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# An In-Depth Study of Crohn's Disease in Two French Families

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***Background:*** **Two French families were investigated. In the first a husband, wife, and 4 children had Crohn's disease; in the second 7 of 11 children had the disease. There was no history of Crohn's disease in antecedent generations and no linkage to HLA haplotypes.** ***Methods:*** **Methods included family interviews; review of medical records, radiographs, and pathology slides; serology; selective stool culture; enzyme-linked immunosorbent assay for fecal viral detection; and immunocytochemistry.** ***Results:*** **In both families multiple cases occurred among siblings in 7–13-month periods. There appeared to be a 4–8-year recurrence of new disease in both families. Radiographs showed a remarkable similarity in the pattern of disease, confined to distal ileum and cecum, in the members of family 1. Examination for pathology showed granulomas in all 8 patients for whom tissues were available. Acid-fast organisms or *Campylobacter*-like organisms were not found in tissue sections, and immunocytochemistry was negative for mycobacteria and *Yersinia*. Stool cultures were negative for mycobacteria, *Yersinia*, and *Mycoplasma*. Torovirus and coronavirus antigens were not found in stool. Serology was negative for antibodies to *Brucella*, *Yersinia*, influenza, and three enteropathogenic viruses of animals.** ***Conclusions:*** **The circumstances and data suggest that an infectious microorganism is responsible for these clusterings of Crohn's disease.**

In 1989, Darchis et al. briefly reported the occurrence of Crohn's disease in a married couple and all of their four children.[1] This was followed by a HLA haplotype linkage study of the above kindred, one other French family, and three Belgian families with a high incidence of Crohn's disease.[2] The first study concluded that the occurrence of three cases within 10 months in members of a family living in the same house was evidence for an environmental etiology; the second study found an absence of HLA linkage in familial Crohn's disease. In view of the rarity of such high frequencies of Crohn's disease among family members, we initiated an in-depth study of the two French families.

## Patients and Methods

Family 1 consisted of a 52-year-old mother and 4 children, aged 20–30, all of whom had Crohn's disease. The father died of complications of Crohn's disease in 1986. Family 2 consisted of a 59-year-old mother and 11 children, aged 22–41 years, 7 of whom had Crohn's disease. The father in family 2 died of unrelated disease in 1986. Medical records, surgery notes, radiographs, endoscopy notes, and pathology slides of biopsy specimens and resections were assembled and reviewed. Photographs were made of representative radiographic changes, and paraffin blocks of all pathology specimens were obtained for special staining and immunocytochemistry.

Three of the authors (H.J.V., J.F.C., and A.C.) met with each family in the presence of their private doctors and conducted a round table interview, at which time questions regarding onset and status of disease and risk factors were explored. We met with family 1 in their private doctor's office and subsequently visited their home. We met with family 2 at their home, where all but two siblings (both with Crohn's disease) were present as well as an affected cousin. All brought recent stool specimens, which they had collected (5–10 mL) into 50-mL capped plastic centrifuge tubes containing 30 mL of Dulbecco's modified Eagle medium, with penicillin (200,000 U/L) and gentamicin (250 mg/L) plus 5 mL of dimethyl sulfoxide (DMSO). At each family meeting a nurse who traveled with us obtained blood from each family member. Whole blood was used for immediate typing, and sera and feces were frozen (−70°C) for later studies.

***Abbreviations used in this paper:*** **CLOs, *Campylobacter*-like organisms; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay.**


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**Table 1.** Historical and Epidemiologic Data of Family 1

| | IA (Father) | IB | IC | ID | IE | IF (Mother) |
|---|---|---|---|---|---|---|
| Birthdate | 12/02/33 | 05/20/59 | 07/02/65 | 05/08/68 | 07/02/69 | 01/08/38 |
| Current age (*yr*) | Deceased | 30 | 24 | 21 | 20 | 52 |
| Sex | M | M | M | F | M | F |
| Occupation | Welder | Stock clerk | Unemployed | Unemployed | Unemployed | Housewife |
| Current condition | Deceased 11/28/86 | Well 3 yr | Well 3yr | Well 1 yr | Well 1 yr | Well 8 mo |
| Blood group | A+ | O+ | O+ | O+ | O− | O− |
| HLA | AB | BC | BD | BC | AD | CD |
| First symptom | Anal abscess | Anal abscess | Anal fissure | Diarrhea, weight loss | Anal fissure | Asthenia, weight loss |
| Mo 1st symptom | January | May | December | May | May | December |
| Yr 1st symptom | 1970 | 1974 | 1974 | 1982 | 1983 | 1988 |
| Age 1st symptom (*yr*) | 37 | 15 | 9 | 14 | 14 | 50 |
| Childhood TB test results | − | + 1972<br>+ 1974<br>− 1981 | − | + 1976 | + 1987 | − |
| TB treatment | − | 7 mo 6/74-1/75 | − | − | − | − |
| Breast fed | | + | − | − | − | + |
| Childhood vaccinations | | Diphtheria<br>Tetanus<br>BCG | Diphtheria<br>Tetanus<br>Polio<br>BCG | Diphtheria<br>Tetanus<br>Polio<br>BCG | Diphtheria<br>Tetanus<br>Polio<br>BCG | Smallpox |
| Childhood diseases | | Measles<br>Whooping cough<br>Varicella<br>Otitis | Measles | Measles<br>Whooping cough<br>Scarlatina | Measles<br>Varicella<br>Otitis | Mumps |
| Closest kin or sibling | − | − | − | − | − | |
| Slept with | IF | IC 1969–1975, age 10–16 | IB 1969–1975, age 4–10 | Roomed with IE | Roomed with ID | IA |
| Shared eating utensils | IF | − | − | − | − | IA |
| Used cigarettes | − | 3 years | − | − | − | − |

NOTE. Patients are arranged from left to right in the order of onset of disease from 1970 to 1988.

