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IBD in Children: The More You Look, the More You Find

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Introduction to the symposium. Inflammatory Bowel Disease (IBD) in paediatric patients was reported as early as in 1932. In Burrill Crohn's first report from 1932 of 14 patients with the disease, a 17 year old adolescent was among them. In 1941 he described two boys with Crohn's disease (CD) and growth retardation and pubertal delay (1). At the Joint meeting of ESPGHAN and NASPGHAN in Paris 1980, there were just two abstracts on IBD, one from Boston, USA, and one from Sweden. Now, 26 years later, the interest in and knowledge of paediatric IBD has almost exploded, so a symposium for two and half days on the subject appears entirely appropriate.

Porto Criteria. The ESPGHAN IBD-working group was initiated in the 1990s by Hans Buller, who at the time was working in Amsterdam in the Netherlands. The working group focused initially on epidemiology and risk factors.

In order to compare incidence and prevalence estimates from different centres and different countries it became obvious that we all had to agree on diagnostic criteria and the work-up of new patients. Jorge Dias invited the group to annual meetings in Porto, Portugal and the fist document was named *The Porto Criteria* (2). Diagnosis was based on macroscopic and microscopic findings at endoscopies and on the result of X-ray examinations. Of special importance was the inclusion of an upper endoscopic investigation and a colonoscopy that visualised the distal ileum. These are both necessary to make a diagnosis of IBD in patients where the evidence is not clear cut and also to discriminate accurately between ulcerative colitis (UC) and CD.

On the issue ''the more you look, the more you find'', this introduction will include some information on faecal tests for intestinal inflammation and two new therapy options for the future.

Faecal tests. Many patients with IBD, in spite of normal activity indices, are obviously not in remission. Laboratory tests like CRP, ESR, platelets and orosomucoids provide some assistance in assessing disease activity. However, they are all blunt tools and can also be influenced by factors other than intestinal disease. Several faecal tests have been introduced to measure intestinal inflammation. Measurement of intestinally produced nitrogen oxide (NO) from samples taken from the rectum has shown that increased concentration of NO is a sign of inflammation in patients with IBD, including children. Analysis of some proteins in faeces, like elastase, lactoferrin and calprotectin, all show associations between high values and inflammation.

In a Swedish study, faecal calprotectin discriminated well between IBD and non-IBD in children investigated on suspicion of IBD, with a predictive value of 95%, compared with predicted values from blood tests; albumin, platelet count, orosomucoid, ESR and CRP (75-61%) (3). Further studies from the same group found a close association between the assessment of calprotectin and the endoscopic grading of both macro- and microscopic changes in inflammation. In daily practice the test can also be used to record the effects of new therapies and for early detection of relapse.

Therapies for the future. Our current therapies include use of immunosuppressive agents and biologicals. Serious side-effects are possible for both types of therapy and some long terms consequences are unknown. This is of great concern for patients, parents and physicians.

Leucocyte adsorbtive aphaeresis. Blood is passed through a column that adsorbs leucocytes during an hour, once weekly for 5 to 11 weeks. Good results in open studies have been reported in severe UC, where a majority of treated patients went into remission and most of them did not relapse during the following year (4). There are indications that this treatment is less effective for CD. The result of a controlled study using sham-therapy in the control group is due to begin soon.

Trichuris suis therapy in CD. CD is common where helminthic colonisation is rare and uncommon in areas where most people carry worms. Helminths have been shown to reduce inflammation in experimental murine colitis. The proposed mechanism is that helminths change the balance between Th1 and Th2, in favour of Th2 and limit Th1-type inflammation.

Trichuris suis (T. suis), the porcine whipworm, is similar to human whipworm. In an open study using this organism, 29 patients with CD drank a solution of ova from *T. suis* every third weeks for a duration of 24 weeks. A total of 23 patients (79%) exhibited a positive response with a decrease in the Crohn's activity index score (5). Similar results have been reported in UC.

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Environmental Risk Factors in Paediatric IBD

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There is accumulating evidence that events early in life may have long term effects on health and disease. Several epidemiologic studies have suggested a role for perinatal or childhood events in the aetiology of inflammatory bowel diseases (IBD). Both non-specific exposures and specific exposures have been studied. Non-specific exposures include gastroenteritis and other non-specific infections. Specific exposures include measles virus infection and vaccines, appendectomy and passive smoking. The gut is constantly exposed to a stream of dietary factors which has been made increasingly complex by modern food processing techniques and chemical preservatives. Despite this evidence, the role of diet in paediatric IBD has almost not been investigated. As for auto-immune diseases, a consistent finding in early observational studies for both Crohn's disease (CD) and ulcerative colitis (UC) was the association between high socio-economic status and an increased risk. An attractive theory to explain this association is that it may result from a decrease in the prevalence of early childhood infections.

A positive family history and appendectomy still remain the most consistent risk factors for IBD. Having a first-degree relative with IBD represents the single most important factor determining an individual's risk of developing disease. Polito et al reported the proportion of patients with relatives affected with IBD according to the age at

diagnosis in the proband[1]. Thirty percent of patients diagnosed under age 20 years had a positive family history, decreasing to 18% for those diagnosed between 20 and 39 years of age, and to 13% among those diagnosed after 40 years. Thirteen published case-control studies were summarized by Koutroubakis et al. concluding that appendectomy reduced the risk of UC by 69% (62%-75%)[2]. This inverse relation between UC and appendectomy is particularly strong in paediatric populations.

Adult cigarette smoking is associated with the development of CD and protection from the development of UC. Studies on exposure to passive smoking in childhood have given controversial results: association with an increased risk of both CD and UC, lower incidence of UC not confirmed in other studies including ours.

Diet has been repeatedly suspected as being a risk factor in IBD[3]. Practically, diet is an important determinant of IBD symptoms and clinical nutrition is an essential component of IBD therapeutics in children. The role of diet as a de-novo risk factor for IBD is less clear, but it remains highly biologically plausible. The incidence trends for IBD seem to be associated with a 'Westernized' lifestyle and diet. Western dietary patterns may predate IBD in countries with traditionally low IBD prevalences. However, retrospective case-control studies examining the role of dietary risk factors in IBD have often shown discrepant results. There are several reasons why this may be so. Firstly, the *pre-illness* diet is of presumed importance in the etiology of IBD, probably over years or decades. However, individuals with IBD modify their diet significantly following onset of disease. This influence of intestinal inflammation on dietary habits is likely to lead to inaccurate pre-illness reporting, especially when the disease has been of long duration. Secondly, there has been great variation in quantification of food consumption in the existing studies, ranging from highly subjective measures such as 'low', 'medium' or 'high' intake, to teaspoon measures to actual weighing of foods, despite the importance of standardized and validated quantification of food intakes for assessing risk. Thirdly, definitions of the foods themselves have varied from study to study. All of these variances have made firm conclusions regarding the relationship of diet to IBD difficult to determine. If previous methodological pitfalls can be overcome however, the argument for revisiting the role of diet as a risk factor remains strong. An emerging area of potential importance is the emergence of foodborne bacteria. Several groups have demonstrated increased counts of adhesive E. coli in the intestines of CD patients, and it is conceivable that modern-day agricultural practices are adversely influencing the emergence of these bacteria. It remains unclear what impact modern agricultural practices including widespread use of antibiotics might be having upon the dynamic epidemiology of IBD. It has recently been hypothesized that widespread dissemination of psychrophilic (''cold-loving'') bacteria through the food chain by the use of refrigeration may be linked to the rising epidemic of IBD, particularly CD[4].

