

## Letter to the Editor

### *Mycobacterium avium* subsp. *paratuberculosis* in Crohn's Disease Is Serologically Positive

Crohn's disease, similar to Johne's disease (a *Mycobacterium avium* subsp. *paratuberculosis*-caused inflammatory bowel disease in ruminants and primates), is an inflammatory bowel disease with suspected mycobacterial etiology (1). The disease emerged perceptibly in Western Europe and North America in the late 1940s and early 1950s. The incidence then increased progressively on both continents to a level which in some areas, such as northeast Scotland (11.6/100,000 per year), now approaches that of an epidemic (4). Granulomas and lymph node alteration in Crohn's disease patients resemble those of tuberculosis, leprosy, sarcoidosis, and bovine paratuberculosis (3). The latter has been suggested as a reservoir for epidemiologic transmission of the microorganism through contaminated dairy and meat products and possibly even in water (5).

In this study, we analyzed the humoral immune responses of Crohn's disease patients compared with those of age-matched controls against two recombinant clones of *M. avium* subsp. *paratuberculosis*. The recombinant clones, designated p35 and p36 and expressing 35- and 36-kDa proteins, respectively, were identified from a previously constructed expression genomic library of *M. avium* subsp. *paratuberculosis* that was screened by Western blotting against rabbit hyperimmune anti-*M. avium* subsp. *paratuberculosis* antibodies (2). A total of 110 human serum specimens, consisting of samples from 61 Crohn's disease patients and 47 controls (35 volunteers with no history or symptoms of gastrointestinal tract disorder and 12 individuals diagnosed with ulcerative colitis) were analyzed by Western blotting (5). The participants who donated the sera used in this study were free of tuberculosis, leprosy, and/or had not received the *Mycobacterium bovis* BCG vaccine. As shown in Table 1, of the 61 Crohn's disease-positive serum samples tested, 48 (79%) reacted with p35, 56 (92%) reacted with p36, 47 (77%) reacted with both antigens, and 57 (93%) reacted with either antigen. As expected, a small portion of Crohn's disease-positive serum specimens, four (7%), did not react with either antigen. Of the 35 serum samples from healthy controls, 5

(14%) reacted with p35, 4 (11%) reacted with p36, none (0%) reacted with both antigens, and 9 (26%) reacted with either antigen. Of the 12 ulcerative colitis-positive serum specimens, only 1 (8%) reacted with p35 or p36, either individually or combined. The only ulcerative colitis patient who reacted positively with these antigens is under further evaluation for possibility of misdiagnosis. Crohn's and ulcerative colitis diseases are inseparable at the early stage of infection. Many physicians refer to them as colitis diseases.

These data confirm that there is a statistically significant difference in reactivity between Crohn's disease-positive serum samples and serum samples from controls ( $P < 0.001$ ). This ultimately adds more evidence to strengthen the proposed association of *Mycobacteria* with Crohn's disease. The data also suggest that there is great potential for using *M. avium* subsp. *paratuberculosis* recombinant antigens (p35 and p36) individually or combined in a serologic test for diagnosis and possibly in vaccine development.

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TABLE 1. Reactivities of serum samples from Crohn's disease patients and controls with p35 and p36 recombinant antigens of *M. avium* subsp. *paratuberculosis*

Antigen(s)	No. (%) of reactive serum samples from:		
	Crohn's disease patients	Ulcerative colitis patients	Controls
p35	48 (79)	1 (8)	5 (14)
p36	56 (92)	1 (8)	4 (11)
p35 and p36	47 (77)	1 (8)	0 (0)
p35 or p36	57 (93)	1 (8)	9 (26)
Total	61	12	35