All H&E-stained pathology slides were reviewed. Later, the paraffin blocks were recut and in some instances re-embedded, and new H&E-stained slides were made, a total of 18 for the 5 patients in family 1 and 40 for 5 patients in family 2. Selected sections, 1–3 per patient, usually including representative lesions and regional lymph nodes when available, were stained by the Ziehl–Neelsen method for acid-fast bacteria and a modified Steiner's silver method[3] for *Campylobacter*-like organisms (CLOs),* *Borrelia*, spirochetes, and *Rickettsia*. Unstained sections of the same selected blocks were mounted on glass slides that had been coated with poly-L-lysine, then processed by a labeled avidin-biotin immunoperoxidase method using primary antibodies directed against mycobacteria (*Mycobacterium paratuberculosis, Mycobacterium bovis, and Mycobacterium duvali*) and *Yersinia enterocolitica*.[5] Positive control tissues were processed along with each run of slides that were specially stained or treated immunocytochemically.

*CLOs were originally reported in swine as *Campylobacter sputorum* subsp. *mucosalis;* recent DNA homology studies indicate a close relationship to the desulfovibrios.[4]

Stool specimens of 10 patients (5 in family 1 and 5 in family 2) and 4 unaffected siblings and the mother of family 2 were thawed, shaken, aliquoted, and then tested for the presence of *Mycobacterium tuberculosis, M. paratuberculosis, Y. enterocolitica, Mycoplasma* sp., group A rotavirus, Breda virus (a torovirus), and bovine coronavirus. Lowenstein–Jensen medium was used for *M. tuberculosis* culture; a centrifugation concentration method and Herrold's egg yolk medium with mycobactin J and 4.1 g/L pyruvate for *M. paratuberculosis;*[6] cold enrichment (25°C) and Schiemann CIN medium (Difco Labs., Detroit, MI) for *Y. enterocolitica;*[7] and Fortified Commercial Medium, G199H and Sp4 for *Mycoplasma.*[8–10] Enzyme-linked immunosorbent assay (ELISA) methods for antigen capture were used for the viruses.[11–13]

Sera from the same 10 patients and 5 unaffected family members were aliquoted and distributed for antibody determinations. An immunoblot method determined specific immunoglobulin A (IgA) and IgG antibodies to plasmid encoded virulence proteins (Yops 2a, 2b, 4a, and 5 and the V-antigen) of *Y. enterocolitica* and *Yersinia pseudotuberculosis;*[14] tube agglutination was used for *Brucella abortus,* which also recognizes *Brucella suis* and *Brucella melitensis,*[15] and mercap-

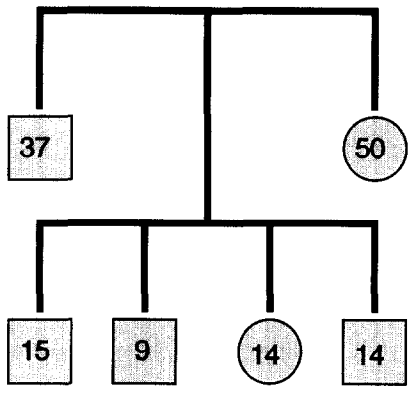


**Figure 1.** Familial Crohn's disease in family 1. Numbers indicate age at onset.

toethanol tube agglutination for *Brucella canis*.[16] A complement-fixation test measured antibody to influenza A and B,[17] and blocking ELISA was used to detect Breda virus and bovine coronavirus.[18] Serum neutralization measured antibody levels to several other enteric animal viruses, the agents of malignant catarrhal fever (a cell-associated herpes virus),[19] bovine virus diarrhea (a pestivirus),[20] and equine viral arteritis (an arterivirus).[21]

## Results

### History and Epidemiology

Details regarding the onset and character of Crohn's disease, as well as pertinent historical data for family 1, are summarized in Table 1 and Figures 1 and 2. Crohn's disease occurred first in the father, at age 37, in 1970. Two sons, aged 15 and 9, developed Crohn's disease during a 7-month period in 1974; they shared a bed for 6 years from 1969–1975, and both had perianal disease as their first symptom. In 1982–1983, a second pair of siblings, a son and a daughter, developed Crohn's disease at age 14; they had shared a room together as youngsters. Subsequently, in 1988, the mother, aged 50, contracted Crohn's disease. In four of the six cases in this household, disease began as anal abscess or fissure. Extraintestinal disease occurred in only one patient, the mother, who experienced aphthous stomatitis, conjunctivitis, arthritis, and erythema nodosum. The patients were cared for with medical measures (corticosteroids, sulfasalazine, metronidazole, 5-aminosalicylic acid, isoniazid, ethambutol, and rifampin), and later three of the six required surgical resection.

The home of family 1 was a small modest cottage (close quarters for a family of six) in a town in Northern France (Valenciennes; population 39,276). The rooms were small but tidy and clean; kitchen and toilet facilities were modern. The family denied household contact with dogs, cats, birds, or other animals. They denied consumption of uncooked clams, oysters, mussels, wild game, beef, horse meat, pork, and vegetables. They had consumed unpasteurized milk commonly and uncooked fish rarely. They did not have a garden. They drank bottled mineral water and had an adequate septic system (septic tank from 1958 to 1968, followed by city sewers thereafter). They denied travel to foreign countries, contact with chronically ill persons, food poisoning events, and other diseases that might have coincided with their bouts of Crohn's disease. The father's sister died of pulmonary tuberculosis in 1954; however, the father had no evidence of tuberculosis.

