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Is Crohn's disease caused by *M. avium* subsp. *paratuberculosis*?

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ABSTRACT

Speculation that *M. avium* subsp. *paratuberculosis* (MAP), the infectious agent of Johne's disease, might be involved in the aetiology of Crohn's Disease (CD) in humans was first voiced in 1913. No supportive evidence was obtained until the organism was identified in cultures from a small number of CD cases in the 1980's. Evidence accumulated over the last 15 to 20 years has left the medical community somewhat polarised about the possible role of MAP in Crohn's disease. Technological advances enabling more sensitive detection of bacteria or their components (e.g. PCR, immunohistochemistry), or immunological responses to them, have not clarified the fundamental question of whether an apparent association between the presence of MAP and CD lesions may have any causal significance. The basis for concluding that MAP has a causal role in the aetiology of CD is unconvincing.

INTRODUCTION

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the cause of Johne's disease, or paratuberculosis, a chronic enteric disease of domestic and wild animals worldwide. Paratuberculosis is characterised by a long incubation period such that clinical signs are not evident until late in the course of disease. Clinical manifestations vary among species, and include diarrhoea, loss of weight, emaciation, and eventual death. Lesions can be found throughout the intestinal tract, but occur most commonly in the terminal ileum and associated lymph nodes, with extraintestinal lesions being rare (Chiodini *et al.*, 1984).

Crohn's disease (CD) is a chronic enteric disease of humans that has clinical and pathological similarities to paratuberculosis in animals. CD is characterised by fever, diarrhoea, vomiting, abdominal pain, progressive anaemia and weight loss. Lesions of CD can occur in any part of the human alimentary tract, with the terminal ileum and large intestine most often involved (van Kruiningen, 1999). Studies of human CD cases, and animal models, suggest that the basic pathophysiological aberration underlying intestinal inflammation in CD is unrestrained cellular immune responses to luminal bacterial flora (Sartor, 1997; Papadakis and Targan, 1999). The factors that induce and maintain these unrestrained immune responses are unclear, and associations with a range of infectious agents have been proposed. However, no infectious agent has been confirmed to be a necessary cause of CD, and a multifactorial aetiology including environmental, genetic, immunological and microbial factors is generally accepted (Chiodini, 1989; Sartor, 1997; Papadakis and Targan, 1999; Andus and Gross, 2000; Hermon-Taylor *et al.*, 2000; Shanahan, 2002). As stated by Shanahan (2002), 'a simple cause and effect relationship probably does not exist for most cases of Crohn's disease'.

Among putative infectious causes for CD, MAP has come under greatest scrutiny since a mycobacterium isolated from 3 patients with CD was confirmed to be MAP (Chiodini, 1989). The level of controversy surrounding these issues is evident in recent scientific reviews (van Kruiningen 1999; Hermon-Taylor *et al.*, 2000; Chamberlin *et al.*, 2001; Harris and Lammerding 2001; El Zaatari *et al.*, 2001). Hermon-Taylor *et al.* (2000)

stated that 'the problem of MAP constitutes a public health issue of tragic proportions for which a range of remedial measures are urgently needed'. In contrast, van Kruiningen (1999) stated that 'the evidence that is reviewed herein provides no support for this association or for MAP as the etiologic agent of CD. The time now seems ripe to lay this hypothesis to rest'.

WHAT IS CROHN'S DISEASE

Initial recognition of CD as a pathological entity was based on its differentiation from intestinal tuberculosis (IT). The key features that distinguish CD from IT are the absence of both acid-fast organisms and caseous necrosis. Progress in understanding CD has been hampered by its similarities to other inflammatory bowel diseases (IBD) of humans, and heterogeneity among cases (Gasche *et al.*, 2000). Like other mycobacterial diseases (e.g. leprosy), IT has a spectrum of pathological types, with lesions classified as ulcerative, ulcerohypertrophic, and hypertrophic ('tuberculoid') lesions. Cases of hypertrophic IT also vary with respect to the degree of caseous necrosis and the numbers of acid-fast organisms (mycobacteria) present. The relatively rare hypertrophic paucibacillary lesions are most similar to CD and the two conditions cannot be differentiated reliably (Pulimood *et al.*, 1999).