Several studies including a recent meta-analysis have suggested a protective effect of breast feeding against the subsequent development of IBD. Certainly, the unexpected finding of a recent case-control study from Northern France was that breast feeding was associated with an increased risk of CD[5]. Breast feeding is known to provide immunological protection to the newborn. Delayed infections occurring at weaning may lead to an inappropriate immune response and persistence of intestinal inflammation. Another hypothesis may be related to breast milk pollution in this highly industrialised area.

A lot has been written concerning the ''hygiene hypothesis'' in IBD as in other auto-immune diseases. It postulates that multiple childhood exposures to enteric pathogens protect an individual from developing IBD later in life, while individuals raised in a more sanitary environment are more likely to develop IBD. Studies by Gent et al. and Duggan et al, showing that CD was more common in children who had access to hot water supply and a separate bathroom, supported this hypothesis. We and others were not able to confirm this finding. Conversely, in our study, bedroom sharing was a risk factor for IBD. This may be a surrogate marker of exposure to infections early in life because the more crowded the living conditions are, the more frequent is the exposure to infections. In recent studies, it was also shown that the number of older siblings conferred an incremental increased risk of developing UC and that lower birth rank, as a possible indicator of increased childhood infection exposure, was associated with a higher risk for IBD. These findings are consistent with the observation that older siblings increase risk and severity of secondary infections in younger siblings. The role of infections early in life has been the focus of many investigations. There has been previously described increased frequency of gastroenteritis, respiratory, and perinatal infections in IBD. A history of frequent childhood infections or exposure to antibiotics has also been proposed as a risk factor for IBD. As of now, the veracity of the hygiene hypothesis for IBD is not confirmed. and it is worth noting that in a recent issue of the *American Journal of Gastroenterology*, two case-control studies, both from Canada, came to diametrically opposed conclusions regarding the hygiene hypothesis for CD[6,7].

One of the most controversial areas regarding risk factors in paediatric IBD has been the role of vaccinations. An increased risk of IBD among persons born during a measles epidemic as well as a close temporal relationship between measles infection and the development of IBD was initially suggested. A high risk of CD was reported in patients whose mothers were infected with measles around the time of birth. Live-attenuated measles vaccination was subsequently implicated. This theory has since been abandoned. In our recent survey, there was no difference between cases and controls regarding exposition to measles as wild or attenuated virus. None of the mothers reported measles disease during pregnancy. MMR vaccination was even negatively associated with a risk of CD, confirming data from four large health maintenance organisations (HMOs) in the United States. In contrast, smallpox and poliomyelitis vaccination were associated with an increased risk of both CD and UC. An association between BCG and an increased risk of CD was particularly convincing because it was dose dependent and remained in the multivariate analysis. BCG is associated with a TH1 immune response. It has been proposed that administration of BCG early in life could protect against the development of allergic disorders with a predominant TH2 response. It is generally accepted that CD is a disorder with a predominant TH1 immune response. Modulation of the immature immune system early in life towards TH1 immune response could thus favour the development of CD.

While family history and appendectomy are the best established risk factors, the changes in risk based on domestic promiscuity, certain vaccinations and dietary factors may provide new etiologic clues. Soundly designed and carefully conducted epidemiological studies will be necessary to help confirm or refute these environmental factors as explanations for the rising incidence of IBD. What is ideally needed is a long-term, prospective cohort study following at-risk children for IBD over several years or decades, to accurately determine and characterize those lifestyle factors which may be associated with future development of IBD.

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BASIC SCIENCE

Immunopathogenesis of IBD: Where Do We Go from Here?

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During the last decade, there has been gradual shift towards consensus concerning the immunopathogenesis of IBD, away from focus on individual pathogens as putative global causes of IBD, towards recognition that immune tolerance mechanisms towards the gut flora are critical determinants of inflammation[1]. There has been broad concordance between experimental models highlighting the importance of the flora and human genetic susceptibility studies identifying innate immune receptors involved in bacterial sensing. Despite this there are large gaps in our understanding, particularly concerning ulcerative colitis.

Insights into pathogenesis through genetic and epidemiological studies. Following the identification of the Nod2/CARD15 gene as an important susceptibility determinant for Crohn's disease, there has been recognition of additional gene loci that modify risk of developing IBD or affect phenotype. Even prior to the identification of susceptibility loci, there was evidence from twin studies that phenotype concordance was far from complete. This implies an important role for environmental factors even in those predisposed to IBD[2]. One challenge, if genetic studies are to provide insight into pathogenesis, is to incorporate recognition of interacting environmental factors which may differ between patients. Recognition of significant links depend on fruitful interaction between molecular geneticists and epidemiologists. It is more likely that such collaboration will unearth true gene-environment interactions in transitional societies where IBD is recently emerging, and where there are substantial social gradients in environmental exposure[3].

Understanding of proinflammatory mechanisms. It has become almost axiomatic that the production of proinflammatory cytokines by immune cells is critical in driving mucosal inflammation in IBD. Amongst a growing array of potential mediators, the major macrophage-derived cytokines TNF-α, IL-1β and IL-6 appear to have predominant roles in IBD. Their downstream effects include impairment of epithelial barrier function, promotion of microvascular thrombosis and alteration of extracellular matrix composition. Much has been learned about the distinct pathways in which production of these mediators may be driven, and an apparently central role for nuclear factor-κB (NF-κB) in their production. The advent of small molecular inhibitors of intracellular activation pathways provides opportunity and challenge. The opportunity is of specific inhibition of pathological cytokine overproduction without leaving the patient globally immunosuppressed. The challenge is of understanding regulatory input in sufficient detail to achieve this happy state. However, therapeutic trials have continued to provide important reality checks for theoretical optimists, and the unexpectedly poor performance of tacrolimus and several TNF antagonists has forced re-evaluation of just what may be central in pathogenesis. If the complement-fixing TNF-α monoclonals and TNF receptor antagonsists are equipotent in rheumatoid arthritis, why should only the former be effective in IBD? We do not really have much idea of the mucosal mechanisms through many agents work, and future studies may need to compare mucosal biopsies before and after novel therapy, using appropriately sensitive techniques.

New basic immunological discoveries may also force novel insight into IBD mechanisms. Previously unrecognised subpopulations of dendritic cells and lymphocytes, notably including T_H17 cells, appear important in inflammatory and autoimmune diseases. It is therefore possible that individual cell types may prove pivotal in driving IBD pathogenesis, and therapeutic attention may shift from neutralising proinflammatory molecules towards inhibition of disease-driving lymphocyte clones.