Details regarding family 2 are summarized in Table 2 and Figures 2 and 3. Seven of 11 children had Crohn's disease. It began in 1971 and 1972 when 4 children, aged 22–13, developed symptoms within a 10-month period. Three of the four had abdominal pain and diarrhea and the fourth had an anal abscess. Three of the 4 siblings were boys who had shared a bed for some overlapping segments of their childhood. Six years after the first cluster of cases, another sister developed Crohn's disease (1978); 6 years later (1983–1984), 2 additional siblings were affected. In contrast to family 1, in family 2, 6 of the 7 siblings with Crohn's disease presented with abdominal pain and diarrhea. None had extraintestinal disease. The patients were cared for with medical measures (corticosteroids, sulfasalazine, metronidazole, 5-aminosalicylic acid, chloraminophene, azathioprine, antibiotics, ethambutol, and rifampin), and ultimately 5 of the 7 required surgical resection.

The home of family 2 was similar to that of family 1, a small modest cottage (close quarters for a family of

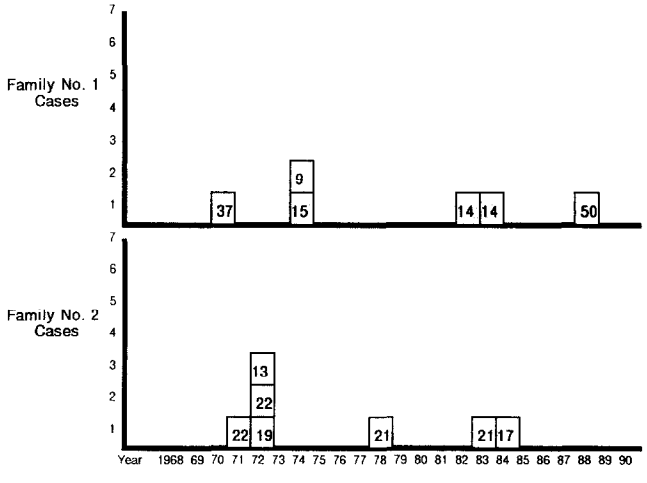


**Figure 2.** Onset of Crohn's disease in families 1 and 2. Numbers in *boxes* indicate age at onset.

**Table 2.** Historical and Epidemiologic Data of Family 2 (Affected Siblings)

| | IIA | IIB | IIC | IID | IIE | IIF | IIG |
|---|---|---|---|---|---|---|---|
| Birthdate | 1949 | 1953 | 1950 | 1959 | 1957 | 1962 | 1967 |
| Current age (*yr*) | 41 | 36 | 39 | 30 | 33 | 28 | 22 |
| Sex | F | M | M | M | F | F | M |
| Occupation | Housewife | Unemployed | Mechanic | Dental technician | Unemployed | Unemployed | Unemployed |
| Current condition | Well 8 yr | Well 1½ mo | Well 12 yr | Well 2 yr | Well on medication | Pain in morning | Has symptoms |
| Blood group | B+ | O+ | O+ | B+ | O− | AB− | AB+ |
| HLA | BC | BC | BC | AC | AC | BC | BD |
| First symptom | Abdominal pain, diarrhea | Abdominal pain, diarrhea | Abdominal pain, diarrhea | Anal abscess | Abdominal pain, diarrhea | Abdominal pain | Diarrhea |
| Mo 1st symptom | December | April | May | September | March | November | August |
| Yr 1st symptom | 1971 | 1972 | 1972 | 1972 | 1978 | 1983 | 1984 |
| Age 1st symptom (*yr*) | 22 | 19 | 22 | 13 | 21 | 21 | 17 |
| Childhood TB test results | + | + | + | + | − (1978) | | + |
| TB treatment | − | − | Streptomycin 6 wk | 8 mo | 5 mo | + | − |
| Breast fed | + | + | + | − | − | − | − |
| Childhood vaccinations | Diphtheria<br>Tetanus<br>Small pox<br>BCG | Diphtheria<br>Whooping cough<br>BCG | Diphtheria<br>Tetanus<br>Typhoid<br>BCG | Diphtheria<br>Tetanus<br>Typhoid<br>BCG | Diphtheria<br>Tetanus<br>Polio<br>BCG | Diphtheria<br>Tetanus<br>Polio<br>BCG | Diphtheria<br>Tetanus<br>Typhoid<br>BCG |
| Childhood diseases | Varicella<br>Measles<br>Mumps | Varicella<br>Measles<br>Mumps<br>Hepatitis | Varicella<br>Measles<br>Mumps<br>Hepatitis | Varicella<br>Measles<br>Mumps | Varicella<br>Measles | Varicella | Varicella<br>Measles<br>Mumps |
| Closest kin or sibling | III | IIC | IIB | − | − | IIJ<br>IIH | IIK |
| Slept with | III<br>IIE | IIC | IIB | IIC<br>IIB | IIA<br>III | IIH<br>IIJ | IIK |
| Shared eating utensils | − | − | − | − | − | − | − |
| Used cigarettes | − | + | + | + | + | − | + |

NOTE. Patients are arranged from left to right in the order of onset of disease from 1971 to 1984.

13) in a small village (Lecluse; population, 1,674) 35 km distant from family 1. The interior was tidy and clean and the kitchen and toilet facilities modern. The family denied household contact with dogs, cats, birds, or other animals. They denied consumption of uncooked clams, oysters, mussels, beef, horse meat, pork, and fish. They had consumed uncooked duck and goose rarely, and unpasteurized milk commonly. They raised their own fresh vegetables in a garden but denied use of night soil. They drank bottled water and some farm spring water and had an adequate septic system (septic tank up to 1980, then city sewage system). They denied travel to foreign countries, contact with chronically ill persons, food poisoning events, and other diseases that might have coincided with their bouts of Crohn's disease. The mother in this family had had surgery for ileocecal tuberculosis in 1955, and one of the patients, IIC, was said to have had lymph node tuberculosis at age 11, in 1961 (11 years before the onset of his Crohn's disease). A sister of the father of this family had ulcerative colitis in 1980 and again in 1990. A cousin (a 35-year-old woman), who attended the interview, also had Crohn's disease.

