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Opinion

The Study of Gastric Mucosa for Possible Bacterial Causative Agent of Crohn's Disease

Dear Editor,

Crohn's disease is an inflammatory bowel disease of unknown aetiology, which may involve any part of digestive tract from mouth to anus, but most commonly involves terminal ileum. In general, the course of the disease is chronic and most commonly characterized with periods of relapses and remissions or chronic course without remissions in a minority of patients.[1] Surgery for bowel stenosis, bowel perforation and other complications of Crohn's disease is required in a significant number of cases.

Despite extensive research efforts, a causative agent which could be responsible for the appearance of Crohn's disease has not been identified yet and, therefore, institution of aetiological therapy for this disease is not possible. In practice, medical treatment of most patients with Crohn's disease consists of 5-aminosalycilic acid derivates, corticosteroids and other immunosuppressive drugs.

There are several theories regarding aetiology of Crohn's disease, including ones that Crohn's disease is caused by a transmissible infective agent or that it could be a result of genetically-determined inadequate immune response to luminal bacteria.[2] The facts, that antibiotic therapy,[3] like dual antibiotic therapy with metronidazole and ciprofloxacine,[4] triple macrolide-based antibiotic therapy,[5] and diversion of the fecal stream from inflamed bowel loops,[6] have favorable effects in patients with Crohn's disease, support the theory of bacterial origin. There are genetic influences in the development of disease, and overall risk for the appearance of Crohn's disease is increased in close relatives of patients with Crohn's disease.[7] People with NOD2/CARD15 gene mutations have an increased risk for the appearance of Crohn's disease.[8] The NOD2/CARD 15 gene is an intracellular element responsible for indirect recognition of bacterial peptidoglycan.[9] Risk of appearance of Crohn's disease is also increased in people who have T300A mutation at ATG16L1 gene, which is responsible for autophagy.[10] As a matter of fact, silorimus (rapamycin), a drug that is used experimentally to induce autophagy may improve Crohn's disease.[11] Certain variants of IL23R gene have also been associated with susceptibility to Crohn's disease or protection against this disease,[12] as confirmed by Cohran-Mantel-Haenszel Chi-square test. Therefore, if Crohn's disease is caused by some bacteria, it is possible that mutations of genes responsible for bacterial recognition, autophagy or inflammatory response against infection increase susceptibility to infection with such bacteria and appearance of Crohn's disease.

Multiple attempts have been made to isolate infectious agent, which might be responsible for appearance of Crohn's disease. According to cold chain hypothesis, psychrotrophic bacteria which are capable to grow at low temperatures inside refrigerators, might contribute to Crohn's disease.[2] Indeed, analysis by multivariate logistic model of data collected in one study pointed that, among other household factors, there was a positive relationship between exposure to domestic refrigeration and rising incidence of Crohn's disease.[13] *Yersinia enterocolitica*,[14] and *Mycobacterium paratuberculosis*,[15] have been most commonly studied as possible causative agents of Crohn's disease. However, the results of such studies are not conclusive, and theory of infectious aetiology of Crohn's disease has never been proved.

A major problem in identification of possible infectious causative factor of Crohn's disease comes from the fact that studies performed so far, including those which utilized bacterial 16S rRNA detection, were generally focused on terminal ileum and colon,[16] which represent sites most commonly affected with Crohn's disease, and ileum-related lymphatic follicles and nodes.[14,17] Since terminal ileum both in healthy people and in Crohn's disease patients is an area of high bacterial density and contains enormous number of different bacterial strains, it is hard to distinguish whether any isolated bacteria represents a pathogen, a saprophyte, or it is the case of superinfection.

