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ORIGINAL PAPER Clinical Mechanisms in Allergic Disease

Cow's milk protein sensitivity assessed by the mucosal patch technique is related to irritable bowel syndrome in patients with primary Sjögren's syndrome

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Clinical and Experimental Allergy

Summary

Introduction Patients with primary Sjögren's syndrome (pSS) are reported to have a variety of gastrointestinal symptoms partly attributed to an overrepresentation of celiac disease. We have observed that irritable bowel syndrome (IBS)-like symptoms are frequent complaints in this patient group. Allergic manifestations to various drugs are also common in pSS. A role of food allergy in IBS has been proposed.

Objective This study is aimed at evaluating the mucosal response to rectal challenge with cow's milk protein (CM) in patients with pSS and relates possible CM reactivity to their intestinal symptoms.

Methods A rectal challenge with CM was performed in 21 patients with pSS and 18 healthy controls. Fifteen hours after challenge the mucosal production of nitric oxide (NO) and the release of myeloperoxidase (MPO) as signs of mucosal inflammatory reaction were measured using the mucosal patch technique.

Results Eight out of 21 patients with pSS had a definite increase of mucosal NO synthesis and the luminal release of MPO after rectal CM challenge. This sign of milk sensitivity was not linked to IgG/IgA antibodies to milk proteins. The symptoms for IBS according to Rome III criteria were fulfilled in 13 patients. All patients who were CM sensitive suffered from IBS. In a small open study, patients reactive to CM reported an improvement of intestinal symptoms on a CM-free diet.

Conclusion A rectal mucosal inflammatory response after CM challenge is seen in 38% of patients with pSS as a sign of CM sensitivity. IBS-like symptoms were common in pSS, linked to CM sensitivity.

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Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune disease characterized by the sicca complex due to lymphocytic infiltration of primarily the lacrimal and salivary glands, resulting in keratoconjunctivitis sicca and xerostomia [1]. Sicca symptoms may also appear in, e.g. the skin and trachea due to affection of other exocrine glands. General accompanying symptoms are fatigue, myalgia and arthralgia. Gastrointestinal symptoms are also frequent and include dysphagia, impaired pancreatic function, gastric antral inflammation and atrophy and autoimmune liver disease [2]. In contrast, reports on small bowel and colonic manifestations are rare but include nutritional deficiencies due to malabsorption, possibly due to associated celiac disease (CD) [3]. However, histological examinations have demonstrated increased amounts of intra-epithelial lymphocytes in the mucosa of pSS patients [4]. We ourselves have observed a high

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incidence of reported intestinal symptoms and in particular diarrhoea in our pSS patients.

A high prevalence (65%) of various allergic manifestations and in particular allergic drug reactions (46%) have been reported previously in pSS patients [5, 6]. In this study, we have raised the question of whether their intestinal complaints may possibly also reflect an allergic manifestation. Up to 20–30% of the adult general population in different European countries report self-experienced adverse food reactions [7–10]. However, using objective measures like dietary exclusion and a controlled food antigen challenge, the prevalence of food allergy and food intolerance is estimated to be much less frequent [7, 9–11]. In food allergy, skin and laboratory tests may detect the presence of IgE-mediated reactions particularly in patients with asthma or eczema especially where the foods are highly allergenic like egg, fish, nuts and milk. However, many patients with proven food intolerance have negative tests, suggesting other mechanisms than those mediated by IgE. Unfortunately, the tests available for non-IgE reactions are unreliable. Elimination diets and placebo-controlled challenge with suspected antigens have until now been the only established way to identify non-IgE adverse food reactions [11].

The recently described mucosal patch technique, which enables simultaneous measurements of nitric oxide (NO) and soluble mediators like myeloperoxidase (MPO), has been used recently in CD to study the mucosal inflammatory reactivity to rectal gluten challenge [12]. Using the same technique, about 50% of adult CD patients have recently been reported to have a mucosal reactivity also to cow's milk protein (CM) [13]. We ourselves have observed that 20% of patients with pSS and with the CD-associated haplotype DQ2 have CD-like reactivity to rectal gluten challenge without showing signs of CD [14]. We have defined such patients as being gluten sensitive with a possible risk of developing CD at a later stage. In this study, we report that pSS patients may have either an isolated CM sensitivity or combined gluten–CM sensitivity. In an attempt to elucidate the possible adverse effect of CM sensitivity, we correlated the CM challenge results to data from self-reported questionnaires about gastrointestinal symptoms and experienced connection between food ingestion and symptoms.

