Environmental risk factors in Crohn’s disease and ulcerative colitis: an update

Place des facteurs environnementaux dans la physiopathologie des maladies inflammatoires chroniques intestinales

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Summary
Rapid increase in Crohn’s disease (CD) and ulcerative colitis (UC) incidence in developed countries, occurrence of CD in spouses and lack of complete concordance in monozygotic twins are strong arguments for the role of environmental factors in inflammatory bowel disease (IBD). Only two environmental factors have an established role in IBD. Smoking is a risk factor for CD and a protective factor for UC; appendectomy is a protective factor for UC. Many other environmental factors for IBD have been investigated. These are infectious agents, diet, drugs, stress and socio-economic factors. They are detailed in this paper. Among them, adherent invasive E. coli, infectious gastroenteritis, oral contraceptives and antibiotics could play a role in CD. To date, three theories integrate environmental factors to pathogenesis of IBD: hygiene, infection and cold chain. Much work remains to be done to identify risk factors for IBD. As exemplified by smoking, research of environmental risk factors of IBD is useful since it may lead to an improved disease course among patients and perhaps, to appropriate prevention among predisposed subjects. Further studies in this field are eagerly awaited.

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Résumé
L’augmentation rapide de l’incidence de la maladie de Crohn (MC) et de la rectocolite hémorragique (RCH) dans les pays développés, l’absence de concordance totale chez les

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Introduction

Inflammatory bowel diseases (IBD) are characterised by a decreased tolerance to intestinal bacteria and an abnormal activation of intestinal cellular immunity. Several mechanisms involved in the loss of homeostasis of intestinal mucosal immunity have been described in animal models of IBD and in clinical settings: increased production of proinflammatory cytokines, dysbiosis of intestinal flora, inactivation of regulatory T cells, loss of the epithelial barrier function, etc. [1, 2].

The description of IBD mechanisms is distinct from the research for its causes or triggering factors. IBD is the result of the combination of both genetic and environmental factors. Major research efforts, have concentrated on genes predisposing to IBD. At the present time, nine genes predisposing to Crohn’s disease (CD) have been found [3]. NOD2-CARD15 is one of them. It is a key component of the innate immune response against bacteria [4]. Two genes IRGM and ATG16L1 play a role in autophagy of intracellular bacteria [5, 6]. Several other genes are involved in the regulation of innate and adaptive immune response (IL23R, PTPN2, TNF AIP3, etc.). Research of genes predisposing to IBD is far from complete and further associations are likely to be described in the coming years.

However, as we will see as we read on, environmental factors are involved in the occurrence of IBD, and their role has been confirmed in a number of observations.

Do environmental factors play a role in IBD?

Time trends in IBD incidence

Several IBD registries have been set up across the globe. Depending on regions, the mean annual incidence rate of CD varies from 0.7 to 14.6 for 100,000 inhabitants and that of ulcerative colitis (UC) from 1.5 to 24.5 per 100,000 inhabitants [7-13]. All registries have noted an increased incidence of both CD and UC. This increase was first noted in the 1940’s in Northern developed countries and in the 1960’s in Southern countries [7-13]. In half of studies, the increased incidence of IBD (either UC or CD) is followed by a plateau [6]. It suggests that environmental factors have progressively spread throughout the entire population, reaching, within a few decades, all individuals at risk. In several registries, incidence of CD is still rising, particularly in children from Sweden [13], Northern France [11] and Denmark [15] during the 1990’s and 2000’s, suggesting that some environmental factors still act to increase the incidence of CD in Western nations. However, one cannot exclude that the increased incidence of IBD is due to its better recognition by patients and physicians.

North-South gradient of IBD incidence

A North-South IBD incidence gradient has been described in Europe, the USA, Scotland and France [12, 16, 17, 18]. All studies conducted have observed a North-South gradient for CD. Two of these studies did not observe a similar gradient for UC [17, 18] and a third study observed a less marked gradient for UC than for CD [12]. In France, the incidence of CD is significantly higher in the north of (Nord-Pas-de-Calais, Picardie, Champagne-Ardenne and Lorraine) and lower in Southern and Western regions (Brittany, Aquitaine, Midi-Pyrénées, Rhône-Alpes, Limousin and Burgundy) [18].

The North-South gradient observed for CD in different regions throughout the world can be explained by genetic or environmental factors. Subjects who have a genetic predisposition to CD may reside more frequently in Northern regions. The higher incidence observed in the north-east of France is consistent with the first (and most important) component which corresponds to the spread of farming from the Levant to the north and west of Europe at the Neolithic age [19]. It is said to account for 28% of the genetic variation in Europe. Environmental factors, in particular improved hygiene in Northern regions, may also explain this gradient. The economic development of Southern regions resulting in the standardisation of way of life should reduce the North-South gradient. In CD, the abnormal activation of intestinal cellular immunity is dependent upon TH1 lymphocytes. In multiple sclerosis, another disease associated with excessive TH1 activity, a South-North gradient has been observed. In Australia, the most Southern regions such as Tasmania have a higher incidence than Northern regions such as Queensland.
appendectomy satisfy these criteria. All the suspected causative factors of IBD only tobacco and Bradford Hill has defined a list of criteria required to identify a factor as causative. These criteria appear in box 1. Among all the suspected causative factors of IBD only tobacco and appendectomy satisfy these criteria.

