ACADEMIC (Ph.D.) DISSERTATION

CLINICAL, IMMUNOLOGICAL AND THERAPEUTICAL ASPECTS OF NON SPECIFIC INFLAMMATORY BOWEL DISEASES

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1. INTRODUCTION AND AIMS

As the incidence of the gastrointestinal and digestive diseases increases their significance rises so they must consider as one of the most important group of diseases affecting human population. This fact is supported by both of the mortality and morbidity indicators and the out- and inpatient turnover data. Gastroenterology developed a discipline which affects and partly unifies others: for example immunology. This means pathophysiological data explored by theoretical and clinical investigations, or bed-side clinical observations. The interdisciplinary subject has particular significance as the investigations of the gastrointestinal diseases can be carried out via different approaches and methodologies. Scientific results of the recent past resulted in significant changes in approaching and effected new identified relationships. They revised our knowledge about certain diseases as modified the traditional conceptions of their evolution, appearance and treatment. Notwithstanding the unarguable results, there are many questions and few answers, moreover new data raise new questions. Investigations of the gastrointestinal diseases may serve as clinical and laboratory scientific issues and may enrich our clinical and theoretical knowledge with new ideas to utilize in practice.

Inflammatory bowel disease (IBD) is an umbrella term as the two main forms are: ulcerative colitis and Crohn's disease. Also microscopic colitides (collagenous colitis and lymphocytic colitis) referred here of late. Notoriously, microscopic colitis as clinicopathological entity consists of three features: chronic watery diarrhea, a normal or near-normal gross appearance of the colonic mucosa, and a specific histological picture. They are well-distinguished by the presence of the thickened subepithelial collagen table and both can cause watery diarrhea. It is likely that they are a spectrum of one disease, but this is yet to be proven. Some have suggested that lymphocytic colitis is an early stage of collagenous colitis. The fact that microscopic colitis may precede or follow IBD outlines nowadays but the interactions between the processes resemble „terra incognita". Microscopic colitis thought to be rare disease but data enhance it's increasing frequency.

At the present time the origin and the exact cause of these diseases are not confirmed. The inflammatory process requires genetical background at the start-up, but

familial/genetical and immunological or inflammatory factors, infections and environmental agents can equally play as triggers.

The overall aim of the dissertation is to review our IBD patients' clinical features, the occurrence of certain antibodies, the coexistence of organ-specific and systemic autoimmune diseases and allergic diseases. It is suggested to apply new or uncommon treatments based upon these observations in regard with immunological considerations.

1. There is no survey in Hungary about microscopic colitides. One goal was to draw all our treated patients with microscopic colitis into my study based upon a retrospective, clinical, histological and demographical data analysis. All data (case history, clinical status, immunserological parameters, etc.) for these patients were abstracted for patient characteristics. The possible immunpathological mechanisms were reviewed and the subtypes of microscopic colitides were defined. The clinical picture of these diseases were analyzed both in male and female patients with or without autoimmune diseases and compared with the literature.

2. There are relatively few observations with immunserological (characteristic) alterations in IBD, so one goal was to investigate ASCAs (anti-Saccharomyces cerevisiae antibodies) in this group of patients and compare my results with the clinical manifestation and outcome.

3. One goal was to observe the coexistence of another immune-mediated inflammatory bowel disease: GSE (gluten sensitive enteropathy) and Sjögren's syndrome. The clinical characteristics and the significances were analyzed.

4. Although the etiology of Crohn's disease remains unclear, in addition to genetic, immunologic and other environmental factors, microorganisms have been discussed as possibly playing an important role. With increasing concern about the transmission of infectious diseases from animals to humans, attention has refocused on Mycobacterium paratuberculosis (MAP) as a candidate organism in the etiology of Crohn's disease. With conflicting scientific data no one can answer the question but a number of experts could prove the bacteria in histological samples, sera or milk in patients with Crohn's disease.

The significance of the strain-off the bacteria-carriers in veterinary medicine has extraordinary consequences as MAP causes Johne's disease which is similar to human Crohn's disease. In these animal cases the serological investigations are routine methods. One goal was to observe our Crohn patients first time in Hungary with an originally veterinary test as we adapted to humans and investigated the features of the occurrence of anti-MAP and/or ASCAs.

