1] 3 patients with both DQ2 and DQ8 alleles (One in CC group, two in LC group)

[2] only cases with duodenal histology available

p value between the study group and the control group

5.3.1 HLA-DR3-DQ2 and HLA-DR4-DQ8 in MC (III)

The HLA-DR3-DQ2 haplotype was more prevalent in patients with MC compared to the control group (OR 3.529; CI 2.251–5.533) and this increased prevalence was found in both CC and LC (Table 12). As predicted, in patients with CD, the frequency of HLA-DR3-DQ2 was significantly higher compared to

controls (OR 29.493, CI 6.64–131.008) and also to MC patients without CD. However, in MC patients without CD, the frequency of HLA- DR3-DQ2 was still elevated when compared to the control group (OR 2.269; CI 1.335–3.857). HLA-DR4-DQ8 haplotype was found in 11 (13.8%) of patients, and 836 (23.0%) of controls (P = 0.062). One patient with CD was negative for both HLA- DR3-DQ2 and HLA-DR4-DQ8, but she carried the combination of DQA1*01 and DQB1*05 encoding for the DQ5 molecule.

5.3.2 Cytokine gene polymorphism (III,IV)

TNFα (-308) gene polymorphism (III)

TNF2 allele carriers were more common among patients with MC than in the controls ($p < 0.0005$; OR 2.864, CI 1.627–5.042; adjusted $p = 0.0035$; Table 13). When compared to controls, the TNF2 –carrier frequency was also higher in all subgroups of MC, including patients with CC, LC, MC with CD and MC without CD. The TNF2-carriage rate tended to be higher in MC patients with CD compared to those without CD ($p = 0.082$).

The linkage between the carriage status of HLA-DR3-DQ2 haplotype and TNFα-2 allele was also analyzed. The association of HLA-DR3-DQ2 haplotype with TNF2 allele was significant in MC patients, as well as in CC, LC and in MC without CD ($p < 0.001$, $p = 0.002$, $p < 0.001$, $p < 0.001$, respectively), thus indicating the presence of a linkage disequilibrium between the two genetic sites. This association did not reach statistical significance in MC patients with CD ($p = 0.095$). The frequency DR3-DQ2/TNF-2 double positivity in patients with MC and CD patients was higher compared to the rest of the patients (66.7% vs. 29.5%; $p = 0.015$).

IL-6(-174) gene polymorphisms and serum IL-6 concentration (IV)

Genotype IL-6-174-GG was significantly associated with MC when compared to controls ($p = 0.030$; OR 1.941; CI 1.078–3.495; adjusted $p = 0.105$; Table 13). The same trend was found in the comparison between the patients with CC and controls ($p = 0.051$; OR 2.466; CI 1.064–5.714). The IL-6-174 allele G frequency was higher in MC patients than in the controls (55% vs. 45%; $p = 0.036$; OR 1.514, CI 1.041–2.203; adjusted $p = 0.126$). A similar tendency was found in the

comparison of the patients with CC and controls (57% vs. 45%; p = 0.086) and between MC patients without celiac disease and controls (54% vs. 45%; p = 0.078).

The serum IL-6 concentrations (medians / interquartile percentiles) did not differ according to IL-6-174 genotypes in MC. Patients with CC had a higher concentration of IL-6 compared to patients with LC (1.73/1.40–4.64 vs. 1.34/0.78–2.02 pg/ml; p = 0.017). Male patients had higher concentrations of IL-6 than female patients (3.73/1.40–4.64 vs. 1.43/0.82–2.14 pg/ml; p = 0.007). Patients who were overweight (BMI over 25) had higher levels of IL-6 compared to patients of normal weight (1.76/1.29–3.87 vs. 1.43/0.74–2.21 pg/ml, p = 0.039). There was no correlation between the IL-6 levels and the age of the patients. The effect of confounding factors such as age, gender and BMI on the IL-6 concentration was tested by conducting of stratified analysis but no critical effect was found.

Table 13. TNFα -308 and IL-6-174 gene polymorphisms in microscopic colitis (MC), collagenous colitis (CC), lymphocytic colitis (LC) and in MC with and without celiac disease (CD)

Cytokine polymorphisms Genotype/allele	MC (all) (%)	CC (%)	LC (%)	MC with CD (%)	MC without CD (%)	Controls N = 178 (%)
TNFα gene polymorphisms						
TNF-2- allele	36/80	12/27	24/51	10/15	24/61	41/178
carriage rate	(46.2)	(44.4)	(47.1)	(66.7)	(39.3)	(23.0)
p value	< 0.001	0.031	0.001	0.001	0.019	
IL-6-174 gene polymorphisms						
GG	27/80	11/28	16/52	6/15	19/63	37/178
	(33.8)	(39.3)	(30.8)	(40)	(30.2)	(20.8)
GC/CC	53	17	36	9	44	141
	(66.3)	(60.7)	(69.2)	(60)	(69.8)	(79.2)
p value	0.030	0.051				

p value comparisons between the study group and the control group

Other polymorphisms studies (IV)

When polymorphisms of IL-1ß +3953, IL- 1RA, IL-10 and CD14 were evaluated, no differences were found between patients with MC and controls and correspondingly between the subtypes of MC. In IL-1ß -511- polymorphism, there was no difference in the genotype distributions between patients with MC

and controls. However the MC patients without CD had more often the IL-1ß -511-CC genotype than the IL-1ß -511-CT/TT genotype when compared to controls (50% vs. 33.1%, $p = 0.024$, adjusted $p = 0.168$) and when compared to MC patients with CD (50% vs. 13.1%; $p = 0.01$, adjusted $p = 0.07$). The frequency of IL-1ß -501-allele T was statistically higher in CD patients in comparison to non-CD patients (0.57 vs. 0.34, $p = 0.023$; OR 2.585, CI 1.15–5.81; adjusted $p = 0.180$).