IBD is a general term used for chronic disorders of unknown aetiology characterised by inflammation or ulceration in the intestines. Based on clinical and histopathological features, IBD is categorised into two major subtypes: CD and ulcerative colitis (UC). CD typically causes ulcers along the length of the small and large intestines, but either spares the rectum, or causes inflammation with drainage around the rectum. In contrast, UC usually induces ulcers in the lower large intestine, often starting at the rectum. Intestinal inflammation in CD is transmural (involving all layers of the intestinal wall), discontinuous, and may contain granulomas or be associated with intestinal or perianal fistulas. In UC, the inflammation is continuous, limited to rectal and colonic mucosal layers, and fistulas and granulomas are not observed (Tytgat *et al.*, 1994). Although epithelioid granulomas are highly specific for CD, they are only observed in approximately 30% of CD

patients (Bayless, 1998). Approximately 10% of IBD cases confined to the rectum and colon cannot be definitively classified as CD or UC, and are designated 'indeterminate colitis' (Tytgat *et al.*, 1994). The difficulties in diagnosis of IBD were illustrated by a study finding that re-evaluation of initial diagnosis resulted in about 10% of previously diagnosed UC and CD patients being reclassified after 1 or 2 years follow up (Moum *et al.*, 1997). Until now, opinion is divided on whether UC and CD are distinct entities or different expressions of the same disease based on the presence or absence of additional risk factors (Delco and Sonnenberg 1999; Shanahan 2002).

Uncertainty in case definition of CD and considerable heterogeneity among CD cases are recognised (Gasche *et al.*, 2000). The extent to which this heterogeneity reflects variability in host responses to specific aetiological factors; is the result of different exposures to related sets of risk factors; or is an artefact of aggregation of separate pathological entities of differing aetiology is unknown. Ambiguity over the role of specific aetiological factors in CD is likely to remain as long as the basis for heterogeneity among CD cases is unexplained. It has been suggested that MAP may be causally involved with only a sub-set of CD patients (Chamberlin *et al.*, 2001).

OVERVIEW OF THE AETIOLOGY OF CD

A complex aetiology for CD is generally accepted, with factors such as abnormalities in the immune system (including autoimmunity and cytokine regulation), genetic risk determinants, and environmental factors including microorganisms likely to play a part (Papadakis and Targan, 1999; Andus and Gross, 2000; Hermon-Taylor *et al.*, 2000; Shanahan 2002). Sartor (1997) listed 3 aspects related to aetiology of IBD:

- 1) reaction to persistent intestinal infection
- 2) existence of a defective mucosal barrier to luminal antigens
- 3) a dysregulated host immune response to ubiquitous antigens.

Combined these features result in pathogenic or resident luminal bacteria stimulating the mucosal and systemic immune systems to perpetuate the inflammatory cascade. Chronic inflammation results from an interaction of the persistent stimulus of microbial antigens with genetically determined host susceptibility factors that determine the individual's immune response or mucosal barrier function (Sartor, 1997). Papadakis and Targan (1999) suggest that several agents may initiate the unrestrained immune responses that, under the right conditions of intestinal microenvironment and genetic disposition of the patient, finally leads to pathology. Similarly, Mishina *et al.*, (1996) and Sartor (1998), proposed the concept of a 'trigger', which may be a microorganism, that initiates mucosal damage leading to a self-perpetuating inflammatory process. Recently Chamberlin *et al.*, (2001) stated that the two leading theories on causation of MAP are the autoimmune theory and the mycobacterial theory. These authors argued that the theories are complementary, with mycobacterial

infection being the aetiology of CD (ie., the proposed trigger) and subsequent autoimmune disease representing the pathogenesis. However there is little evidence to claim that MAP have more significance than ubiquitous intestinal microflora. The interplay between normal enteric flora, specific microbial agents and mediators of inflammation is not well understood, and many bacteria and viruses agents have been investigated. Cartun *et al.*, (1993) used immunohistochemistry to screen CD cases for more than 20 putative agents, and proposed that some granulomas in CD may result from immunologic processing of luminal bacterial antigens (particularly *E. coli* and streptococci) following their penetration through a compromised mucosa. Recently, specific *E. coli* were shown to survive and replicate within macrophages, without inducing death of the infected cells which released high levels of TNF-alpha. The authors concluded that these properties could be related to granuloma formation in CD (Glasser *et al.*, 2001).