Understanding regulation of tolerance to the flora. The recognition of specific regulatory lymphocyte populations such as CD4+ CD25+ cells, of regulatory cytokines such as TGF-β and IL-10, and of the pivotal role of the transcription factor Foxp3 in the regulatory pathway has been of immense importance in understanding the pathogenesis of IBD[4]. Much remains to be learned about the programming of immune tolerance in early human life, particularly in respect of IBD. While tolerance to self antigen may be determined by thymic selection in utero, tolerance to acquired commensal antigens necessarily happens after birth. There is evidence of intense immune activation and transient systemic cytokinaemia in normal human infants after birth. In the light of experimental data showing the obligatory role of the indigenous flora in promoting the primary acquisition of immune tolerance to dietary antigens, it appears that much more basic data needs to be gathered about initial tolerance events to the flora in infancy. Is it safe to assume that the development of IBD at age 10, 15 or 20 reflects the sudden loss of previously acquired tolerance at that stage? One equally possible scenario would be that the primary sensitisation event may have occurred much earlier, and that potentially pathogenic T cell clones have remained clinically silent until unmasked by later challenge[5]. This latter scenario is reminiscent of that thought to underlie many cases of autoimmunity, in that thymic selection fails to delete potentially autoreactive cells, but autoimmunity supervenes only when peripheral tolerance mechanisms fail or pathogens initiate inflammation through molecular mimicry. The equivalent of central tolerance to self antigen for the gut flora may develop within extrathymically maturing intestinal lymphocytes rather than the thymus. An intriguing question is whether similar tolerance-inducing mechanisms may apply. Almost nothing is known about such mucosal events in human infancy, and this appears a fundamental gap in our knowledge of potential sensitisation mechanisms in IBD.

Understanding of the gut flora. Given the importance of the gut flora in the overall pathogenesis of inflammatory bowel disease, it is daunting to recognise the complex constitution of the flora. If indeed there turns out to be a Helicobacter pylori-equivalent for IBD, a previously overlooked dominant pathogen responsible for disease development in many patients, its discovery may be a matter of great difficulty. Recent comprehensive molecular analysis has identified a massively complex normal flora, most of whose components cannot be cultured. Components of the flora induce specific genes within the host's epithelium and immune system. Unravelling the interactions of this profoundly complex ecosystem in health and disease will require input from mathematicians as well as biologists, but may shed new light on disease mechanisms in IBD.

Location and timing are key issues in many immunological mechanisms. Uptake of gut bacteria by dendritic cells or penetration into mucosal lymphoid tissue and specific interaction with the immune system are potentially fundamental events in acquisition or loss of tolerance to the flora, and are still poorly understood in humans. It is notable from mouse models that pathophysiological responses to the flora are set in train in very early life, but remain clinically silent for a considerable period. The comprehensive change in initial colonisation patterns of human infants within developed societies may yet turn out to be important in determining early-life sensitisation events that may only be unmasked much later after additional environmental challenges[5].

Build a better mouse model and the world will beat a path to your door. The major importance of murine models of IBD has been to change basic concepts of disease pathogenesis, notably to emphasis the central role of the luminal flora and the importance of a balanced immunological response to the flora, in which regulatory lymphocytes are generated. Where they are arguably less good is their precise equivalence to human disease, and there is no shortage of therapeutic agents that work spectacularly well in mouse models but are ineffective in human disease. Another area of weakness has been lack of clinical relevance in experimental design, although recent reports of the modulatory effects of psychological stress in IBD models have been an important step in the right direction. Clustering of relapses of human IBD and serological evidence of recent exposure to various viruses, suggest that attempts to modulate presentation and phenotype of mouse models by ordinarily non-pathogenic viruses may be a worthwhile target. Study of the presymptomatic phase in early life, when the animal has passed a silent event horizon towards inevitable later disease, is another target that may be particularly relevant to paediatric IBD.

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Early Immune Responses in IBD

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Traditionally, aberrant adaptive mucosal immune responses by T and B cells have been regarded as the main contributors to the pathogenesis of Inflammatory Bowel Disease (IBD). Recently, various defects in the function of innate immune cells have been implicated. As such, both hypo- as well as hyper-responsiveness of these cells have been put forward. The two main hypotheses on the contributions of the innate immune system to the pathogenesis of IBD are: (1) The innate immune system has a *loss of function*. This type of defect is associated with a failure of microbial eradication at the mucosal surfaces (2). The innate immune system has a *gain of function*. The mucosal innate immune cells display exaggerated responses to harmless bacterial stimuli.

Loss of function. The discovery of an association between mutations in the intracellular microbial pattern receptor NOD2 and Crohn's disease (CD) have unequivocally established a role for the innate immune system in disease pathogenesis. NOD2 is expressed by monocytes and epithelial cells such as the Paneth cells. As Paneth cells are mainly found in the terminal ileum it is not surprising that genotypic defects in NOD2 seem to be linked to a disease phenotype with severe inflammation at that specific location. Mice with a mutated NOD2 gene expressed significantly less antimicrobial peptides (cryptdins) within the Paneth cells and displayed impaired eradication of *L. monocytogenes* upon oral administration (1). Studies with NOD2 mutant mice explain how a defect in clearance of certain bacteria may lead to a perpetuative, albeit insufficient, mucosal immune response and therefore chronic inflammation. The latter paradigm has been extended to human disease by showing strongly diminished expression of the human variant of these peptides (defensins) in CD patients with a NOD2 mutation.

A second innate cell type that may contribute to the chronic inflammation in IBD is the mucosal macrophage. Similar to the mechanism for Paneth cells, a loss of function in macrophages has been described. As such, monocyten-derived macrophages from CD patients with a NOD2 mutation produced less of the chemo-attractant IL-8 upon stimulation with the NOD2 ligand MDP. In turn, this could lead to a reduced and delayed recruitment of neutrophils, and suboptimal clearance of bacteria from the intestine, again leading to chronic inflammation (2).

Gain of function. It has been proposed that innate cells may become hyper-responsive due to a defect in physiological anti-inflammatory mechanisms. In a group of pediatric CD patients it was shown that buccal epithelial cells derived from these patients produced higher levels of IL-8 and other chemokines either spontaneously or upon microbial stimulation (3). The enhanced chemokine production was specifically associated with pediatric Crohn's disease and appeared restricted to cells derived from the epithelial barrier. Although a definitive mechanism has not been established yet, these data suggest that besides a defect in antimicrobial immunity, hyper-responsiveness may also be involved.

A typical example of the gain of function theory was offered by research performed in NOD2 deficient mice. Microbial peptidoglycan (PGN) can be recognized by the extracellular receptor TLR2 as well as the intracellular located NOD2 protein. When macrophages derived from NOD2 deficient mice were stimulated with PGN, this resulted in a over expression of pro-inflammatory cytokines compared to wild-type macrophages (4). It was suggested that NOD2 may act as a negative regulator for TLR2 signaling. This way, defective signaling through NOD2 may lead to exaggerated TLR2 sensitivity through the absence of dampening mechanisms.

Loss or Gain? In the light of the complexity of mucosal homeostasis that is geared for tailored antimicrobial responses in the absence of overt inflammation, it is astonishing that the IBDs are relatively rare diseases. The typical tolerant state of both the mucosal adaptive as well as the innate immune system implies vigorous anti-inflammatory mechanisms. Various novel regulatory mediators and associated mechanisms have been described: Tollip, sTLR2, IRAK-M, SIGGR, A20 and SLPI (5). Furthermore, it is becoming clear that the IBD patients represent a very heterogeneous group of patients that may need a novel classification, based on specific gene-defects, phenotypical appearance and perhaps responsiveness to specific drugs. Therefore, we predict that many of the proposed mechanisms may only apply for certain subsets of patients. On the other hand we cannot exclude that both loss and gain of function may actually occur at the same time in the same patient.