6 Discussion

6.1 Methodological aspects (I-IV)

Although at the time of current study was started, both subtypes of MC were relatively new entities, they were well known by the pathologists in Oulu University Hospital. The first diagnosis of CC in this study population was made in the year 1985. Since there is no registry of MC, and no compherensive clinical database available, it was only possible to identify the patients with the diagnosis of MC, CC and LC by exploring the locally available pathological databases. While endoscopies are performed both in communal health centres and in private practice, it was not possible to collect all patients with a certain diagnosis and to perform a comprehensive epidemiological study. Nonetheless, most of the patients with MC in the city of Oulu region were likely identified, only some of those who were colonoscopied in the private practices may have been overlooked. Although the current study is not representative in an epidemiological sense, the series of patient was unselected and systemically assessed, and the diagnosis was based on uniform criteria and represent the majority of patients diagnosed with MC in a geographically defined region. This is the first study of MC evaluating the upper gastrointestinal endoscopical and histological changes in MC and in particular, this is the first systematic analysis of gastric morphology of MC, only case reports so far have been published of the special type of gastritis found in MC. This is also the most comprehensive study investigating the relationship between MC and HLA-DR3-DQ2 status and the first study to report the results of IL-6, IL-1 ß, IL-1RA, IL-10 and CD-14 gene polymorphisms in MC.

This study, in contrast to most reports in MC, included an age- and sex-matched series of 84 controls (Table 7) and thus it was possible to show a characteristic pattern of clinical features and associations in MC and compare the histology of upper gastrointestinal channel (I, II). The reference group represents the normal population of Caucasian origin from northern Finland. The age was standardized according to the study time i.e. the time when the patients were included in the study and interviewed and for the controls the time of inclusion to the study after colonoscopy. Since the aim of the study was to obtain a population sample without any significant inflammatory conditions affecting the intestinal mucosa and associated with a significant abnormality in the upper gastro-intestinal tract, no subjects with diarrhoea were included. Therefore in this study,

it is not possible to evaluate whether some of the observed features were connected with MC or whether they would be connected with chronic diarrhoea even without MC. In addition, no direct comparisons of MC, with IBD or IBS can be made, because the patients with IBD were excluded and IBS patients were not characterized in our series.

In the immunologic studies, two control groups from the same northern Finnish population were used as described in section 4.1.2. The first of these control groups consisted of 3627 school children, of which the prevalence of CD was known (III, group 1) and the other control group of 178 subjects was made up of university staff and students with no information about their state of health or diseases (III, group 2; IV) (Maki *et al.* 2003). Although the age structures of these control groups (III; IV) were not similar to the patients, this should not influence the results obtained in the genetic testings, but as the prevalence of CD increases with age, the frequency rates of CD are not comparable (Vilppula *et al.* 2009). However, the frequency of CD in the control group 1 (1%) is similar to the values described at that time in the entire Finnish population (Maki *et al.* 2003, Lohi *et al.* 2007).

Due to the retrospective nature of the current study, the endoscope data extracted from the endoscopic statements is not uniform, and possibly some information was missing from the statements depending on the endoscopists. In addition, the histologic samples and laboratory parameters were not similarly available from every patient. However, the histologic evaluation was uniform, as the histologic samples of colon, ileum, gastric mucosa and duodenum were re-evaluated blindly by the same experienced pathologist and the diagnosis of MC, CC and LC was based on the generally accepted criteria described in methods. No grading about histologic remarks in colon and ileum was included in this study. Samples of upper gastrointestinal channel were systematically assessed and graded as described in study II. The HLA- typing was performed to investigate the connection of CD to MC because of the high frequency of CD. In order to exclude latent CD in patients carrying the HLA-DR3-DQ2, TTG- antibody testing was performed but no positive cases were found. The analyzed cytokines and their polymorphisms (TNF-α-308, IL-6-174, IL-1β-511, IL-1β+3954, IL-1RA, IL-10 and CD-14-260) were chosen because of their known biological significance in the pathogenesis of various inflammatory and infectious diseases.

6.2 Clinical characteristics of MC (I)

In most of the epidemiologic studies, the prevalences for CC and LC have been similar, only in an American study was a trend of about a two times higher incidence of LC found when compared to CC (Agnarsdottir *et al.* 2002, Olesen *et al.* 2004b, Pardi *et al.* 2007, Fernandez-Banares *et al.* 2010). In the current study, LC was more common than CC, especially in the prospective series of patients (76.9% vs. 23.1%). The age of the patients with MC at the diagnosis (Table 8) has a trend to be even lower compared to most of the reported ages, with both CC and LC typically presenting in the 6th to 7th decades (Baert *et al.* 1999, Olesen *et al.* 2004b). The diagnostic delay was shorter in the prospective group compared to the retrospective group (12 vs. 4 months; $p = 0.025$), which may reflect a better colonoscopy capacity and a greater awareness of these diseases.

The reported pattern of symptoms and the clinical course did not differ from observations in the current study (Baert *et al.* 1999, Fernandez-Banares *et al.* 2003, Olesen *et al.* 2004b). Interestingly, the BMI was lower in patients compared to controls, although the mean BMI was in the higher region of the normal ranges of BMI and controls were mildly overweight (Table 8). However, the BMI difference could reflect the severity of the disease i.e. nearly 70% of patients with MC had lost weight at least temporary, although no signs of malnutrition and dehydration were found.