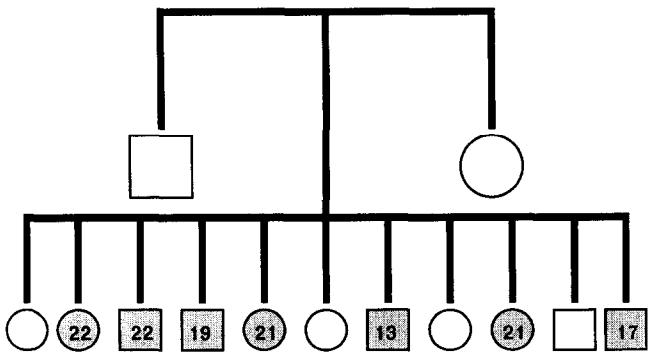


**Figure 3.** Familial Crohn's disease in family 2. Numbers indicate age at onset.

## Radiology

Radiographic features of the six patients from family 1 and seven from family 2 are illustrated in Figures 4–9 and summarized in Table 3 and 4. The patients in family 1 had strikingly similar location and extent of disease. Five of the six had cobblestoning of the surface and marked reduction of lumen diameter of terminal ileum, accompanied by significant contraction of the cecum, referred to as "*retraction en bourse.*" They appeared to have identical disease. Three of the

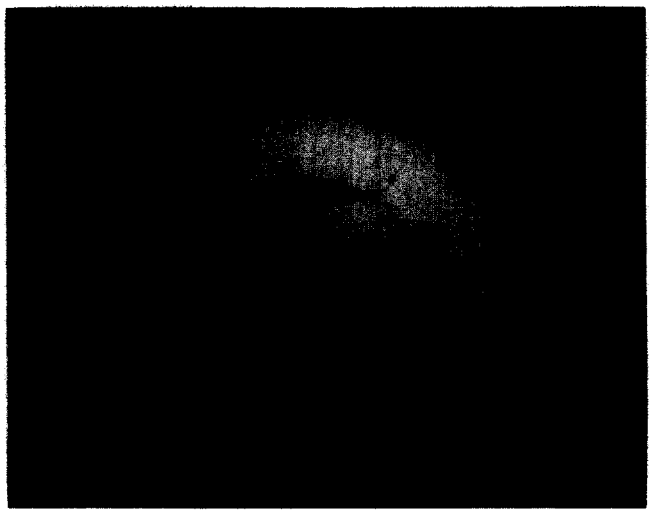

**Figure 4.** Disease of the distal ileum, with fistulas, in patient IA 5 years after onset of symptoms.

five had fistulas. One of the five subsequently developed a 17-cm segment of disease in the left colon, and the mother had ileal involvement exclusively.

In family 2, for one reason or another, retraction of the cecum was not often documented; all seven patients had terminal ileal disease. Three of the seven had fistulas. One patient, IIB, presented initially with diffuse mucosal colitis and may have had ulcerative colitis as well as Crohn's disease. Colonic involvement in two other siblings was clearly segmental.

## Pathology

Pathological features that are important in Crohn's disease are summarized in Table 5. Resection specimens were available from three of the six members of family 1 and from five of seven patients in family 2. Forty tissue blocks were recut and re-examined, 37 from the resection specimens and 3 from biopsy specimens. Lesions were essentially the same in the patients of both families. Mucosal ulcerations occurred in all 8 resection specimens, fissures or fistulas in 6 of the 8, follicular lymphocytosis (submucosal and transmural) in 8 of 8, lymphocytic lymphangitis in 5 of 8, granulomas in 8 of 8, and caseation necrosis in none of 8. Lymph nodes were not available from the three patients in family 1; granulomas were present in the lymph nodes of two of five patients from family 2. All of the eight patients who had undergone resection had the histological criteria for a diagnosis of Crohn's disease, and there were no points of disagreement between these new descriptions of the lesions and those originally rendered by hospital and private pathologists years earlier. Caseation necrosis was not present in any of the 40 slides examined, and a Ziehl–Neelsen stain applied to 16 selected blocks failed to reveal any acid-fast bacteria. A modified Steiner's stain failed to show CLO, spirochetes, or rickettsia in 10 selected early mucosal lesions. Immunocytochemistry failed to show mycobacteria or *Yersinia* in 16 blocks.

## Microbiology

Bacteriologic culture of stool specimens from 10 patients and 5 unaffected family members was negative for *M. tuberculosis* and *M. paratuberculosis* after 6 months' incubation. Cold enrichment and cultivation on Schiemann CIN at 25°C failed to grow *Y. enterocolitica*. *Mycoplasma* media were either overgrown with bacteria or failed to grow *Mycoplasma*. (The addition of antibiotics and the freezing of stools in transport media may have hampered attempts to cultivate *mycobacteria, Yersinia,* and *Mycoplasma.*) Antigen capture ELISA for Breda virus and bovine coronavirus were routinely

