Hypothesis

Crohn's disease: the cold chain hypothesis

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Crohn's disease is the result of an abnormal immune response of the gut mucosa triggered by one or more environmental risk factors in people with predisposing gene variations, including *CARD15* mutations. Epidemiological data allow assessment of familial environmental risk factors related to western lifestyle, diet, bacteria, and domestic hygiene. All findings point to refrigeration as a potential risk factor for Crohn's disease. Furthermore, cold-chain development paralleled the outbreak of Crohn's disease during the 20th century. The cold chain hypothesis suggests that psychrotrophic bacteria such as *Yersinia* spp and *Listeria* spp contribute to the disease. These bacteria have been identified in Crohn's disease lesions and we discuss their pathogenic properties with respect to our knowledge of the disease. From a molecular perspective, we postulate that the disease is a result of a defect in host recognition by pathogenic bacterial components that usually escape the immune response (eg, Yop molecules), which results in an excessive host response to these bacteria.

Crohn's disease is characterised by a relapsing inflammatory process in the digestive tract.[1] Since its first description in 1913, the cause of Crohn's disease is still largely unknown, although it is recognised to be a complex trait that is the result of an interaction between environmental and genetic risk factors. Although some genetic factors are known,[2,3] uncertainty still exists around environmental factors, except for cigarette smoking.[4]

Knowledge of causative environmental factors is crucial if disease mechanisms are to be understood and a specific treatment developed. We postulate that Crohn's disease is the result of an excessive response to pathogenic psychrotrophic bacteria in some genetically predisposed people. Such bacteria can exist and grow at temperatures between –1°C and 10°C, and we believe that the advent of domestic refrigeration contributed to the outbreak of Crohn's disease in the 20th century.

Cold chain and Crohn's disease

Crohn's disease is common in Europe and North America, where incidence has risen in the second half of the 20th century.[5] Thus, environmental factors, probably related to the modern occidental way of life, might have a role in Crohn's disease.[6–8] From the first to the second half of the 20th century, many crucial changes occurred with respect to food, housing, transport, leisure, and clothing. Thus, to tease out the relevant causative risk factors for Crohn's disease from the mesh of inter-related variables is difficult.

It is beyond the scope of this paper to discuss epidemiological data in detail, but of note is that Crohn's disease has been positively associated with good standards of domestic hygiene (such as hot running water),[9–11] and that environmental risk factors contribute to familial aggregations of the disease.[12] Because inflammation occurs in the digestive tract, antigens present in the gut lumen, including components of food and intestinal bacteria, have been implicated in the development of Crohn's disease. However, no specific dietary component has been identified, suggesting that many foods contain putative contaminants. Several infectious agents have been proposed, including species of *Mycobacterium, Listeria,* and *Yersinia,* and *Escherichia coli,* but none has been proven as a causative factor. However, the link between bacterial components and Crohn's disease was lent strong support by the discovery that mutations in *CARD15*, a gene involved in innate immunity, predisposes people to the disease.[2,3]

In view of the observation that Crohn's disease is linked to a familial environmental risk factor related to modern western lifestyles, domestic hygiene, diet, and infectious agents, we propose a specific candidate for the development of Crohn's disease: the refrigerator.

Domestic refrigeration began with the advent of ice containers in the 19th century. The first refrigerating machines were built around 1875, and during the first part of the 20th century, refrigeration methods were more widespread in the USA than in other countries.[13] The first domestic refrigerator was developed by Kelvinator in 1918 in the USA. It became more and more popular and in 1921, 5000 refrigerators were produced, with the yearly production rate rising to 75 000 in 1925, 850 000 in 1930, and 1 700 000 in 1935. By 1937, 49% of Americans had a refrigerator.

In 1930s' Europe, only wealthy families had refrigerators, and ownership became more common only after World War II with improvements in living standards, and with the supply of electricity to homes. Even in 1958, only 10% of French and 12% of British families had a refrigerator—ownership was as low as 2% in Spain, the USSR, and Japan. The European exception was Sweden, where at the same time just over half of families had a refrigerator developed by Electrolux.

Although difficult to define precisely, population-based data suggest that the increase in prevalence of Crohn's disease was in the 1940s or before in the USA,[8] in the 1950s or before in Sweden,[14,15] in the 1960s in the UK,[6,7] and later

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in southern Europe. These examples show temporal and geographical coincidences between the development of the refrigerator and the outbreak of Crohn's disease. Of course, the cold theory for the development of Crohn's disease cannot be limited to the domestic refrigerator, which is the last part of the cold chain. The cold chain is much more complex: of the 520 kg of food eaten every year per person in France, 320 kg are, at some time, conserved at a low temperature. Furthermore, external and complex factors could affect the quality of the cold chain including machine maintenance procedures, food conservation habits, and the availability of electricity.