Diseases which have a suspected autoimmune origin are common in MC with frequencies of 18–45 in MC reported in previous studies (Table 9) (Ayata *et al.* 2002, Barta *et al.* 2005). In the current study, the prevalence of autoimmune diseases was also statistically higher in MC patients than in controls and even more prevalent in CC when compared with LC. CD was the most common disease of autoimmune origin (18%); the relationship between CD and MC will be discussed in section 6.7.

Starting a new drug treatment seems to increase the risk of MC (Fernandez-Banares *et al.* 2007.) In all, 13% of patients with LC and 6.7% of patients with CC had started taking a new drug shortly before the onset of the diarrhoea in this study population. Some of the drugs they had started such as ticlodipine, NSAID, ASA, simvastatin have been listed as possible compounds with high or intermediate causality for MC (Beaugerie & Pardi 2005). In this series the use of both NSAIDs and ASA was remarkable though it was not different from controls. In addition, initiation of therapy with certain antidepressants such as mianserin and fluoxetine was associated to the onset of diarrhoea. Antidepressants were

used by 14.3% of patients which may be attributable not only to some co-existing depression, also by the presence of chronic pain which is often treated with those types of compounds. Since the use of the NSAIDs and antidepressants is not rare in MC, it has been suggested that drugs like NSAIDs or SSRIs may be a trigger for colonic inflammation or worsen the self-evolving microscopic colitis, but often clear causality could not be shown as the symptoms still persisted even after stopping the medication. (Fernandez-Banares *et al.* 2007).

The prevalence of lactose intolerance has not been reported in MC before. In Finland, the prevalence of hypolactasia has been estimated at 17%, which is lower than in the patients and controls in this study (Sahi 1994). The hypolactasia had been tested by an oral lactose test, and the data has been collected retrospectively with about 70% of patients and 40% of controls having been tested. The high frequency in controls may reflect a bias because only subjects with symptoms had been tested and about 30% of controls had IBS-like symptoms. The high frequency of CD does not explain the difference between patients and controls, because hypolactasia is often a secondary condition in untreated CD, and also the frequency of hypolactasia in CD patients was not significantly higher compared to non-CD patients (Bode & Gudmand-Hoyer 1988). The majority, 62%, of patients with MC did not tolerate lactose containing food and avoided lactose compared to 38% of controls. In IBS, lactose-related symptoms have been described as being more common compared to controls (38–40%) although no differences have been found in hypolactasia (4.1 vs. 3.8%) (Farup *et al.* 2004). Also in MC, lactose can cause symptoms without there being any underlying lactose malabsorption, although in the patients in this series, the frequency of hypolactasia was also high. Genetic testing for hypolactasia might provide more exact information if there is an independent association of hypolactasia with MC (Rasinpera *et al.* 2004).

6.3 Colonoscopy and histology of the colon and ileum (I)

Minor abnormalities such as erythema, oedema or abnormal vessel-pattern in colonoscopic examination have been described 20–34% in MC which is in accord also with our observations (Ayata *et al.* 2002, Olesen *et al.* 2004a). The prevalence of colonic diverticulosis is not known in MC, only in one publication was a frequency as high as 66% (17/27) in LC reported (Mullhaupt *et al.* 1998). In our material, DD was less prevalent in patients with MC than in controls (16% vs. 39%; $p = 0.001$), the prevalence of DD in controls being close to the expected

prevalence in this age group (Jung *et al.* 2010). The relationship of IBS and DD has been controversial, with prevalence rates varying from 9% to 51% of patients with non-constipated IBS in recent studies (Chey *et al.* 2010). In IBD the frequency of DD has been reported to be lower than in normal population even in patients older than 50 years (Lahat *et al.* 2007). It would be interesting to examine, if there is any mechanism providing protection for the development of diverticula in IBD and also in MC, or vice versa. However, the lesser occurrence of DD in MC needs also more evidence because the present study was not extensive and there is possibility of bias because the detection and registration of diverticula in colonoscopy may vary between endoscopists.

Colon histology was revaluated in this study, and the final diagnosis was confirmed. However, because the diagnosis of MC was more often used as an umbrella term in the histologic statements, the diagnosis was specified from MC to CC or LC in 35 patients, but a prior diagnosis of CC or LC was seldom changed (2 in each group). So called mixed-form of colitis with both excess of IEL and broad SCL was found in 16/30 of patients in CC (53%), which is only 19% of all the patients. This observation is in line with previous reports with IEL count over 20% having been described in 28–81% of patients with CC (Veress *et al.* 1995, Baert *et al.* 1999, Fernandez-Banares *et al.* 2003).

Ileal histologic changes such as villous atrophy, intraepithelial lymphocytosis and abnormal SCL has been reported in MC (Marteau *et al.* 1997, Sapp *et al.* 2002). In the current study, the ileal biopsies were re-evaluated and 18% of patients with MC with ileal histology had atrophic changes or ileitis in ileal mucosa, although no endoscopic abnormalities in mucosa of ileum had been reported. In a study by Sapp *et al.* (2002) ileal changes were found up to 64% of patients with MC (29/45) the numbers being similar in both CC and LC, but there also a slight elevation of IEL over 5 included. In the current study, the limit value of IEL count was 15/100 ECL which may partly account for the lower prevalence found. When comparing ileal histology with the histologic changes observed in the upper gastrointestinal channel, there was a clear trend for higher IEL counts through the mucosa of the gastrointestinal channel, especially in duodenum. According to these findings, it seems obvious that there is a remarkable proportion of MC patients who experience a more widespread inflammatory changes throughout the gastrointestinal channel. The common pathogenetic mechanism behind this phenomenon is not known and no conclusion could be drawn in the current study about how the symptoms correlate to the extensiveness of the disease.