### Family aggregation in IBD

In monozygotic twins, the concordance level is only 50% for CD and 19% for UC [24]. In families with CD, affected children are more often consecutive than may be expected by chance alone. Hugot et al. studied 102 relatives who belonged to families with three or more children, with at least two affected with CD. They observed 58 consecutive cases, whereas chance predicted only 46 (P = 0.005) [25]. This difference could be attributed neither to a cohort effect, nor to a de novo mutation of germinal cells after the birth of the first child. The shared exposure by siblings to common environmental factors was the preferred explanation.

Moreover, IBD in spouses are significantly more frequent than chance would predict [26]. CD among disease-free spouses at time of marriage occurs after a median of 8.5 years (3.5-14) of conjugal life [26]. These conjugal forms of CD suggest that shared exposure to environmental factors within the same family household favours the development of CD.

### Clusters of CD

Seven observations of CD have been reported among non-related adolescents having studied at the Mankato College (Minnesota-USA) from 1976 to 1991 [27]. Such an aggregate of CD within this small community would result in the extremely high prevalence of 2,400/100,000 inhabitants. A detailed investigation showed that patients had commonly bathed in a pool polluted with Coliform bacteria [27]. However, caution is needed in interpreting these results. Indeed, the occurrence of a spatio-temporal aggregate could be the result of pure chance.

The abovementioned studies suggest that environmental factors play a role in IBD pathogenesis. Many studies have attempted to identify these environmental factors. Numerous epidemiological studies, either ecological studies, case-control studies, or more recently, cohort studies have been performed. Such research is painstaking, since factors “having emerged in Western living conditions since the Second World War” are numerous and probably, incompletely recorded. Several systematic case-control studies have attempted to identify them. Others have targeted a single factor. Environmental factors may also be studied in the laboratory: detection of microorganisms on human tissue using immunohistochemistry or PCR, effect of an environmental factor on the immune response. Based upon the philosophy of David Hume (1711-1776), the British epidemiologist Austin Bradford Hill has defined a list of criteria required to identify a factor as causative. These criteria appear in box 1. Among all the suspected causative factors of IBD only tobacco and appendectomy satisfy these criteria.


- The association is strong.
- The association is constant and reproducible.
- Cause must precede the effect.
- There is a dose effect relationship.
- Cause is biologically plausible.
- Cause is in line with present knowledge about natural history of the disease.
- A conclusive randomized controlled trial is a strong argument.

### Systematic case-control studies

The first study of this type focused on 14 centres throughout 9 different countries (Northern America, Northern Europe and the Mediterranean) [28]. One hundred and ninety-seven patients presenting with UC and 302 patients with CD were compared with two controls per patient, matched for both age and sex, with or without gastrointestinal disease. Family history increased the risk of UC and of CD. Eczema in a patient or his/her parents, respiratory infections, antibiotic prescription and being the youngest sibling, all increased the risk of CD. On the contrary, consumption of vitamins and minerals during pregnancy by the mother, small pox vaccination and belonging to a high-level socio-economic group, all diminished the risk. Respiratory infections and the presence of pet rodents at home increased the risk of UC. In the contrary, appendectomy, consumption of vitamins and minerals during pregnancy by the mother, small pox vaccination and belonging to a high-level socio-economic group, all diminished the risk. Respiratory infections and the presence of pet rodents at home increased the risk of UC.
water decreased this risk. A possible explanation is that tap water may contain bacteria, in particular non-pathogenic mycobacteria, which may play an immunoregulatory role. Family history of IBD, preterm birth and sharing a bedroom with another family member (suggesting the involvement of a transmissible infectious agent) all increased the risk of UC, whereas appendectomy played a protective role.

A systematic environmental factor study was conducted among 21 families from Northern Belgium with at least three first degree relatives presenting with CD [31]. These 21 families were compared with 10 control families via a questionnaire including 176 items concerning childhood and adolescence for children and concerning the ten-year period immediately prior to the onset of disease for adults. This study evidenced several CD risk factors among patients and/or their families. Factors favouring CD were: periodontitis, consumption of raw pork, of non-pasteurised dairy products, of well water and of tobacco. On the contrary, history of mumps, consumption of oats, bran, rye and tap water, together with domestic contact with a bird, dog or cat all played a protective role against CD. The increased risk of CD induced by the consumption of raw pork, non-pasteurised dairy products and well water is in support of a transmissible infectious agent.

