Expression of LTC_4-synthase in small intestinal mucosa of patients with celiac disease and controls

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1. Acknowledgements

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Abbreviations used in the text:

aa:	amino acids
ab:	antibody
APC:	antigen presenting cells
CD:	celiac disease
Cox:	cyclooxygenase
CysLT1:	cysteinyl leukotriene receptor 1
DC:	dendritic cells
DIC:	differential interference contrast microscopy
FLAP:	5-lipoxygenase activating protein
HLA:	human leukocyte antigen
IEL:	intraepithelial lymphocyte
IFN:	interferon
Ig:	immunoglobulin
kb:	kilo base pairs
LTC_4S:	leukotriene C_4 synthase
MC:	mast cells
MGST2:	microsomal glutathione transferase 2
PG-:	prostaglandin …
TG-2:	tissue transglutaminase-2
TXA_2:	thromboxane A_2
5-LO:	5-lipoxygenase

2. Introduction

2.1 Leukotriene formation

Leukotrienes are a group of eicosanoids produced from arachidonic acid through 5-lipoxygenase (5-LO). The enzyme may be expressed by myeloid cells such as neutrophils, macrophages, monocytes, mast cells and B-cells. 5-LO translocates from cytosol to the nuclear envelope after cell activation. The enzyme catalyzes two following steps in the leukotriene formation; First the conversion of arachidonic acid to 5-HPETE (5-hydroperoxy-eicosatetraenoic acid) and the subsequent formation of leukotriene A_4. LTA_4 is further metabolized to LTB_4 by LTA_4 hydrolase or to LTC_4 by LTC_4 synthase.(1) FLAP (5-lipoxygenase activating protein) is needed as cofactor for both 5-LO, LTA_4-hydrolase and LTC_4-synthase. (2)

LTC_4 synthase (LTC_4S) is the committed step in the generation of the cysteinyl-leukotrienes. The metabolites LTD_4 and LTE_4 are generated by the enzymes γ-glutamyl transpeptidase and dipeptidase respectively (figure 1).

LTA4 is a short-lived intermediate, but is in interest because of its role in transcellular metabolism in vascular inflammation of atherosclerotic plaques. (3)
LTB_4 is produced mainly by the neutrophils and attracts neutrophils and macrophages, stimulates activation and recruitment of inflammatory cells, migration of T-cells and vasoconstriction. (1;4)
LTC_4, LTD_4 and LTE_4 – are called the cysteinyl leukotrienes (cys-leukotrienes) or referred to as "slow reacting substances of anaphylaxis". Their biological effects are closely similar and consist of dose dependent protracted bronchial constriction, increased secretion of mucus, eosinophil recruitment, increased blood flow and local vascular permeability. (1)

It has been reported two different receptors for the cys-leukotrienes: $CysLT_1$ and $CysLT_2$, and there is evidence for at least one more. (5)

2.1.1 Inhibiting leukotriene-synthesis – therapeutic applications

Inhibiting leukotriene synthesis has been of interest for the treatment of asthma and rhinitis, and hopefully in the future also atherosclerosis. In Norwegian market montelukast (Singulair) – a $CysLT_1$ receptor antagonist, is the only available preparation, and recommended as supplement in the treatment of bronchial asthma. The effects vary between patients and this may relate to LTC_4S-gene polymorphisms. (4)

2.1.2 Microsomal glutathione transferases

Glutathione transferases belong to a superfamily of proteins involved in cellular detoxification. They encompass a diversity of different catalytic functions as: conjugation, reduction, isomerase reactions, and non-catalytic actions as ligands or signal transduction modulation. The subgroup microsomal GSTs, also called MAPEGs (membrane-associated proteins involved in eicosanoid and glutathione metabolism) include both LTC_4S, 5-LO, FLAP and microsomal glutathione transferase 2 (MGST2). Although LTC_4S has only 44% amino acid identity with MGST2 they are functionally similar and may catalyze the same reaction producing LTC_4 from LTA_4 with FLAP as cofactor. (2)