Methods

Study subjects

Twenty-one adult patients (two males) with pSS according to the revised American European Consensus Criteria were included in this study [1]. Patients with secondary Sjögren's syndrome, previously diagnosed CD or IgE-type reaction to CM were excluded. The patients were included consecutively from the outpatient clinic at the Department of Rheumatology, Uppsala University Hospital. The mean age of the patients was 56 years (range 34–73) and the median duration of sicca symptoms was 9 years (range 1–37). Eight of the patients also had other symptoms besides sicca manifestations. One of the pSS patients had high IgA antibody levels against gliadin, endomysium as well as tissue-transglutaminase (tTG) and a duodenal biopsy in this patient showed a total flat mucosa consistent with CD. Another patient had IgA deficiency but the duodenal biopsy was normal. Three patients were treated with corticosteroids in low dosages and four with chloroquine phosphate. Eighteen adult healthy control subjects (13 males) were included. The mean age of the control subjects was 34 years (range 19–58). All participants were asked about gastrointestinal symptoms, the existence of eczema, allergic rhinitis, asthma or anaphylaxis and self-experienced adverse food or drug reactions before challenge studies. The pSS patients also answered a questionnaire for support of the diagnosis of irritable bowel syndrome (IBS) according to Rome III criteria [15]. The Ethics Committee of the Medical Faculty, Uppsala University, approved the study. All the participants gave their informed consent to participate in the study.

Serum antibodies to cow's milk proteins

Serum IgA and IgG antibodies to casein, β-lactoglobulin and α-lactalbumin were measured according to the manufacturer's instructions (Phadia AB, Uppsala, Sweden).

Human leucocyte antigen typing

The HLA-DQB1 typing was performed with PCR-SSOP using the Luminex flow bead platform (One Lambda Inc., Canoga Park, CA, USA) at the Department of Clinical Immunology at our hospital.

Rectal challenge

The pSS patients and control subjects were challenged with 6.5 g dried milk powder (Semper AB, Stockholm, Sweden) suspended in 25 mL of 0.9% NaCl solution. The solution was instilled into the rectum with a syringe with the participant lying in the left lateral position. The subjects were then allowed to move about as they wished and were instructed to retain the enema for at least 60 min. Rectal challenge was performed between 4 and 6 p.m. and samplings were made 15 h later, between 7 and 9 a.m. The subjects were told to fast for 1 h before and 1 h after the challenge and also from midnight until the samplings were made. A placebo rectal challenge was performed with 6.5 g soya bean protein (Sigma-Aldric Sweden AB, Stockholm) suspended in NaCl solution. The patients were not on a specific diet and did not change medication during the baseline and CM challenge studies.

Subject preparation

All patients and controls were given a rectal enema (Klyx 120 mL; Ferring, Copenhagen, Denmark) within 1 h before being tested with the rectal mucosal patch technique.

Mucosal patch technique and analytical measurements

The instrument used is a plastic catheter with a silicon balloon at the end of the catheter. Three patches made of highly absorptive cellulose material (Phadia AB) are attached to the balloon. After the instrument is positioned in the rectum with the subject lying in the left lateral position with submaximal flexed hips, the balloon is inflated with air, bringing the patches in contact with the mucosa for 20 min. The balloon was then deflated and the catheter was removed. The patches were cut off and immediately placed into 2 mL of 0.3% CTAB (*N*-cetyl-*N,N,N*-trimethyl ammonium bromide; Merck, Darmstadt, Germany) solution to extract the content. The extraction solution was squeezed out of the patches, pooled, centrifuged and frozen at $-70\,^{\circ}C$. The extraction solutions were analysed for the concentrations of granule constituents from neutrophils (MPO) and eosinophils (eosinophil cationic protein; ECP) at the Department of Clinical Chemistry, University Hospital, Uppsala. The intraindividual variation of separate measurements on two occasions for 14 unchallenged individuals was on average 21% for MPO ($P = 0.6$) and 5% for ECP ($P = 0.80$).

Air samples were collected with glass syringes during deflation of the balloons and analysed for NO using a chemiluminiscence NO analyser (Sievers NOA 280; Ionic Instrument Business Group, Boulder, CO, USA) as described previously [16].

Statistics and calculations

The results are presented as means and SD within brackets. χ^2 (between groups), Mann–Whitney *U*-test between groups and Spearman's rank correlation were used for the statistical calculations.