A case-control study of newly-diagnosed cases of CD \((n = 194)\), less than 20 yr of age has been reported recently [32]. In multivariate conditional logistic regression, family history of IBD, age, and owning a pet were associated with risk for CD, whereas regular use of a personal towel and lesser crowding in homes were protective. Day-care attendance during the first 6 months of life and “physician-diagnosed infections” between 5 and 10 yr of age were associated with increased risks for CD. This study concluded that “infection-related exposures seem to enhance risk for CD in children”.

Conversely, a multicenter case control study performed in children with CD \((n = 444)\) and UC \((n = 304)\) has shown that regular contact with farm animals during the first year of life was inversely associated with CD and UC [33]. Regular contact with cats during infancy was also associated with CD but not UC. Allergic rhinitis was correlated significantly with CD but not UC.

**Targeted studies on specific risk factors**

**Smoking**

**Smoking and UC**
The role of smoking is crucial in CD and UC [34]. It is clearly established that smoking has a strong protective effect against UC. One meta-analysis has calculated a pooled odds ratio of 0.41 (95% confidence intervals 0.34-0.48) for current smokers compared with non-smokers [35]. The protective effect of smoking is abolished in those who stop smoking. Ulcerative colitis is also less severe in smokers than in non-smokers: flare-up and hospitalization rates, the need for oral steroids and, colectomy rates, are lower in smokers than non-smokers [36]. Stopping smoking increases the risk of developing UC when compared to neversmokers. This increased risk, about 1.64 (1.36-1.98) persists during the 2 to 3 years following smoking cessation [35]. Likewise, smokers with UC who quit experience an increase in disease activity, hospital admissions and the need for oral steroids, and immunosuppressants (but seemingly not colectomy), within the first years following the cessation of smoking [37].

**Smoking and CD**
It is clearly established that smoking increases the risk of having CD. One meta-analysis has calculated a pooled odds ratio of 2.0 (1.65-2.47) in current smokers compared to non-smokers [35]. CD is also more severe in smokers than in non-smokers: flare-up, penetrating complications, surgery and postoperative recurrences are higher in smokers than non-smokers [34]. The detrimental effect of smoking is dose dependent, however a light consumption remains harmful. Smoking increases the risk of having ileal CD and increases its severity in patients affected with CD but has little effect upon colonic CD [34]. CD activity in ex-smokers is not different to that of non-smokers, and less marked than in current smokers. One prospective study performed in a selected group of 59 patients who stopped smoking following a smoking cessation intervention examined the disease course from one year after the quit date [38]. Regarding the flare-up rate and therapeutic needs, disease severity was similar in patients who had never smoked and in those who stopped smoking, and both had a better course than continuing smokers. Quitters had a 65% decreased risk of flare-up compared with paired continuing smokers. They were less likely to require corticosteroids, start immunosuppressive therapy, or require an increased dose of immunosuppressants (Figure 1). Additional intervention studies of smoking cessation in smokers with CD are required.

**Figure 1** Risk of flare up of Crohn’s disease in active smokers, ex-smokers and in non-smokers (according to Cosnes et al. [41]).

*Risque de poussée de maladie de Crohn chez des malades fumeurs actifs, anciens fumeurs, ou non-fumeurs (d’après Cosnes et al. [41]).*
Mechanisms of the effects of smoking in IBD
The reasons why smoking has an opposite effect in CD and UC remain obscure. Tobacco contains hundreds of different substances including nicotine, free radicals and carbon monoxide. The targets of smoking are plentiful: mucus layer, macrophage function, adaptive immunity, cytokine production, vascular effects, motility and intestinal permeability to macromolecules... In CD the mucus layer is thicker whereas it is thinner in UC; nicotine increases mucin synthesis in UC patients. Smokers with IBD have a significant reduction in mucosal cytokine levels, specifically, IL-1b and IL-8 for patients with UC and IL-8 for patients with CD [39]. Smoking also targets macrophage function. Macrophages from smokers express a selective functional deficiency in their ability to kill intracellular bacteria [40] and carbon monoxide at low concentrations inhibits the lipopolysaccharide-induced expression of proinflammatory cytokines (TNFα, IL-1b and macrophage inflammatory protein-1b) and increases that of IL-10 [41]. Smoking increases the risk of ileal CD and decreases macrophage ability to kill intracellular bacteria. Likewise, some of the CD-predisposing genes are involved in autophagy (ATG and IRGM) and innate immune response against bacteria (NOD2); these genes increase the risk of ileal CD but not colonic CD. Therefore, it can be suggested that ileal CD results from innate immune deficiency against intracellular bacteria, either inherited or due to environmental factors (including smoking) or both.

Therapeutic implications
The effects of smoking upon IBD course have important therapeutic implications. Smokers with UC who plan to stop should be informed about the potential risk of increase in disease activity. Smoking cessation has become a major therapeutic goal in the management of CD, particularly in young women and in patients with ileal involvement. However, the proportion of CD patients who stop smoking remains low—about 10%—and relapse rate is high. Smoking cessation programs should be developed in IBD centers.