5. The management of steroid refractory, severe inflammatory bowel disease (IBD) is yet an unsolved problem. The officially recommended drugs (immunosuppressant: 6-MP, metothrexate, cyclosporine-A, etc. and certain biological agents: i.e. infliximab) may have beneficial effects but in some cases have ambiguous impact and/or they are expensive.
Based on the previous observations of improvement in systemic autoimmune diseases (i.e. vasculitides) cyclophosphamide is known as „rescue" therapy, but there is no experience in Hungary and sporadic worldwide at all. The goal was to evaluate this type of treatment as a potential therapy in IBD.

6. Allergic diseases (especially food allergy/increased antibody titers against food allergens) can be associated both with IBD and irritable bowel disease (IBS). One goal was to analyze the associated incidental diseases and verifiable antibodies to characterize these diseases and to contribute to the treatment options based upon my observations.

2. PATIENTS AND METHODS

I. Attended/treated patients

1. Clinical and immunological aspects of microscopic colitides.
The Department of Pathology of the University of Debrecen certified in 53 cases the diagnosis of the MC (46 with CC, 7 with LC) in the analyzed colonic biopsy specimens between 1994 and 2004. All of these paraffin blocks were collected together to the Department of Pathology. Two independent pathologists verified the subsequent sections and completed the check with additional investigations (intraepithelial lymphocytes,

tenascin labeling of the collagen layer, mast cells and other lamina propria cell components). To avoid observer bias, all microscopic slides were coded prior to analysis by one observer and read blind. With this, the histologically verified patients were re-examined at the 3rd Department of Medicine of the University of Debrecen (detailed history taking and symptoms). Adult patients of both sexes were included. None of the patients had an infectious disease. The medical records for these patients have previously been reviewed by investigators and abstracted for patient characteristics.

2. Seroreactivity against Saccharomyces cerevisiae in patients with Crohn's disease and their association with clinical manifestation.

A cohort of patients with IBD (42 patients with CD and 10 patients with UC) and GSE (16 patients) from Debrecen, Hungary were enrolled in the study. Adult patients of both sexes were included. The diagnosis of CD, UC or GSE was made using the formally accepted criteria. The medical records for these patients have previously been reviewed by investigators and abstracted for patient characteristics. The blood samples for the study were collected between January 2000 and March 2000. Their sera were separated and stored at -70 . Determination of serum values was performed by individuals blinded to the clinical data for the patients in our Regional Immunology Laboratory.

3. Celiac disease in Sjögren's syndrome: relevance of the clinical picture.

One hundred and eleven consecutive patients with SS were recruited from the outpatient clinic of the Division of Clinical Immunology, Third Department of Medicine, University of Debrecen Medical and Health Science Centre in Hungary, where they are receiving follow-up care. The diagnosis of SS was established according to the revised American-European Consensus Group classification criteria. Informed consent approved by the university ethics committee was given by the patients. Detailed history from each patient was taken involving gastroenterological anamnesis and dietary habits.

4. Mycobacterium avium subspecies paratuberculosis antibodies in inflammatory bowel diseases.

The entire cohort of patients with IBD (42 patients with patients with CD and 34 healthy patients from Debrecen, Hungary were enrolled in the study. Adult patients of both sexes were included. The diagnosis of CD was made using the formal accepted criteria. The medical records for these patients have previously been reviewed by investigators and

abstracted for patient characteristics. The median age of the patients, 36 men and 40 women, was 42 yr with the range of 19 to 76 yr. All CD patients had been followed up for several years. None of the patients had received antimycobacterial therapy. The blood samples were collected and sera were separated and stored at –70°C. Determination of serum values was performed by individuals blinded to the clinical data for the patients in our Regional Immunology Laboratory.

5. Pulse cyclophosphamide therapy in inflammatory bowel disease

All patients were recruited in this prospective uncontrolled pilot study from our out-patient clinic specializing in chronic inflammatory bowel diseases (at the 3rd Department of Medicine, University of Debrecen). Between September 2002 and December 2003 we included in our cohort eight patients with (moderate/severe) steroid refractory IBD (four patients with CD and four with UC. All patients were diagnosed according to the standard criteria and had undergone endoscopy and/or radiological studies, including ileo-colonoscopy, CT and/or double-contrast barium air enteroclysis, within 6 months of the start of the study. Video endoscopy of the large intestine, including the terminal ileum, was performed before the first cyclophosphamide pulse and at weeks 12. They did not respond to conventional therapy (this was the only selection criteria for this study). We used the Modified Truelove and Witts activity index for Ulcerative Colitis and Best index (Crohn's Disease Activity Index) for Crohn’s disease as others.