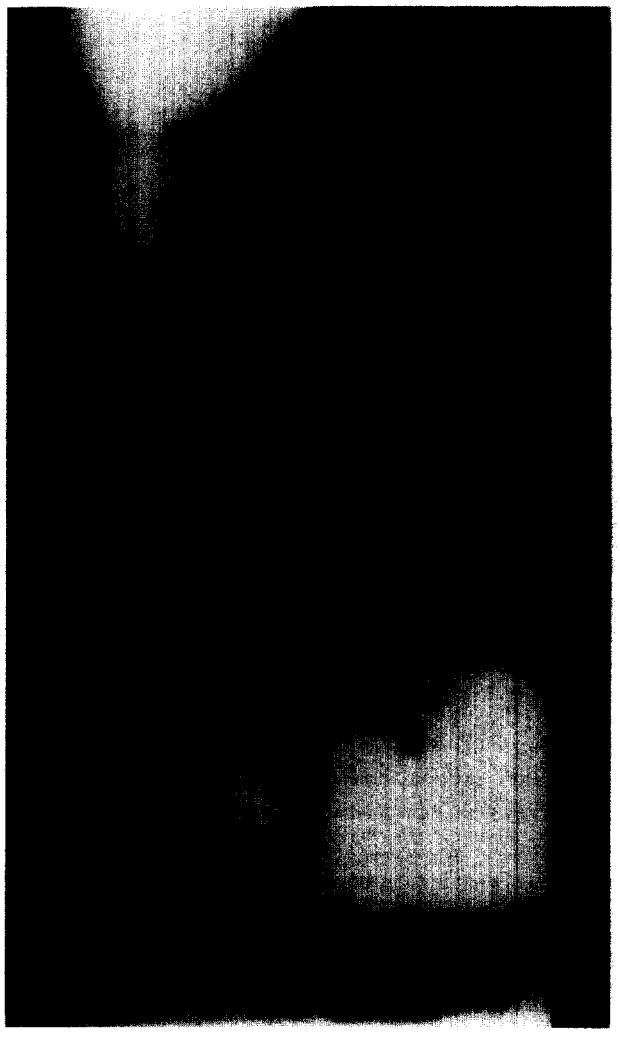

**Figure 5.** Disease of the distal ileum, cecum, and proximal right colon in patient IB 1 month after onset.

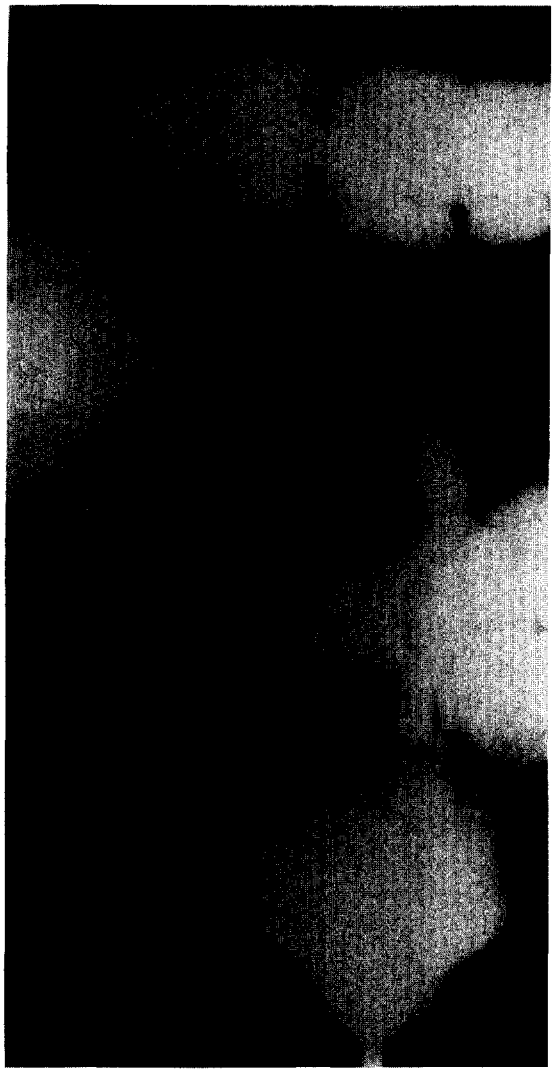

**Figure 6.** Disease of the distal ileum with circumferential involvement of the cecum in patient IC 2 years 8 months after onset.

negative. One of the 15 stool specimens was positive for human group A rotavirus antigen, a specimen from patient IIG, in family 2; the other 14 were negative.

### Serology

None of the 10 patients and 5 unaffected family members showed specific IgA or IgG antibodies against plasmid encoded virulence proteins of *Y. enterocolitica* and *Y. pseudotuberculosis*. Tube agglutination testing for *B. abortus, B. suis,* and *B. melitensis* and microagglutination testing for *B. canis* were uniformly negative. Complement fixation testing for antibodies to influenza A and B revealed no elevated titers. Test results for antibodies to, or antibodies that might cross-react with the agents of malignant catarrhal fever, bovine virus diarrhea, and equine viral arteritis were uniformly negative. One of the 10 patients had an elevated anticoronavirus titer (>1280 in patient IIB) that was fourfold greater than any seen in the other 9 patients and the 5 unaffected family members. A blocking ELISA method for the demonstration of antibody to Breda virus (or related toroviruses) discovered elevated titers in 6 of 10 patients and in 2 of 5 unaffected family members.

## Discussion

By all accounts, history, physical findings, radiography, and pathology, the 6 patients in family 1 and the 7 in family 2 had Crohn's disease. The special stains, immunocytochemistry, microbiology, and serology indicate that neither tuberculosis nor *Y. enterocolitica* infection complicated these cases. The families thus represent the most concentrated clusterings of Crohn's disease ever reported, an entire family of 6 in one small home and 7 of 11 children in another. Although HLA haplotype linking is not the only way to test for a genetic basis for a disease, when it was performed in these two families, no linkage was found.[2] There is no history of Crohn's disease in antecedent generations in either family. Extensive pedigree studies are currently underway and will be published separately; no consanguinity between husband and wife has been recognized through five generations in either family or between families dating back to 1825 in family 1 and 1800 in family 2 (A. Chaventre, Institute National d'Etudes Demographiques, Paris).

The coincidence of cases in these families, in time and in bed partners, and the overwhelming similarity of distribution and extent of disease in family 1 suggest

**Figure 7.** Disease of the distal ileum, cecum and proximal colon in patient ID 6 years 9 months after onset.

**Figure 8.** Disease of the distal ileum with circumferential involvement of the cecum in patient IE 5 years after onset.

an infectious etiology. In family 1, two cases occurred in a 7-month period (1974) and two others in a 13-month period (1982–1983); in family 2, 4 of 11 children developed Crohn's disease in a 10-month period (1972–1973) and 2 others in a subsequent 10-month time frame (1983–1984). In two of these four aggregates, the children affected were bed partners before and at the time they developed Crohn's disease; in one other aggregate, the children shared a room. In each of these four aggregates, the children were not twins and they were not of the same age. In family 1, nonsanguinous husband and wife contracted Crohn's disease.