Appendectomy
Several case control and cohort studies have found that appendectomy reduces the risk of having UC. In 2002, a meta-analysis pooled the results of 17 case control studies. The overall pooled OR was 0.312 (0.261-0.373) for having UC in appendectomized patients vs non appendectomized patients [42]. However, the methodological quality of some of these studies has been questioned [43]. There have been two national cohort studies on this subject. The Swedish study has followed 212,963 people who had undergone an appendectomy and the same number of matched controls [44]. It concluded that the incidence of UC was 26% lower in appendectomized patients (relative risk 0.42; 0.31-0.57). The Danish study included 154,434 appendectomized patients and found that the incidence of UC was 13% lower (relative risk 0.87; 0.69-1.07) in appendectomized patients [45]. In both studies, the risk of UC was lower for patients who had been operated on before the age of 20 and for a confirmed appendicitis. There is thus good evidence to support the association between appendectomy and UC. UC is also less severe in appendectomized patients, the disease course is less active and colectomy rate is lower [46].

However, in view of the abovementioned results, it seems likely that the protective effect is due to appendicitis not appendectomy. Appendectomy is often inflamed in UC, even in patients with distal disease [47]. Immunohistochemical studies have shown that in UC, there is an accumulation of activated macrophages closely linked to epithelial cells which harbor HLA class II antigens on their surface [48]. TCRα knock out mice develop a spontaneous inflammatory colitis between 24 and 31 weeks of life [49]. Neonatal appendectomy prevents these mice from having a subsequent inflammatory colitis [50]. It has been suggested that, in this model, autoreactive T cells which cross react with bacterial antigens are activated within the appendix.

The role of appendectomy in CD onset is less well defined than for UC. A recent population-based study in Sweden and Denmark has shown an increase in CD frequency in the first 6 months after appendectomy but this excess risk disappears thereafter, suggesting that the association is due to a diagnostic bias [51]. Appendectomy does not seem to affect severity in patients with CD [52].

Infectious agents
Several infectious agents have been suspected to have a causative role in IBD.

Perinatal infections
One study demonstrated a higher risk of IBD among patients having suffered acute gastroenteritis in the days following their birth [53]. Furthermore, it has been demonstrated that IBD was more frequent among children presenting with perinatal infection, all localisations combined [54]. In this case-control study including 257 cases of CD, 257 UC and 514 controls matched for age, sex and maternal age or parity, the authors found an OR of 3.8 [CI: 2.6 to 5.8] to develop CD among patients who had had a perinatal infection noted in their medical file. This risk was higher for viral than for bacterial infections.

The measles virus
The measles virus has been evidenced in immunohistochemistry and in situ hybridisation in endothelial intestinal cells among patients presenting with CD [55]. These initial results were not subsequently confirmed [56, 57]. Simultaneously, several epidemiological studies have looked into the association between, on one hand the occurrence of IBD and, on the other hand, anti-measles vaccination, measles infection during pregnancy or infancy. An epidemiological study of a historical cohort of 3,545 patients vaccinated against measles in 1964, found an odds ratio (OR) of 3 for CD (1.45–6.23) and 2.53 (1.15-5.58) for UC in the vaccinated group compared with a control group of 11,407 non-vaccinated individuals [58]. A further study concerning 7,616 individuals born in 1970 found no significant association between anti-measles vaccination before the age of 5 years and the occurrence of CD (OR = 0.67[0.27-1.63]) or the occurrence of UC (OR = 0.57 [0.20-1.61]). On the contrary, a trend to develop CD was observed among individuals vaccinated after the age of 2 years (OR = 1.05; CI [1-1.10]) [59]. Two other case-control studies on approximately 150 patients matched with 2 or more controls per patient, found no association between
anti-measles vaccination and IBD [60, 61]. A hospital cohort study (1,959 CD and 2,018 UC) over a period of 20 years also failed to identify an association between vaccination, CD and UC [62]. These data do not support the role of measles vaccination in IBD. One study focused on the occurrence of IBD among children whose mothers had been infected with measles during pregnancy. By analysing data from 25,000 births over a period of 10 years, the authors found four cases of measles occurring during pregnancy; three of the four infants involved developed CD [63]. They suggest that measles exposure during pregnancy represents a major risk factor for CD. A further study, however, failed to reproduce this association [64]. Two population-based studies revealed an increased risk of IBD among children having suffered from measles before the age of five [65, 66]. These results were not confirmed by a serological study [67]. Furthermore, the incidence of IBD continues to rise in children, whereas measles is on the decline, thanks to vaccination.

From these studies, the role of measles virus or vaccine in the pathogenesis of IBD can be reasonably dismissed.