We propose a different approach for isolation of bacteria, which may cause Crohn's disease. The approach include identification of such bacteria in inflamed gastric mucosa in patients who suffer from Crohn's gastritis. Crohn's gastritis is an uncommon form of Crohn's disease. Although it is estimated that symptomatic involvement of upper gastrointestinal tract is present in less than 4% of patients, who suffer from Crohn's disease,[18] histological changes of gastric mucosa, including those consistent with gastric Crohn's disease may be present in more than 40% of patients with the disease.[19,20] Contrary to terminal ileum, human stomach is a place where very limited number of bacteria may survive, so that finding of a bacteria other than *Helicobacter pylori* in gastric mucosa of patients with gastric Crohn's disease may point that such bacteria is a pathogen.

The detection of 16S bacterial rRNA by PCR represents a convenient method for identification of bacteria. This gene is present in bacteria and has remained conserved during evolution. The method has proved its usefulness in the discovery of another intestinal pathogen, Trophyrema

Whipplei in 1992,[21] as well as identification of new Helicobacter species.[22] Therefore, with utilization of this method may identify bacteria responsible for appearance of Crohn's disease, providing that they are still present in gastric mucosa at the time of the study.

We believe that it would be best to take gastric biopsies from two groups of people, who did not receive any prior therapy with proton pump inhibitors, since such therapy may result in decreased gastric acid secretion and gastric bacterial colonization which might adversely affect the results of the study. One group would consist of patients who have clinical signs of gastric involvement with Crohn's disease with appropriate symptoms such as upper abdominal pain, vomiting and nausea and consistent endoscopic findings and who are not infected with H. pylori. The other group would include Crohn's disease patients who have no clinical symptoms attributable to gastric Crohn's disease and no H. pylori infection, but have signs consistent with gastric Crohn's disease like focally enhanced gastritis at gastric biopsy. Biopsies, stored in paraphin blocks, would be deparaffinized, and DNA would be extracted and examined with universal primers for 16S bacterial rRNA. The polymerase chain reaction would be carried out according to standard protocols.

If the presence of consensus bacterial 16S rRNA is detected with this method, philogenicity of bacteria would be studied further with species specific primers. Helicobacter pylori is a common gastric bacteria, which may also be detected with the above mentioned 16S rRNA method, and H. pylori gastritis may mask gastritis due to Crohn's disease.[23] Therefore, if the presence of bacteria is detected with this method, search with H. pylori specific primers will be performed first to rule out Helicobacter pylori infection. If there is no H. pylori infection, biopsies would be further studied and search for bacteria would be performed with primers specific for each bacterial species which might play a role in the development of Crohn's disease like Yersinia, Mycobacterium tuberculosis, or TM7 bacteria.[24] Bioinformatics could also be used to study this.

If one sort of bacterium other than H. pylori is detected in gastric mucosa of patients in studied groups, obtained results should be statistically compared with Chi square test. In case that this test can not be applied, Fischer's test would be used. However, if two or more groups of bacteria are present in gastric mucosa of studied patients, multivariate logistic model should be used for analyses.

If specific sort of bacterium is identified in the studied patients, a further study would include determination of this bacterium in biopsies taken from affected intestinal areas in previously studied patients with intestinal involvement. If this sort of bacteria is identified in affected intestinal areas of studied patients, the last stage of the study would be performed in significant number of patients with Crohn's disease. It would consist of the determination of this bacterium in affected intestinal areas in Crohn's disease patients and corresponding sites in healthy persons. Obtained results would be statistically compared to demonstrate whether the infection with this bacteria is related to Crohn's disease.

Conclusion

We proposed search for possible causative bacteria of Crohn's disease with advanced molecular techniques at the site, which is not commonly involved in this disease. Symptomatic gastric involvement with Crohn's disease occurs in less than 4% of patients. Any medical center willing to test such hypothesis in the proposed way should have at least 750 patients with Crohn's disease in order to find 30 patients with symptomatic gastric Crohn's disease.

As gastric Crohn's disease is an uncommon form of the disease, we guess that rarity of this form of presentation is the reason why so many researchers have overlooked gastric mucosa as the organ where the search for aethyology of Crohn's disease should be performed. However, our hypothesis might be tested if several large centers who deal with Crohn's disease perform a multicenter study.

Conflict of interest: none declared

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