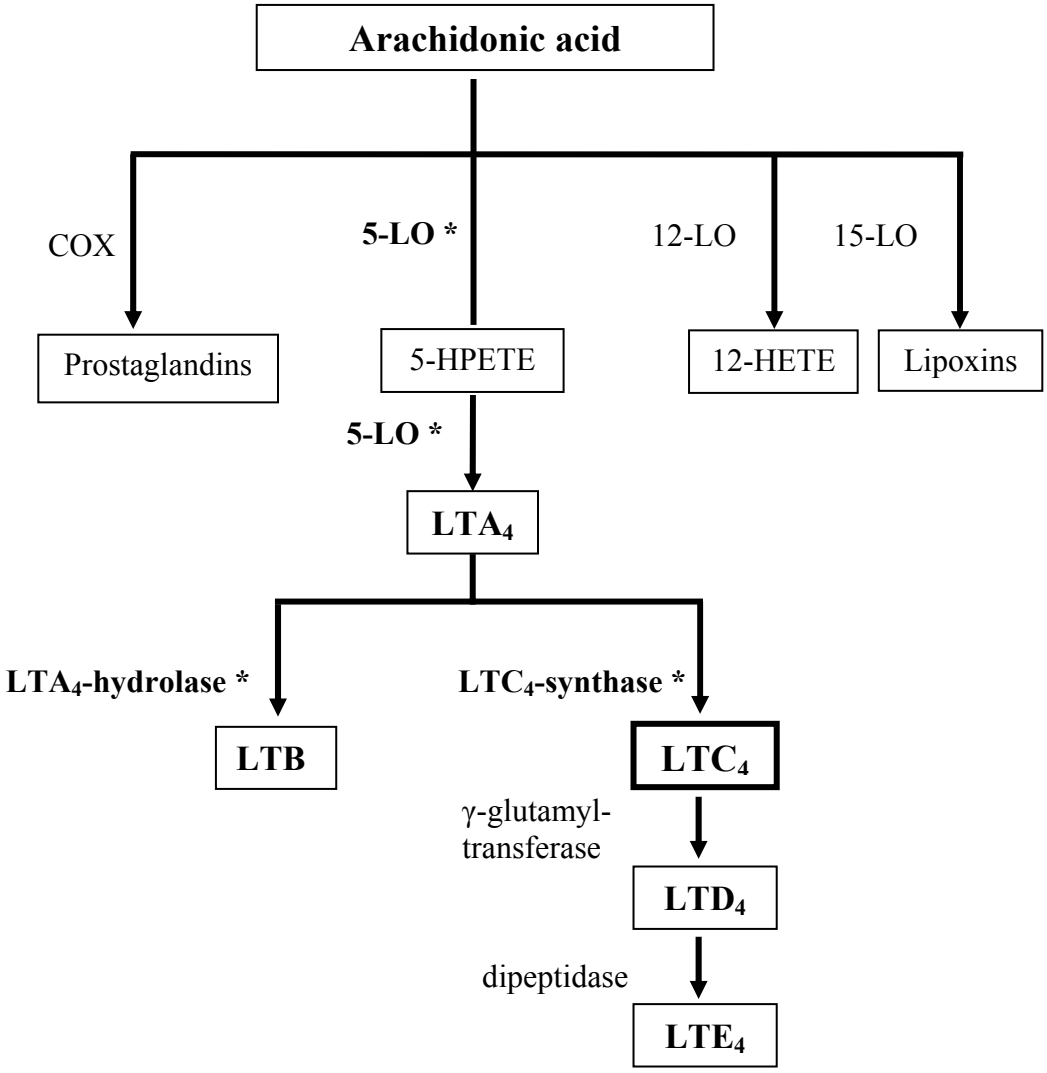


Figure 1: Arachidonic acid is the source of the eicosanoids and the leukotrienes result from the 5-LO pathway. LTC_4-synthase is the committed step in the production of the cysteinyl-leukotrienes and the enzyme investigated by immmunohistochemical methods in this study.
*steps with FLAP as coenzyme

2.2 Celiac disease

Celiac disease is an enteropathy in which genetically susceptible individuals can develop small intestinal inflammation when exposed to gluten. Gluten-proteins are found in wheat and there are similar proteins in barley and rye. The disease is reckoned as a food-allergy, but includes autoimmunity.

2.2.1 Epidemiology and diagnostics

With the availability of serum-tests for IgA anti-transglutaminase 2 (TG-2), one have discovered that what was thought to be a rare disease is actually quite common with a prevalence of 1/100 – 1/300 in Caucasian populations. (6-8)

Disease development requires both a genetic predisposition and environmental factors. Although the environmental factor is gluten, viral gastroenteritis activating Th1-immune responses may be a precipitating factor. The majority of the genetic predisposition appears to be linked to the HLA-region on chromosome 6. More than 90% of the celiac patients have HLA-DQ2 compared to 25% in the general population. The rest of the patients carry HLA-DQ8. (9;10) The risk is increased for homozygous compared to heterozygous. (11) Also non-HLA-genes are associated with celiac disease, but they are less thoroughly investigated and no single genes of greater significance are identified. (12;13)

2.2.2 Histology

Histological changes in celiac disease include invasion of leukocytes and especially T-cells in lamina propria and the epithelium. Cell-division increases and lead to crypt hyperplasia, while there is villous atrophy. This leads to a reduced absorptive area covered by immature enterocytes with a reduced capacity to degrade food. The morphology of the gut will normalize on a gluten free diet though a moderate increase in leukocytes usually remains. (14)

2.2.3 Clinical manifestations and complications

The disease has a wide range of clinical symptoms, some considered classical and some atypical. The classical picture of celiac disease includes fatigue, diarrhoea, abdominal distension, flatulence and failure to thrive for children, or weight loss for adults. The patients have different degree of malabsorption depending on the intensity of the intestinal inflammation. Atypical presentations are actually more common than classical, (15) and the most common symptoms are fatigue, anaemia, depression, osteoporosis and diffuse musculoskeletal pain. (16) The symptoms often worsen when life is stressing and can easily be misunderstood as irritable bowel disease both by the doctor and the patient. (16;17)

Some patients diagnosed with CD do not improve on a gluten free diet, and are referred to as refractory celiac disease or refractory sprue. This condition is reckoned as pre-malignant and associated with poorer prognosis due to development of intestinal T-cell lymphoma. (18)

Patients with celiac disease have more often autoimmune endocrinologic disorders, and diabetes mellitus type I, autoimmune thyroid disorder and Sjogren's syndrome are the most common ones (19). The coexistence might relate to common predisposing genes and investigations have found connection to Th1-cells for both celiac disease, diabetes mellitus type I and autoimmune thyroid disorder. (20)

2.2.4 Pathogenesis

The gastrointestinal digestion of gluten creates a peptide resistant to both gastric acid and intestinal enzymes which pass the intestinal barrier without further break-down. In the celiac patients this leads to intense inflammatory reactions in and beneath the epithelial line. (14) The enzyme TG-2 reacts with gluten resulting in deamidation. Deamidated gluten binds with high affinity to the HLA-DQ2/ -DQ8-molecules on antigen presenting cells (APCs). (10;21)

The APCs in the celiac mucosa are mainly dendritic cells and macrophages. The dendritic cells are the most effective APC concerning activation of gluten-specific T-cells. (22) Gluten fragments are linked to TG-2 when binding to TG-2 specific Ig on B-cells. (23) Antibodies mainly of IgA-subtype are made both against gluten and TG-2. (9;24) The T-cells are most thoroughly investigated, but also mast cells, eosinophils (25;26) and plasma cells producing IgA (26) are shown to increase during the celiac inflammation. Gluten is proven to induce activation of mast cells and eosinophils (27), but their role in the process of pathogenesis is not fully understood.