Listeria monocytogenes
An immunohistochemical study of 16 intestinal resection samples for CD revealed the presence of Listeria monocytogenes in macrophages and in giant cells in 12 cases out of 16. The presence of E. coli and of Streptococcus was also observed, respectively, 57 and 44% of resection samples [68]. Other studies looking to identify Listeria monocytogenes in intestinal tissue, by PCR or immunohistochemistry, observed no difference between CD and controls [69-71].

Mycobacterium avium subspecies paratuberculosis (MAP)
The role of MAP in CD is controversial [72]. Several recent studies have showed a significant association between MAP and CD. A PCR study evidenced the presence of MAP in the blood of 40% of CD or UC patients and 20% of controls [73]. Furthermore, the bacteria was found in culture following a 12-week incubation period, exclusively among IBD patients [73]. Another study has showed that a specific DNA fragment of MAP called IS900 was more frequently found in the small intestinal or colonic tissue of CD patients, than UC patients or controls (52% versus 2% and 5% respectively) [74]. A recent meta-analysis analysed 28 case-control studies comparing MAP in patients with CD, UC or controls. The pooled OR comparing CD patients and controls was 7.01 (95% CI 3.95-12.4) in studies using PCR in tissue samples and 1.72 (1.02-2.90) in studies using ELISA in serum. ORs were similar for comparisons between CD and UC patients [75]. The specificity of this association can be questioned however. Shanahan’s group has evidenced MAP DNA in micro-dissected tuberculoid granulomas in intestinal tissue taken from CD patients [76]. Using the same technique, the same group also observed E. coli DNA within granulomas [77].

Recently a multicenter randomized placebo controlled trial has compared the efficacy of triple anti-MAP therapy (clarithromycin, clofazimine, rifabutin) to that of placebo [78]. Primary end points were the proportion of patients experiencing at least 1 relapse at 12, 24, and 36 months. The combination antibiotic therapy for up to two years failed to induce a sustained benefit. Although the results of this study do not support the role of MAP in CD, the hypothesis cannot be totally refuted however. Firstly, in this study, MAP status (either culture, ELISA or PCR in blood or intestines) was not assessed. Therefore, it cannot be excluded that antibiotics were active in the subset of MAP-positive patients; the study may have lacked adequate power to detect a difference in the patients infected with MAP. Furthermore, dose of antibiotics may have been insufficient. The controversy concerning the role of MAP is not ended yet.

Adherent-invasive Escherichia coli
Adherent-invasive E. coli (AIEC) can be found in the ileal mucosa of 20-30% of CD patients versus 5% of controls [79, 80]. These bacteria do not possess the virulence factors of species responsible for acute infectious diarrhoea but adhere to and invade intestinal epithelial cells. Moreover, AIEC are phagocytosed by macrophages and persist within their cytoplasm rather than being destroyed [81]. Their ecology and transmission mode are, as yet, unknown. The receptor of adherent-invasive E. coli upon the enterocyte mucosal surface has been found recently. It is a member of the CEA family protein called CEACAM6 [82]. It is abnormally expressed by ileal enterocytes in CD patients. In addition, interferon-γ or tumor necrosis factor-α stimulation and infection with AIEC bacteria increase CEACAM6 expression in cultured intestinal epithelial cells, indicating that AIEC can promote its own colonization in CD patients.

The presence of MAP or AIEC within the intestinal mucosa of CD patients could be the result of abnormally high intestinal permeability and/or a defect in bacterial clearance. Indeed, NOD2 mutations observed in CD lead to a loss of function and therefore, to an inborn innate immune deficiency [83]; this could prevent the clearance of intracellular bacteria which, consequently persist in intestinal mucosa, inducing chronic stimulation of adaptive immunity. Moreover, it has been showed recently that NOD2 mutations lead to increased intestinal permeability to E. coli in Peyers’patches [84]. Finally, IRGM a gene predisposing to CD encodes a GTP-binding protein which induces autophagy and is involved in elimination of intracellular bacteria, including Mycobacterium tuberculosis [5]. Interactions between NOD2 mutations, MAP and AIEC presence in CD patients should be looked for.

Infectious gastroenteritis
Two large-scale case control studies have shown that the risk if IBD, and particularly CD, is higher in patients with a prior episode of infectious gastroenteritis [85, 86].

Dietary factors
Several studies have considered the role of dietary factors in the genesis of IBD.

Sugar consumption
Most case-control studies have observed a significant association between simple sugar consumption and CD [87]. This result could be interpreted in two different ways. The first interpretation is that simple sugars favour the onset of CD, the second is that patients presenting with CD consume higher quantities of rapidly absorbed sugars to alleviate their symptoms. A possible confounding factor is
that simple sugar consumption is associated with tobacco consumption [88]. A review of the literature conducted in 1998 concluded that methodological problems prevented any conclusion concerning the role of simple sugars in the onset of IBD [87].

Other foods
Other dietary factors: proteins, fat, margarine, fruit and vegetables, dairy products, coffee, coca cola and “fast food” have all been studied in case-control studies or retrospective cohort series [89-98]. As for rapidly absorbed sugars, methodological problems prevent any conclusion from being drawn [97].