More systematic histologic studies will be needed to characterize the full spectrum of changes in the entire gastrointestinal channel in MC. In the future, a more detailed evaluation, a more extensive grading and follow-up of the histologic changes of the colon and ileum in MC should be made. It would also be interesting to study if there is any correlation between the colon histology in patients with respect to severity of symptoms and disease course.

6.4 Gastroscopy and histology of gastric and duodenal mucosa (II)

The MC patients with *H. pylori* infection were diagnosed for MC at an age about 9 years older than the patients without the infection. On the other hand, there was a trend towards a lower prevalence of *H. pylori* infection found in MC compared to controls (Table 10). In a Finnish study, IBD patients with *H. pylori* infection were similarly diagnosed for IBD at about a 10 year older age than the patients without the infection (Vare *et al.* 2001). In addition in IBD and particularly in Crohn`s disease, the seroprevalence of the *H. pylori* infection has been reported to be lower than in age-matched controls (Halme *et al.* 1996b). It has been speculated that certain factors, e.g. environmental or pathogenetic, may protect against *H. pylori* infection but increase the susceptibility to IBD. These findings may indicate that *H. pylori* infection confers some protective effect against intestinal inflammation, delaying the age of onset of IBD, or possibly *H. pylori* is simply a marker of unknown environmental conditions which confer protection. The so-called hygiene hypothesis states that when children are protected from microbial infections, the development of their immunological system may be affected in such a way that they are more prone to develop autoimmune conditions (Rook & Brunet 2005). Accordingly poorer living conditions and a lower standard of hygiene have been suggested to protect also from CD e.g. there is a lower prevalence of CD detected in schoolchildren of Russian Karelia compared to their counterparts in Finland (Kondrashova *et al.* 2008). The present finding is the first evidence supporting the hygiene hypothesis also in MC. However, the observed time trends of MC pointing to an increased incidence in recent decades would also fit well with the protective role *H. pylori* infection or some factors which have undergone a similar change in prevalence. An alternative explanation for the difference in the age of onset of MC between *H. pylori* negative and positive patients is that the latter simply represents a group of older subjects, belonging to the birth cohort with a high prevalence of *H. pylori* infection.

LG is a relatively common type of gastritis with a reported prevalence of about 4–6% in gastric biopsy material (Niemela *et al.* 1995). In this series, the overall prevalence of LG in MC (5.6%) did not differ from that in the controls (10%) (Table 10). However, as in previous studies, LG was found only in LC and no cases were detected in CC (Wu & Hamilton 1999). LG has been linked to *H. pylori* and this association was also found in the current study (Hayat *et al.* 1999a). LG showed a clear association with non-treated CD in the current study. This kind of association of LG and CD is in line with previous reports, with the prevalence of LG having been described to be in the range of 10–61% in untreated CD (Karttunen & Niemela 1990, Fine *et al.* 1998). Previously also patients with both MC and CD have been reported to exhibit coexisting LG, though more often this association is found in RCD (Verkarre *et al.* 2003).

Although the prevalence of the endoscopic gastric erosions was similar in patients with MC as in controls, *H. pylori* positive patients with MC had more often erosions than controls. Furthermore, patients with MC and the *H. pylori* infection suffered less severe antral gastritis than controls. The use of NSAIDs did not differ between these groups, but patients using NSAIDs had more often gastric erosions than other patients. According to these findings it can be postulated that subjects with MC are especially prone to experience complications of NSAID both in the upper gastrointestinal tract and probably also in the colon. The mechanism for this sensitivity is not clear, but certain features such as more active antral gastritis were detected in patients with erosions.

Foveolar height in the body mucosa was significantly lower and fundic gland polyps less prevalent in MC than in the controls (Table 11). Since increased gastrin secretion induces glandular growth, a mechanism to account for the fundic gland polyps has been claimed to be related with the use proton pump inhibitors (Freeman 2008). However, no association was detected with the use of these drugs and the occurrence of fundic gland polyps, and the pathogenetic mechanism for this difference is unknown. It could be speculated that the failure of the foveolar growth and absence of fundic gland polyps in the gastric body mucosa in MC may be related to a decrease in the secretion of some growth factor from the ileal and colon mucosa, but this kind of factor has still to be identified.

The duodenal villous height, although within the normal limits, was lower in MC patients than in the controls, even when patients with CD were excluded, Interestingly,the IEL count in duodenum was even lower in patients without CD carrying the HLA-DR3-DQ2. Reduced villous height in MC without CD was not associated with any signs of immune system activity i.e. it was not associated

with either higher IEL count or with increased enterocyte loss. Therefore it could be speculated that the reduction in villous height might also be related to a deficiency of critical growth factors similarly to the situation with the decrease with the foveolar height in the body mucosa.

6.5 Immunological aspects (III, IV)

A familial occurrence has been described in MC as also in the current series (Jarnerot *et al.* 2001). There are few studies which have examined the genetic background of MC. The first study investigating HLA antigens found an increased frequency of HLA-A1 and a decreased frequency of HLA-A3 only in LC as compared to controls (Giardiello *et al.* 1992). Although no HLA class II typing was reported in that article, HLA-A1 is known to be in linkage disequilibrium with HLA-DR3 and -DQ2, as part of an extended ancestral HLA haplotype (Price *et al.* 1999). In the study of Fine *et al.* (2000a), an increased prevalence of HLA-DR3-DQ2 in MC was reported (64% pts vs. 31% controls), while 4% of patients had evidently CD. A Spanish group reported the association of HLA-DQ2 only in LC (48%, OR 2.83), not in CC (32.3%), and in that series, CD was found in 4 of 33 patients with HLA-DQ2 positivity (Fernandez-Banares *et al.* 2005). In the present study, even though MC patients with CD were excluded, the prevalence of HLA-DR3-DQ2 was significantly increased in MC (Table 12; 33.3% vs. 18.8%). In contrast to the studies of Giardiello *et al.* (1992) and Fernandez-Banares *et al.* (2005), an association of HLA-DQ2 with CC was found and also CD was more common in the current series. Remarkably none of the MC patients with the HLA-DR3-DQ2 haplotype but without duodenal villous abnormalities displayed TTG- antibodies, which should exclude the possibility that CD was present in these patients. The discrepancies in different studies may be attributable to differences in the diagnostic criteria or geographical differences in the prevalence of CD. The evidence of these HLA-studies points also to a discrete linkage between the HLA-DR3-DQ2 allele and susceptibility to MC.