COMPARATIVE PATHOLOGY OF CD AND PARATUBERCULOSIS

Similarities between lesions of CD and paratuberculosis diseases prompted early workers to hypothesise a mycobacterial aetiology for CD (Chiodini, 1989). However, van Kruiningen (1999) listed many dissimilarities and stated 'the morphological similarities are superficial and ... are more suggestive of differing etiopathogenesis'. Like CD, heterogeneity is evident in lesions of paratuberculosis both among and within species, and it impossible to resolve the question of common aetiology based on pathological similarities and differences. However, the following issues merit consideration:

- 1) The uniform absence of acid-fast bacilli from CD lesions is not inconsistent with a mycobacterial aetiology. Advocates for an aetiological role for MAP propose that CD may be analogous to tuberculoid leprosy and paucibacillary tuberculoid forms of paratuberculosis in which acid-fast bacilli are often not observed.
- 2) Mishina *et al.*, (1996) postulated that a spectrum of pathological types (perforating, non-perforating, and intermediate lesions) of CD was analogous to the immunopathological spectra of mycobacterial diseases (tuberculoid to lepromatous types). However, the invariable absence of acid-fast bacilli from CD lesions makes this hypothesis untenable. In all animal species, tuberculoid lesions of paratuberculosis have their lepromatous (multibacillary) counterpart. Despite pathological heterogeneity among CD cases, there is no multibacillary counterpart. Proponents of MAP as a cause of CD tacitly accept that humans respond uniformly to MAP infection with a 'tuberculoid' type response. This would constitute an anomalous phenomenon among known mycobacterial diseases.
- 3) MAP infection of macaques confirmed the ability of MAP to infect primates, but the lesions were consistent with multibacillary paratuberculosis and not CD (McClure *et al.*, 1987; Chiodini 1989). Again, the contention that CD results from MAP infection

implies a uniform 'tuberculoid' response of humans to MAP infection that would be distinct from that of all other species, including primates.

- 4) Efforts to reconcile the occurrence of strictures, fissures and intestinal perforation in CD, but not paratuberculosis, include statements that these features occur in 'regional ileitis and colitis' of dogs and pigs (Hermon-Taylor *et al.*, 2000). Van Kruiningen concurred that lesions described for regional ileitis in pigs are much more similar to CD than is paratuberculosis. However, 'regional ileitis' (proliferative enteropathy) of pigs is now well characterised and is caused by *Lawsonia intracellularis*, an obligate intracellular organism unrelated to mycobacteria.

These issues support the conclusion of van Kruiningen (1999) that paratuberculosis is not a 'naturally occurring animal counterpart to CD'. However, this does not preclude a possible role of MAP in the aetiology of CD that is distinct from that of a primary infectious agent.

PRESENCE OF MAP IN CD LESIONS

Results of efforts to demonstrate MAP in lesions of CD have been regularly reported and reviewed (e.g. van Kruiningen *et al.*, 1999; Hermon-Taylor *et al.*, 2000; Chamberlin *et al.*, 2001; El-Zaatari *et al.*, 2001), and only key points will be listed here

- Despite extensive efforts, the number of live MAP recovered from CD lesions remains very few (van Kruiningen *et al.*, 1999; El-Zaatari *et al.*, 2001)
- Detection of DNA from MAP using PCR or immunohistochemistry has not yielded consistent results, but overall detection has become more frequent with more sensitive methods of sampling and testing tissues.
- Data generally suggest that when detected, MAP organisms are not abundant in CD lesions (El-Zaatari *et al.*, 2001).
- Most papers report data on small numbers of patients and controls, without giving explicit details of selection of these groups.