2.2.5 Treatment and prognosis

The treatment is life-long gluten-free diet. Also symptom-free patients will benefit from a gluten-free diet by feeling increased well-being. (28) Because gluten contamination in gluten-free products can not be totally avoided, investigations have been made to try to find a safe threshold. (29) Oat is considered safe if the process of production avoids contamination. (30;31) Strict adherence to the diet is important to avoid symptoms and reduce the risk of malabsorption or malignancies. (32;33)

2.3 Cytokines in celiac disease

A classical Th1-dominated immune-reaction would contain abundant IFN-γ in addition to the cytokines listed in table 1. Such immune responses induce predominantly macrophage activation. Th1-cells do have a certain capacity to stimulate B-cells, but modest compared to Th2-cells.

Table 1: Cytokine profiles

	IL-2	IL-4	IL-6	IL-10	IL-12	IL-15	IL-18	IFN-γ	TNF-α
Th1	+	-	-	-	+	+	+	+++	+
Th2	+/-	+	+	+	-	?	-	-	-
Celiac	+/-	+/-	-	-	-	+	+	+++	+

The Th2-cytokines predominantly stimulates B-cells to produce antibodies. (20) Celiac disease has abundant IFN- γ and the T-cells are of Th1 or Th0-type. (34) There are no IL-5 or IL-12 and low/ undetectable levels of IL-2, IL-4, IL-6 and TNF-α. (35) This mostly fit in with the Th1-profile. The Th1-skewing seem to take place without the classic Th1-directoring IL-12, but rather induced by IFN-α and IL-18. (36) There are interesting reports that therapeutic administration of IFN-α for malignancy or viral-hepatitis can trigger celiac disease. (37)

IFN-γ increases the expression of HLA-II molecules, while IFN-α will increase expression of HLA-I. (20) The result is an increased number of cells that may act as APCs.

2.4 Aims of the study

Leukotrienes are important mediators of the Th2-dominated asthmatic reaction, but also found to increase in celiac disease which apparently is strongly Th1-dominated. The aims of this study were to identify the LTC_4-synthase producing cells and immunohistochemically examine their distribution in small intestinal mucosa of controls and celiac patients.

3. Materials and methods

3.1 Materials

The non celiac controls (median age 10 years; range 1 – 59, n = 15) consisted of food intolerance or food allergy (n = 4), non-celiac malabsorption (n = 4), atopic disease (n = 1), multiple sclerosis (MS, n = 1), B12-deficiency (n = 1) and Downs syndrome (n = 1). All controls had some kind of abdominal discomfort, but celiac disease was excluded based on immunohistochemical stereo microscopy, serology and HLA-typing.

We have 14 biopsies from diet-treated patients (median age 6; range 5 - 19) and 14 untreated (median age 6; range 1 - 10). Biopsies from untreated patients are both from before start of treatment or on a provocation diet. Patients are referred in table 2. A few sections could not be counted due to bad quality of the staining.

Parents/ patients have given written consent concerning scientific use of remnants of the biopsies after the diagnostic procedures.

3.2 Tissue processing

All specimens were fixed in 1% paraformaldehyde (1% PLP) at 4ºC and brought to the laboratory within 24 hrs. At arrival the specimens were infiltrated in Histocon (Histolab, Göteborg, Sweden) for 1 h. Specimens were oriented on a thin slice of carrot and embedded in OCT (Tissue-Tek, Miles Laboratories, IN) snap-frozen in liquid nitrogen and stored at – 20˚C.

Cryosections were cut serially at 4 µm and dried overnight in room temperature, then enwrapped in aluminum foil and stored at - 20˚C until use. All biopsies were stained for haematoxylin-eosin for morphological evaluation

3.3 Immunohistochemistry

We used multicolor immunohistofluorescence-staining basically as described elsewhere. (38) Sections were pretreated with BSA (bovine serum albumin) and blocked with 10% dry milk powder in 20% horse serum for 20 min. at room temperature. Thereafter the sections were stained with two or three primary antibodies from table 3 and visualized by corresponding antibodies from table 4. Differential interference contrast microscopy-technique (DIC) was used as support for identification of the eosinophils. (39)

For negative control we used mouse-serum 1/1000, rabbit-serum 1/1000 and the secondary antibodies as listed in the table. The negative controls occasionally contained a few unspecific staining cells which could be recognized by their strong and evenly cytoplasmatic staining.

Table 3: Primary antibodies

Antigen	Subtype	Conc.	Time	Temp.	Producent
human-EG2	mouse IgG1	1/500 or 1/2000	1 hr 20 hrs	20ºC 4ºC	Pharmacia
human c-kit/ CD117	mouse IgG1	1/500 or 1/5000	1 hr 20 hrs	20ºC 4ºC	DAKO
human LTC4S	rabbit	1/2000 or 1/5000	1 hr 20 hrs	20ºC 4ºC	Dr. Jilly Evans
human LTC4S	rabbit	1/1000	20 hrs	4ºC	Dr. Frank Austen
human CD68, KP1	mouse IgG1	1/400	20 hrs	4ºC	DAKO
human HLA-DR	mouse IgG2a	1/200	20 hrs	4ºC	BD Biosciences
human CD3	Mouse IgG1	1/10 000	20 hrs	4ºC	DIATEC
ds DNA		1/200	30 min	20ºC	Molecular probes