Polymorphisms in TNF genes have been associated with susceptibility to several autoimmune diseases, e.g. CD and DH (Wilson *et al.* 1992). The presence of the allele TNF2 is associated with enhanced TNF production and TNFα is one of the key cytokines participating in the regulation of inflammatory responses (Wilson *et al.* 1997). The carriage rates of the TNF2 allele were clearly increased in all subgroups of MC (Table 13). The presence of HLA-DR3-DQ2 and TNF2 strongly correlated with each other in MC, which is not surprising as the TNF2

allele is known to be in strong linkage disequilibrium with HLA-DR3 and HLA-DQ2 alleles (Price *et al.* 1999).

The IL-6-174-GG genotype was associated with MC (Table 13). The possible association of G allele with MC is in agreement with previous studies in which the IL-6-174-G allele has been linked with several chronic inflammatory and autoimmune diseases and with the activity of diseases such as rheumatic diseases and chronic periodontal disease (Pawlik *et al.* 2005, Raunio *et al.* 2007). The IL-6-174-G allele or IL-6-174-GG genotype have been associated with elevated serum levels of IL-6 in several diseases (Fishman *et al.* 1998, Hulkkonen *et al.* 2001b, Raunio *et al.* 2007). However, the genotype of IL-6-174 did not have any influence on the serum IL-6 concentration in MC in the current series. Since a correlation between the serum level of IL-6 and the activity of the inflammatory disease has been reported in several conditions, a potential confounding factor in the evaluation of serum levels of IL-6 and genotypes could be the heterogeneous activity of MC since the disease course is variable exhibiting both relapses and remissions (Gross *et al.* 1992, Pawlik *et al.* 2005, Raunio *et al.* 2007). At present, the correlation of the disease activity and outcome of MC to IL-6 genotype and IL-6 levels could not be thoroughly explored because of a lack of validated methods for monitoring the activity of MC either by symptoms scoring or through its histological parameters. Other factors potentially influencing the IL-6 production such as gender, age or body weight did not influence the results (Jones *et al.* 2001). Since also other polymorphic sites in IL-6 gene have been described, the contradictory results might be explained by the synergistic effects of the different SNPs and their function in linkage equilibrium (Fife *et al.* 2005). Accordingly, further studies in a larger population with different ethnic groups may be necessary to clarify whether IL-6-174- polymorphism would be associated with susceptibility and/or outcome of MC and whether it is involved in regulating IL-6 secretion in MC. One could even speculate that monitoring serum IL-6 concentration could mirror the activity of the disease.

When studying multiple gene polymorphisms, a correction for multiple testing such as that devised by Benjamini-Hochberg (1990) may be useful, even necessary, but then the observed significant associations may simply disappear as was the case with IL-6 polymorphisms. However as suggested by Rothman (1990), those kinds of adjustments are not always considered necessary before one can make a conclusion. Accordingly, since this is the first study into the cytokine gene polymorphisms in MC and since there was no previous data available, it was felt important to scrutinize all possible leads for further

exploration. However, as the experience with IBD shows, the results of the studies of the cytokine polymorphisms are controversial, this subject has been discussed in section 2.3.3. It is apparent, that in MC, more immunogenetic studies of these SNPs in larger populations are required before one cane make more reliable conclusions of both positive and negative associations.

6.6 Comparisons between CC and LC (I-IV)

There has been much debate about whether CC and LC are related but not identical diseases, or totally separate conditions or perhaps even different phases of the same disease. (Giardiello *et al.* 1992, Baert *et al.* 1999.) The histological changes in colon mucosa are often patchy, which sometimes cause confusion in the diagnosis of MC especially when only a few biopsies are taken. This was evident also in the current patient material, but the diagnosis was changed only in two patients with CC diagnosis and two patients with LC. In follow-up studies, a conversion of LC to CC or the opposite has been reported (Fernandez-Banares *et al.* 2003, Olesen *et al.* 2004a).

The proposal for a greater female preponderance in CC is not so obvious as several studies in addition to this present study have detected a clear female preponderance also in LC (Fernandez-Banares *et al.* 1999, Agnarsdottir *et al.* 2002.) As in previous studies, the age at diagnosis did not differ remarkable in patients with CC as compared to LC (Table 8). As in most studies, the diagnostic delay displayed a trend to be longer in CC compared to LC (Baert *et al.* 1999, Fernandez-Banares *et al.* 2003, Olesen *et al.* 2004b). The reason for this difference is not clear because clinically, these colitides are indistinguishable. The difference in the diagnostic delay in current series is partly attributable to the greater proportion of LC in the prospective study group and thus may reflect the better colonoscopy capacity and the awareness of these diseases. The clinical courses of CC and LC may differ, as according to some reports more chronic and severe symptoms have been described in CC compared to LC though the evidence is not consistent. (Baert *et al.* 1999, Fernandez-Banares *et al.* 2003, Sveinsson *et al.* 2008). Here, no differences in symptoms and disease course could be found between CC and LC.

