

Seroprevalence of *Mycobacterium paratuberculosis* in Patients with Crohn's Disease

Although the etiology of Crohn's disease remains unclear, in addition to genetic 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* as a candidate organism in the etiology of Crohn's disease.

In a recent population-based case control study of seroprevalence of *M. paratuberculosis* in patients with Crohn's disease and ulcerative colitis, the authors could not prove the difference between inflammatory bowel disease patients and healthy volunteers (1). The rate of positive enzyme-linked immunosorbent assay results was significantly higher for all study groups. In conclusion, in this study, the *M. paratuberculosis* seropositivity rate was approximately 35% for all groups, and there was no difference in rates among Crohn's disease patients, ulcerative colitis patients, healthy controls, and unaffected siblings (1).

We also examined the seroprevalence of *M. paratuberculosis* in 42 Crohn's disease patients and 34 healthy, randomly selected volunteers in Debrecen, Hungary. The relatively small number of cases limited our consideration of the possibilities. Adult patients of both sexes were included. All patients had previously received routine *Mycobacterium bovis* bacillus Calmette-Guérin vaccinations in childhood but had no evidence of tuberculosis in their case histories. The diagnosis of Crohn's disease was made using the formally accepted criteria. We used the same adapted enzyme-linked immunosorbent assay (IDEXX Laboratories) as Dr. Bernstein did for serum antibodies to *M. paratuberculosis*, as Dr. Collins advised (he suggested that we test human sera from healthy blood donors to establish, by analysis of frequency distributions, what a reasonable approximate cutoff level might be for Hungarian patients). We used sera from the healthy controls to set the upper limit of normal with 95% confidence. A positive test for a human serum sample was defined, based on the results with these sera, by a test result equivalent to the mean S/P ratio (0.079) plus 2 standard deviations (2×0.123), i.e., 0.325. We proved that only approximately 9% of the controls were seropositive for *M. paratuberculosis*, in contrast to 33.3% of Crohn's disease patients.

Even given the small number of subjects in our study, these observations invite further surveys to elucidate the results, as we think they are definitely more than accidental.

Our findings may enrich other findings and suggest that *M. paratuberculosis* has certain importance in Crohn's disease after all, but further multicenter research is required for a comparison of results.

REFERENCE

1. Bernstein, C. N., J. F. Blanchard, P. Rawsthorne, and M. T. Collins. 2004. Population-based case control study of seroprevalence of *Mycobacterium paratuberculosis* in patients with Crohn's disease and ulcerative colitis. *J. Clin. Microbiol.* **42**:1129–1135.

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Authors' Reply

We appreciate Dr. Barta's interest in our paper. The Hungarian seroprevalence of *Mycobacterium paratuberculosis* raises some important issues. First, Dr. Barta is reporting a prevalence in controls comparable to that which has been reported previously in Denmark and Wisconsin (less than 10%) (1) but much lower than that of a control population in Manitoba (2). This may suggest that the population of Manitoba is truly more exposed to *M. paratuberculosis* in general than three other populations from disparate areas of the world. Second, the prevalence of 33% among Crohn's disease patients was comparable to the seroprevalence in Manitoba but much higher than that reported in Denmark and Wisconsin (1). Two strengths of our study in Manitoba are that we studied a reasonably large sample (nearly 1,000 subjects) and that both cases and controls were population based. The interesting findings from Hungary underscore the need for larger studies with

well-matched cases and controls to determine if seropositivity to *M. paratuberculosis* varies by region, is associated with Crohn's disease, or is different in population-based versus selected populations. While our results are different than those from Hungary, our findings are mutually provocative, and we agree with Dr. Barta's plea for further data to help clarify the issues.

REFERENCES

1. **Collins, M. T., G. Lisby, C. Moser, et al.** 2000. Results of multiple diagnostic tests for *Mycobacterium avium* subsp. *paratuberculosis* in patients with inflammatory bowel disease and in controls. *J. Clin. Microbiol.* **38**:4373–4381.
2. **Bernstein, C. N., J. F. Blanchard, P. Rawsthorne, and M. T. Collins.** 2004. Population-based case control study of seroprevalence of *Mycobacterium paratuberculosis* in patients with Crohn's disease and ulcerative colitis. *J. Clin. Microbiol.* **42**:1129–1135.

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