Results

The results of rectal challenge with CM in patients with pSS and healthy controls are presented in Fig. 1. The figure shows the individual findings of the increase of rectal mucosal NO and MPO concentrations (ΔNO and ΔMPO) in the patient group and the healthy controls after rectal CM challenge. As illustrated, 8/21 patients ($P < 0.01$, χ^2 test) had a mucosal reaction to CM defined as the level of two SD above the mean of the control subjects. No significant increase of ECP was seen in either patients or controls after challenge (data not shown). None of the patients reacted to challenge with soya bean. No relation to age and sex was seen in patients or controls.

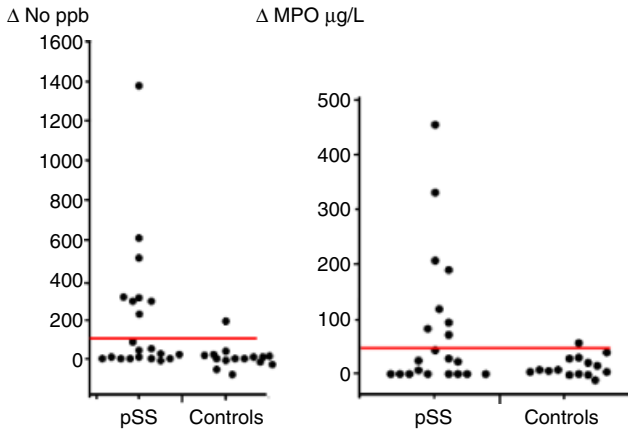

Fig. 1. Increase of rectal luminal nitric oxide (ΔNO) and myeloperoxidase (ΔMPO) in patients with primary Sjögren's syndrome (pSS) after rectal cow's milk protein (CM) challenge. The level of two SD above the mean of the control subjects ($n = 18$) is marked by a line (ΔMPO = 49 μg/L and ΔNO = 123 p.p.b.)

Table 1. Mean serum levels (SD) of IgA and IgG antibodies to casein, α-lactalbumin and β-lactoglobulin in patients with primary Sjögren's syndrome and healthy controls, with and without cow's milk (CM) sensitivity defined by the inflammatory response to rectal challenge with CM protein

	Sjögren's syndrome		
	CM-protein sensitive	CM-protein non-sensitive	Controls
IgG anti-casein (mg/L)	8.2 (7.9)	7.1 (4.6)	7.2 (7.1)
IgA anti-casein (mg/L)	1.7 (1.1)	1.9 (0.63)	2.8 (3.1)
IgG anti-α-lactalbumin (mg/L)	2.5 (0.3)	7.0 (12.2)	2.4 (0.7)
IgA anti-α-lactalbumin (mg/L)	1.1 (0.2)	1.4 (0.5)	1.2 (0.3)
IgG-β-lactoglobulin	11.1 (11.6)	12.9 (18.2)	4.2 (3.0)
IgA-β-lactoglobulin	1.4 (0.7)	1.7 (0.7)	1.2 (0.08)

SD, standard deviation.

The serum levels of IgA and IgG antibodies to casein, β-lactoglobulin and α-lactalbumin were similar in the patients with pSS compared with the controls (Mann–Whitney *U*-test). Neither were any differences observed between CM-reactive and non-reactive pSS patients (Table 1). Fifty-two percent of our pSS patients had the HLA alleles DQ2/DQ8. None had both haplotypes. Among those who had a definite CM reactivity ($n = 8$), three patients were DQ2 positive and one was DQ8 positive. In the group of 13 patients defined as non-CM reactive, we found seven DQ2-and DQ8-positive individuals. Thus, no association between CM reactivity and these haplotypes was seen. The patients of the present study had been challenged previously with gluten and 5/21 were gluten reactive and DQ2 positive [14]. We observed that 2/21 of