Table 4: Coloring/ secondary antibodies, temperature: 20ºC for all

Antigen	Specie	Conc.	Time	Producent
mouse IgG	horse, biotin conj.	1/800	1,5 hrs	Vector
	streptavidin,	1/4000	30 min	Molecular probes
rabbit	goat, Alexa 488 conj.	1/800	45 min.	Molecular probes
mouse IgG1	goat, Alexa 594 conj.	1/1000	45 min	Molecular probes
mouse IgG2a	goat, biotin conj.	1/200	45 min	Southern biotechnology ass.
	avidin, Cascade blue conj.	1/1000	30 min	Molecular probes

Table 2:

Patient	Diagnosis	Age	Atrophy	Mast cells, identified by c-kit				Eosinophils identified by EG2				% MC of
		(yrs)	(0-4)	Nr. of cells LTC_4S-	Nr. of cells LTC_4S +	% LTC_4S+	Int. score	Nr. of cells LTC_4S-	Nr. of cells LTC_4S +	% LTC_4S+	Int. score	total LTC_4S+
1	normal	10	0	2	90	98 %	2,6	42	2	5 %	1,0	98 %
2	Downs	2	1	6	171	97 %	2,8	55	7	11 %	1,3	96 %
3	normal	13	0	21	479	96 %	2,5	180	65	27 %	1,0	88 %
4	atopic	14	0	8	233	97 %	2,8	163	10	6 %	1,0	96 %
5	food intol.	6	1	18	84	82 %	1,8	102	6	6 %	1,0	93 %
6	normal	3	0	1	23	96 %	2,3	55	20	27 %	1,7	53 %
7	malabs.	1	1	9	107	92 %	2,9	0	0			100 %
8	MS	40	1	10	154	94 %	2,5	97	5	5 %	1,0	97 %
9	food allergy	39	0	6	111	95 %	2,2	47	2	4 %	1,0	98 %
10	B12-def.	59	1	6	156	96 %	2,3	62	2	3 %	1,0	99 %
11	food intol.	10	1	7	99	93 %	2,3	103	14	12 %	1,1	88 %
12	malabs	10	1	18	229	93 %	1,8	84	9	10 %	1,1	96 %
13	normal	7	2	5	127	96 %	2,8					
14	normal	15	1	4	267	99 %	2,4	45	1	2 %	1,0	100 %
15	malabs.	4	1	20	146	88 %	2,5	201	16	7 %	1,2	90 %
16	food intol.	1	1,5	15	96	86 %	2,4	92	13	12 %	1,2	88 %
17	malabs.	7	2	4	138	97 %	2,8	29	6	17 %	1,0	96 %
18	treated cd	10	1	4	47	92 %	2,6	82	11	12 %	1,0	81 %
19	treated cd	8	3	10	103	91 %	2,2					
20	treated cd	6	1	13	95	88 %	2,9	46	11	19 %	1,0	90 %
21	treated cd	9	2	2	126	98 %	2,9	109	23	17 %	1,2	85 %
22	treated cd		1					18	0	0 %		
23	treated cd	7	0	0	164	100 %	2,8	90	5	5 %	1,0	97 %
24	treated cd	6		2	63	97 %	2,8	73	4	5 %	1,0	94 %
25	treated cd	5	2	5	86	95 %	2,4	66	3	4 %	1,0	97 %
26	treated cd	5		12	112	90 %	2,3	126	2	2 %	1,0	98 %
27	treated cd	5		0	90	100 %	2,9	82	7	8 %	1,0	93 %
28	treated cd	5	1	2	171	99 %	2,8	56	6	10 %	1,2	97 %
29	treated cd	19	1	2	40	95 %	3,0	89	3	3 %	1,0	93 %
30	treated cd	7	1	17	95	85 %	2,4	83	37	31 %	1,2	72 %
31	treated cd	11	1	1	111	99 %	2,7	76	1	1 %	1,0	99 %
32	treated cd	6	1	1	84	99 %	2,2	74	1	1 %	1,0	99 %
33	untreated	6	4	74	228	75 %	2,8	148	63	30 %	1,3	78 %
34	provocation	6	4	78	97	55 %	2,8	103	90	47 %	1,3	52 %
35	provocation	10	3	26	230	90 %	2,9	103	16	13 %	1,0	93 %
36	untreated	2	3	23	135	85 %	2,6	166	17	9 %	1,0	89 %
37	provocation	9	4	14	77	85 %	2,8	63	15	19 %	1,0	84 %
38	provocation	8	3	6	181	97 %	2,7	78	25	24 %	1,4	88 %
39	provocation	6	3	9	65	88 %	1,9	86	3	3 %	1,0	96 %
40	untreated	4	3,5	5	99	95 %	2,8	99	8	7 %	1,1	93 %
41	provocation	5	4	34	118	78 %	1,7	178	9	5 %	1,0	93 %
42	untreated	5	4	3	47	94 %	3,0	27	13	33 %	1,2	78 %
43	untreated	1	4	16	56	78 %	2,1	56	18	24 %	1,2	76 %
44	untreated	9	4	10	34	77 %	2,2	49	21	30 %	1,3	62 %
45	untreated	9	4	19	64	77 %	2,0	64	26	29 %	1,1	71 %
46	untreated	6	4	2	24	92 %	2,3	45	21	32 %	1,1	53 %

3.4 Microscopy and evaluation

The sections were examined by the same investigator in a Zeiss axioplan 2 microscope equipped with a plan-neofluar x40 x1.25 oil lens and appropriate fluorochrome filters including single- (red/ green/ blue), double- (red + green) and triple-color (red + green + blue) that allows simultaneous examinations of different colors.