The uniformity of ileal and cecal disease in family 1 is akin to that which might be expected had a uniform dose of an enteric pathogen been given to a genetically uniform group of experimental subjects, e.g., a litter of mice or piglets. There is the suggestion in this family that some microbial agent specifically targeted the terminal ileum and cecum, segments we know to be abundant in lymphoid follicles and M cells.

Figure 2 shows an apparent 4–8-year pattern of resurgence of Crohn's disease in both families. This pattern is certainly not suggestive of the emergence of an inherited disease. On the contrary, it may reflect an increase in environmental risk in the home, or alternatively an age susceptibility to an ever present exposure. In family 1 siblings acquired their diseases at ages 9–15, in family 2 at ages 13–22. The apparent long latent period between new cases among household contacts is similar to that described by Bennett et al. in spouses with inflammatory bowel disease (IBD).[22] When one partner had IBD before marriage, symptoms appeared in the spouse an average of 6.4 years after marriage; when neither had IBD before marriage, the second spouse developed disease 6.8 years after the first. Earlier, Reilly and Robinson noted a long latent period in four cases of Crohn's disease that occurred in unrelated adult women, aged 21–30, who had been close high school friends for 7 years, from the ages of 11 through 18.[23] Allen et al. described a clustering of 12 patients with Crohn's disease in Gloucestershire, England.[24] In the small parish of Blockley, from

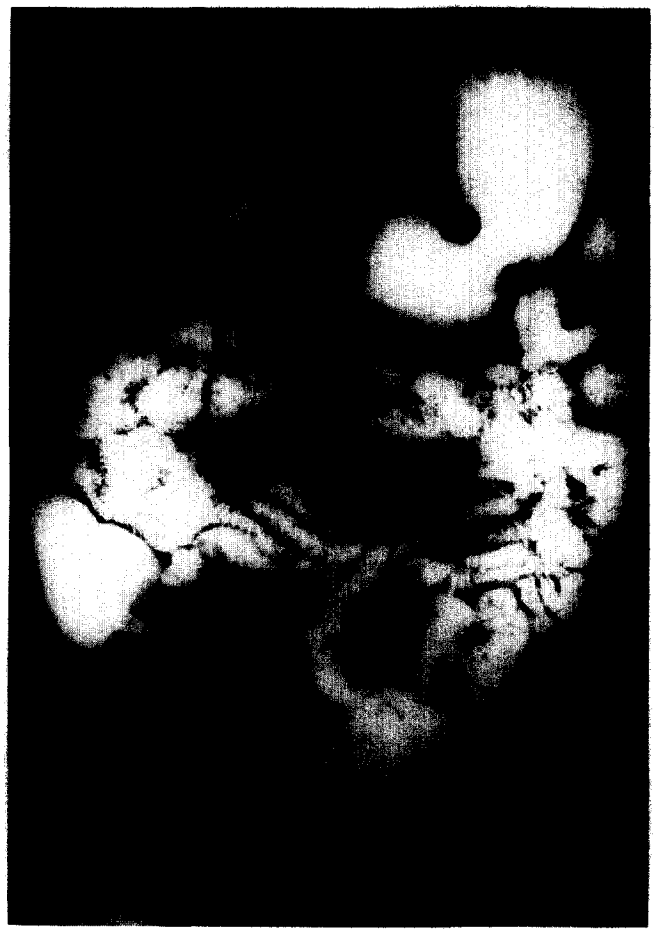

**Figure 9.** Long segment (25 cm) disease of the ileum in patient IF 2 months after onset.

**Table 3.** Historical and Epidemiologic Data of Family 2 (Unaffected Members)

| | IIH | III | IIJ | IIK | IIL (Mother) | IIM (Father) |
|---|---|---|---|---|---|---|
| Birthdate | 1958 | 1948 | 1961 | 1965 | 1931 | |
| Current age (*yr*) | 31 | 42 | 29 | 24 | 59 | |
| Sex | F | F | F | M | F | M |
| Occupation | Unemployed | Unemployed | Hospital aid | Forklift driver | Housewife | Factory worker |
| Current condition | – | – | – | – | – | Deceased 12/01/86 |
| Blood group | B– | A+ | A– | O+ | A+ | B– |
| HLA | BC | ABC | AD | BD | CD | AB |
| First symptom | | | | | | |
| Mo 1st symptom | | | | | | |
| Yr 1st symptom | | | | | | |
| Age 1st symptom (*yr*) | | | | | | |
| Childhood TB test results | | + | + | + | – | |
| TB treatment | – | + | – | – | + | – |
| Breast fed | – | + | – | – | + | |
| Childhood vaccinations | Diphterhia<br>Tetanus<br>BCG | Diphtheria<br>Tetanus<br>BCG | Diphtheria<br>Tetanus<br>BCG | Diphtheria<br>Tetanus<br>BCG | – | |
| Childhood diseases | Varicella<br>Measles<br>Mumps | Varicella<br>Measles<br>Mumps | Varicella | Varicella<br>Measles<br>Mumps | Measles<br>Mumps | |
| Closest kin or sibling | IIF<br>IIJ | IIM | IIH<br>IIF | IIG | – | – |
| Slept with | IIF<br>IIJ | IIM<br>IIE | IIH<br>IIF | IIG | – | |
| Shared eating utensils | – | – | – | – | – | – |
| Used cigaretts | – | – | – | + | – | + |

1968–1982, there occurred almost one new case per year in a population of approximately 2000, an incidence of 36.5 per 100,000 per year. The latent period for Crohn's disease after leaving this community was 3–5 years.