Several longitudinal cohort studies of middle aged women are ongoing in USA, Europe and France. These women have had a dietary and lifestyle questionnaire at baseline and at regular visits. Some of these women have developed UC or CD during their follow up. It is hoped that the pre-illness dietary and lifestyle risk factors for CD or UC will be determined.

Food microparticles
Foodstuffs in developed countries contain increasing quantities of microparticles such as titanium dioxide and aluminium silicate. According to a recent theory, microparticles act like antigen transporters from the lumen to the intestinal mucosa. In vitro, complexes formed by antigens and microparticles are powerful stimuli of T lymphocytes and macrophages [98]. One study failed to observe any difference between CD patients and controls with regard to the quantity of food microparticles contained in diet [99]. However, the role of microparticles is extremely difficult to analyse via a dietary questionnaire. A therapeutic trial including 18 corticosteroid dependant patients, randomized to receive a normal and a microparticle-reduced diet concluded to the efficacy of the latter [100]. This was not confirmed in a subsequent multicentric trial [101].

Maternal breastfeeding
Several studies have shown that exclusive artificial feeding (as opposed to maternal breastfeeding, exclusive or not) favours the occurrence of IBD [102-105] whereas others have failed to demonstrate this association [106, 107]. Data from 17 studies conducted on 2,577 patients and 3,551 controls for UC, together with 3,190 patients and 4,026 controls for CD were pooled in a meta-analysis [108]. Despite the small number of studies involving high methodological quality (4 for UC and 4 for CD), authors observed an OR in favour of the protective role of maternal breastfeeding towards IBD: OR = 0.56 [0.38-0.81] for UC and 0.45 [0.26-0.79] for CD. This protective effect remains when analysing all studies. Nevertheless, this meta-analysis did not include the EPIMAD study, in which maternal breastfeeding was found to increase the risk of CD [30]. As already seen, allergy is more frequent among IBD patients. It has been commonly acknowledged that breastfeeding protects against allergy. However, a recent longitudinal study demonstrated that, when administered on a long-term basis, maternal breast-feeding increased the risk of allergy [109]. This detrimental effect could be due to toxic substances in maternal milk or to a hindered acquisition of infection during infancy due to the immunity provided by maternal breastfeeding. Such hindrance could lead to an immunoregulatory deficiency and, consequently, to the loss of homeostasis of intestinal mucosal immunity.

Oral contraceptives
Oral contraceptives have been recently introduced into Western lifestyle. Furthermore, whilst disease is more frequent before puberty among boys, it is more frequent at adult age among women. A meta-analysis has been published on this subject in 1995 [110]. It combined the results of nine studies. The pooled relative risk (adjusted for smoking) associated with oral contraceptive use was 1.44 (1.12-1.86) for CD and 1.29 (0.94-1.77) for UC. Since this paper two case control studies have found a significant association between oral contraceptives and CD [111, 112] and UC [112]. These results suggest modest associations between the use of oral contraceptives and the development of CD and UC. We have assessed the effect of oral contraceptive use on the clinical course of CD and found no effect [113]. The results of this study are discordant with those of Timmer et al. [114] who reported an increased risk of relapse in patients receiving or having received oral contraceptives (both groups were pooled). In our study, the absence of a significant effect of current use of oral contraceptives was probably linked to the low oestrogen dosage of most of the pills used. Although smoking did increase the flare up rate, the use of oral contraceptives did not potentiate the effect of smoking and there was no interaction between the two exposures. Other studies also failed to show any significant impact of oral contraceptives on the course of CD [115, 116].

Vaccinations

BCG
BCG vaccination induces TH1 stimulation, whose excessive activity is one of the essential mechanisms involved in CD. The EPIMAD registry study observed an OR of 3.6 to develop Crohn’s disease among individuals exposed to the vaccine [CI at 95%: 1.1-11.9] with a dose effect [30]. This is the first study to demonstrate such an effect and it needs to be confirmed in further studies.

Combined Measles Mumps Rubella vaccines (MMR)
A major North American epidemiological study conducted based on statistics from “Health Maintenance Organizations” demonstrated that the combined MMR vaccine does not increase the risk of IBD. On the contrary, a reduction if the risk of IBD was observed in patients vaccinated before the age of 18 months [117].