We are aware that the suggested association between the cold chain and Crohn's disease needs confirmation by more detailed analyses before being retained. Additionally, even if firmly established, temporal and geographical coincidences cannot prove a causal relation by themselves, and we cannot rule out the possibility that the cold chain is a confounding risk factor that occurred in parrallel with the true cause.

Psychrotrophic bacteria

If our hypothesis is valid, it points to the existence of bacteria capable of surviving or developing at low temperatures.[16] Such microorganisms, known as psychrotrophic bacteria, have optimum growth at temperatures higher than 30°C but are able to grow, at a slower rate, at temperatures between –1°C and 10°C—ie, the temperature inside refrigerators.

The most frequently encountered psychrotrophic bacteria with pathogenic properties are *Listeria monocytogenes, Yersinia enterocolitica, Clostridium botulinum* and *Bacillus cereus*.[16] The pathogenicity of *B cereus* and *C botulinum* is mainly related to toxin production. To our knowledge, these bacteria have not been studied in Crohn's disease. *L monocytogenes* is a gram-positive bacillus that induces acute diarrhoea. It has been identified in Crohn's disease lesions by immunohistochemical analysis, but not by PCR.[17–21] *Y enterocolitica* is an ubiquitous gram-negative bacillus that has been detected in the digestive tract of many wild and domestic animals. In addition to *Y enterocolitica*, less virulent species of the *Yersinia* genus are also encountered in food, but their pathogenic role is less clear. *Yersinia* species are commonly found in humans and in foods such as milk, beef, pork, chicken, sausages, hamburgers, cheese, and lettuce.[22]

People with Crohn's disease have been tested for the presence of Yersinia with conventional methods. Sera from patients were reactive to *Y pseudotuberculosis* more often than were control sera in one study,[23] but most studies report negative results. More interestingly, with PCR-specific primers, *Yersinia* spp were detected by two independent groups in 63% (13/19) and 31% (17/54) of people with Crohn's disease, respectively.[24,25] Intestinal samples were positive for *Y enterocolitica*, *Y pseudotuberculosis*, or both. The clinical and histological features of the yersinia-positive cases were indistinguishable from yersinia-negative cases.[25] Disease misclassification is unlikely, since median follow-up in the two study groups was 9 and 10 years, respectively. Thus, the data clearly indicate that *Yersinia* spp can exist in lesions associated with Crohn's disease. Moreover, the large number of yersinia-positive cases and the absence of specific clinical or histological features suggest that a large proportion of people with the disease have chronic contact with the bacteria.

However, *Yersinia* spp might be present at only very low rates in Crohn's disease lesions, in view of the negative results from studies that use conventional methods for bacterial detection. In accordance with this point of view, yersinia was not a predominant microorganism in biopsy samples from ten patients with the disease,[21] lending support to the theory of a chronic bacterial infestation that is limited by an intense host reaction.

Because data from microbiological studies are compatible with the cold chain hypothesis, we have further developed the theory of chronic infestation by psychrotrophic bacteria.