6. Food allergy in inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).

A cohort of patients with IBD (both with CD and UC) and IBS from Debrecen, Hungary were randomly enrolled in the study. Adult patients (age 18-60) of both sexes were included. They did not take steroids, antihistamines or SSRI. The diagnosis of CD, UC or IBS was made using the formally accepted criteria. The medical records for these patients have previously been reviewed by investigators and abstracted for patient characteristics. The blood samples for the study were collected; sera were separated and stored at -70. Determination of serum values was performed by individuals blinded to the clinical data for the patients in our Regional Immunology Laboratory. The control group was free of gastrointestinal complaints, had no evidence of allergic and/or autoimmune disease, IBD or IBS.

II. Laboratory methods

ASCA IgG and IgA: Medizym® ASCA IgA is an enzyme immunoassay for the quantitative determination of IgA antibodies to Saccharomyces cerevisiae in human serum. Autoantibodies of the diluted patient samples and calibrators reacted with mannan (cell surface component of baker's yeast) immobilized on the solid phase of a microtiter plate. Following an incubation period of 60 min at 37 , unbound serum components are removed by a washing step. The bound antibodies react specifically with anti-human-IgA-antibodies conjugated to horseradish peroxidase (HRPO). Within the incubation period of 30 min at 37 , excessive conjugate was separated from the solid-phase immune complexes by the following washing step. Horseradish peroxidase converted the colorless substrate solution of 3, 3', 5, 5'-tetramethylbenzidine (TMB) added into a blue product. This enzyme reaction was stopped by dispensing an acidic solution (H2SO4) into the wells after 10 min at room temperature turning the solution from blue to yellow. The optical density (OD) of the solution at 450 nm was directly proportional to the amount of specific antibodies bound. The standard curve was established by plotting the concentrations of the antibodies of the standards (x-axis) and their corresponding OD values (y-axis) were measured. The concentration of antibodies of the specimen was directly read off the standard curve.

Mycobacterium avium subspecies paratuberculosis antibodies: Mycobacterium avium subsp. paratuberculosis antibody HerdChek® M. pt (IDEXX) was used in this study according to the manufacturer's instructions. HerdChek® M. pt. is an enzyme immunoassay for the detection of Mycobacterium paratuberculosis-specific antibodies in serum and plasma of cattle. We followed the kit instructions treating human sera as though they were cows. First of all the ELISA was adapted for use on humans. There were no official cut-off but Dr. Collins (Johne's Testing Center School of Veterinary Medicine, Madison, WI) suggested us to test human sera from healthy blood donors to establish (by analysis of frequency distributions) what a rational approximate cut-off might be for Hungarian patients (24). The specificity of the HerdChek® M. pt. Antibody Test Kit is enhanced by a pre-treatment step with Mycobacterium phlei. The capture antigen (Map strain VRI 316/102-2 crude protoplasmic antigen) is adsorbed within the wells of 96 well polystyrene plates, which are blocked and air-dried. The kits are with serum diluents that contain adsorbing Mycobacterium phlei antigen, anti-bovine IgG conjugated to

horseradish peroxidase, enzyme substrate solution with hydrogen peroxide/tetramethyl benzidine and stop solution. Protein G-HRP conjugate (Protein G bound with peroxidase) is a specialty of the kit. With this kit, optical density (OD) values were transformed to S/P ratios based on the OD for the serum sample together with those for the negative and positive controls provided with the kit by using the following equation: S/P ratio = (OD of sample - OD of negative control)/(OD of positive control - OD of negative control). All assays were run in duplicate. Any assay with a between-well coefficient of variation of >10% was repeated, and the second result was used for data analysis.