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Host–Flora Interactions in Inflammatory Bowel Disease

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The mucosal immune system of the intestine senses luminal bacterial populations via multiple cell types such as epithelial cells, M cells, dendritic cells and lamina propria macrophages. These sentinel cells then initiate different innate and adaptive immune responses depending on whether it is a pathogen or a commensal that has been the trigger. While immunity to pathogens is well characterized, the underlying mechanisms governing the response to commensals are more poorly understood. ''Danger'' signals generated by pathogens induce the production of inflammatory/chemotactic mediators and thereafter, activate adaptive immune mechanisms. Although these processes cause some damage to host tissues, the bacterial infection is cleared. In contrast, the response to commensals allows the bacteria to persist in the intestine in the absence of an exaggerated inflammatory reaction. Indeed, host-commensal flora interactions are cytoprotective for the epithelial layer and assure immune homeostasis in the mucosa (1).

Bacterial recognition in mucosal compartments depends on the expression of germ-line inherited pattern recognition receptors (PRR) that recognize conserved bacterial motifs in both pathogens and commensals. One example is the Toll-like receptor (TLR) family which detects microbial motifs, such as lipopolysaccharide (LPS) and lipoteichoic acid, in the extracellular compartment. Upon TLR ligation by bacterial products, there is activation of NF-κB and the genes for inflammatory mediators. However, since TLR ligands are common to both pathogens and commensals, it is not fully understood how the host

can simultaneously distinguish between the two types of bacteria. It appears that all bacteria induce an initial inflammatory response but with commensal-derived products, this reaction is limited in severity, is transient in time and is followed by an immunological unresponsiveness called tolerance that involves components of both the innate and adaptive immune response. In contrast, encounter with pathogenic organisms generates a more intense response which is triggered by the ''danger'' signals liberated from damaged host cells or the detection of bacterial products by intracellular, most probably cytosolic, molecular platforms. An example of the latter is the recently described inflammasome, a cytosolic protein complex of NALP1, ASC and caspases (2). Inflammasome initiated responses follow detection of intracellular muramyl dipeptide (MDP) or LPS but also stress signals or endogenous danger signals such as ATP or the uric acid crystals which are released from dying cells during inflammation. Interestingly, NALP1 shares structural homology with the nucleotide-binding oligomerization domain (NOD) family of intracellular signaling molecules. NOD1 and NOD2 are intracellular PRR that bind bacterial products in the cytosol and activate NF-κB and MAP kinases via the receptor-interacting protein (RIP)-2.

Taken together, the host response to bacteria can be considered as a two-tiered process which in a first instance, involves pro-inflammatory genes that are triggered by most bacteria, pathogenic or not, and subsequent activation of a second cluster of genes that is defined by specific virulence traits that are present only in pathogens (3). Apparently, the primary gene cluster activation is a prerequisite for the subsequent anti-inflammatory response and tissue homeostasis (4) that occurs in the presence of commensals or after infection has been resolved.

There is accumulating evidence that an abnormal immune response to enteric bacteria underlies the inflammation and tissue injury observed in IBD, that members of the commensal flora are perceived as surrogate pathogens by the mucosal immune system. The capacity to distinguish pathogens from commensals is apparently impaired and it has been postulated that the abnormal innate response which ensues, leads to an altered adaptive response to commensals and loss of immunological tolerance. Exactly how or why this comes about is not known though several mechanisms have been proposed. Altered gut epithelial barrier function and permeability to bacterial products is one proposition. Indeed, mutation of two genes implicated in such functions of epithelial cells, are associated with susceptibility to IBD. Mutation of the gene encoding the organic cation transporter (OCTN) on chromosome 5q31, affects the ability to transport xenobiotics and amino acids, specifically carnitine, across the epithelial cell membrane. While maintenance of epithelial cell polarity is lost with mutation of the guanylate kinase DLG5 gene on chromosome 10q23 (5). Such mutations may lead to an increased exposure of lamina propria immune cells to bacterial molecules. Certainly, the increased expression of CD14, TLR2 and TLR4 on lamina propria macrophages, exposed to a larger bacterial load in IBD patients, may explain a hyperreactivity to bacterial products.

Other disease susceptibility genes for IBD are allelic variants of NOD2. Most of the identified alleles associated with higher susceptibility appear in the leucine reach repeat of the molecule that enables the molecule to recognize the bacterial conserved molecular motifs. Thus impaired recognition of bacterial MDP and NF-κB activation is observed. The most common disease-associated variants of NOD2 result in hyporesponsivenss to intracellular peptidoglycans and results in reduced NF-κB activation (6) upon bacterial challenge. This may contribute to lower the capacity in the clearance of translocating bacteria. Abnormal high bacterial load can activate antigen presenting cells that subsequently may activate the CD4+, Th1 lymphocytes that are involved in the pathogenetic mechanisms of IBD.

Finally, TLR-mediated innate responses and immunological tolerance to commensals are regulated by a multitude of mechanisms including production of transforming growth factor (TGF)-β and interleukin-10 and stimulation of peroxisome proliferator-activated receptor (PPAR)-γ and de-ubiquitinating enzymes, all of which are altered in IBD.

In conclusion, environmental, genetic and immunological factors determine susceptibility to IBD. There is increasing evidence that inappropriate recognition of and/or response to commensal-derived products explains the interplay of these factors.

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Opportunities in Pediatric IBD Research: Updating the Scientific Agenda

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Pediatric inflammatory bowel disease (IBD) is finally coming of age. After decades of having been relegated to a distant second position compared to adult IBD, and having only indirectly and marginally benefited of the remarkable progress that has occurred with the adult disease, pediatric IBD is now being recognized as an entity in its own right. This means that pediatric IBD is not just the adult disease in ''little people'', but a condition with features characteristic of each individual component of its pathogenesis, i.e., epidemiology, genetics, microbiology and immunology. Regrettably, progress in each of these areas has been slow and uneven, primarily due to the short time frame since when pediatric IBD has been recognized as a distinct entity, but the pace of investigation is undeniably speeding up.

Few studies have seriously tackled the difficult task of collecting hard evidence demonstrating that children with Crohn's disease (CD) and ulcerative colitis (UC) constitute an increasingly larger proportion of the overall IBD population. Daily clinical practice, where children with newly diagnosed IBD appear to be increasingly common, supports this notion, but it is far from scientifically solid data. An intriguing aspect of pediatric IBD epidemiology is that this newly diagnosed population seems to differ from similar groups of several decades ago, where certain ethnicities and social or economical classes were traditionally more afflicted. Recent studies indicate that the ethnicity and racial background of children with newly diagnosed IBD perfectly match the background of the general population (1), strongly suggesting that environmental factors may be more dominant than genetics in today's pediatric IBD. In view of this evidence, what is needed are large population-based studies that should be initiated and prospectively followed up with the support of local and federal agencies.

Genetics of IBD have experienced a truly impressive progress in less than a decade, and have catapulted IBD at the forefront of genetic studies among all other autoimmune and chronic inflammatory disorders. Again, most of the progress derives from observations in adult IBD populations, both for CD and UC, and few studies have been targeted to pediatric IBD. It could be argued, and some investigators claim, that there is no reason to expect that the assessment of genetics of children with IBD should yield results different from those of adults. Countering this argument are two important facts. The first is that appearance of IBD early in life may very well define a peculiar subset of individuals where disease-predisposing genes are different or have a different penetrance. The second is that, in complex disease like IBD, genes alone are not sufficient to induce clinical manifestations and that gene-environment interactions are essential for clinical phenotypes to emerge (2). Therefore, the investigation of pediatric IBD genetics

should be encouraged, not only in populations where IBD is well established and with known genetic associations, like for Caucasians or Jewish people with NOD2/CARD15 mutations, but also in those where such associations do not exist, as in the Asian Pacific rim region.