As previously discussed, autoimmune disorders were more commonly found in CC than in LC (Table 9). However, the prevalence of CD did not differ between CC and LC. The patients with LC suffered more often from bronchial asthma than those with CC, this association has not been reported before. In

previous studies, the prevalence of asthma in MC has been 4–7% as it was for CC in this study, these figures being at the same level as the frequency of asthma in the adult Finnish population (4–6%) (Arinen S-S *et al.* 1998, Baert *et al.* 1999, Olesen *et al.* 2004a, Haahtela *et al.* 2006). It is possible that the different associations in CC and LC could reflect the importance of immunological mechanisms in their pathogenesis and indicate that the balance of immunological activation is different in these two conditions.

Lactose-containing food was more poorly tolerated by patients with CC than LC, although no significant difference was found in the prevalence of hypolactasia. The co-existence of CD and IBS-like symptoms can not explain this difference in subjective lactose intolerance, because they were as frequently found in CC and in LC. In this study, starting a new drug was more commonly associated with the onset of the diarrhoea in patients with LC as compared to the situation in patients with CC. In previous studies, a correlation has been found between CC and the use of NSAIDs and SSRIs and correspondingly between LC and SSRIs, but no such associations were found in this study (Fernandez-Banares *et al.* 2007).

LG was found only in patients with LC, though the difference was not statistically significant (Table 10). The IEL count in the antral mucosa was higher in LC as compared to CC and a similar trend was found in the body mucosa (Table 11). In addition, ileal changes have a tendency to be more common in LC and they were associated with higher numbers of duodenal and antral IEL. No differences in IEL count were found in the duodenum mucosa. However, our findings indicate that an increase in the number of gastric IELs is a characteristic feature of LC.

Endoscopic gastric erosions were more often detected in CC than in LC and there was also a trend that duodenal endoscopical inflammatory changes were more common in CC compared to LC. The features found in gastric morphology representing more active gastritis and higher glands in antral mucosa in CC compared to LC especially in *H. pylori* positive subjects emphasize the importance of the antral predominance of the gastritis in the pathogenesis of gastric erosions. In addition, the glands in the gastric body were higher in CC than in LC. Thus the higher erosion rate in CC could be related to the antral predominance of gastritis and the potentially higher acid secretion in CC (Toljamo *et al.* 2005).

It has been reported that there is a difference in the HLA-types between CC and LC as discussed earlier (Giardiello *et al.* 1992, Fernandez-Banares *et al.*

2005). However, in the present material, HLA-DR3-DQ2 was similarly prevalent in both subtypes of MC (Table 12), a similar association was also found by Fine et al (2000a). The trend towards a higher frequency of the IL-6-174 –GG genotype compared to the controls was detected in CC which was not found in LC (Table 13). No statistically significant difference was found in IL-6-174-GG genotype frequencies between CC and LC (31 vs. 39%), but it is possible that the difference could have become statistically significant if a larger study population had been available. Interestingly, the serum IL-6 concentration was higher in patients with CC than in those with LC. This may point to differences in the pathogenesis of CC and LC. Alternatively, a high IL-6 level may indicate that CC is a more severe disease, as has been found in some studies, but these correlations could not be made because there was no validated way to evaluate the disease activity (Baert *et al.* 1999, Fernandez-Banares *et al.* 2003).

6.7 Comparisons concerning the association of CD to MC (I-IV)

The overall prevalence of CD was 16.7% which is a significantly higher frequency than the reported prevalence of 1–2% in the adult population and 2.7% in elderly people in Finland (Vilppula *et al.* 2009). The diagnosis of CD was uniformly based on typical histological findings in duodenum before starting the gluten-free diet and to the response to the diet as assessed in follow-up biopsies. In previous studies, the prevalence of CD in subgroups of MC had varied considerably, partly depending on the population being studied, the number of patients tested and on the diagnostic criteria, with frequencies in CC varying between 0–40% and in LC from 0% up to 27%, the highest frequencies normally being reported in studies with small sample sizes (Armes *et al.* 1992, Gillett & Freeman 2000, Fernandez-Banares *et al.* 2003). However, according to most of these studies there seem to be higher proportion of subjects with CD in MC than in the normal population. CD was as common in both subtypes of MC in our study. On the other hand, the prevalence of MC in CD patients is higher than in the normal population, with frequencies varying from 2.7% up to 36% depending on the timing of the colonoscopy (Fine *et al.* 1998, Abdulkarim *et al.* 2002, Hopper *et al.* 2005). Thus, in a clinical setting, the co-existence of CD in MC patients should be screened at least by serological tests and if CD patients in spite of strict GFD and improved duodenal histology are still suffering chronic diarrhoea, then a colonoscopy should be performed to exclude MC.

Patients with CD were younger than patients without CD at the time of the diagnosis of MC and the diagnostic delay was longer in patients with CD compared to other patients with MC. Nine patients with CD had been on a GFD already for years before the MC was diagnosed. Many patients with previously diagnosed CD are often more accustomed to the intestinal symptoms such as diarrhoea, and often their symptom relapses may be thought to have been due to other reasons i.e. gluten contamination in diet. However, colonoscopy may be also more readily performed in the early stages after the diagnosis of CD in those patients whose symptoms do not disappear with a GFD. Nonetheless, it is possible that the spectrum of MC (symptoms, disease course etc.) is different in CD patients although this was not clarified in the present study.

At the time when the gastroscopy was counducted in this study, three patients who had already been on a GFD for years, had mild villous atrophy, but no criteria of RCD were fulfilled. However, also in this study, six patients with CD and on a GFD had normal duodenal villous structure when MC was diagnosed, i.e. gluten was not be the noxious agent in MC. The presence of LG in CD is discussed in section 6.4.

