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Immunoproliferative small intestinal disease associated with *Campylobacter jejuni*

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A B S T R A C T

Background. Immunoproliferative small intestinal disease (IPSID, also known as alpha-chain disease) is a form of lymphoma that arises in small intestinal mucosa-associated lymphoid-tissue (MALT), and is associated with the expression of a monotypic truncated immunoglobulin α-heavy chain without associated light chain. Early stage IPSID responds to antibiotics, suggesting a bacterial origin. Previous attempts to identify a causative agent have failed.

Methods. Polymerase chain reaction (PCR), DNA sequencing, fluorescence *in situ* hybridization (FISH) and immunohistochemical studies were performed on intestinal biopsies obtained from a series of patients with IPSID.

Results. Analysis of frozen intestinal tissue obtained from an index patient with IPSID who exhibited a dramatic response to antibiotics revealed the presence of *Campylobacter* jejuni. A follow-up retrospective analysis of archival intestinal biopsies disclosed *Campylobacter* spp. in four of six additional patients with IPSID.

Conclusions. These results indicate an association between *Campylobacter* spp. and IPSID, and suggest that *C. jejuni* should be considered a candidate for addition to the growing list of human pathogens responsible for immunoproliferative states.

Immunoproliferative small intestinal disease (IPSID) is a mucosa-associated lymphoid-tissue (MALT) lymphoma characterized by infiltration of the bowel wall with a plasma cell population that secretes a monotypic truncated immunoglobulin alpha-heavy chain (aHC) lacking an associated light chain.[1-3] Lymphoid infiltration leads to malabsorption and protein-losing enteropathy. IPSID can be manifested by a spectrum of histopathologic changes, ranging from seemingly benign lymphoid infiltration to malignant diffuse large B-cell lymphoma. Since its initial description,[1] the majority of IPSID cases have been reported from the Mediterranean basin, Middle and Far East, and Africa. In the Middle East, the most common site of extra-nodal lymphoma is the gastrointestinal tract: IPSID accounts for around one third of gastrointestinal lymphomas.[4] The restricted geographic distribution of IPSID has led to the hypothesis that environmental factors may play a role in its pathogenesis. Remarkably, patients with early stage IPSID typically respond to antibiotics, suggesting that it may be triggered by bacterial infection.[5,6] This hypothesis has been reinforced by three additional observations made since the description of the response to antibiotics of IPSID: (i) the description of the pathological entity *MALT lymphoma*, which allowed to classify in the same histopathologic category the gastric MALT lymphoma and intestinal MALT lymphoma (IPSID), (ii) the discovery of the association between gastric MALT lymphoma and *Helicobacter pylori,* and (iii) the response of gastric MALT lymphoma to antimicrobial therapy aimed at eradicating *H. pylori* chronic infection. However, previous attempts to identify a causative agent of IPSID using standard culture methods have failed.[5-7] Given the similarities between *H. pylori*-associated gastric MALT lymphoma and IPSID, it has been proposed that *H. pylori* be considered as a potential causative agent of IPSID.[8] However, this has not been supported by the results of a retrospective study of 21 IPSID cases.[9]

Association between C. jejuni and IPSID

In order to identify a possible bacterial species associated with intestinal tissue from patients with IPSID, we decided to use an alternative method to the traditional culture-based techniques that had previously failed. This method was to be able to circumvent the putative non-cultivatable nature of microbial species and not to privilege any bacterial species *a priori.* We used a strategy based on the amplification and sequencing of the coding region of ribosomal ARN sequences (16S rDNA) from unfixed frozen intestinal tissue samples obtained from a patient with IPSID and having not received any previous antimicrobial therapy.

This method combines the sensitivity of the PCR technique and the specificity of the phylogenetic information contained in the gene sequence encoding the 16S rRNA subunit. Indeed, the percentage of identity between two 16S rDNA sequences directly correlates with the evolutionary distance separating the bacterial isolates from which they originate. The intraspecific variability of 16S rDNA sequences is considered lower to 1%. One thus considers that a bacterial isolate belongs to a given species if its 16S rDNA sequences shares more than 99% identity with the reference sequence of the considered species. The existence of very highly conserved sequences within the collection of 16S rDNA genes identified to date makes it possible to design so-called *universal* oligonucleotide primers hybridizing with 16S rDNA sequences common to all known bacterial species. These oligonucleotide primers thus make it theoretically possible to PCR-amplify 16S rDNA sequences from any bacterial species. The sequence of a given amplicon can be compared with the sequences published in public databases and the bacterial species to which it belongs determined. In the absence of significant identity with previously described bacterial species, a new species can be named and positioned in the Bacteria phylogenetic tree built from the known 16S rDNA sequences. The power of this approach was first illustrated by the identification of *Tropheryma whippelii*, the bacterial species associated Whipple's disease,[10] or of *Bartonella henselae*, the species associated with cat-scratch disease and bacillary angiomatosis.[11]

The use of this technique led to the identification of an association between IPSID and *Campylobacter* jejuni infection in an index patient for which frozen tissue sample was available.[12] A PCR assay using universal Bacterial 16S rDNA primers and DNA templates prepared from proximal small intestinal biopsies collected before initiation of antimicrobial treatment yielded amplicons encompassing the entire 16S rDNA gene that were identified as *C. jejuni* by sequencing. *C. jejuni*-specific PCRs were also positive in diseased intestinal samples, but no other enteropathogen was detected by PCR. The relevance of these results was confirmed by FISH and immunohistochemical analyses, which both detected *C. jejuni* in diseased small intestinal biopsies. Importantly, *C. jejuni* eradication with antimicrobial therapy was associated with rapid remission of IPSID (i.e. resolution of diarrhea and lymphoplasmacytic infiltration of the intestine, disappearance of the leukemic component and the monotypic truncated immunoglobulin alpha-heavy chain in serum).