It is currently accepted that components of the normal enteric flora represent the target of the abnormal immune response responsible for inflammation at the gut mucosal level. Experimental and clinical evidence supports this notion, both in humans and animal models. However, which bacterial components provide the dominant antigens being abnormally recognized and when the development of this inappropriate response occurs are still undefined. This ignorance is partly due to the limited attention paid to gut microbial ecology by the community of IBD investigators for several decades, and partly due to the fact that most studies of the gut microbiota are being carried out in adult IBD patients with long-standing disease. Its is well established that, even though the composition of the gut microbiota is roughly the same among humans, each person has his or her individually unique flora, and this may considerably change depending on age, diet, geography and concurrent infections. Since the starting point of IBD is always unknown, it is impossible to know what the flora was when the mucosal immune system began to recognize it abnormally and react against it. The immune system early in life is extremely adaptable and certainly starts recognizing the gut flora immediately after birth (3). Thus, it seems both logical and potentially more rewarding to study the gut microbiota in children at risk of developing IBD and immediately after the clinical diagnosis or even suspicion of IBD is made.

The immunology of IBD is the best characterized component of IBD pathogenesis. This is certainly true in adults and, to some degree, even in children with IBD, although many fewer studies have been conducted in the pediatric population. Some of the same immunoregulatory and pro-inflammatory abnormalities described in adult patients have also been reported in children with CD or UC. However, exciting new evidence is emerging to suggest that some peculiar aspects of the immune response are unique to children with early onset IBD. Mucosal T cells derived from children with early, but not late, CD and UC appear display a Th1 pattern similar to that observed in infectious colitis (4). This is obviously different from what is traditionally observed in adult subjects with typical CD and UC, who display a well defined Th1 and an atypical Th2 cytokine profile, respectively. Studies in animal models lend support to the existence of different immune response profiles in the early and late phases of experimental colitis. The immune response observed in the early stages of colitis in IL-10-deficient mice is a typical Th1 response dominated by IL-12 and IFN-γ, but in the chronic stage of disease both cytokines return to normal levels and inflammation becomes associated with an elevation of IL-4 and IL-13, prototypical Th2 cytokines (5). This is not unique to IBD, and differences in early and late immune response have been documented in other immune-mediated conditions (6). In addition, new immunological paradigms are constantly being uncovered, like the newly described Th17 cells (7), and they must be evaluated and possibly integrated in pediatric IBD pathogenesis. Based on this evidence, the concept that early and late IBD may be pathogenically diverse entities is starting to consolidate. As a corollary, pediatric/early and adult/late IBD might also be fundamentally diverse, and this could have profound therapeutic implications.

Ideally, to obtain a comprehensive and realistic pictures of pediatric IBD, one should try to integrate data from genetic, microbial and immune experimentation to reproduce the pathogenic events occurring in children with CD or UC. This is obviously impossible to do in humans, and more so in children, who still suffer from a more restricted approach in regard to human testing. To compensate this limitation, the development of a large number of animal models has come to the rescue, allowing to investigate the evolution of experimental IBD from birth to adulthood, analyze changes in the gut flora during the progression of disease, evaluate the associated immunological changes, and test rationale- and time-based therapies aimed at modulation of the enteric microbiota and the mucosal immune response (8).

In summary, it is clear that pediatric IBD is at a critical juncture, when enough data are accumulating to validate the notion that children with IBD represent a unique patient population deserving to be studied as one different and potentially more relevant than the adult IBD population, both from a pathogenesis as well as a therapeutic point-of-view. This situation has been appreciated not only by the community of academic investigators interested in pediatric IBD, but also the Crohn's & Colitis Foundation of America, that has recently put forward a "Challenges in Pediatric IBD Agenda" (9). This agenda, based on a consensus reached by several task forces composed by leading pediatric investigators, lists a series of areas for priority investigation in pediatric IBD, including genetics, epidemiology, microbiology, immunology, pharmacogenomics, nutrition and diet, growth and skeletal health, psychological issues, clinical trials and quality improvement and safety. The implementation of this comprehensive approach by the research IBD community will realize the opportunities offered by serious and well designed studies, and will inevitably result in improvement not only in knowledge, but also treatment of children with IBD.

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NIH Funding Opportunities and IBD Research

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The National Institutes of Health (NIH), a part of the U.S. Department of Health and Human Services (DHHS), is the primary federal agency for conducting and supporting medical research. Its mission is science in pursuit of fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to extend healthy life and reduce the burdens of illness and disability. To accomplish this, it funds research across the world via various mechanisms, such as research grants, contracts, NIH roadmap initiatives, and NIH loan repayment programs, etc.

About 180 research grants have been funded for Inflammatory Bowel Disease (IBD) research since 1972. Research areas expand from infectious pathogens, immunology, therapeutics as well as genetics and microflora study, etc. Of which, about 100 research grants related

to the research of Crohn's disease, 50 to ulcerative colitis, and 30 to both. There are about 82 clinical trials currently registered at http://www.clinicaltrials.gov. Of which, 9 are sponsored by NIH with focuses on immune regulation, therapeutics as well as pediatric IBD researches, etc.

National Institute of Allergy and Infectious Diseases (NIAID) is the component of the National Institutes of Health (NIH) charged with conducting and supporting research on diseases of the immune system and infectious diseases, and developing better means of preventing, diagnosing, and treating these illnesses. The Food and Waterborne Diseases Integrated Research Network (FWD IRN) is one of its contracting networks which facilitates the integration of research programs to address food and waterborne pathogens included in NIAID Category A, B, C priority list, as well as Enteric and Hepatic Diseases Branch (EHDB) research priorities for other enteric pathogens, such as *Helicobacter pylori* and etiology of Crohn's disease. It has funded research in a clinical trial of *Mycobacterium Avium ssp Paratuberculosis* in Crohn's disease patients and development of animal model for Crohn's disease.

In summary, Crohn's disease and ulcerative colitis affect nearly one million Americans. Crohn's disease tends to affect the small intestine while ulcerative colitis usually causes an inflammation in all or part of the large intestine. People with inflammatory bowel disease (IBD) such as Crohn's disease and ulcerative colitis frequently suffer from diarrhea, abdominal pain, fever, and weight loss. The cause(s) of Crohn's disease and ulcerative colitis are unknown and there is no cure for either condition. Medications can only control the symptoms of IBD and, in some cases, surgical removal of the involved intestine may be necessary. In the past few years, IBD research has experienced tremendous progress, including identification of Crohn's disease susceptible gene, development of infliximab, an anti-TNF monoclonal antibody, etc. NIH will continue its effort to advance research in these debilitating diseases through its strong support and scientific leadership.

Neuroplasticity of the Enteric Nervous System Induced by Inflammatory Conditions of the Gut

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The enteric nervous system (ENS), the third component of the autonomic nervous system, is composed of a wide collection of neuronal cells distributed along the length of the alimentary tract and organized in two major ganglionated plexuses, the myenteric (Auerbach's) and submucosal (Meissner's) plexus. Both plexuses contain many different type of functionally distinct neurons, including primary afferent neurons, interneurons and motor neurons, synaptically linked to each other in microcircuits which virtually control, independently from central nervous system inputs, all gastrointestinal functions (i.e., motility, secretion, blood flow, mucosal growth and aspects of the local immune system).

