JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2007, p. 3883–3890 Vol. 45, No. 12
0095-1137/07/$08.00+0 doi:10.1128/JCM.01371-07

Mycobacterium avium Subspecies *paratuberculosis* Infection in Cases of Irritable Bowel Syndrome and Comparison with Crohn's Disease and Johne's Disease: Common Neural and Immune Pathogenicities[▽]

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Received 9 July 2007/Returned for modification 9 September 2007/Accepted 25 September 2007

***Mycobacterium avium* subsp. *paratuberculosis* causes Johne's disease, a systemic infection and chronic inflammation of the intestine that affects many species, including primates. Infection is widespread in livestock, and human populations are exposed. Johne's disease is associated with immune dysregulation, with involvement of the enteric nervous system overlapping with features of irritable bowel syndrome in humans. The present study was designed to look for an association between *Mycobacterium avium* subsp. *paratuberculosis* infection and irritable bowel syndrome. Mucosal biopsy specimens from the ileum and the ascending and descending colon were obtained from patients with irritable bowel syndrome attending the University of Sassari, Sassari, Sardinia, Italy. Crohn's disease and healthy control groups were also included. *Mycobacterium avium* subsp. *paratuberculosis* was detected by IS*900* PCR with amplicon sequencing. Data on the potential risk factors for human exposure to these pathogens and on isolates from Sardinian dairy sheep were also obtained. *Mycobacterium avium* subsp. *paratuberculosis* was detected in 15 of 20 (75%) patients with irritable bowel syndrome, 3 of 20 (15%) healthy controls, and 20 of 23 (87%) people with Crohn's disease ($P = 0.0003$ for irritable bowel syndrome patients versus healthy controls and $P = 0.0000$ for Crohn's disease patients versus healthy controls). One subject in each group had a conserved single-nucleotide polymorphism at position 247 of IS*900* that was also found in isolates from seven of eight dairy sheep. There was a significant association ($P = 0.0018$) between *Mycobacterium avium* subsp. *paratuberculosis* infection and the consumption of handmade cheese. *Mycobacterium avium* subsp. *paratuberculosis* is a candidate pathogen in the causation of a proportion of cases of irritable bowel syndrome as well as in Crohn's disease.**

Mycobacterium avium subsp. *paratuberculosis* is a well-defined subspecies of the *Mycobacterium avium* complex. *Mycobacterium avium* subsp. *paratuberculosis* (GenBank accession no. NC_002944) is an established multihost pathogen with the specific ability to cause Johne's disease, a systemic infection and chronic inflammation of the intestine of a range of histopathological types which can affect many animals, including primates (12, 14, 41). *Mycobacterium avium* subsp. *paratuberculosis* infection in cases of Johne's disease is associated with a chronic enteric neuritis (6, 29), together with immune activation and dysregulation (15, 16, 61, 65, 74, 75).

Subclinical *Mycobacterium avium* subsp. *paratuberculosis* infection is widespread in farm animals (38). Infected animals shed large numbers of *Mycobacterium avium* subsp. *paratuberculosis* cells into the environment, and there are wildlife reservoirs (1). These robust pathogens can survive for a long time in the environment and within environmental protists (43, 52). In some localities people are at risk of exposure from sources of environmental contamination (53, 71). People are also exposed to *Mycobacterium avium* subsp. *paratuberculosis* in retail milk supplies (20). A systematic review and meta-analysis of research from many laboratories demonstrated a significant and specific association between *Mycobacterium avium* subsp. *paratuberculosis* infection and chronic inflammation of the intestine of the Crohn's disease type in humans (21).

Irritable bowel syndrome (IBS) (18) is a widespread abdominal condition that affects about 10 to 15% of people in the industrialized economies of Europe, North America, Australasia, and Japan, with a rising prevalence among the populations in the developing economies of Asia. The onset can be triggered by incidental enteric and systemic infections (66). IBS results in a substantial impairment of quality of life and has a major impact on health care costs and resource utilization (40, 50). The causes of IBS are unknown.