Dysbiosis
Disturbed equilibrium between protective and harmful species, so called dysbiosis, has been suspected to play a role in IBD [118]. The recently developed metagenomic approach allows to investigate the composition of bacterial intestinal flora. It has shown a reduced proportion (particularly Clostridium leptum) and diversity of Firmicutes
bacteria as well as the presence of new bacterial species in patients with CD [119]. Composition of bacterial flora is genetically determined but environmental factors such as mode of childbirth, weaning practices, and local environmental variables (hospital bacteria, level of hygiene, etc.) may influence it. Also, antibiotics may intervene, as it has been showed recently by a study based upon a prospective cohort of patients (representing 5% of the United Kingdom population) monitored by British general practitioners, the General Practice Research Database [120]. At each visit, the physicians noted their observations, diagnoses and prescribed treatment. Within this database, 587 patients were identified as suffering from CD and were compared with 1,460 matched controls. CD patients were more frequently smokers (28% of cases versus 20% of controls) and had more often received antibiotic treatment in the 2 to 5 year prior to CD diagnosis (71% of cases versus 58% of controls). Such an association is not necessarily causal and it can be hypothesized that antibiotics were prescribed for symptoms erroneously interpreted as intestinal infections, whilst they were in fact revealing the onset of CD. However, if the analysis was restricted to patients with neither gastrointestinal symptoms nor gastrointestinal targeted treatment in the 3 to 5 year period immediately prior to diagnosis, the excessive risk induced by antibiotics was unaltered. The detrimental role of antibiotics was recently evidenced in a model of sodium dextran sulphate-induced inflammatory colitis in Myd88 gene invalidated mice (Myd88 is a protein activated by the binding of TLR4 with its bacterial ligands such as LPS) [121]. Colonic lesions were more severe among mice treated with antibiotics, suggesting the role of the intestinal flora in the maintenance of epithelial integrity.

**Psychological stress**

It is clearly established that patients with IBD have more often anxiety and depression disorders than matched controls [122]. While it is likely that IBD leads to anxiety and depression, the reverse is controversial. The role of stress in IBD onset or relapse is frequently evoked by patients. Stress plays a role in certain animal models of inflammatory colitis [123]. It has been shown that stress may disturb gut permeability in patients with IB [124]. Furthermore, two prospective longitudinal studies have shown that life events and/or perceived stress may increase the risk of relapse in IBD patients in remission [125, 126]. One recent study performed in a larger cohort of patients failed to reproduce these results [127]. A recently published case-control study observed an excess of life events in the 6-month period immediately prior to the onset of CD and UC symptoms [128]. However this result was no longer significant in the multivariate analysis. The anxiety and depression scores were higher in CD and UC patients and probably accounted for the perceived increase of life events prior to the diagnosis of IBD. This study did not support an independent association between stress and onset of IBD [128]. Finally, the prevalence of IBD was assessed among 21,062 parents having lost a child aged under 18 years and 293,745 matched parents [129]. No difference in the prevalence of IBD was observed between the two groups: OR = 0.97 (CI 95% [0.76-1.34]). These studies do not support the role of life events and/or perceived stress in the onset or relapses of either CD or UC.

**Socio-economic factors**

The majority of studies have showed that IBD is more common among the most socially advantaged groups [16, 130]. For example, Sonnenberg et al. demonstrated, via analysis of German social security figures, that the incidence of IBD was higher among white-collar workers than among blue-collar workers [131]. A Danish prospective study observed that sedentary professions were exposed to an excess risk of IBD [132]. Most epidemiologic studies have also found that CD incidence was higher in urban than in rural areas [131, 133].

The higher frequency of IBD among more sedentary, urban and upper class subjects may be secondary to other factors such as physical exercise, sunlight exposure, domestic hygiene and environment.

**Environmental factors and theories of IBD**

**The hygiene hypothesis**

The hygiene hypothesis is the leading theory of IBD. Originally raised for allergic diseases, this hypothesis has also been advocated in IBD. It theorizes that a lack of exposures to enteric pathogens makes one susceptible to CD. Childhood infections and poor hygiene may protect one from developing CD by allowing the host to develop tolerance or immunity to agents that could trigger CD later in life. There are numerous results consistent with the hygiene hypothesis in IBD. Firstly, two case-control studies revealed a more frequent hot water supply among patients presenting with CD than among controls [134, 135]. In one study, separate bathrooms were more frequent among CD patients than among controls. However, no difference between patients presenting with UC and controls was evidenced. Nowadays, all, or almost all, households in developed countries have a hot water supply; this factor can consequently no longer provide significant results in case-control studies, but it may well have encouraged the increased incidence of CD in previous decades. Secondly, three studies have demonstrated a lower frequency of anti Helicobacter pylori IgG among patients presenting with CD compared to controls [29, 136, 137]; however a further study failed to reproduce this result [135]. Thirdly, allergy is more frequently observed among CD patients than among controls. However, no difference between patients presenting with UC and controls was evidenced. Nowadays, all, or almost all, households in developed countries have a hot water supply; this factor can consequently no longer provide significant results in case-control studies, but it may well have encouraged the increased incidence of CD in previous decades. Secondly, three studies have demonstrated a lower frequency of anti Helicobacter pylori IgG among patients presenting with CD compared to controls [29, 136, 137]; however a further study failed to reproduce this result [135]. Thirdly, allergy is more frequently observed among CD patients than among controls, and it is reasonable to conclude that both diseases, whose incidence has simultaneously increased, commonly involve environmental factors. Yet, it has been demonstrated that contact with stables and consumption of non-pasteurised milk before the age of 1 year protects against the onset of allergy and IBD [138], whilst the level of endotoxins in the environment is inversely correlated to the incidence of allergy among children [137]. Fourthly, IBD is rare and helminthiasis frequent in developing countries [140]. An open trial suggests that helmint eggs could have a therapeutic effect in CD [141]. A controlled phase II study also suggests that helmint eggs have a beneficial effect in UC [142].
The hygiene hypothesis has been theorized by Rook et al. [143]. According to these authors, hygiene has led to decreased infectious mortality and morbidity, but has distanced man from the micro-organisms which, hitherto, played a regulatory role in the immune system function (“old friends”): *Lactobacillus* (abundantly present in fermented dairy products), environmental saprophytic mycobacteria (which thrive in untreated water, but are absent from chlorinated water), helminths. The price to pay for decreased infectious mortality would appear to be the increase in allergic disease and inflammatory diseases such as IBD.