LTC_4S staining cells in the lamina propria down to lamina muscularis mucosae were counted except for vessels and smooth muscles. Mast cells identified by c-kit (a total number of 6 102 cells), were counted and registered according to LTC_4S-positivity by double filter and if positive, scored for LTC_4S-staining intensity on a visual scale from 1+ to 3+ in single filter. The average intensity score for the positive staining cells in the section is listed in table 2.

Eosinophils (a total number of 4 329 cells) were identified by EG2 and DIC light-microscopy. Double identification was especially helpful in the untreated patients where eosinophil degranulation spread abundant EG2-containing granula in lamina propria masking the single cells. Intensity-scoring was performed by the same procedure as for the mast cells.

3.5 Cross reacting LTC_4S-antisera

The first antiserum we used to identify LTC_4S, were against full length LTC_4S-protein and cross-reacted to MGST2 (microsomal glutathione S-transferase-2). Later Dr. Frank Austen kindly provided a specific LTC_4S antiserum against a C-terminal peptide with no amino acid sequence similarity between LTC_4S and MGST2. The counting and scoring of cells were done using the full length protein antiserum, but an additional 10 sections were investigated by both antisera. The results both concerning cell-types and staining intensity were the same for the two antisera except structures resembling smooth muscles. The structures staining for the full length protein antiserum and not for the specific LTC_4S-antiserum, was assumed to express MGST2 only. Corresponding investigations in asthma show that smooth muscles were the only structures containing MGST2 and not LTC_4S. (40) There were some weak background staining for the specific antiserum, but practically none for the specific antiserum.

3.6 Statistical analysis

Median and standard deviation were analyzed with SPSS version 14.0 using descriptive statistics, and differences between the investigated groups tested with the non-parametric Mann Whitney with significance-level of $p<0,05$.

3.7 Methodological considerations

Inflammation caused by non-celiac conditions in our control-group could influence our interpretation, because leukotriene production in the mucosa is not restricted to celiac pathology. Another factor could be lack of expected histological normalization in the treated biopsies either due to insufficient compliance or the refractory nature of the condition.

Getting more quantitative information was abandoned because of difficulties in measuring the area/ volume of lamina propria. The morphology is strongly irregular and multiple small tears in the tissues that developed during preparation contribute to this. Cells in and beneath the lamina muscularis mucosae are not counted, but not all biopsies are cut this deep. Moreover the volume is no constant but a result of the inflammation itself. Counting a specified number of squares in a grid is possibly the best method but also this is problematic due to the mentioned morphology and the following need to assume that the cells are evenly distributed through mucosa.

4. Results

4.1 Distribution and identification of LTC_4S-containing cells

LTC_4S-containing cells were numerous and scattered through lamina propria without clustering. The staining pattern within the cells was predominately perinuclear, tough occasional cells had cytoplasmatic staining.

The LTC_4S-containing cells were mainly mast cells (figure 2) and eosinophils (figure 3). In addition there were some weak LTC_4S-expressing cells resembling macrophages, but difficult to identify because they were few in number.

The mast cells constitute > 90% of the LTC_4S-containing population in controls and treated patients while the proportion shrink to 81% in the untreated due to an increase in the eosinophils (table 5). While the mast cells tend to dominated in the deep parts of lamina propria, the eosinophils seem to accumulate closer to the epithelium.

Table 5: Median values of LTC_4S expression in Mast cells (MC) and in eosinophils (EG2+).

	n	Age (yrs)	Percentage LTC_4S+ MC	LTC_4S intensity in MC	Percentage LTC_4S+ EG2	LTC_4S intensity in EG2	Percentage MC in LTC_4S+
Controls	15	10	96%	2,4	7%	1,0	96%
Treated	13	6	96%	2,7	5%	1,0	94%
Untreated	14	6	85%	2,7	19%	1,1	81%

LTC_4S intensity: average intensity of LTC_4S-expression in the double-positive cells, + =1, ++ =2, +++ =3

4.2 Most mast cells contain LTC_4S

Most of the c-kit+ mast cells expressed high levels (2 – 3+) of LTC_4S (table 5, figure 2). However the proportion was lower in the untreated group (median 85%; range 55 – 97%, n = 14, p< 0,003) than in the treated (median 96%; range 85 – 100%, n = 14) and in the controls (median 96%; range 82 – 99%, n = 17, figure 3).

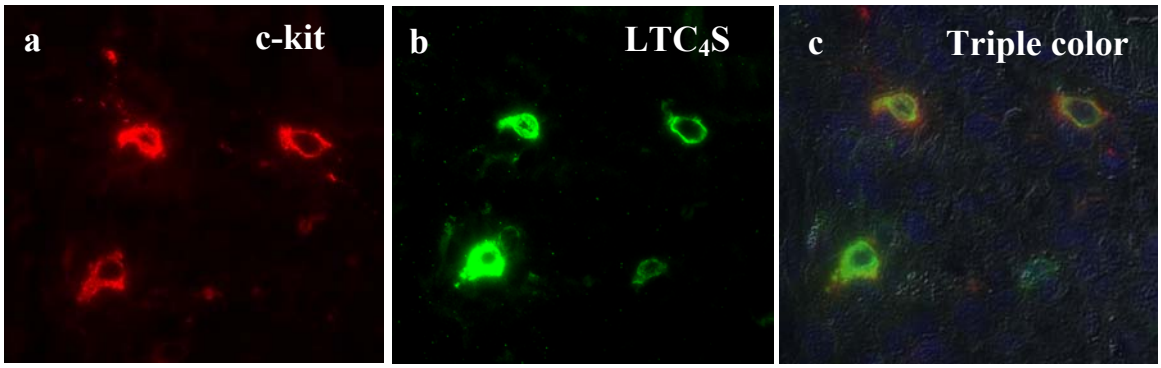


Figure 2: Most mast cells – identified with c-kit (a) had strong perinuclear staining for LTC_4S (b). Nuclear staining in blue and DIC-filter are added i c.