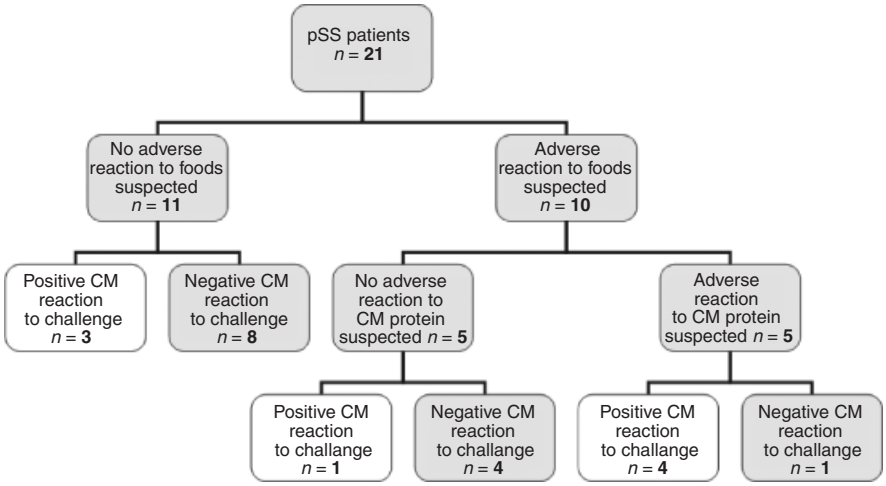

Fig. 2. Prevalence of self-reported food intolerance and suspected adverse reactions to cow's milk protein (CM) in primary Sjögren's syndrome (pSS) patients and results of rectal challenge with CM.

our patients were only gluten reactive (one of the gluten-positive patients was diagnosed as having coeliac disease), 5/21 were only CM reactive while 3/21 were both gluten and CM reactive. The two patients with the highest values of NO and MPO after CM protein challenge were DQ2/DQ8 negative and had no reaction to gluten challenge.

Allergic reactions (rhinitis, itching, urticaria, dyspnoea, anaphylaxis) to intake of avocado, apple, peanut, strawberry and shellfish or exposure to pollen, dust, furred animals or mould were reported by 13/21 patients. Allergic drug reactions (urticaria, itching, dyspnoea) were frequently reported ($n = 9$, 43%) and in particular against antimicrobials and salicylates. Gastrointestinal symptoms (obstipation, diarrhoea, flatulence, pain, dyspepsia) were reported by 16 patients (76%), and 10 patients (48%) attributed such symptoms to an adverse food reaction against particular CM products ($n = 5$) and wheat gluten ($n = 4$). The flow chart (Fig. 2) shows that the majority of our patients who suspected CM intolerance were also in fact reactive to rectal CM challenge. However, CM reactivity also appeared in patients who had no suspicion of food intolerance. Thirteen of our patients (62%) fulfilled the criteria of IBS according to the Rome III criteria [15]. Four of them were classified as diarrhoea-predominant, two as constipation-predominant, six with alternating diarrhoea–constipation and one with unsubtyped IBS. All patients except one with CM reactivity fulfilled the criteria for diarrhoea-prominent or alternating IBS. Those patients who were CM reactive were offered help from a dietist to identify foods containing CM, and seven patients excluded CM products for at least 6 months, and six reported improved well-being and less abdominal problems assessed by symptoms reported according to the IBS protocol. One patient no more fulfilled the criteria of IBS.

Discussion

In this study, we measured the local mucosal production of NO and the mucosal release of MPO after instillation of CM into the rectum of patients with pSS. The results were obtained using a newly developed diagnostic system, the mucosal patch technique [16, 17]. The major finding was that rectal challenge with CM frequently induced a local inflammatory mucosal reaction reflected by enhanced mucosal NO production and MPO release in patients with pSS but not in healthy controls. A minority of our pSS patients was treated with low-dose corticosteroids, which may have reduced an inflammatory response to CM. The pronounced NO production observed in some of our pSS patients is probably a result of activation of the major inducible isoform of NO synthase NOS IIa [18], which produces NO in high concentrations for as long as it is activated due to inflammatory principles [19]. Gut mucosal granulocyte activation, defined as MPO release, precedes NO production in patients with CD challenged with gluten but, 15 h after the challenge, we found both MPO and NO responses [16]. Based on this knowledge, we designed the timing of post-challenge measurements in the present study. We found no increase in ECP concentrations after the challenge, suggesting an absence of eosinophil activation induced by CM challenge, at least 15 h after challenge. The challenge and/or sampling procedures have not caused the inflammatory response observed because the CM challenge response was corrected for baseline values and furthermore none of our patients reacted to challenge with soya bean protein. Thus, patients with pSS are obviously apt to react with a mucosal inflammatory reaction due to the exposure of not only gluten [14] but in particular CM.

The prevalence of all kinds of adverse reactions to food reported by our pSS patients was quite high (48%). A few

population studies in Europe have assessed the prevalence of adverse reactions to food and report a high frequency of perceived food intolerance [7, 9]. In the United Kingdom, 20% of the population attributes atopic, intestinal and joint symptoms and migraine to certain foods including CM products and wheat. However, the results of double-blind placebo-controlled food challenges (DBPCFC) estimated the prevalence of adverse food reactions to be 1.8% [7]. In a representative cross-sectional study in Germany, the prevalence of all kinds of adverse reactions to food was estimated to be 35% of the population [9].