IBS is defined symptomatically by the persistence of abdominal discomfort or abdominal pain relieved by defecation, together with diarrhea, constipation, or a mixture of both, in the absence of detectable organic disease and with normal appearances at endoscopy. IBS is frequently accompanied by systemic symptoms, such as lethargy, back and muscle aches, headache, and urinary disorders. IBS overlaps symptomatically with microscopic colitis (37, 70). In recent years evidence of abnormalities affecting the enteric nervous system and its neurotransmitters in patients with IBS (2, 4, 11, 17, 19, 25, 69),

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▽ Published ahead of print on 3 October 2007.

together with histopathological and functional changes in the intestine consistent with a low-grade immune activation (35), has accumulated.

The present study was designed to look for a potential association between *Mycobacterium avium* subsp. *paratuberculosis* infection in the intestine and IBS to confirm the significant association with Crohn's disease and to make comparisons between the pathophysiological features of IBS and Crohn's disease in humans and Johne's disease in animals.

MATERIALS AND METHODS

Patients. Patients attending the Institute of Clinical Surgery, University of Sassari, Sassari, Sardinia, Italy, for ileocolonoscopy as part of their routine medical care and with a diagnosis of IBS were invited to participate in the study. Since the *Mycobacterium avium* subsp. *paratuberculosis* infection rate to be expected in endoscopic mucosal biopsy specimens from people attending the Institute of Clinical Surgery in Sassari with a diagnosis of Crohn's disease had previously been established (83%) (59) and since we wished to make a simultaneous comparison with Crohn's disease, a second test group comprising Crohn's disease patients was also included. A healthy control group of subjects without IBS or any evidence of inflammatory bowel disease (non-IBS/IBD), most of whom were attending the Institute of Clinical Surgery for screening ileocolonoscopy, was also recruited. Patients were recruited sequentially at random. The inclusion criteria for IBS were conformity with the Rome II criteria; and those for Crohn's disease were the established clinicopathological, radiological, and endoscopic features associated with a diagnosis of Crohn's disease. Patients on anticoagulation medications were excluded. Informed written consent was obtained from each subject. The study was approved by the Bioethics Committee of the University of Sassari.

In a structured interview, information was obtained from each patient on the date and the place of birth, whether there was a family history of IBD, where the patient lived at the time of the interview (urban or rural), whether the patient was exposed to farm animals, and whether the patient consumed raw milk or "hand-made" cheese normally obtained directly from farms. For the IBS group, additional information comprised the length of IBS history; whether the patient had an IBS subtype in which diarrhea, constipation, or the mixed type predominated; and whether they received postinfection treatment and what drug treatment they were receiving, if any. For the Crohn's disease group, additional information comprised the length of Crohn's disease history, the site of macroscopic inflammatory disease (the ileum, colon, or both), whether the disease was active or inactive (Crohn's disease activity index, >150 or <150), the presence or absence of microscopic granulomas, and current drug treatment. Routine hematology and blood biochemistry were done for all patients. In addition, antigliadin immunoglobulin G and M antibodies, serum transglutaminase, stool culture and microscopy for enteric pathogens and parasites, and abdominal ultrasound or computed tomography scan were done for all IBS patients.

Mucosal sampling. At ileocolonoscopy two mucosal biopsy specimens were taken from each of the terminal ileum (samples T1 and T2), the ascending colon (samples A1 and A2), and the descending colon (samples D1 and D2). For the Crohn's disease group, this sampling regimen was applied regardless of the site of gross inflammatory disease. Each biopsy specimen was immediately placed in an individual blue-capped Lysing Matrix B ribolyser tube (catalogue no. 6911; Qbiogene, Nottingham, United Kingdom) containing 600 μl buffer (400 mM NaCl, 10 mM Tris HCl, 2 mM sodium EDTA, and 0.6% sodium dodecyl sulfate containing 33 μg proteinase K [Sigma] [final concentrations]). The tubes were sealed, coded, and immediately transferred to the Microbiology Department, University of Sassari, where further processing was carried out by investigators blinded to any knowledge of the clinical diagnosis. Mucosal biopsy specimens for routine histopathology were also taken from all patients.