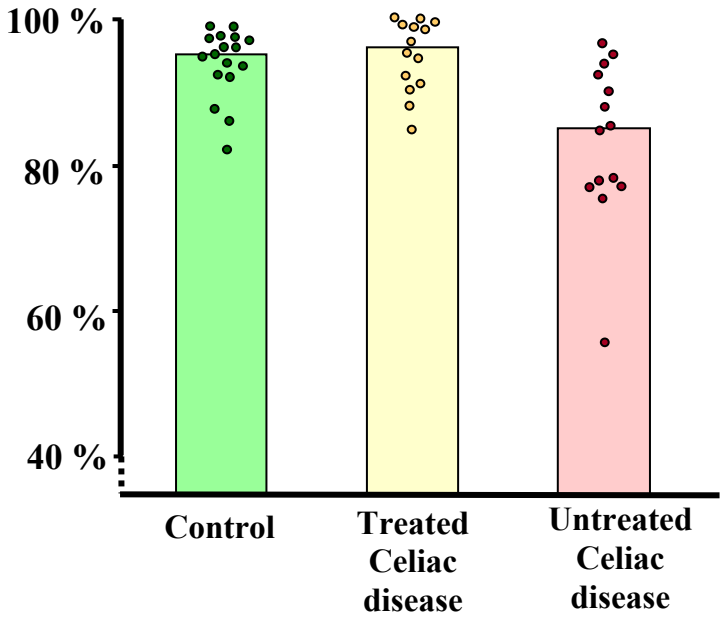


Figure 3. Proportion of subepithelial mast cells that express LTC_4S in celiac disease is decreased in untreated patients compared to the treated and controls.

4.3 LTC_4S-containing eosinophils are increased in untreated patients

The eosinophils were scattered quite evenly in the lamina propria, sometimes a few (2-4) cells clustered close to each other (figure 4).
Few eosinofils contained weak (1+) LTC_4S staining intensity, but the fraction was raised in the untreated (median 19%; range 3 – 47%, n = 14, $p< 0,02$) compared to the treated (median 5%; range 0 – 31%, n = 14) and the controls (median 7%; range 2 – 27%, n = 15, figure 5). Average intensity score judged after the same scale as for the mast cells gave scores around 1 for all 3 groups.

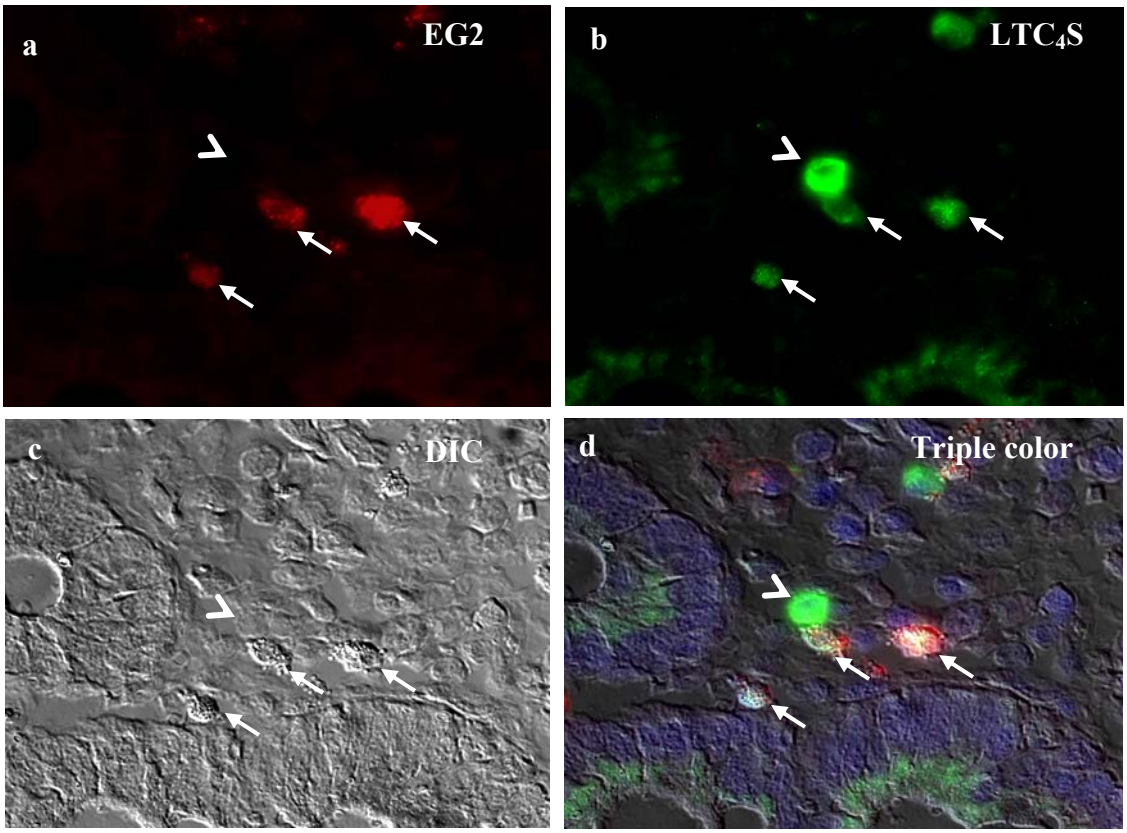


Figure 4: EG2+ eosinophils (arrows) (a), also contained LTC_4S (b), The proportion of LTC_4S+ cells increased in the untreated group compared to the controls and treated. Eosinophils could easily be seen in DIC due to their granula (c).
Note that the strongly LTC_4S-staining cell (arrowhead) is not an eosinophil (d).