Pathogenesis of Crohn's disease

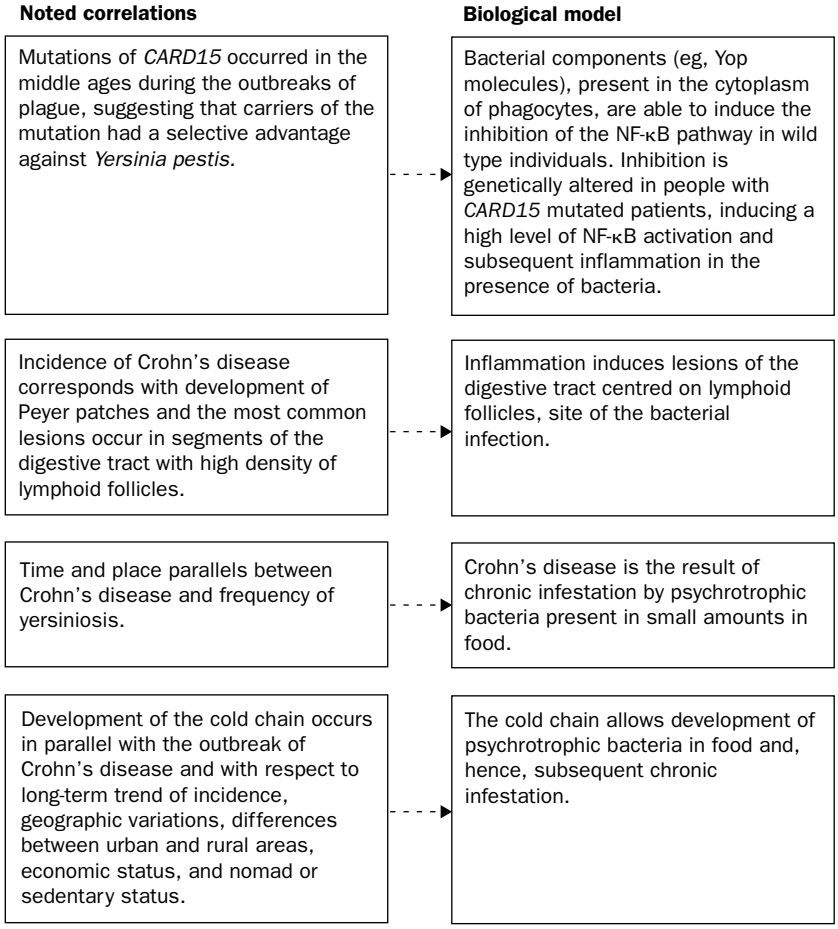
Crohn's disease lesions occur predominantly where there is a high density of lymphoid follicles, in the small bowel where the follicles are grouped to form Peyer patches, and in the colon where they are isolated. In the small intestine, lesions are more frequent in the distal ileum where Peyer patches are most common. In addition to this spatial relationship, a temporal relationship has also been suggested by Van Kruiningen and colleagues.[26] Peyer patches develop from birth to age 10–15 years, and then undergo involution. Of note is that the age-dependent incidence curve for Crohn's disease is roughly parallel to the number of Peyer patches with a peak in the third decade of life. A delay of 5–10 years is then seen between the development of Peyer patches and the occurrence of symptoms. Such a delay is compatible with chronic infection. Conversely, the ileal location of Crohn's disease is less frequent in elderly people after the involution curve of Peyer patches.[27] In the colon, by contrast with the small intestine, the number of lymphoid follicles is less subject to variation. This observation can account for the fact that colonic Crohn's disease can occur at any age. Furthermore, aphthoid ulcerations (thought to be the first lesions in development of Crohn's disease) are centred on lymphoid follicles.[28]

Together, these data suggest that lesions initially develop on lymphoid follicles and accord with a theory of specific infection. Many infectious agents exhibit a specific tropism for M cells including *Salmonella* spp, *Shigella* spp, *M paratuberculosis*, *E coli*, *Vibrio cholerae*, *Campylobacter jejuni*, reovirus, poliovirus, HIV, and the psychrotrophic bacteria *Y enterocolitica* and *L monocytogenes*.

Yersinia spp are able to produce an invasin, which allows M cell penetration. This specific tropism for M cells makes yersinia pathogenic for the lymphoid follicles but not for other parts of the mucosa. In mice infected with yersinia, vesicles appear on the lymphoid follicles, which are progressively destroyed with micro-abscess formations.[29] Inflammation of the gut is discontinuous and only 30–50% of Peyer patches are involved.

In human beings, *Yersinia* spp are able to induce a clinical ileitis or ileocolitis, and a mesenteric adenolymphitis, most often in children and young adults. They might also induce reactive arthritis and erythema nodosa. Histological analysis shows that intestine and lymph-node lesions can exhibit granuloma formations. All these findings are common to Crohn's disease and yersiniosis, explaining that these two disorders have to be classically differentiated during the diagnosis procedure.

Additional features are relevant for Crohn's disease. Like *Helicobacter pylori*, yersinia produces a urease that is known to be essential in gastritis formation, a finding which could explain the high frequency of focally enhanced gastritis in Crohn's disease. *Y enterolitica* produces a heat-shock protein that might cause diarrhoea and autoimmune colitis in animal models.[30] The bacteria has been implicated in spondylarthropathies, which are known to be associated with Crohn's disease. Finally, antibiotics active against *Yersinia* spp such as ciprofloxacin have a limited effect in Crohn's disease.[31]



Links between Crohn's disease, the cold chain, and psychrotrophic bacteria

Historical perspective

The three main mutations of the *CARD15* gene associated with Crohn's disease in white people (G702R, R908W, and 1007*FS*) have probably occurred only recently in human history. There were large outbreaks of plague in Europe that occurred periodically between the 6th and the 14th centuries.[32] Thus, in the Middle Ages, *CARD15* mutations might have provided carriers with a selective advantage during plague outbreaks and it could be postulated that mutation carriers have a more intense reaction against *Yersinia* spp.