In our search for potential pathogens in these families, we eliminated some important agents from consideration, e.g., mycobacteria, *Yersinia,* and *Brucella,* and did not give consideration to others, such as *Campylobacter, Salmonella,* or Shigella, because they more than likely would have produced other symptoms or would have been detected in the original patient workups. Although the selective bacteriologic culture for *M. paratuberculosis* was conducted on feces (not on tissues as

**Table 4.** Extent of Crohn's Disease as Shown Radiographically

| | Ileum | Cecum | Right colon | Transverse colon | Left colon | Anal region |
|---|---|---|---|---|---|---|
| Family 1 | | | | | | |
| IA (father) | 8 cm F | R | | | | Abscess |
| IB | 18 cm | R | 8 cm F | | 17 cm | Abscess |
| IC | 12 cm F | R | | | | Fissure |
| ID | 15 cm | R | 6 cm | | | |
| IE | 7 cm | R | | | | Fissure |
| IF (mother) | 25 cm | | | | | |
| Family 2 | | | | | | |
| IIA | 20 cm F | | | | | Fistula |
| IIB | 20 cm | 10 cm F | Diffuse MC | Diffuse MC | Diffuse MC | Fistula |
| IIC | 30 cm | | | | | Abscess |
| IID | 20 cm | | | Diffuse MC F | 10 cm MC | Abscess |
| IIE | + | | + | | + | |
| IIF | 14 cm | | | | | Ileopsoas abscess |
| IIG | 14 cm | | | | | |

NOTE. Radiographs of IIE were not available for review. Lesions of the anal region, although not demonstrated radiographically, are included for completeness. F, fistulas present; R, cecal "retraction en bourse"; MC, mucosal disease.

**Table 5.** Selected Pathological Elements in Familial Crohn's Disease

| | Ulcers | Fissures | Abscess | Fistulas | Follicular lymphocytosis | Lymphangitis | Granulomas | Caseation necrosis | Lymph node granulomas |
|---|---|---|---|---|---|---|---|---|---|
| Family 1 | | | | | | | | | |
| IA (father) | + | | | | + | + | + | – | ND |
| IB | + | | | + | + | | + | – | ND |
| IC | + | | | + | + | | + | – | ND |
| ID (biopsy) | | | | | | | | – | ND |
| IE (ND) | | | | | | | | | |
| IF (mother) (biopsy) | + | | | | + | | | – | ND |
| Family 2 | | | | | | | | | |
| IIA | + | | + | + | + | + | + | – | – |
| IIB | + | + | | + | + | + | + | – | – |
| IIC (ND) | | | | | | | | | |
| IID | + | | | | + | | + | – | + |
| IIE (ND) | | | | | | | | | |
| IIF | + | + | | | + | + | + | – | + |
| IIG | + | + | + | + | + | + | + | – | – |

ND, not done.

in previous studies) that had been frozen and was terminated after 6 months, we failed to find acid-fast organisms or immunostained mycobacteria in the lesions. We also tested for evidence of other pathogens that produce enterocolitis or regional enteritis in animals, i.e., pestiviruses, toroviruses, and CLO.[25] Antibodies to pestiviruses have been shown in children with enteric disease[26] and in animal handlers and veterinarians;[27] none occurred in these patients. Normally human sera do not block the signal in the Breda virus ELISA; therefore, the titers seen in six patients and two unaffected family members are remarkable. However, we found no evidence of Breda virus antigen in the stools of these individuals. Lamouliatte et al. reported aggregation of a bovine Breda virus with convalescent serum from a human patient with diarrhea as tested by immunoelectron microscopy.[28] With the exception of this one case, the patients described here are the first humans showing serologic evidence of torovirus infection, even though several investigators have studied that possibility.[29,30] CLOs were never demonstrated.

We tested for a possible serologic response to influenza A and B because of a reported association with flare-ups of IBD[31] and because early-in-life viral infections, especially influenza, represent a risk factor for Crohn's disease (odds ratio, 18.0).[32] Our patients showed no elevated titers; however, acute and convalescent phase sera were not available for comparison.

During the summer of 1991, we became aware of gastrointestinal illness in the wife of one of the sons in family 1. Pedigree analysis showed no evidence of consanguinity between this spouse and family 1 or with family 2 over three generations, dating to 1880. Now a woman of 30 years of age, she met patient IB in 1977 (at age 16), 3 years after his first symptoms. They were married in 1983, and there is evidence that her first intestinal symptoms were noted in September 1984 (at age 23). A diagnosis of Crohn's disease was made in September 1991, for which surgical resection was performed October 24, 1991. Histopathology confirmed the diagnosis. The other 3 children in family 1 are unmarried; there are 3 grandchildren. In family 2, 9 of the children were married, and thusfar no spouse is affected; there are 17 grandchildren.

The circumstances and data reported here suggest that a transmissible infectious agent is at work in these two clusters of Crohn's disease; however, we have not yet been successful in identifying one. Additional genetic studies have not been neglected. Pedigree reviews will be completed and DNA analyses will follow.