Allergenspecific-IgE:

The ALLERgen kits (Adaltis Italia SpA, Bologna, Italy). are based on the EIA immunocapture principle and exploit the same solid phase, i.e. wells coated with anti-human IgE, for both the calibration curve and all allergen-specific IgE tests. To run the calibration curve the calibrators are pipetted into the wells of the first strip whereas the samples, each in different replicates as many allergens are to be tested, are pipetted into the subsequent wells. During the first incubation, the coated anti-IgE capture the IgE of the calibrators and of the sample, either specific or non-specific. The washing performed at the end of the incubation eliminates any possible interference from other immunoglobulin, i.e. allergen-specific IgG, eventually present in the sample. In the next step, the anti-IgE-Biotin conjugate is added into the calibrator wells and the Allergen-Biotin conjugates into the sample wells. The IgE captured in the first step reacts with the conjugates, and while the anti-IgE-Biotin forms in the calibrators wells the sandwich (solid phase anti-IgE : IgE : anti-IgE-Biotin), the allergen-Biotin conjugates form the immuncomplex (solid phase anti-IgE : IgE : Allergen-Biotin) in the sample wells. After the washing, the Streptavidin-Peroxidase conjugate is added into all the wells. During the incubation the anti-IgE-Biotin remained in the calibrators wells as well as the Allergen-Biotin remained in the sample wells, are bound by the Streptavidin-Peroxidase conjugate. The last washing eliminates non reacted species and the incubation with the substrate allows the detection of the Streptavidin-Peroxidase conjugate remained bound to the solid phase immuncomplex. Optical densities (O.D.s) are read on a microplate reader and the samples O.D.s are an index of specific IgE concentration in the sample. The quantization, in terms of units and classes, can be done by interpolation of sample O.D.s on the calibration curve.

III. Statistical Analysis

The relations were concluded from the evidences with statistical methods and summarized them in tables and figures. Correlations were estimated by applying the Fisher, Student, Mann-Whitney and Spearman rank correlation tests. All these data were analyzed with SPSS v11.0 and Microsoft Excel soft packs.

3. NEW RESULTS AND THEIR UTILIZATION.

1. Clinical and immunological aspects of microscopic colitides.

- A computerized database was worked out of the clinical and laboratory parameters of the patients with microscopic colitis. Based upon this database, stated out that the traits of the observed cohort of patients (distribution of sex, age and age at the diagnosis, following term, frequency of manifestations, laboratory alterations) not differ significantly in general from other authors' observations (in cases of the "classic" type: diarrhea). On the other hand, the "atypical" constipation type patients' has different characteristics and this is novelty.
- Age at the diagnosis did not influenced the spectrum of the clinical and laboratory findings and the clinical course had no dependence on age as well.
- Sex of the patients had effect on the clinical picture, the affected organs and laboratory alterations.
- This was the first Hungarian observations with a relatively large group of MC patients.
- My results strengthened the idea that microscopic colitis an umbrella term so as to IBS and suggested the „Janus face" of MC.
- Based upon my results I call the attention on the question of the correct co-work of the clinician and the pathologist as the diagnosis depends on this relationship in contempt of the up-and-up diagnostic methods.
- I determined the occurrence of associated autoimmune diseases and their characteristics respectively MC in Hungarian patients and established that the seriousness of MC was not influenced by these autoimmune diseases.
- Underlined that the outcome and the response to budesonide of both type (constipation and diarrhea) is similar.

- I could not find antibodies specific to MC (with our test sin the Regional Immune Laboratory). It has to be emphasized the associated allergic diseases and/or increased antibody titers against food antigens, but without any specific linkage.
- I proved the efficacy of longstanding oral budesonide therapy (at least 6 months, 9mg/day) accordingly to the international observations but proved in the constipation form of the diseases. This is brand new observation.

2. Seroreactivity against Saccharomyces cerevisiae in patients with Crohn's disease and their association with clinical manifestation.

- This was the first Hungarian observations with ASCAs.
- I think that both the international observations and my results suggest ASCAs to differentiate a unique group between Crohn' patients with expectedly serious outcome (prospective surgical intervention).
- Maybe there are different pathogenetic mechanisms in the background of the different forms of Crohn's disease, but further investigations need to prove this idea.

- Frequent ASCA positivity is known in IBD, but no data in celiac disease in contempt of the probable role of them. My control group was a celiac patient cohort and I found similar alterations as in IBD.

3. Celiac disease in Sjögren's syndrome: relevance of the clinical picture.

- This was the first Hungarian observation with celiac disease in a relatively large group of Sjögren's patients.
- I must place emphasis on the fact that the occurrence of celiac disease is more frequent between Sjögren's patients in contrast to the average population. This could be presumed both in case of patients with manifest and latent celiac disease. This is why Sjögren's patients need an accentuated interest of their GI complaints and signs.
- Our result suggest that GI complaints and increased sedimentation and immunoglobulin titers may indicate celiac disease. So, the first suggested step is to do the serological investigations an din case of positivity the invasive procedures (endoscopy and histological samples) are the next step.