Despite neurons have been long thought to be structurally and functionally stable cells, with a well-defined phenotype, data acquired in the last twenty years have led to the concept of enteric neuronal plasticity. This phenomenon encompasses a wide range of structural and/or functional changes of the ENS in response to a wide array of stimuli coming from the enteric microenvironment in order to maintain gut homeostasis.

Herein, we will briefly focus on neuronal plasticity evoked by gastrointestinal inflammation (e.g. in inflammatory bowel disease, IBD) and major aspects underlying the mechanisms responsible for neuronal changes such as neurotrophic factors and the role exerted by enteroglial cells. For a more detailed analysis of inflammatory evoked changes of the ENS, the readers are referred to extensive works (for example, see reviews 1,2).

Structural changes, accompanied by altered gastrointestinal function, such as motility and secretion, have been identified in experimental animal model of enteritis/colitis (3) and in patients with IBD (4). Specifically, in patients with IBD, enteric neuron abnormalities include neuronal hyperplasia and hypertrophy and enteroglial cells hyperplasia, often accompanied by ganglion cell and axonal degeneration and necrosis. The pathological abnormalities of IBD appear to be related to the type of disease (Crohn's vs. ulcerative colitis, UC), the region of the intestinal wall and whether or not the tissue was from a site of active disease. Likely, the observed damage is immunologically mediated since it has been shown that both neurons and enteric glia in tissues from Crohn's disease (CD) patients display the major histocompatibility class II antigen on their surface, which exerts an important role in the antigen presentation to T-lymphocytes (2,3). These findings suggest the existence of an active cross-talk between nerves and inflammatory cells. Moreover recent data indicate that during inflammation enteroglial cells synthesize and release inflammatory cytokines, thereby contributing to neural changes (5).

Several studies reported specific changes in enteric neuronal coding and receptor expression in IBD patients and in animal models of inflammation; these neurochemical changes are observed in neurons, nerve fibres and glial cells. The most widely studied peptides have been substance P (SP) and vasoactive intestinal peptide (VIP) because of their well known effect on gut function (1-4). SP is mainly expressed by intrinsic enteric neurons and primary afferent nerve fibres mainly projecting from dorsal root ganglia. Immunohistochemical analysis of tissue specimens from patients affected by UC revealed a threefold increase in SP-positive myenteric neurons. Other studies reported an increase in SP-positive nerve fibres and neuronal SP level, suggesting that this neurochemical change may be interpreted as an hallmark of UC. In fact, SP appears to be relatively unaffected in CD. SP and other related tachykinins mediate their effects via the interactions with three neurokinin receptors, namely NK-1, NK-2 and NK-3. In line with changes to SP neural network, several data showed expression abnormalities to these receptors in patients with IBD. VIP is up-regulated during intestinal inflammation. An increase in VIP submucosal and myenteric ganglion cells and nerve fibers has been documented in inflamed and non-inflamed tissues of CD patients. In contrast, however, a marked decrease of VIP-immunoreactive nerve fibres in the colonic lamina propria and submucosa of patients with IBD has been also reported. In support of a reduced VIP neuronal content, Duffy et al. measured VIP plasma levels and found a positive correlation between peptide levels and disease activity. In active disease, VIP levels nearly doubled, indicating a massive and sustained release of VIP, consistent with decreased levels detected in tissues. Consistent with evidence showing neurochemical abnormalities, several alterations of intestinal motor function have been identified during intestinal inflammation. *In vitro* muscle responses to electrical field stimulation in colonic samples of patients affected by UC suggests a selective up-regulation of non-cholinergic, non-adrenergic inhibitory innervation of the colon compared to controls. A significant amount of data is available in experimental models of intestinal inflammation showing motility abnormalities due to either neuronal or smooth muscle changes.

Neurotrophins (i.e., nerve growth factor [NGF], brain derived neurotrophic factor [BDNF] and neurotrophin-3 [NT-3]) are essential for the development and the maintenance of nervous system, promote neuronal differentiation and survival and modulate neurotransmitter and neuropeptide synthesis and release. Several studies provide evidence for the presence of neurotrophic factors and their receptors in the adult gastrointestinal tract, where they are predominantly localized to ENS and exert a modulatory role in synaptic and morphological neuroplasticity (1,2). Evidence indicates that NGF and NT-3 are up-regulated in various inflammatory processes and their neutralization exacerbate the severity of experimental colitis, suggesting a regulatory and protective role of these neurotrophic factors in inflammation.

Enteroglial cells share morphological, structural and functional properties with CNS astrocytes (5). Further to their well-known protective function, glial cells provide a trophic, metabolic and functional support to neurons by synthesizing and releasing trophic factors and cytokines. In addition, studies showed that ablation of enteric glial cells led to fulminating enteritis with severe inflammation and hemorrhagic tissues necrosis. These results suggest that enteric glia also play an important role in maintaining structural and functional integrity of the

ENS. These data provides support the concept that enteric glia exert an important role in eliciting and regulating the inflammatory response in experimental conditions and likely IBD (2-5).

In conclusion, a better comprehension of ENS plasticity during inflammation could lead to the discovery of potential therapeutic targets to restore functional and neurochemical abnormalities of IBD. In this view, therefore, it is plausible to pave the way to compounds able to favour repair and regeneration of injured gut.

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NOD2/CARD15 and NF-κB Expression in Mucosal Biopsies in Children with Active Crohn's Disease

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Background: The chronically relapsing inflammatory bowel disease (IBD), including Crohn's Disease (CD) and ulcerative colitis (UC), appear to be the consequence of overly aggressive immune responses to enteric bacterial components in genetically predisposed individuals. A significant advance in the understanding of CD was achieved by the identification of NOD2/CARD15 as the first susceptibility gene for this disease in the Caucasian populations. NOD2/CARD15 is a specific pattern recognition receptor protein which plays a main role in the regulation of inflammatory processes in mammals. It detects a specific microbial cell wall component (peptidoglycan) and induces the activation of the NF-κB signalling pathway, affecting the downstream cytokines production.

Aims: To investigate the mRNA expression of NOD2/CARD15 in fresh intestinal mucosal specimens of children with CD in comparison with normal controls in order to validate the hypothesis of a NOD2 up-regulation in CD, as previously experimentally suggested. To assess changes of the nuclear expression and activity of transcription factor NF-κB between patient and control tissues in order to establish a possible relationship between the induction of NOD2 and that of NF-κB. Finally, to measure the mRNA expression level of IL-1beta following NOD2 induction and NF-κB activation.

Methods: 20 children (mean age:12) with active CD plus 10 age matched controls entered into the study; mRNA was extracted from unfixed, frozen biopsy specimens and expression levels of NOD2/CARD15 and IL-1beta were detected by using RT-PCR reactions. NF-κB p65 protein expression was detected by Western Blot analysis, while NF-κB activity was assessed by electromobility gel shift assay (EMSA). Image analysis and quantifications of the bands were performed by a densitometer GS-700 model using the Software Quantity One.