With time, *Y pestis* disappeared—probably because it was replaced in Europe by less pathogenic strains such as *Y pseudotuberculosis* (and later *Y enterocolitica*), which probably induced a cross-immunisation to *Y pestis* in rodents carrying the plague agent.[32] However, the intense reaction against *Yersinia* spp developed by mutation carriers might have become inadequate for these less virulent strains and the intestinal site of the infection, which is usually characterised by an immune tolerance.

Y enterocolitica was isolated for the first time in 1934 in the USA, 2 years after the complete description of the regional enteritis by Burrill B Crohn, Leon Ginzburg, and Gordon D Oppenheimer. In Europe, *Y enterocolitica* infections increased after the 1960s—the same time as the Crohn's disease outbreak.[32] Because Crohn's disease is a complex genetic trait, progressive population exposure to *Yersinia* spp might have allowed more and more genetically predisposed people to be affected. Interestingly, the present stabilisation of the disease incidence in North America and in some European countries suggests that almost all people who are genetically at risk are now affected by the disease, because of the widespread dissemination of the risk factor.

In addition to the temporal coincidence in Crohn's disease, a geographical relationship can also be noted: yersinia are more common in northern countries. This finding can be related to the North-South gradient seen for Crohn's disease in Europe and America.[33,34] Finally, seasonal variations have been reported for both yersinia infections and relapses of Crohn's disease, and this finding warrants further investigation.[35]

Molecular explanations

The *CARD15* gene has been shown to predispose people to Crohn's disease.[2,3] This gene is mainly expressed in phagocytic cell lines including monocytes, which are the target cells of *Yersinia* species. *CARD15* encodes for a protein that is able to induce NF-κB activation (and potentially apoptosis), after recognition of intracytoplasmic peptidoglycan.[36] A defect of NF-κB activation in the presence of peptidoglycan has been shown in the case of mutations associated with Crohn's disease.[3,36] This loss-of-function model is consistent with an observed genetic model of inheritance at the *CARD15* locus, which is characterised by a dosage effect of the Crohn's disease mutations.[2] However, the model does not accord with the increased NF-κB activation noted in Crohn's disease lesions.[37]

Of note is that *CARD15* is also mutated in another granulomatous condition characterised by an inflammation of the eyes, joints, and skin but not the digestive tract—Blau syndrome.[38] In this mendelian dominant trait, a gain-of-function model has been established with an excess of NF-κB activation at the basal level.[36]

To relate the two disorders because of a common activation of the *CARD15* pathway that induces an inflammation with granulomas is tempting. Several bacteria are usually able to inhibit NF-κB activation through virulence factors. We can, thus, postulate that Crohn's disease mutations might induce not only a loss of NF-κB activation by the peptidoglycan, but also a loss of NF-κB inhibition in the presence of bacterial agents of virulence. As a result, hosts carrying *CARD15* mutations should have a more intense reaction toward virulent bacteria.

The virulence factors of the *Yersinia* species are mainly carried by a plasmid (pYV) encoding for 12 secreted Yop and Ysc proteins forming the secretory apparatus type III. Some of the Yop proteins are involved in the inhibition of phagocytosis and NF-κB activation. NF-κB inhibition has been shown via YopJ/P, which is able to interfere with IKK proteins.[39,40] However, the mechanism of inhibition is not completely understood, Yop proteins might also interact physically with *CARD15* to induce an inhibition of the NF κB by an alternative pathway. This NF-κB inhibition could be due to YopJ/P or other molecules, including YopM which is characterised, like *CARD15*, by leucine rich repeats (LRRs). A similar model can also be proposed

for other psychrotrophic strains such as *Listeria* spp that carry LRR proteins at their surface.

Our hypothesis could be tested in experimental models in which cells expressing the wild type or mutated CARD15 protein were analysed in the presence of Yop proteins.

Conclusions

We propose a link between Crohn's disease, the cold chain, and chronic infestation of the digestive tract by psychrotrophic bacteria. Our model provides a structured and unified explanation, based on biological evidence and a network of statistical relationships (figure). By themselves, each line of evidence is quite convincing, but their association is even more persuasive.

However, much more testing is needed before our model can be retained or discarded. Furthermore, some intrinsic limitations can be raised. For example, because our hypothesis takes into account only one genetic risk factor and one environmental component from several expected factors, it cannot elucidate the clinical heterogeneity seen between patients with Crohn's disease.

The cold chain has produced many benefits for western societies, including the prevention of enteric infections, allowing more people access to a well-balanced diet, and the economic development of agriculture and fishing. These advantages clearly outweigh the putative risks discussed here and, in the absence of experimental evidence, practical conclusions should not be drawn.

Conflict of interest statement

None declared.

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