- The gluten-free diet after the correct diagnosis can prevent malnutrition and/or GI malignancies.

4. *Mycobacterium avium subspecies paratuberculosis antibodies in inflammatory bowel diseases.*

- This was the first observation with Mycobacterium avium subspecies paratuberculosis antibodies in IBD patients in Hungary.
- Potential use of a simple test (similar to the test sin veterinary medicine) emerges to prove the sensitization against MAP in our patients.
- This was the first observations with ASCAs and jointed MAP antibodies tests.
- ASCA positivity and the anti-MAP seropositivity correlated and this observation indicate further surveys to elucidate it as I think it is definitely more than accidental.
- These findings foreshadow that MAP has certain importance in Crohn's disease. Further research is required to understand the pathogenic mechanisms and to enable development of future novel diagnostic and/or therapeutic possibilities to roll back the spreading of Crohn's disease by chance.

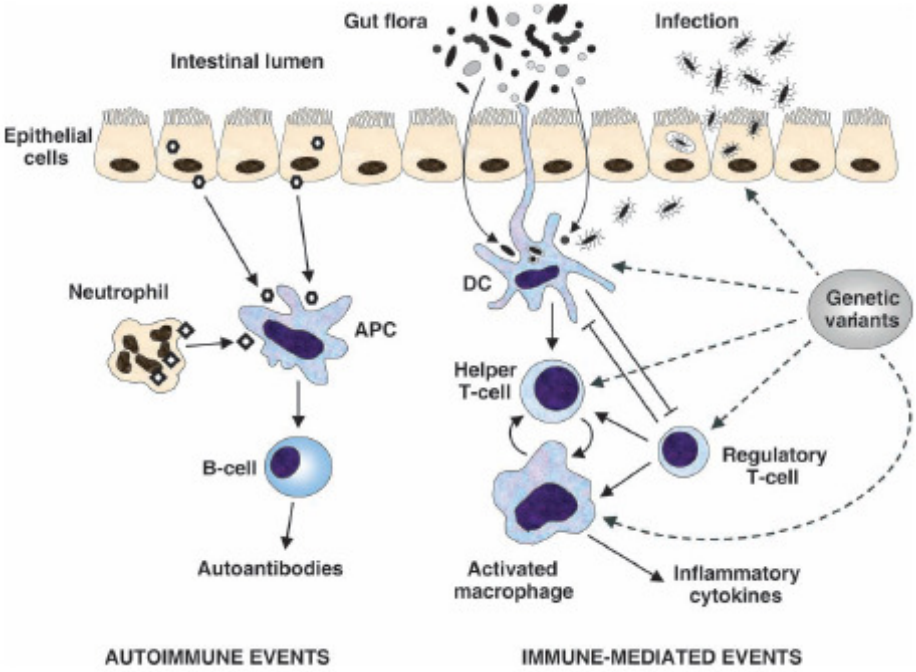
5. Pulse cyclophosphamide therapy in inflammatory bowel disease

- This was the first documented intravenous pulse cyclophosphamide therapy in inflammatory bowel disease in Hungary.
- Intravenous pulse cyclophosphamide may be a safe and effective treatment in patients with severe IBD unresponsive to "conventional" treatment and it is also recommended as a first-line adjunct to or replacement of systemic corticosteroids in the treatment of IBD.
- After remission induction, for the maintenance, patients with CD must be treated with „conventional" therapy (methotrexat or azathioprin).
- Disease activity decreased, there was no side effects, no toxicity, and all the patients went into long lasting remission.
- Last but not least another aspect is that costs of this kind of treatment are relatively low.

6. Food allergy in inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).

- We proved increased titers of food specific IgE antibodies in IBD patients in contrast to the normal controls.
- These observations were proved in patients with irritable bowel syndrome as well.
- The usefulness of these observations may be in the question of the treatment. In case of IBS it is well known that these patients cannot treat easily and satisfactorily.
- I would like to take the attention to the idea that we must allow for food allergy in case of an IBS patient. The verification is simple and non-invasive.
- A correct diet (elimination of the suspected food) can be a part of the treatment of an IBD or IBS patient. Antihistamines and/or disodium-chromoglicate are also recommended in these cases.
- Our IBS patients with proved food allergy keep diet and their complaints improved.