Collins *et al.*, (2000) used 5 methods to detect MAP infection in 439 inflammatory bowel disease patients and 324 control subjects. Bacteriological results were uniformly negative for all groups. PCR for IS900 on resected bowel and lymph node tissues was positive for a higher proportion of CD (19%) and UC patients (26%) than controls (6%), and positive PCR results occurred more often in American (32%) than Danish (13%) patients. Collins *et al.*, (2000) concluded that MAP, or a similarly fastidious mycobacterial species, infects at least a subset of IBD patients but it remains unknown whether the organisms have any causal role in these diseases. This paper (Collins *et al.*, 2000) presents the strongest evidence yet for an association of MAP with CD. However, the similar proportion of PCR positives for CD and UC patients requires explanation as MAP has not been seriously implicated in the aetiology of UC. Presence of granulomas is the cardinal pathological feature of paratuberculosis, while lack of granulomas is a basic criterion for distinguishing UC from CD. Again, the

uniform failure to detect MAP by culture implies that the organism is present in low numbers or in an unculturable form. The possibility remains that the observed association of MAP with CD and UC represents opportunistic colonisation of diseased tissue rather than a causal role for MAP in inflammatory bowel disease.

IMMUNE RESPONSES TO MAP IN CD

Interpretation of serology is generally problematic in mycobacterial diseases and serological studies have provided minimal insight into the possible involvement of MAP in CD. Likely cross-reactivity with antibodies to other mycobacterial antigens, apparently low abundance of organisms when present in lesions, and the possibility that the organisms exist in a poorly immunogenic form (e.g. spheroplasts) could explain failures of serology to demonstrate a valid association. On the other hand, because CD is an ulcerative disease, tissues are exposed to a wide array of luminal antigens that may induce immunological responses in the absence of any primary aetiological role. For example, antibodies to *Saccharomyces cerevisiae* (brewer's yeast) have been detected in about 80% of CD patients (compared with 10% of UC patients), and elevated seroreactivity to numerous enteric organisms has been described (Vasiliaskas *et al.*, 2000).

Cell mediated immune (CMI) responses to MAP are detectable in the early stage of paratuberculosis (Stabel 2000). Although there is evidence that disturbed regulation of CMI responses may play a role in the aetiology of CD there has been little investigation of CMI responses to MAP in CD patients, and the sparse data available have failed to demonstrate the presence of specific CMI response to MAP (Rowbotham *et al.*, 1995). CMI is associated with 'tuberculoid' immunopathological types of mycobacterial lesions in which acid-fast organisms are usually sparse or not detectable. Because acid-fast organisms are uniformly absent from CD lesions, and other evidence points to a low abundance of organisms if present, analogies are frequently drawn between CD lesions and paucibacillary paratuberculosis and tuberculoid leprosy (Hermon-Taylor *et al.*, 2000). For such analogies to be valid, specific CMI responses to MAP should be detectable in CD patients, but have yet to be demonstrated. On the other hand, the absence of granulomas in most CD patients has been likened to lepromatous leprosy in which CMI responses are not detectable; lesions do not have fully differentiated granulomas, but organisms are abundant (Kaplan, 1998). The dilemma in CD is that the lack of CMI **and** the lack of organisms does not fit with either tuberculoid or lepromatous models of mycobacterial disease.

In summary, MAP organisms appear to be present in low abundance in some CD patients, but it appears that neither a detectable CMI response nor a strong humoral response to MAP occurs. This suggests that CD is not analogous to either paucibacillary paratuberculosis or lepromatous leprosy. Hermon-Taylor (1998) acknowledged that this pattern cannot be reconciled with current models of infectious diseases, and that 'we have to think creatively to come up with a new model'.

RESPONSE TO THERAPY

Response to therapy has been an important factor contributing to acceptance of the causal role of *Helicobacter pylori* in peptic ulcer disease, and it is suggested that similar data will be required for acceptance of the MAP hypothesis in CD (El Zaatari *et al.*, 2001). A recent review of antibacterial therapy in CD concluded that there were some encouraging preliminary reports of multiple drug therapies, but that large controlled studies are needed before these therapies could be recommended (Hulten *et al.*, 2000b). Because of the limited efficacy of antimycobacterial agents in paratuberculosis and against other *M. avium* organisms, the questionable response of CD patients to antimycobacterial agents cannot be considered a strong argument against a possible role of MAP in CD. On the other hand, remission following treatment with broad spectrum agents is very tenuous evidence for any involvement of MAP in CD. Collectively, information from therapeutic studies of CD has provided little clear direction to either support or refute involvement of MAP in CD.

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