DNA extraction, PCR, and amplicon sequencing. The sealed ribolyser tubes were incubated at 37°C overnight for 18 h and then placed in a FastPrep ribolyser instrument (Qbiogene) for mechanical disruption at a setting of 6.5 m/s for 45 s. Subsequent DNA extraction, nested IS*900* PCR with L and AV primers in duplicate with DNA extracts from each of the biopsy specimens, and visualization of the amplification products were carried out as previously described in detail (7). Stringent precautions, as described, were used throughout to exclude contamination, and the reactions were monitored by the use of four separate control tubes with TE (Tris-EDTA) buffer and one process control tube with tissue lysis buffer for each patient tested. Full-length DNA sequencing in both directions was performed with the amplicons of the AV1 and AV2 primers obtained by the second-round nested PCR and was repeated by going back to the original tissue DNA extract from each patient in whom a single-nucleotide polymorphism had been identified.

Characterization of *Mycobacterium avium* subsp. *paratuberculosis* isolates from Sardinian dairy sheep. In order to provide a comparator with the *Mycobacterium avium* subsp. *paratuberculosis* organisms identified in humans in Sardinia, we amplified and sequenced the amplicons of the AV1 and AV2 primers obtained by PCR using as the template DNA extracted from *Mycobacterium avium* subsp. *paratuberculosis* cultured from the feces of eight *Mycobacterium avium* subsp. *paratuberculosis*-infected Sardinian dairy sheep on different farms. All *Mycobacterium avium* subsp. *paratuberculosis* isolates were also genotyped by PCR for mycobacterial interspersed repetitive units (MIRUs), as described previously (8).

Statistics. Fisher's exact test was used to determine the significance of differences in *Mycobacterium avium* subsp. *paratuberculosis* infection rates between the clinical groups and the significance of the factors potentially associated with infection. The Mann-Whitney U test was used to compare the ages of the clinical groups.

RESULTS

A total of 63 patients were recruited to the study: 20 in each of the IBS and the non-IBS/IBD control group and 23 in the Crohn's disease control group. In the IBS group, patients with celiac disease and infection or infestation with common enteric pathogens, as well as any lesion visible on abdominal scanning, were excluded in all cases. The histopathological appearances of mucosal biopsy specimens in the IBS and the non-IBS/IBD groups were assessed and were found to be normal for all subjects.

The mean ages of the patients in the different groups were 52.8 years (range, 25 to 70 years) for the IBS group, 60.3 years (range, 45 to 79 years) for the non-IBS/IBD control group, and 34.7 years (range, 14 to 71 years) for the Crohn's disease group. The Crohn's disease group was significantly younger than the IBS group ($P = 0.001$) and the non-IBS/IBD group ($P < 0.0001$). The ages of the IBS group and the non-IBS/IBD group were not significantly different ($P = 0.14$). Details of the patients and the results of their *Mycobacterium avium* subsp. *paratuberculosis* tests are summarized in Table 1. An example of the normal endoscopic appearances in a 47-year-old female patient with IBS with constipation associated with an extensive *Mycobacterium avium* subsp. *paratuberculosis* infection in all three regions of the gut tested is shown in Fig. 1.

Fifteen of the 20 (75%) IBS patients and 3 of the 20 (15%) non-IBS/IBD control subjects tested positive for *Mycobacterium avium* subsp. *paratuberculosis* in one or more biopsy specimens ($P = 0.0003$; odds ratio [OR] = 17; 95% confidence interval [CI] = 0.037 to 58.89). Twenty of the 23 (87%) Crohn's disease patients tested positive for *Mycobacterium avium* subsp. *paratuberculosis* ($P = 0.0000$ compared to the non-IBS/IBD group; OR = 37.8; 95% CI = 5.47 to 302.8). There was no significant difference ($P = 0.44$) in the *Mycobacterium avium* subsp. *paratuberculosis* detection rates between the IBS and the Crohn's disease groups.