4. SUMMARY



Schematic diagram of autoimmune and immune-mediated events in IBD pathogenesis. Left: Self-antigens derived from intestinal epithelial cells (hexagons), neutrophils (diamonds), and other host cells are internalized and processed by antigen-presenting cells (APC), and presented to B-cells which produce autoantibodies such as epithelial cell-associated components (ECAC), pANCA, lymphocytotoxic antibodies, anti-pancreas antibodies etc. Right: Loss of tolerance to the commensal autologous flora results in an enhanced reactivity against gut bacterial antigens and the inappropriate activation of effector CD4þ helper T-cells which induce macrophage activation and production of pro-inflammatory cytokines. Possible causes for the loss of tolerance include infections, excessive dendritic cell (DC) stimulation by the gut flora, inadequate regulatory T-cell function, or genetic factors, such as the CD-associated NOD2/CARD15 variants, that affect both epithelial and immune cell function.

It seems to be that immune-mediated events are the prominent besides autoimmune events in the pathogenesis of IBD. However in case of ulcerative colitis autoantibodies against

colonic epithel cells and in case of Crohn's disease antibodies against commensal gut flora refer autoreactivity.

With increasing knowledge of these processes the old questions are not answered: what is the explanation to the „explosion" of IBD in the last century? What is the reason of the increase of their incidence? What is the cause of the prevalence in certain "new" populations "healthy" previous to now? These questions urge correct answers.

The most case presentations and summaries describe microscopic colitis with an incipient diarrhea. Other well known clinical signs are abdominal dyscomfort and pain with tenesmus. I proved in the half of the cases constipation in histologically proved microscopic colitis patients therefore I suggest MC as a "Janus-faced" disease with two clinical form with the same histological picture. In the cases of our patients sex dominancy was not demonstrable. I think that the principle of the diagnosis is the histological picture. Our data support the hypothesis that patients with MC may have laboratory and/or clinical evidence of allergic diseases and/or food allergy and these mean a possible connection between MC and food allergy and suggest a possible reason for the paradox of diarrhea-constipation. As remodelling of the gut wall is thought to be a result of chronic inflammation, since steroids reduce or reverse inflammation, they may also reduce or reverse remodelling. The etiology of MC is still unknown and more research is needed to determine the cause as well as the treatment. Whether MC (both CC and LC) is an autoimmune disease has not been conclusively established. Autoimmune diseases have been reported to occur in patients with MC so an association with various autoimmune diseases has been suggested, but no serological findings have supported such a theory. Overviews and case reports discuss the reason that the collagenous colitis and autoimmune diseases can associate. Half of the patients has autoimmune disease and their diagnosis always pre-dated the diagnosis of MC.

Examining the ASCA's occurrence in patients and compared it with the clinical picture of the Crohn's disease the results supported the theory that ASCA positivity correlated with small intestinal Crohn's disease and in these cases both IgG and IgA type antibodies were proved. The relatively high incidence of ASCA in GSE was unexplained but indicated further surveys to elucidate it as it was definitely more than accidental. The antibodies in the sera of the analyzed ASCA positive cases proved a systemic immune response against

Saccharomyces cerevisiae and suggested the end of the oral tolerance against the yeast's antigens. The diet restriction (elemental diet, total parenteral nutrition, and fecal diversion) may ameliorate the status of the patients with Crohn's disease. It can also be speculated that the yeast-free diet as a part of the therapy for the ASCA positive patients can be reasonable, moreover the permanent "forbidding" of the yeast can be an acceptable alternative in case of getting well.

Our results confirmed that celiac disease appears more frequently among Sjögren's patients that in the general European population. Although a general, epidemiological survey on the prevalence of celiac disease has not been done yet in Hungary, the prevalence there—according to local data of medical university departments and hospitals—is at the European average. In the European population, the average frequency of celiac disease is 4.5–5.5:1,000, while our survey showed a ratio of 4.5:100 in Hungarian Sjögren's patients, which means a tenfold celiac disease frequency over the non-Sjögren's population. Our findings further described that the mean age of Sjögren's patients with celiac disease is significantly lower than that of the non-celiac Sjögren's population ($P<0.001$). In Sjögren's syndrome, the latent/silent form of celiac disease is more frequent, which makes it difficult to obtain the correct diagnosis. The most relevant gastrointestinal symptom was abdominal discomfort. In Sjögren's disease, various gastrointestinal manifestations occur (e.g. epigastric pain, nausea, dyspeptic symptoms, malnutrition) due to chronic atrophic gastritis, with the possible involvement of the small and large intestines. Also, a great variety of bowel diseases with autoimmune origin frequently appear in Sjögren's syndrome (e.g. collagenous and lymphocytic colitis), which makes problems in differential diagnosis and treatment.