**Infectious theory**

Several studies suggest that infection plays a prominent role in IBD. Firstly, IBD in spouses is more frequent than chance would predict. Secondly, neither the Gilat study nor the EPIMAD registry study have provided data in favour of the hygiene hypothesis in CD and UC [28, 30]. On the contrary, the EPIMAD study concludes an increased risk of UC among children having shared their bedroom with their brothers and sisters. Thirdly, several case-control studies have evidenced an increased incidence of respiratory, intestinal or perinatal infections among patients compared to controls [53, 54]. Fourthly, two case control studies have showed that having a pet was associated with an increased risk of IBD [28, 32].

Both hygiene and infectious hypotheses are not mutually exclusive. It can be hypothesized that disturbed mucosal and/or systemic immunoregulation induced by a high level of hygiene in early infancy may be a risk factor for IBD triggered by an infectious agent in adolescence or early adulthood.

**The cold chain hypothesis**

The cold chain theory unifies both the hygiene and the infectious hypotheses [144]. It can be summarized as follows: “The NOD 2 mutation are thought to have occurred during the plague epidemic which struck Europe in the 14th Century. It provided increased resistance to this disease. The arrival of the refrigerator in the early 1940s in the United States and in Scandinavia coincides with an increase in the incidence of Crohn’s disease. Some bacteria referred to as psychrotrophic (*Listeria, Yersinia*) survive in refrigerators. One of them, *Yersinia* contains a protein named Yop which normally inhibits the activation of NFkB via an interaction with NOD2 (wild phenotype). Yet, it has been demonstrated that NOD2 mutations leading to an increased risk of CD have a loss of function of this protein. In other words, in individuals with NOD2 mutations, Yop could induce a loss of inhibition, i.e. an activation of NFkB”.

**Conclusion**

IBD is the result of the combination of predisposing genes and environmental factors. All of the results presented herewith suggest that the increased incidence of IBD is related to a change introduced within our living environment during the decades following World War II. It could be an altered composition of faecal flora or the involvement of a low pathogenic agent transported by a vector (water from a well, natural source or a swimming pool, non-pasteurised milk, refrigerator) and which may trigger CD among genetically vulnerable individuals. Studies showing an association between MAP, AIEC and IBD are accumulating. Furthermore, the recent discovery that genes that control autophagy (IRGM and ATG16L1) are predisposing factors for CD support the idea that subgroups of patients may harbour genetic mutations which lead to the persistence of specific microorganisms within the intestinal mucosa and may contribute to the chronic inflammation of the intestinal mucosa. However, criteria of causality have not been reached for any of these infectious agents. This three partner scenario (genetic predisposition, low pathogenic bacteria, environmental vector) does not exclude the role of cofactors such as tobacco (which reduces the bactericidal activity of macrophages towards intracellular bacteria), oral contraception or food composition. Many factors have been studied. Nevertheless, the results of one study are often contradicted by another and, consequently, to date, only the role of tobacco and appendectomy has been firmly established and reproduced by many studies. Smoking modifies the risk of developing IBD, increasing the risk of CD and decreasing that of UC. In addition, smoking exerts a considerable effect on the course of the disease, improving UC and worsening CD, and smoking cessation is followed rapidly by reversal of the effect. In CD, smoking is harmful and persuading patients to stop smoking is probably the most cost-effective strategy for controlling disease activity. As exemplified by smoking, research of environmental risk factors of IBD is useful since it may lead an improved disease course among patients and perhaps, to appropriate prevention among predisposed subjects. Further studies in this field are eagerly awaited.

**Conflict of interest:**

Franck Carbonnel did expert reports for UCB. He attended conferences organised by Ferring, Schering and UCB. UCB, Abbott, Ferring and Schering offered travels to congresses. Prévost Jantchou has no conflict of interest. Elisabeth Monnet has no conflict of interest. Jacques Cosnes has no conflict of interest.

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