In the whole cohort of 63 patients, there was a highly significant association ($P = 0.0018$; OR = 5.7) between the presence of *Mycobacterium avium* subsp. *paratuberculosis* infection and the consumption of unpasteurized hand-made cheese. An association between a family history of IBD and *Mycobacterium avium* subsp. *paratuberculosis* infection did not reach statistical significance ($P = 0.089$; OR = 4.7). There was no association between *Mycobacterium avium* subsp. *paratuberculosis* infection and urban or rural living ($P = 0.59$), exposure to

TABLE 1. Clinical characteristics and results of IS*900* PCR testing for *Mycobacterium avium* subsp. *paratuberculosis* in participants

Group and sex[a]	Age (yr)	History (yr)	IBS type[b]	Disease location	Disease activity	Drug(s)	IS*900* PCR result[c]	Reason for endoscopy
IBS								
F	55	8	M			None	NEG	
F	59	2	C			None	NEG	
F	25	2	D			None	NEG	
F	53	2	C			Prednisone	A1, A2, D2	
F	47	5	C			Prednisone	T1, T2, A1, A2, D1,[d] D2	
F	70	1	D			None	NEG	
F	33	2	C			Prednisone	A2	
F	56	4	C			None	NEG	
F	69	9	C			None	T2, A1, A2	
M	50	2	C			None	D2	
F	64	3	C			None	D1	
M	58	1	D			None	A1, A2, D1, D2	
F	38	2	M			None	T1, D1, D2	
F	48	5	C			None	T1, T2, A2, D1, D2	
F	36	15	D			None	A1, A2	
F	64	7	C			None	T1, T2, A1, D1	
F	68	1	C			None	D1	
F	69	2	D			None	T2	
F	50	4	C			None	T2	
M	45	2	D			None	D2	
Non-IBS/IBD								
M	67						NEG	Screening
M	59						NEG	Screening
F	63						NEG	Tumor markers
M	77						NEG	Abdominal pain
M	45						NEG	Screening
F	47						NEG	Follow-up
M	60						A1, A2,[d] D1, D2	Follow-up
F	45						NEG	Abdominal pain
F	67						NEG	Follow-up
M	54						A1	Screening
M	64						NEG	Screening
M	67						NEG	Screening
F	46						NEG	Abdominal pain
M	51						T1, T2	Screening
M	59						NEG	Tumor markers
M	67						NEG	Screening
M	74						NEG	Screening
F	49						NEG	Screening
M	79						NEG	Screening
F	67						NEG	Screening
Crohn's disease								
M	58	3		Ileum/colon	Inactive	Mesalazine	D1	
M	14	3		Colon	Active	Mesalazine	T1, T2, A2[e]	
F	54	1		Ileum/colon	Active	None	A1, D2[e]	
F	27	0.5		Ileum/colon	Active	None	T1, T2[e]	
M	31	10		Ileum	Active	Olsalazine	T1, A1[e]	
F	61	15		Ileum	Inactive	Mesalazine	T1,[d] D2	
M	30	4		Ileum	Inactive	Mesalazine	NEG[e]	
F	51	0.08		Ileum/colon	Active	None	T1, T2[e]	
M	35	0.5		Ileum	inactive	None	T2, A2	
M	14	2		Colon	Active	Mesalazine, rifampin, clarithromycin	NEG[e]	
F	25	0.25		Ileum	Active	Mesalazine	A1[e]	
F	18	6		Ileum	Active	Azathioprine, mesalazine	A1, D1[e]	