There were 15 patients with MC and CD, and 13 of them carried the HLA-DR3-DQ2 haplotype associated strongly to CD in northern Europe, and one displayed the HLA-DR4-DQ8 haplotype. In the study of Karell *et al.* (2003) of 61 CD patients without HLA-DR3-DQ2 or DR4-DQ8, 60 patients encoded either one half of the DQ2 heterodimer (DQA1*0501; DQB1*02 alleles) and/or carried DQ5 (DQA1*01-DQB1*05). Accordingly also in this study, one of the 15 MC patients with CD, who was negative for both HLA-DR3-DQ2 and HLA-DR4-DQ8 carried the DQ5 molecule.

In CD, the HLA-DR3-DQ2/TNF-2 double positivity was higher than in MC patients without CD (66.7% vs. 29.5%). There was also a trend for homozygotic TNF2 to be more frequent in patients with CD than in controls. There are previous reports suggesting that the TNF2 allele might be an additional marker for CD or be able to act independently in CD (de la Concha *et al.* 2000, Garrote *et al.* 2002). Unfortunately this aspect could not be studied in this material since there was no available information on both HLA-DQ2 and TNF2 for the control group.

MC patients with CD carried more often the IL-1ß -511-CT/TT genotype than those without CD and correspondingly the frequency of the IL-1β-511-T allele was statistically higher in CD patients compared to non-CD patients with MC. However, IL-1RA-related polymorphisms did not exhibit the same

difference though the present patient population was too small i.e. this finding needs to be re-examined in larger patient groups.

6.8 Comparisons of MC with IBD (I-IV)

The relationship of MC and IBD is unclear. There are several reported cases of MC developing into IBD during the follow-up (Bohr *et al.* 1996a, Fernandez-Banares *et al.* 2003). It is not known whether these two disorders occur only independently in the same individual or whether there could be a common genetic predisposition or shared immunological pathways.

In contrast to the association between MC and CD, in IBD the prevalence of CD is not clearly higher than in the normal population, although not many large studies have been published. (Leeds *et al.* 2007.) The prevalence of IBD in treated CD patients has been reported to be higher than in controls (1.6% vs. 0.33%).

Previously an association between *H. pylori* and a later onset of IBD has been speculated and this seems to occur also in MC. FG is known to associate with IBD, especially in Crohn`s disease (Halme *et al.* 1996a, Sharif *et al.* 2002, Kundhal *et al.* 2003). FG was only rarely found in the present series of MC patients and its frequency did not differ from that found in the controls. This observation is in agreement with previous findings in a small series of MC patients where focal cryptitis (analogous to FG) was found at a similar frequency as in controls (2/8 vs. 2/5) (Danelius *et al.* 2009). The absence of any association between FG and MC favours the concept that MC and IBD are different diseases in terms of their pathogenesis.

In the current material ileal histological changes were found as also previously described in MC (Sapp *et al.* 2002). In Crohn`s disease, the inflammatory changes of ileum and colon are often endoscopically visible, although also only histologic findings with ileitis and granulomas in ileum mucosa have been described (Nikolaus & Schreiber 2007). However, the typical histology of colon in MC may help in the differential diagnosis.

IBD often clusters in families, and also in MC a familial occurrence has also been reported as the patients examined here (Jarnerot *et al.* 2001, Baumgart & Carding 2007). UC and Crohn`s disease are polygenic diseases, and there are conflicting results in the published genetic studies. The studies of cytokine polymorphisms in IBD have been conflicting and the possible connection found is often associated with variations in IBD phenotype, though the samples studied have been small and the results often not reproducible in different populations

(Balding *et al.* 2004.). According to the current findings, it seems likely that MC and IBD do not share a genetic background involving HLA-DR3-DQ2 and IL-6 polymorphisms (Cho & Weaver 2007).

6.9 Comparisons of MC with IBS (I-IV)

It is not uncommon for MC patients to display IBS-like symptoms. In this study as many as 80% of patients suffered those symptoms and frequencies from 30 to 70% have been noted in previous studies (Bohr *et al.* 1996a, Baert *et al.* 1999, Fernandez-Banares *et al.* 2003, Limsui *et al.* 2007). However, as often in other retrospective studies of MC, the prevalence of IBS symptoms in the present study was only an estimate since the diagnosis of IBS was not based on the Rome criteria (Longstreth *et al.* 2006). On the other hand, MC had been found in 1.5%-13% of patients with a prior diagnosis of IBS (Kao *et al.* 2009, Chey *et al.* 2010). In contrast to IBS, patients with MC often report weight loss also in the present study (Baert *et al.* 1999). A female preponderance is also common in IBS but the onset of symptoms of IBS is usually at younger ages than in MC and often prevalence of symptoms often decreases with age (Drossman *et al.* 2002).

The prevalence of asthma was higher in LC than has been previously reported and compared to Finnish adult population (Arinen S-S *et al.* 1998). IBS patients have also been reported to suffer more often from bronchial asthma (15.9%) than the normal population (Yazar *et al.* 2001). It remains to be determined whether this shared connection to asthma is evidence of some further etiopathogenetic relatedness between LC and IBS. Other common phenomena in IBS and MC are lactose related symptoms, although in MC the frequency of hypolactasia was also higher than in the normal population (Sahi 1994, Farup *et al.* 2004).

Cytokine studies in IBS have revealed alterations in cytokine secretion i.e. in diarrhoea prominent IBS, higher levels of pro-inflammatory cytokines including IL-6 have been demonstrated, although no studies examining the IL-6-polymorphisms have been published (Liebregts *et al.* 2007). With respect to the TNFα-308 polymorphisms, TNF2 has been detected more frequently in the patients with IBS than in controls which was also found in MC patients of the current study (O'Mahony *et al.* 2005).