With increasing concern about the transmission of infectious diseases from animal to man, attention has refocused on MAP as a candidate organism in the etiology of Crohn's disease. Mycobacteria and cell wall deficient organisms were first isolated from tissues from patients with Crohn's disease in the 1970's. Paratuberculosis was first described in Germany in 1895 by Johne and Frothingham. They demonstrated the presence of acid-fast bacilli in affected animals and thought that the disease was an atypical form of tuberculosis. The disease later became known as paratuberculosis or Johne's disease and the causative agent MAP. We examined both the ASCA's and anti-MAP occurrence at our patients. The results support the theory that ASCA positivity correlates with small

intestines' Crohn's disease and in these cases both the IgG and IgA type ASCA proved. Moreover, ASCA positivity and the anti-MAP seropositivity correlate and this observation indicate further surveys to elucidate it as we think it is definitely more than accidental. In one hand, this pilot study suggests the utility of a simple test to diagnose the presence of paratuberculosis in an individual with Crohn's disease. On the other hand the correlation between ASCA and Mycobacterium paratuberculosis antibodies seems to be more than a simple coincidence. These findings foreshadow that MAP has certain importance in Crohn's disease. Further research is required to understand the pathogenic mechanisms and to enable development of future novel diagnostic and/or therapeutic possibilities.

Current therapy of IBD is unsolved. Many drugs are prescribed for inflammatory bowel disease, either for treating active disease or for maintaining remission. However, for a high percentage of patients, no drug provides a completely satisfactory response. Corticosteroids (steroids) in dosages equivalent to prednisone 40-60 mg/day are commonly prescribed for acute exacerbations of Crohn's disease and ulcerative colitis. Steroids are not effective for maintenance of remission in either disease. Some patients require long-term steroids for suppression of disease, particularly Crohn's disease. Taking steroids for prolonged periods can cause adverse effects, both reversible (e.g., acne, hypertension, moon face, dyspepsia, mood disturbances, and glucose intolerance) and irreversible (e.g., avascular osteonecrosis, osteoporosis, posterosubcapsular cataracts, abdominal striate, growth retardation, and myopathy). These adverse effects are of sufficient concern to prompt consideration of alternative treatment strategies. Infliximab, human growth hormone, and other novel biotechnology treatments have been investigated as therapy for patients with inflammatory bowel disease. Although these biotechnology-derived treatments are promising, their cost is prohibitive for many patients. Because cyclophosphamide has been effective for other inflammatory conditions, is relatively less expensive than some other agents, and requires infrequent administration, it is being considered for treatment of inflammatory bowel disease. The cost of the treatment is far under the cost of the biological therapy and the frequency of the side effects is similar.

In this study our patients entered into remission after the second/third cyclophosphamide pulse. Disease activity decreased; there were no side effects, no toxicity, and all the patients went into long lasting remission. For the maintenance, patients with CD were treated with methotrexat (15mg/week) and patients with UC were treated with azathioprin

(2.5 mg/kg body weight/day). Remission seems to be stable (except for case 5: relapse after 6 months). These findings suggest that aggressive immunosuppressive therapy may be useful in some refractory patients and further controlled study should be considered in order to full evaluate this type of treatment as a potential therapy in IBD.

Food allergy and/or increased titers of antibodies against food antigens can be associated with both IBD and IBS. We could prove antibodies against milk-protein in both Crohn's disease and ulcerative colitis. We proved increased titers of food specific IgE antibodies in IBS patients in contrast to the normal controls.

Even though an immunotherapeutic approach to IBD addresses the mechanisms rather the cause of disease, the continued investigation of autoimmune and immune-mediated phenomena in IBD is the best hope to gain a therapeutic handling on these devastating conditions, but this must be done in parallel with a better understanding of the interactions, both symbiotic and pathologic, that occur between mucosal immunity and the commensal enteric flora.

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