Continued on following page

TABLE 1—*Continued*

Group and sex[a]	Age (yr)	History (yr)	IBS type[b]	Disease location	Disease activity	Drug(s)	IS*900* PCR result[c]	Reason for endoscopy
M	22	8		Ileum	Active	Mesalazine	T2[e]	
F	28	0.25		Ileum	Active	Mesalazine	T1, A1[e]	
F	11	0.08		Ileum	Active	None	T2[e]	
F	71	9		Ileum	Active	Mesalazine	A2	
F	51	10		Duodenum/ileum/colon	Active	Prednisone	D1, D2[e]	
F	27	2		Ileum	Active	Mesalazine	T2, A2, D2[e]	
F	23	6		Ileum	Active	Prednisone	T1	
M	46	6		Ileum	Active	Mesalazine, rifampin, clarithromycin	NEG	
F	35	8		Colon	Active	Mesalazine, prednisone	A2, D2[e]	
F	40	20		Ileum	Active	Mesalazine	A1, A2	
M	26	5		Duodenum/ileum	Active	Mesalazine, budesonide	T1, T2, A1, A2, D1, D2[e]	

[a] M, male; F, female.
[b] D, diarrhea; C, constipation; M, mixed type.
[c] T, terminal ileum; A, ascending colon; D, descending colon; NEG, negative.
[d] A C-to-T transition was identified at position 247 in this biopsy specimen.
[e] Granuloma positive.

farm animals ($P = 0.54$), or a history of raw milk consumption ($P = 1$). Within the IBS group there was no relationship between *Mycobacterium avium* subsp. *paratuberculosis* infection and IBS with diarrhea, constipation, or a mixture of both ($P = 0.43$). Within the Crohn's disease group there was no relationship between *Mycobacterium avium* subsp. *paratuberculosis* infection and disease activity ($P = 0.45$) and *Mycobacterium avium* subsp. *paratuberculosis* infection and the presence or absence of granulomas ($P = 1$). There was also no relationship ($P = 0.32$) between the distribution of *Mycobacterium avium* subsp. *paratuberculosis*-positive tests (ileum, colon, or both) and the location of gross inflammatory disease (ileum, colon, or both).

Amplicon DNA sequences were obtained from all 15 *Mycobacterium avium* subsp. *paratuberculosis*-positive patients in the IBS group, in 2 of the 3 *Mycobacterium avium* subsp. *paratuberculosis*-positive non-IBS/IBD control subjects, and in a representative 10 of the 20 *Mycobacterium avium* subsp. *paratuberculosis*-positive patients with Crohn's disease. All sequences were identical to those of the AV1 and AV2 amplified regions of IS*900* in the sequenced genome of *Mycobacterium avium* subsp. *paratuberculosis* bovine strain K10 (GenBank accession no. NC_002944), with the exception of a sequence obtained from one patient each in the three clinical groups (Table 1). All three of these patients had a previously undescribed sequence ambiguity characterized by a predominant C peak and a smaller T peak at position 247 of the IS*900* element (Fig. 2). The DNA sequences of the IS*900* AV1 and AV2 amplicons obtained from seven of the eight *Mycobacterium avium* subsp. *paratuberculosis*-infected dairy sheep were identical, including the same C/T ambiguity at nucleotide 247. These seven *Mycobacterium avium* subsp. *paratuberculosis* isolates from dairy sheep were also found to have a previously undescribed MIRU type 3971. The IS*900* AV1 and AV2 amplicons from the *Mycobacterium avium* subsp. *paratuberculosis* isolate from the eighth sheep were identical to the reference sequence with GenBank accession no. NC_002944 and had a MIRU type 3951 commonly associated with bovine strains.