## References


1. Darchis I, Colombel JF, Cortot A, Devred M, Paris JC. Crohn's disease in a married couple and their four children (letter). Lancet 1989;1:737.
2. Colombel JF, Guillemot F, VanGossum A, Dufosse F, Cortot A, Dupont E, Paris JC. Familial Crohn's disease in multiple siblings: no linkage to the HLA system. Gastroenterol Clin Biol 1989;13:676–678.
3. Swisher BL. Modified Steiner procedure for microwave staining of spirochetes and nonfilamentous bacteria. J Histotech 1987;4:241–243.
4. Gebhart CJ, Lin GF, McOrist SM, Lawson GHK, Murtaugh MP. Cloned DNA probes specific for the intracellular *Campylobacter*-like organisms of porcine proliferative enteritis. J Clin Microbiol 1991;29:1011–1015.
5. Cartun RW, Van Kruiningen HJ, Pedersen CA, Berman MM. An immunocytochemical search for infectious agents in Crohn's disease. Mod Pathol (in press).
6. Whitlock RH, Rosenberger AE, Spencer PA. Laboratory culture techniques for Johne's disease: a critical evaluation of contami-

nation and incubating times. Proc 93rd Ann Mtg US Animal Health Assoc 1989;383–386.
7. Schiemann DA. Synthesis of a selective agar medium for *Yersinia enterocolitica*. Can J Microbiol 1979;25:1298–1304.
8. Macy ML. Tests for mycoplasmal contamination of cultured cells as applied at the ATCC. Tissue Culture Association Manual 1980;5:1151–1155.
9. Gabridge MG, Singer SE, Esposito RA. Cultivation of mycoplasmas in a modified tissue culture medium. Appl Environ Microbiol 1976;31:986–989.
10. Tully JG, Rose DL, Whitcomb RF, Wenzel RP. Enhanced isolation of *Mycoplasma pneumoniae* from throat washings with a newly modified culture medium. J Infect Dis 1979;139:478–482.
11. Herrmann JE, Blacklow NR, Perron DM, Cukor G, Krause PJ, Hyams JS, Barrett HJ, Ogra PL. Monoclonal antibody enzyme immunoassay for detection of rotavirus in stool specimens. J Infect Dis 1985;152:830–832.
12. Koopmans M, Cremers H, Woode G, Horzinek MC. Breda virus (toroviridae) infection and systemic antibody response in sentinel calves. Am J Vet Res 1990;51:1443–1448.
13. Ellens DJ, van Balken JAM, de Leeuw PW. Diagnosis of bovine coronavirus infections with hemadsorption-elution-hemagglutination assay (HEHA) and with enzyme-linked-immunosorbent assay (ELISA). Proceedings of the 2nd International Symposium on Neonatal Diarrhea. Acres SD, ed. Saskatoon, Canada: VIDO, 1978:321–330.
14. Heeseman J, Eggers C, Schroder J. Serological diagnosis of yersinosis by the immunoblot technique using virulence associated antigens of enteropathogenic Yersiniae. Contrib Microbiol Immunol 1987;9:285–289.
15. Alton GG, Jones LM, Angus RD, Verger JM. Techniques for the Brucellosis Laboratory. Paris: Institut National de la Recherche Agronomique, 1988:123–126.
16. Flores-Castro R, Carmichael LE. Canine Brucellosis: current status of methods for diagnosis and treatment. Gaines Vet Symp 1977;27:17–24.
17. Ronalds CJ, Hardiman AE, Griffiths PD. Hotting up the complement fixation test. J Hyg (Camb) 1983;90:127–134.
18. Koopmans M, van den Boom U, Woode GN, Horzinek, MC. Seroepidemiology of Breda virus in cattle using ELISA. Vet Microbiol 1989;19:233–243.
19. Heuschele WP, Castro AE, Wan SK, Metz C, Worley MB, Fletcher HR, Plowright W. Recommended standard serologic methods for malignant catarrhal fever. Proceedings of the 28th Annual Meeting of American Association Veterinary Laboratory Diagnosticians 1985:331–335.
20. Corapi WV, Donis RO, Dubovi EJ. Characterization of a panel of monoclonal antibodies and their use in the study of the antigenic diversion of bovine viral diarrhea virus. J Am Vet Med Assoc 1990;51:1388–1394.
21. Senne DA, Pearson JE, Carbrey EA. Equine viral arteritis: a standard procedure for the virus neutralization test and comparison of results of a proficiency test performed in five laboratories. Proceedings of 89th Meeting of the US Animal Health Association, 1985:29–34.
22. Bennett RA, Rubin PH, Present DH. Frequency of inflammatory bowel disease in offspring of couples both presenting with inflammatory bowel disease. Gastroenterology 1991;100:1638–1643.
23. Reilly RP, Robinson TJ. Crohn's disease: is there a long latent period? Postgrad Med J 1986;62:353–354.
24. Allan RN, Pease P, Ibbotson JP. Clustering of Crohn's disease in a Cotswold village. Q J Med 1986;59:473–478.
25. Van Kruiningen HJ. Gastrointestinal system. In: Thomson RC, ed. Special Veterinary pathology. Toronto: BC Decker, 1988:133–227.
26. Yolken R, Leister F, Almeido-Hill J, Dubovi E, Reid R, Santosham M. Infantile gastroenteritis associated with excretion of pestivirus antigens. Lancet 1989;I:517–519.
27. Giangaspero M, Wellemans G, Vanopdenbosch E, Belloli A, Verhulst A. Bovine viral diarrhoea (letter). Lancet 1988;2:110.
28. Lamouliatte F, DuPasquier P, Rossi F, Laporte J, Loze JP. Studies on bovine Breda virus. Vet Microbiol 1987;15:261–278.
29. Weiss M, Steck F, Kaderli R, Horzinek MC. Antibodies to Berne virus in horses and other animals. Vet Microbiol 1984;9:523–531.
30. Brown DWG, Beards GM, Flewett TH. Detection of Breda virus antigen and antibody in humans and animals by enzyme immunoassay. J Clin Microbiol 1987;25:637–640.
31. Kangro HO, Chong SKF, Hardiman A, Heath RB, Walker-Smith JA. A prospective study of viral and mycoplasma infections in chronic inflammatory bowel disease. Gastroenterology 1990;98:549–553.
32. Ekbom A, Adami HO, Helmick CG, Jonzon A, Zack MM. Perinatal risk factors for inflammatory bowel disease: a case-control study. Am J Epidemiol 1990;132:1111–1119.

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