IBS and MC also share some similarities also in their colon histology. The inflammative histology of colon with the increased cellularity of the colonic mucosa and lamina propria in some IBS patients resembles but does not fulfil the histologic criteria of MC (Chadwick *et al.* 2002, Drossman *et al.* 2002). No

pathogenetic links have been found so far; more comparative studies into these states are need to provide more information. One could argue that at least a subset of patients with IBS may form a part of a continuum with MC.

7 Summary and Conclusions

1. The most common symptoms were watery diarrhoea associated with nocturnal diarrhoea and incontinence, and 80% of patients suffered symptoms resembling those of IBS. Women were more likely to suffer both CC and LC. The high susceptibility to autoimmune diseases in patients with MC points to a possible autoimmune involvement in MC. CD was found in 18% of patients with MC. Nine patients with MC had started a new drug shortly before they experienced symptoms related to MC but there were no differences between the patients and controls in the use of drugs. For the first time, evidence was found for a positive association with lactose intolerance and a negative association with DD with MC, and a possible association of LC with bronchial asthma.
2. The present findings suggest that mucosal abnormalities in MC might involve not only the colon but also ileum, gastric and duodenal mucosa. However, no diagnostic hallmarks were found in gastric mucosa. FG, previously linked with IBD, was only rarely found in MC. The older age of onset of MC in *H. pylori* positive subjects suggests that the infection may delay the development of MC. The decrease of foveolar height in the gastric body mucosa and villous height in the duodenum in patients with MC, the latter even without evidence of CD, points to the presence of some previously unknown links between colorectal inflammation and the gastroduodenal mucosal structure. More systematic histological studies would be needed to characterize the full spectrum of changes in the whole gastrointestinal channel in MC. Furthermore, evaluation of the sequence of appearance of anatomically diverse features, and, more importantly, their pathophysiological relationship, is essential if one wishes to understand the pathogenesis of MC.
3. A clear association between HLA-DR3-DQ2 carriage and both subgroups of MC was found even when CD patients were excluded. The TNF2 gene was also more frequently seen in MC patients, but this was probably due to linkage equilibrium between DQ2 and TNF2. The IL-6-174 –G allele was common in MC and may be a predisposing factor or a marker for some other susceptibility gene. However, this genotype, which is repeatedly associated with the elevated production of IL-6, was not related with increased serum levels of IL-6 in MC. The finding of IL-6 polymorphism needs to be confirmed in other ethnic and national patient groups with MC.

In summary, LC and CC seem to share many clinical features. Although autoimmune type disorders were common in both CC and LC, their prevalence was higher in CC. CD was as common in both entities as was also the prevalence HLA-DR3-DQ2. In contrast, bronchial asthma showed an association with LC. The latter findings point to a possible involvement of immunological mechanisms in MC pathogenesis and suggest that the balance of immunological activation may be different in CC and LC. The association of CC with the antrum predominant gastritis and gastric erosions and the relationship between LC and LG suggests that the pathogenesis of these two diseases, and also their genetic background, might be different. No differences in cytokine polymorphisms were found between the patients with CC and LC. However the difference in serum IL-6 levels between CC vs. LC possibly reflects differences in the pathogenetic mechanisms underpinning these conditions

The common association of CD and MC, the high prevalence of HLA-DR3-DQ2 in MC found in the current study and the similar inflammatory characteristics in colonic mucosa in MC and in duodenal mucosa in CD (mononuclear inflammatory cell infiltration of the lamina propria and intraepithelial lymphocytosis), provide evidence that these syndromes might share some pathogenetic mechanisms. It has been speculated that MC is a T-cell-mediated immunological reaction to a luminal antigen under the control of class II HLA genes, though the putative antigen has not been identified so far. In the clinical setting, the possible co-existence of CD should be considered in patients with MC and vice versa.

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Original publications

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals.

I Koskela RM, Niemela SE, Karttunen TJ & Lehtola JK (2004) Clinical characteristics of collagenous and lymphocytic colitis. Scand J Gastroenterol 39: 837–845.

II Koskela RM, Niemelä SE, Lehtola JK, Bloigu RS & Karttunen TJ (2011) Gastroduodenal mucosa in microscopic colitis. Scand J Gastroenterol 46: 567–576.

III Koskela RM, Karttunen TJ, Niemela SE, Lehtola JK, Ilonen J & Karttunen RA (2008) Human leucocyte antigen and TNFα polymorphism association in microscopic colitis. Eur J Gastroenterol Hepatol 20: 276–282.

IV Koskela RM, Karttunen TJ, Niemelä SE, Lehtola JK, Bloigu RS & Karttunen RA Cytokine gene polymorphisms in microscopic colitis. Association with IL-6-174 GG genotype. Eur J Gastroenterol Hepatol, in press.

Original publications are not included in the electronic version of the dissertation.

1079. Hietasalo, Pauliina (2010) Behavioral and economic aspects of caries control

1080. Jääskeläinen, Minna (2010) Apoptosis-regulating factors in developing and adult ovaries

1081. Alahuhta, Maija (2010) Tyypin 2 diabeteksen riskiryhmään kuuluvien työikäisten henkilöiden painonhallinnan ja elintapamuutoksen tunnuspiirteitä

1082. Hurskainen, Merja (2010) The roles of collagens XV and XVIII in vessel formation, the function of recombinant human full-length type XV collagen and the roles of collagen XV and laminin α4 in peripheral nerve development and function

1083. Rasi, Karolina (2010) Collagen XV as a matrix organizer : its function in the heart and its role together with laminin α4 in peripheral nerves

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