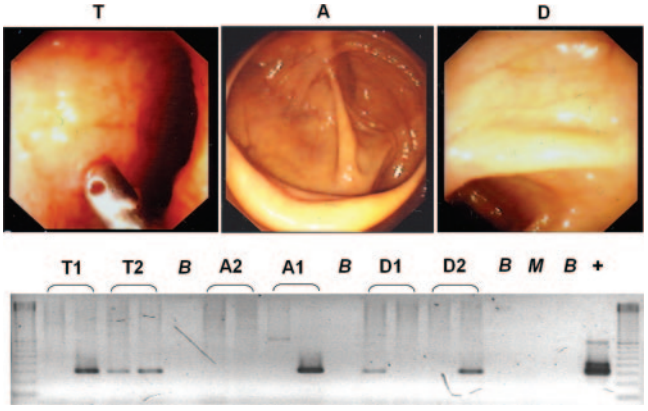

FIG. 1. (Upper panel) The normal appearances at endoscopy of a female patient aged 47 years with constipation-predominant IBS in the IBS group. T, terminal ileum; A, ascending colon; D, descending colon. (Lower panel) Results of IS*900* PCR testing for *Mycobacterium avium* subsp. *paratuberculosis* in the same patient at each of the three sites sampled. The results are consistent with an extensive *Mycobacterium avium* subsp. *paratuberculosis* infection in her gastrointestinal tract. Lanes: *B*, negative buffer control; *M*, negative process control; +, positive PCR control.

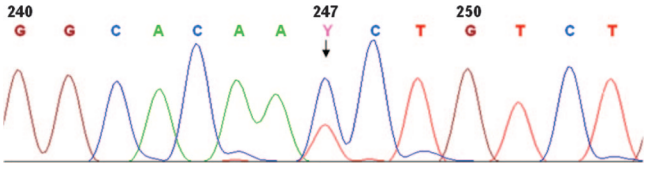

FIG. 2. Sequence electropherogram spanning the previously undescribed C/T ambiguity (code Y) found in the AV1 and AV2 amplicons at position 247 of the IS*900* element and identified in *Mycobacterium avium* subsp. *paratuberculosis* isolates from three humans and seven of eight Sardinian dairy sheep in the present study. It is consistent with a situation in which a proportion of the 14 to 18 copies of the IS*900* elements in the affected *Mycobacterium avium* subsp. *paratuberculosis* isolates underwent a C-to-T transition from the characteristic bovine genotype at this locus.

DISCUSSION

We found a highly significant association between *Mycobacterium avium* subsp. *paratuberculosis* infection in the intestine and IBS. People with a *Mycobacterium avium* subsp. *paratuberculosis* infection were 17 times more likely to have IBS than people without a *Mycobacterium avium* subsp. *paratuberculosis* infection. The validity of the methods and the results of *Mycobacterium avium* subsp. *paratuberculosis* detection in IBS are supported by the finding of a *Mycobacterium avium* subsp. *paratuberculosis* detection rate of 87% in the Crohn's disease control group in this blinded study, in close agreement with the findings of previous work (59). The finding of *Mycobacterium avium* subsp. *paratuberculosis* colonization of the intestinal mucosa of a minority proportion of subjects in the non-IBS/IBD group is entirely in keeping with the population biology of multihost pathogens (34, 76). *Mycobacterium avium* subsp. *paratuberculosis* has been cultured from the blood of people with Crohn's disease (45). In subsequent work it will be interesting to see if the systemic symptoms that occur in individuals with IBS are associated with the presence of *Mycobacterium avium* subsp. *paratuberculosis* in blood.

The identity of the sequenced AV1 and AV2 amplicons with the reference *Mycobacterium avium* subsp. *paratuberculosis* genomic sequence (GenBank accession no. NC_002944) in all but three of the *Mycobacterium avium* subsp. *paratuberculosis*-positive patients was consistent with the results seen after infection of people in the present study with bovine strains of these pathogens (60). This finding also agrees with other work which showed that the human isolates of *Mycobacterium avium* subsp. *paratuberculosis* typed so far have all been similar to bovine type strains (26). There are, however, about 3.5 million dairy sheep in Sardinia, in which *Mycobacterium avium* subsp. *paratuberculosis* infection is widespread. The finding of an identical previously unreported C-to-T transition corresponding to nucleotide position 247 of the IS*900* element in three *Mycobacterium avium* subsp. *paratuberculosis*-infected patients (one in each clinical group) and in seven of the eight *Mycobacterium avium* subsp. *paratuberculosis* isolates from infected dairy sheep could be consistent with the acquisition of the *Mycobacterium avium* subsp. *paratuberculosis* infection from sheep in a proportion of *Mycobacterium avium* subsp. *paratuberculosis*-infected people. The *Mycobacterium avium* subsp. *paratuberculosis* strains infecting the sheep were all bovine type strains, suggesting that the sheep had in turn acquired the infection from cattle. Further studies on the molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis* infection in domestic livestock and in humans are indicated.