Further support for an association between *C. jejuni* and IPSID is provided by four additional observations. Our retrospective study of a monocentric series of archival biopsies yielded four additional cases of *Campylobacter*-associated disease among six IPSID patients.[12] Another group published a case report of an individual who developed culture-positive *C. jejuni* diarrhea 2-3 days after the initiation of an antineoplastic chemotherapy for IPSID[13] - a sequence of events arguing for an exacerbation of a pre-existing *C. jejuni* infection triggered by the chemotherapy. Finally, IPSID occurs almost exclusively in developing countries where *C. jejuni* infection is hyperendemic, often chronic, and asymptomatic.[5, 14-16] Finally, antimicrobial regimens reported to be effective in treating IPSID are active against *C. jejuni.*[5-7]

Our FISH and immunohistochemical studies detected *C. jejuni* in small intestinal and gastric biopsies recovered from the index case, but not in her intestinal lumen. This result is in agreement with her negative stool cultures and may account for the failure of previous studies to link IPSID with this organism using standard culture-based studies.[5-7] Moreover, *C. jejuni* is microaerophilic and may exist in a viable but non-cultivatable state.[17]

Discussion

The results of our study do not allow us to conclude that *C. jejuni* is the only bacterial species associated with IPSID. Nonetheless, *C. jejuni* is a good candidate for addition to the growing list of human pathogens responsible for chronic infection that are also implicated in antigen-driven immunoproliferative states.[18–24] *C. jejuni* association with IPSID is reminiscent of the linkage between *H. pylori* infection and gastric MALT lymphoma.[18, 19] However, contrary to a previous case report,[8] and in agreement with a more recent study based on a series of 21 IPSID cases,[9] we found no evidence that incriminates *H. pylori* in the development of IPSID.

Association is not proof of causation. Nevertheless, association is usually the first step in proving the microbial cause of a disease.[10, 11, 25] With regard to the association between *H. pylori* and gastric lymphoma involving mucosa-associated lymphoid tissue (MALT), further evidence of causation has been provided by

the observation that eradication of *H. pylori* correlates with regression of the lymphoma (which is similar to what we report for *C. jejuni* and immunoproliferative small intestinal disease) and that *H. pylori*–specific T-cell clones stimulate B-cell proliferation in MALT lymphoma.[18, 19, 26] With regard to the association between *C. jejuni* and immunoproliferative small intestinal disease, several lines of evidence support a causal link,[12] but to demonstrate definitively that *Campylobacter* is the cause of immunoproliferative small intestinal disease (i.e., to fulfill Koch's postulate), several questions remain to be addressed. First, is *C. jejuni* detectable in the infected host in early stages of the disease? Second, is it possible to cultivate *C. jejuni* from the diseased tissue? Third, can *C. jejuni* trigger the disease in an animal model? Fourth, if so, can *C. jejuni* be isolated from the diseased animal? We are currently working on these issues.

As for *H. pylori* in the stomach, demonstration of long-term *C. jejuni* intestinal persistence is important for incriminating this bacterial species in IPSID development. It should be noted that *C. jejuni* epidemiology in developing countries, in which IPSID is exclusively observed, sharply contrasts with that in developed countries[27] and may be an important clue for understanding the putative causal role of *C. jejuni* in IPSID development. Up to 15% of asymptomatic children in developing countries carry *Campylobacter* organisms in their stools, whereas in developed countries, fecal *Campylobacter* is present in less than 0.5%[27] *C. jejuni* is also responsible for long-term fecal shedding in developing countries: in a longitudinal study conducted in rural South Africa involving 73 apparently healthy school children, *C. jejuni* was isolated intermittently from 6 children for at least 9 months and from 3 children for more than 1 year.[28] Nothing is known about the ability of *C. jejuni* to establish a persistent colonization of the small intestinal mucosa without concomitant detectable fecal shedding, although this bacterial species may also exist in a viable but non-cultivatable state.[17] Future studies need to focus on the extent of the asymptomatic and long-term intestinal carriage (which is already known to be highly prevalent in poultry; a *C. jejuni* reservoir in close contact with the rural population in developing countries), its putative correlation with prelymphomatous lamina propria infiltration, and ultimately its causal relationship with IPSID. The host factors underlying *C. jejuni* persistence also require investigation. *C. jejuni* has been shown to persist in Peyer's patches and mesenteric lymph nodes in a gnotobiotic mouse model,[29] and to secrete a toxin, CdtB, that mediates DNA damage.[30] These properties could be critical in the pathogenesis of IPSID. *C. jejuni* is able to elicit a strong IgA mucosal response, and chronic *C. jejuni* infection leads to a sustained stimulation of the mucosal immune system.[15] This persistent stimulation could eventually lead to expansion of IgA-secreting clones, and selection of an αHC-secreting clone that has escaped antibody-antigen Fc-dependent down-regulation.[31, 32] Eradication of the antigenic source with antimicrobial treatment may stop proliferation of the ·HC-secreting lymphoplasmacytic population.

As with *H. pylori*-associated gastric MALT lymphomas, understanding the role of *C. jejuni* in the pathogenesis of IPSID will require further mechanistic analysis, and the development of suitable animal models. The association between *C. jejuni* and IPSID may allow improved diagnosis, management, and prevention of this disease in at least a subset of patients.

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