One intriguing finding among our patients was the high incidence of reported intestinal symptoms and in particular IBS-associated symptoms. IBS is considered to be a functional bowel disorder with abnormal gut patterns of motility, secretion and sensation with prevalence estimates ranging from 10% to 20% [15, 20]. IBS has also been reported to be associated with sicca complex, sicca syndrome without an autoimmune component [21]. The aetiology is largely unknown although different mechanisms for its symptoms have been proposed including adverse food reactions to CM products [22–24]. Many of our patients believed that dietary intolerance and in particular intolerance to CM products contributed to their intestinal symptoms. In fact, half of our patients with CM sensitivity discovered by rectal challenge had suspected that their gastrointestinal symptoms were an adverse CM reaction (Fig. 2). In rigorous elimination diet studies in IBS food intolerance is frequently reported [23, 25, 26], and some patients with diarrhoea-predominant or alternating bowel habit responded to an exclusion diet [26], but attempts with various laboratory tests to identify non-IgE food sensitivity have been disappointing [11].

The most common adverse reaction to CM is an IgE-mediated allergy, at least in childhood, with a typical clinical picture and a positive skin prick test and/or increased IgE antibody levels to CM [27–29]. The non-IgE-mediated food allergies are more difficult to diagnose and have until now required procedures with food elimination and food challenges [30]. The best-known non-IgE food protein-mediated immune damage is the gluten-induced enteropathy seen in CD. Cow's milk protein may also induce – as observed among children – an enteropathy with more discrete histopathological findings compared with CD [31, 32]. Many adult patients with CD do not recover completely on a gluten-free diet [8], And the question has been raised as to whether other food antigens may contribute to their enteropathy and symptoms. In this context, CM proteins have come into focus [33]. Recently, we reported that rectal CM challenge in 40% of adult CD patients on a gluten-free diet and without increased serum antibodies to tTG and gliadin and various CM proteins induced an inflammatory reaction similar to that produced by gluten [13]. This finding has led to the possibility that CM sensitivity may contribute to persistent intestinal symptoms in some celiac patients despite a gluten-free diet. Our observed association between CM sensitivity and IBS-like symptoms in our pSS patients may suggest that food allergy against CM might play a role in their intestinal symptoms. The pathways by which mucosal inflammation is induced by rectal challenge with gluten and CM in CD or pSS have not been identified until now. In contrast, the pathophysiology of CD is well characterized. tTG 2 has been identified as the auto antigen in CD and IgA anti-tTG autoantibodies are very sensitive and specific markers for this disease. However, both the expression of CD and the serum antibodies to tTG are strictly dependent on dietary exposure to gluten. Activation of the adaptive immune system is one pre-requisite for the occurrence of CD and is reflected by the development of gliadin and tTG antibodies [34]. Recently, it has been demonstrated that certain gluten peptides may also elicit an innate immune response [35, 36]. One may suspect that an innate immune response is a more prominent factor behind the mucosal inflammation induced by rectal CM and gluten challenge in patients with pSS because they have no increased serum antibody levels against gliadin [14] or immunogenic CM. The same is true for the CM reactivity in celiac patients because they also have normal levels of IgG/IgA antibodies against CM proteins [13]. Celiac disease is strongly associated with the HLA alleles DQ2/DQ8. Those patients with pSS who are gluten sensitive but not suffering from CD also carry these alleles [14]. However, the present findings in pSS patients show that CM sensitivity is not associated with DQ2/DQ8, suggesting that additional genes may relate to CM sensitivity.

The high prevalence (65%) of various allergic manifestations and in particular allergic drug reactions (46%) have been reported previously in pSS patients [5, 6]. In the present study, our observations are in accordance with such findings. The high propensity in pSS to any kind of allergy including CM and gluten sensitivity may reflect an allergic hypersensitivity that may be primarily due to genetic factors or secondarily induced by the pSS disease process. Nevertheless, our anecdotal results after CM withdrawal merit further research on the possible impact of adverse food reactions on symptoms and perhaps disease activity in pSS. Hopefully, future studies, which will require inter alia DBPCFC, will support our expectation that the rectal challenge procedure may be of clinical help to identify foods causing non-IgE-mediated adverse reactions.

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