All three patients with the C-to-T transition at position 247 had a positive history of consumption of hand-made cheese. Our finding of a significant association between the consumption of hand-made cheese and *Mycobacterium avium* subsp. *paratuberculosis* infection in the present study appears to be more prominent, since none of the other potential risk factors for *Mycobacterium avium* subsp. *paratuberculosis* infection, such as a history of the consumption of raw milk, came up positive. Further work is necessary to confirm this association. The consumption of unpasteurized cheeses has been reported to be a significant risk factor for familial Crohn's disease in Belgium (71).

An obvious question is what is the role of *Mycobacterium avium* subsp. *paratuberculosis* infection in the intestine of people with IBS? Exposure to *Mycobacterium avium* subsp. *paratuberculosis* appears to be widespread, so the presence of the organism might merely reflect an incidental colonization, favored in comparison with healthy people by the preexisting pathophysiology of IBS. This would not, however, be in accord with the established status of *Mycobacterium avium* subsp. *paratuberculosis* as a proven multihost chronic enteric pathogen, so it is perhaps more likely that *Mycobacterium avium* subsp. *paratuberculosis* infection may be related to the causation of the syndrome.

There are many parallels between the features of *Mycobacterium avium* subsp. *paratuberculosis* infection and disease in animals and IBS and Crohn's disease in people. *Mycobacterium avium* subsp. *paratuberculosis* infection and clinical Johne's disease in cattle and sheep are frequently associated with a chronic enteric neuritis (6, 29). In naturally infected cattle, myenteric ganglionitis with infiltration, particularly by mast cells, is seen. In both naturally and experimentally infected sheep, there were aggregations of mononuclear cells around enteric nerves in the ileal submucosa and myenteric plexus. Such lesions were not seen in sheep that were challenged with *Mycobacterium avium* subsp. *paratuberculosis* orally but in which infection did not subsequently develop. Although additional detailed work on the impact of *Mycobacterium avium* subsp. *paratuberculosis* infection on both the peripheral and central nervous systems in Johne's disease is desirable, it is clear that *Mycobacterium avium* subsp. *paratuberculosis* infection and disease in animals may reflect a specific mycobacterial neuropathogenicity. Microscopic inflammation affecting the enteric nervous system, together with abnormalities affecting its function and regulation, is well described in cases of IBS (2, 4, 11, 19, 25, 51, 69). Those studies have led to advances in our understanding of how these features integrate into the underlying pathophysiological mechanisms of the syndrome (3, 32, 48). Abnormalities of the enteric nervous system affecting neurons and enteric glial cells are well established in Crohn's disease (5, 22–24). Glial cells express receptors for neurotransmitters and serve as a link between the enteric nervous and immune systems (56). Their selective ablation experimentally results in the loss of the integrity of the mucosal barrier and intestinal inflammation (10, 57, 72). Thus, a chronic enteric neuropathy caused by *Mycobacterium avium* subsp. *paratuberculosis* infection in humans could contribute an important component of the underlying pathophysiology of both IBS and Crohn's disease.