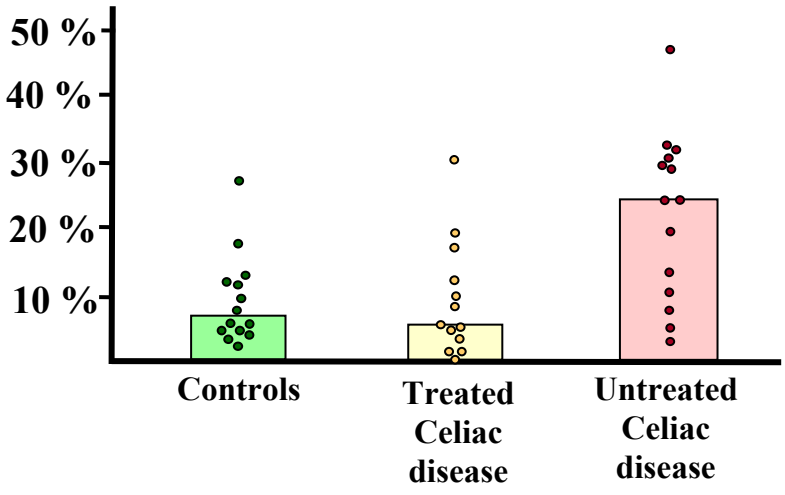


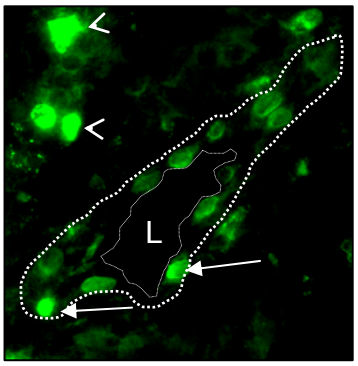
Figure 5. The Proportion of EG2+ eosinophils expressing LTC_4S is increased in the untreated group compared to the treated and the controls. However the staining intensity of the single cells remains weak (1+).

4.4 MGST2 versus LTC_4S

MGST2 was observed presumably in smooth muscles located to lamina muscularis mucosae, stretching up from lamina towards the epithelial surface and in the vessel walls (Figure 8). All the other cells appeared identical for the two antisera. Staining of smooth muscles appeared the same way in controls, treated and untreated patients.

Some vessels contained perinuclear LTC_4S in the endothelium (specific C-terminal antiserum) (figure 6).

Figure 6: Perinuclear LTC_4S staining in endothelial cells (arrows). Staining intensity corresponds to the level observed in eosinophils while mast cells (arrowheads) have considerably stronger staining. (L indicates lumen and the bold dotted line the basal lamina.)



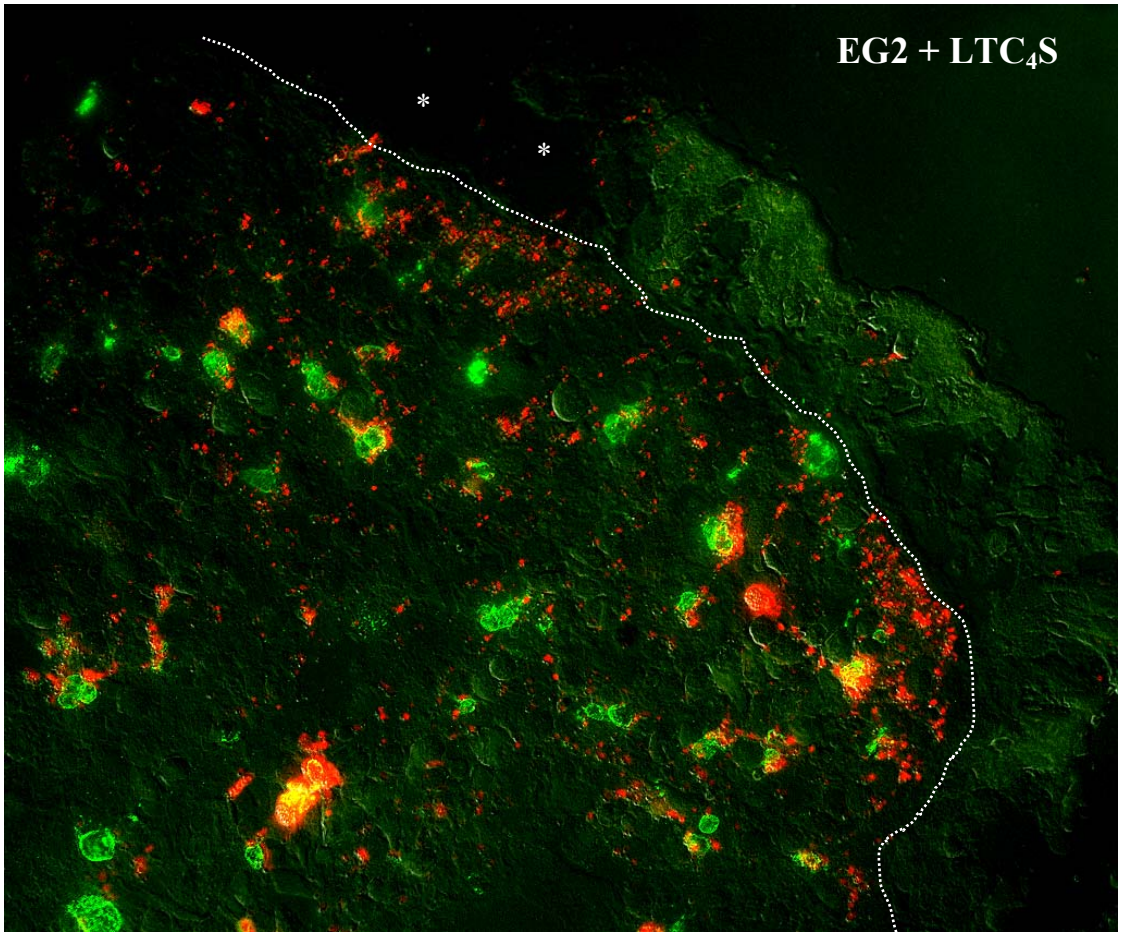


Figure 7: The epithelium was often seen to detach* over areas of massive eosinophil degranulation in the specimens from the untreated patients. (EG2 in red and LTC_4S in green.)