Results: densitometrical analyses showed that CD patients expressed markedly higher levels of NOD2/CARD15 and IL-1beta mRNA as compared to controls. Moreover, significantly enhanced NF-κB protein expression was found in inflamed tissues of CD patients as compared to normal controls. Other analyses are under investigation.

Conclusions: We have shown for the first time that under inflammatory conditions NOD2/CARD15 is up-regulated in intestinal mucosal biopsies from children with active CD. This over-expression is accompanied by a marked nuclear protein expression of the transcription factor NF-κB and a strong secretion of IL-1beta as compared to controls. Our data strengthen the view that NOD2/CARD15 has a crucial role in the etiopathogenesis of CD.

A Model for Bacterial-Mediated Changes of the Mucosal Barrier in IBDs: *Campylobacter jejuni* Infection Impairs the Integrity of Polarized Epithelial Cells

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Background: The pathogenesis of chronic inflammatory bowel diseases (IBD) involves the interaction of host genetic susceptibility, luminal bacteria, and an aberrant immune response. Increased intestinal permeability has been suggested to contribute to IBD pathogenesis; whether this is genetically determined or the result of environmental factors is not known. Several pathogens, including the most common bacterial cause of enterocolitis in humans, *Campylobacter jejuni*, are implicated as possible contributors to IBD.

Aim: The purpose of this study was to define the effects of *C. jejuni* infection on mucosal permeability in model polarized epithelia.

Methods: MDCK1 cells were grown in Transwells and infected with prototype *C. jejuni* strains (TGH 9011; ATCC 81-176; and NCTC 11168) at a multiplicity of infection of 100:1 for up to 48 hours at 37°C. Transepithelial electric resistance (TER) was measured before, and at 18, 24, and 48 hours after infection. Dextran, a macromolecular (10-kDa) permeability probe, was inserted into the apical compartment and sampled 5 hours later from the basolateral compartment. Transmission electron microscopy (TEM) and confocal microscopy were used as complementary methods to assess epithelial monolayer integrity.

Results: In contrast to other enteral pathogens, such as enterohemorrhagic *Escherichia coli*, only a mild reduction in TER was demonstrated after 18 hours of *C. jejuni* infection. However, time dependent reduction in TER was observed at 24 and 48 hours for *C. jejuni* strains 9011 and 11168 (66% of control) in MDCK1 cells ($N=4$-5, $p>0.05$). Introducing the bacteria to the basolateral side of the monolayer resulted in a further drop in TER for strain 11168 (36.1%) ($N=4$, $p<0.05$). Prolonged infection with strain 11168 also caused a 7- and 60-fold increase in permeability to dextran, relative to uninfected controls, with apical and basolateral infections, respectively ($N=4$, $p<0.05$ for basolateral infection). TEM demonstrated areas of tight junction separation and loss of normal polarity, most prominent with strain 11168 infection. The effects of *C. jejuni* were also demonstrated by an abnormal, punctate and interrupted distribution of the tight junction protein ZO-1 using laser confocal imaging.

Conclusions: *C. jejuni* infections result in disruption of the epithelial barrier. *C. jejuni*- induced changes in monolayer integrity were demonstrated by a reduction in TER, an increase in macromolecular permeability, destruction of apical junctional complexes, and a redistribution of junction proteins. These findings provide new insights into the interactions between enteric pathogens linked to IBD and the host epithelial cell barrier.

Diagnostic Work-up and Disease Phenotype in Pediatric IBD: 2-year Results from the ESPGHAN Registry

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Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands, on behalf of the ESPGHAN "Porto" IBD working group (members listed in Table 1)

Introduction: A diagnosis of inflammatory bowel disease is made on the basis of clinical signs and symptoms, endoscopic and histological findings, and radiological assessment. The ESPGHAN Pediatric IBD working group has recently published guidelines on the diagnostic

work-up in children and adolescents (1). According to these Porto criteria, all children with suspected IBD should undergo colonoscopy with inspection of the terminal ileum, as well as upper gastrointestinal endoscopy. In addition, radiological imaging of the small bowel by small bowel follow through (SBFT) or enteroclysis is advised in all children except when a diagnosis of ulcerative colitis is definitive. In order to evaluate to what extent these guidelines are practiced in Europe, the working group has prospectively collected data on the diagnostic work-up of new IBD patients during the two years after agreeing on the Porto criteria.

Materials and methods: The ESPGHAN pediatric IBD working group consisted of 23 paediatric gastroenterologists from 19 centres in 11 European countries and Israel: the Netherlands, Denmark, Sweden, United Kingdom, France, Portugal, Italy, Croatia, Israel, Poland, Czech Republic and Germany. In order to audit the Porto Criteria, the group started to prospectively collect anonymous data on new IBD patients from the participating centres, as from May 1, 2004, using an agreed database sheet. The 2-page electronic datasheets contained items on demographics, family history, diagnostic work-up, diagnosis and findings at first diagnostic endoscopy, histology and radiology throughout the gastrointestinal tract. For each centre in each country, approval was sought from the local Ethics Committee (EC). In most countries, the EC and/or Registration office for databases released a statement of no objection, because the data to be collected were anonymised. Only in the United Kingdom, Sweden and Poland, full review by the local Ethics Committee was required. Datasheets were emailed to the coordinating center in Rotterdam (the Netherlands), entered into a SPSS database and analysed. While data registration is currently ongoing, the two year results (collected from May 2004 till May 31st 2006) are presented below.

Results: During the two years, 800 patients were included (409 patients in May 2004-April 2005, 391 patients in May 2005-April 2006) by the 11 European countries and Israel (Figure 1). Mean age at diagnosis was 12 years for the whole group, with no significant mean age difference between the children with Crohn's disease (CD, n=465; 59%), ulcerative colitis (UC, n=250; 32%) and indeterminate colitis (IC, n=68; 9%). A diagnosis was made in very young children (aged 0-8 years) in 16%, 21% and 24% of children with CD, UC and IC, respectively. Children were aged 9-12 years in 35%, 34% and 35%, and of adolescent age (13-18 years) in 48%, 48% and 41% of the patients with CD, UC and IC, respectively.

Diagnostic work-up: The diagnostic work-up included upper GI endoscopy in 91%, 72%, 76% of patients with CD, UC and IC. Colonoscopy was performed in 99%, 100% and 100% of patients with CD, UC and IC. The terminal ileum however was successfully inspected in only 66%, 63% and 55% of patients with CD, UC and IC. Imaging of the small bowel by SBFT was done in 72%, 36% and 67% of the children with CD, UC and IC, respectively. Altogether, 42% of children with Crohn's disease, 55% of the children with ulcerative colitis and 64% of the children labeled as indeterminate colitis had undergone a complete work-up according to the Porto criteria (as described above). The datasheets in year 2 were adjusted as to incorporate reasons for non-adherence to the Porto guidelines. Failure to inspect the terminal ileum was reported to be due to technical problems in 33%, to risk of perforation in severe disease in 15%, or to ileocecal stenosis in 15% of the failed ileoscopy attempts in 54 CD patients diagnosed in year 2. For 33 UC patients in year 2, ileoscopy failed because of technical problems in 18%, of risk of perforation in 21%, while in 18%, the endoscopist did not feel it was necessary. In the 12 IC patients who did not undergo ileoscopy, technical reasons were the explanation in 50%.