Mycobacterium avium subsp. *paratuberculosis* infection and Johne's disease in animals are accompanied by local and systemic immune dysregulation affecting cells in the gut, the mesenteric lymph nodes, and the blood. There are many examples of this: the downregulation of major histocompatibility complex class I and II molecules in *Mycobacterium avium* subsp. *paratuberculosis*-infected bovine macrophages (74), the hyporesponsiveness in ileal lymphocytes from *Mycobacterium avium* subsp. *paratuberculosis*-infected cows (75), abnormalities in the regulation of cytokine expression (9, 13, 16, 30, 31, 65, 73), the perturbation of macrophage activation and apoptosis (13, 15), and the impairment of nitric oxide responsiveness (61). Together, these and other changes selectively weaken immune

responsiveness in animals and favor the persistence of intracellular *Mycobacterium avium* subsp. *paratuberculosis* infection. IBS is associated with a low-grade immune activation (33, 35, 68). Local and systemic immune dysfunctions in humans are well-described features of Crohn's disease (39, 46).

In patients with Crohn's disease the gross macroscopic inflammation tends to occur in segments, whereas the observed pathophysiological features are found to be distributed throughout the gut. Examples of these are the distribution of T-lymphocyte aggregates (44), the status of tight junctions (49, 62) and epithelial permeability (63, 64), the neurotransmitter coding of enteric neurons (58), the expression of substance P and its receptor (27, 42, 54), the expression of tumor necrosis factor alpha by mast cells and of its inducer (lipopolysaccharide-induced tumor necrosis alpha factor) by macrophages (36, 67), the composition of the intestinal flora (28), and the reduction in antimicrobial activity in the colon (47). An interesting feature of the present study was that there was no statistically significant association between the distribution of positive *Mycobacterium avium* subsp. *paratuberculosis* tests in the gut and the distribution of the gross inflammation in the Crohn's disease patients. This is in close agreement with the results of previous work (55). *Mycobacterium avium* subsp. *paratuberculosis* is present in patients with Crohn's disease in a Ziehl-Neelsen-negative phenotype which minimizes immune recognition. A model for the pathogenesis of *Mycobacterium avium* subsp. *paratuberculosis* in the causation of the gross inflammation in Crohn's disease is one in which *Mycobacterium avium* subsp. *paratuberculosis* infection widely distributed throughout the gut causes a primary immune dysregulation and damages the fine structure and function of enteric neural networks. Mucosal integrity and other critical functions in the intestine are compromised. The gross inflammation results from the perturbed neuroimmune response to the secondary penetration into the gut wall of microbial copathogens and food residues from the gut lumen.

Mycobacterium avium subsp. *paratuberculosis* is a proven multihost chronic enteric pathogen to which humans are widely exposed. It has neuropathogenic and immune dysregulatory properties. It is a strong candidate pathogen for the causation of Crohn's disease in *Mycobacterium avium* subsp. *paratuberculosis*-infected people. Despite differences between the common pluribacillary form of Johne's disease in animals and the paucimicrobial nature and Ziehl-Neelsen-negative phenotype of *Mycobacterium avium* subsp. *paratuberculosis* strains infecting humans, the pathogenesis of *Mycobacterium avium* subsp. *paratuberculosis* infection and disease in animals is a good match for the observed pathophysiological features of IBS and Crohn's disease. The present research suggests that *Mycobacterium avium* subsp. *paratuberculosis* infection may also be a candidate for the causation of IBS in a proportion people with this common condition. Further studies in this field are, of course, indicated. Where these involve the detection of *Mycobacterium avium* subsp. *paratuberculosis*, particular attention should be paid to the use of tissue processing and laboratory methodologies that are optimized for the accurate detection of the phenotype of these difficult versatile pathogens infecting humans.

ACKNOWLEDGMENTS

Funding for the work was provided by the University of Sassari, together with a grant from Regione Autonoma della Sardegna, fondi PES2006, and Italian MIUR no. 2005064503, 2550. In London, funding was contributed by many private donors and charitable trusts, to whom we express our appreciation.

We are most grateful to Paola Molicotti for her contribution to the laboratory work. We thank Giovanni Sotgiu of the Department of Statistics and Hygiene of the University of Sassari Medical School and Jan Poloniecki of the Department of Community Health Sciences at St George's University of London, who provided statistical analyses. Alessandra Manca of the Department of Pathology, University of Sassari, kindly reviewed the histopathology.

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