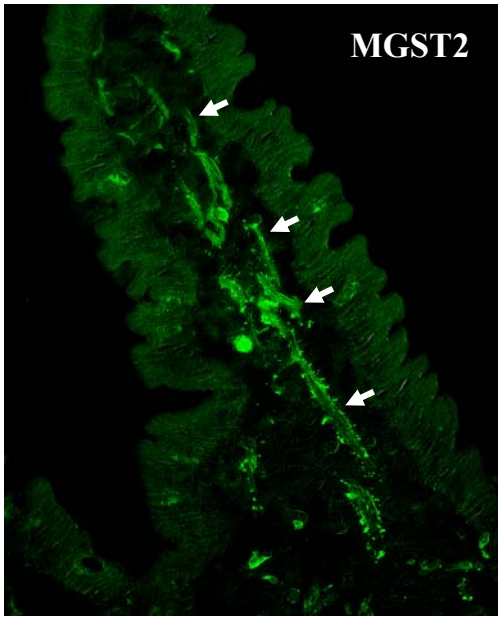


Figure 8: MGST2-only containing smooth muscle cells in a control. This staining pattern was not observed with the specific LTC_4S-antiserum.

5. Discussion

5.1 LTC_4S in mast cells

There is documentation of increased amounts of leukotrienes in celiac intestine (41;42) but the role is not fully understood and its cellular sources was unknown.

Our investigations point out mast cells and eosinophils as the main LTC_4S-containing cells and mast cells dominate both in number and staining-intensity. This is similar to asthmatic bronchi's. (40) We found a reduced proportion of LTC_4S-containing mast cells in the untreated patients compared to the treated and the controls. This may be due to the IFN-γ dominated cytokine-milieu in celiac disease.

IL-4 is known as a potent stimulator of LTC_4S-production in mast cells (43). However IL-4 is low in celiac intestinal mucosa (35), though we can not exclude that it may be produced only at its exact site of action as low inducible amounts were observed. (35) Otherwise umbilical cord stem cell derived mast cells express LTC_4S independently of IL-4 stimulation when they are given sufficient time for maturation (Halstensen et al. - not yet published). Thus IL-4 can not be mandatory for the production.

According to Sjöström et al. mast cells (grown on SCF and IL-6) express both LTC_4S and MGST2 as well as the receptor CysLT1. (44) It may seem strange that the mast cell should need two enzymes catalyzing the same reaction; however it is well-known that other enzymes of the arachidonic cascade; i.e. the cycloxygenases appear as isoenzymes capable of the same actions but differently regulated. One may speculate that MGST2 – LTC_4S share these qualities, but there is limited knowledge about the role of MGST2 in general.

5.2 LTC_4S in eosinophils

A larger proportion of eosinophils contain LTC_4S in the untreated patients compared to the treated and the controls. It is likely that eosinophils are responsible for some of the increase in leukotriene-production in a state of acute inflammation, but the low levels per cell and modest number compared to the mast cells indicates that the increase is not totally provided by the eosinophils.

Massive degranulation of eosinophils was observed in some sections especially in the untreated celiac group, although some of the controls showed a similar pattern. The degree of degranulation differed from scarce to massive within the mucosa of the same biopsy. Eosinophil activation has formerly been described in celiac disease (26;45). The metabolite LTD_4 is an attractant for eosinophils (47), but also other substances present in celiac disease as IL-3, IL-5 and GM-CSF (25) share this possibility.

In areas with abundant extracellular EG2-containing granula, the epithelium was often detached from the mucosa (Figure 7). This detachment could be an artefact from the processing of the tissue, but may nevertheless be related to the subepithelial inflammatory reactions impairing the epithelial connection i.e. by complement-activation. (46)

5.3 Leukotrienes in intestine

The leukotrienes have an important role in the pathogenesis of allergic asthma and thus much of our knowledge about leukotrienes origins from this condition. For some time the eosinophils were thought to be the main producer of cys-leukotrienes (48;49), but they are actually making a smaller contribution while the mast cells dominates. (40) Concerning direct intestinal effects LTD_4 may inhibit apoptosis in enterocytes (50), presumably by inhibiting killing by the intraepithelial lymphocytes. (51) Moreover CysLT1-expressing colorectal adenocarcinomas have poorer prognosis as leukotrienes may have a role in enterocyte survival. (52)

5.4 Symptomatic relief from CysLT1-antagonist

An interesting case report claim that asthmatic patients with nontropical sprue (celiac disease) experience that their diarrhoea improves markedly when treated with the CysLT1-antagonist montelukast, and that reintroducing gluten in the diet gave no relapse. (53) There are also some reports that montelukast can improve intestinal conditions where eosinophils play a considerable role in the inflammation. (54) Leukotrienes are known to increase the secretion of fluids in small intestinal tissue (55), but a direct proportionality towards diarrhoea does not exist. (42)

The leukotrienes seem to have an impact on intestinal immunological reactions but their role is not fully understood and more investigations are needed to clarify this.

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