Disease location: Location of disease in Crohn's disease was labeled (based on endoscopic and radiological findings) as isolated ileal disease in 7% of children, as ileocolitis in 46% or as colitis (without ileitis) in 51%. Age-specific disease distribution revealed that children in the youngest age group (0-8 years) had less isolated ileal disease (3%), and more colitis (62% as compared to the older age groups (6% and 52% of 9-12 year-olds; 10% and 47% of 13-18 year-olds). Upper gastrointestinal abnormalities consistent with a diagnosis of Crohn's disease (aphthous ulcers in the stomach, or granuloma(s) in biopsies taken from the oesophagus, stomach or duodenum) were reported in 48% of the children with CD. Perianal fistulae were seen in 9%, and stenosis in 11% of the children with CD at the time of diagnosis. Location of disease in ulcerative colitis was labeled (based on colonoscopy findings) as proctitis in 14%, left-sided colitis in 14% or pancolitis in 64% of the

TABLE 1. *Members of the ESPGHAN "Porto" IBD Working Group*

	Institution	City and Country
Escher JC	Erasmus MC-Sophia Children's Hospital	Rotterdam, The Netherlands
Amil Dias J	Hospital S. João	Porto, Portugal
Bochenek K	Medical University of Warsaw	Warsaw, Poland
Buderus S	University Children's Medical Centre	Bonn, Germany
Bueno de Mesquita M	University of Rome La Sapienza	Rome, Italy
Bujanover Y	Edmond & Lili Safra Children's Hospital, Sheba Medical Centre	Tel Hashomer, Israel
Büller HA	Erasmus MC-Sophia Children's Hospital	Rotterdam, The Netherlands
Chong SKF	Queen Mary's Hospital for Children, St. Helier NHS Trust	Surrey, United Kingdom
Cucchiara S	University of Rome La Sapienza	Rome, Italy
Fell JME	Chelsea and Westminster Hospital	London, United Kingdom
Henker J	Children's Hospital, Technical University	Dresden, Germany
Hildebrand H	Karolinska Institute	Stockholm, Sweden
Hugot J-P	Hospital Robert Debré, AP-HP	Paris, France
Jedynak U	Polish-American Children's Hospital, Jagiellonian University.	Cracow, Poland
Jenkins H	University Hospital of Wales	Cardiff, United Kingdom
Kolacek S	Children's Hospital Zagreb	Zagreb, Croatia
Koletzko S	Dr. v. Haunersches Kinderspital, Ludwig-Maximillians-University	Munich, Germany
Lazowska I	Medical University of Warsaw	Warsaw, Poland
Levine A	E. Wolfson Medical Centre	Tel Aviv, Israel
Lionetti P	Meyer Hospital	Florence, Italy
Maly J	Charles University Teaching Hospital	Hradec Kralove, Czech Republic
Montgomery SM	Karolinska Institutet	Stockholm, Sweden
Murch SH	Royal Free Hospital	London, United Kingdom
Murphy MS	Birmingham Children's Hospital	Birmingham, United Kingdom
Paerregaard A	Hvidovre Hospital	Copenhagen, Denmark
Sandhu BK	Royal Hospital for Children	Bristol, United Kingdom
Sawczenko A	Royal Hospital for Children	Bristol, United Kingdom

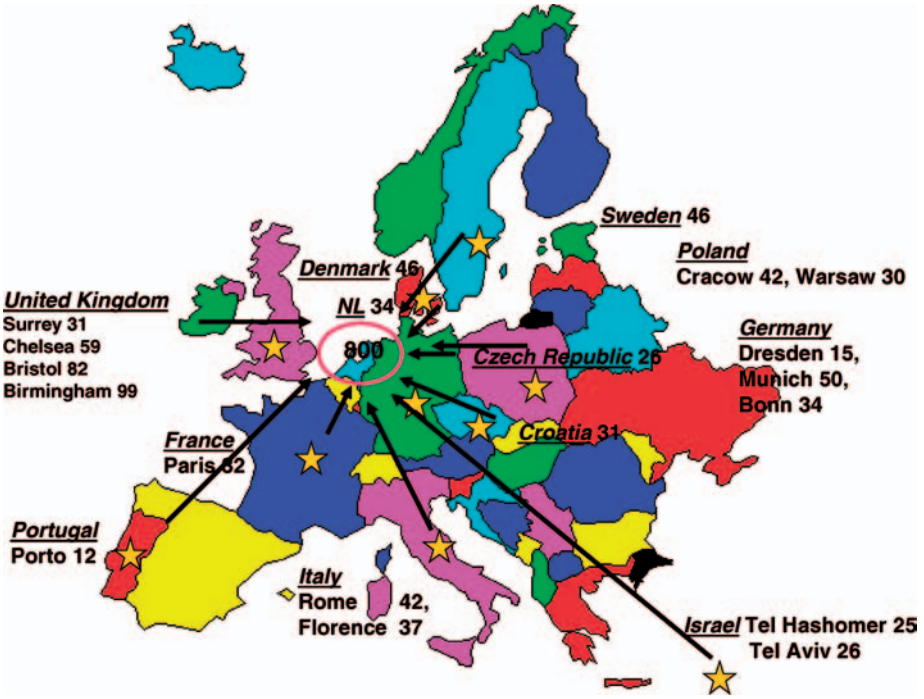

FIG. 1. Collaborative data collection of new IBD patients in 20 centers during 2 years (May 1, 2004- April 30, 2006).

children. In both UC and IC, pancolitis was predominant in all age groups.

Web-based data registration: The data presented here were reported on electronic forms that were sent by email to the coordinating center. Ideally, patients were reported directly after completion of the diagnostic work-up. In practice however, the majority of forms were sent at the end of each year. Because of this, immediate feedback on missing or incorrect data was not given to the participating centers.

In order to improve the quality of data, a web-based dataregistration system was developed in Sophia Children's Hospital, Rotterdam, the Netherlands. This web-based registration gives automatic warnings when incomplete or incorrect data are attempted to submit. In addition, online updates are provided to the participants by graphs or tables showing i.e. numbers of patients, age distribution, diagnostic work-up, and disease characteristics at presentation for each individual center. After online data-entry, data are stored in a central SPSS database, and available for further detailed analysis by the coordinating center. Web-based dataregistation will start after extensive testing, in January 2007.

Conclusions and future plans: In this first report, the results of a collaborative effort to collect data on a well-phenotyped cohort of newly-diagnosed paediatric IBD patients are presented. The database is unique because of its prospective quality as well as participation of 11 European countries. After presentation of the 2-year results during the 39th annual meeting of ESPGHAN in Dresden (June 2006) (2), representatives from Turkey, Greece, Latvia, Switzerland, Scotland, Czech Republic, Belgium and France requested to join the ongoing data-registry. As a next step, the research group will set out to prospectively register treatment of paediatric IBD patients during the first 12 months after diagnosis. This ongoing registry will serve as a nucleus for investigators studying genetics, drug therapy, health outcomes, and the socio-economic impact of these diseases. The ultimate goal envisioned by the working group is to perform several collaborative European studies on this unique cohort of uniformly phenotyped patients, gathered in a core database. Furthermore, this large and well organised database will greatly facilitate the collection of sufficient amounts of human material (specimens), enabling research on the aetiology and pathophysiology of early onset IBD.

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Disease Behavior in Children with Crohn's Disease: The Effect of Disease Duration, Ethnicity, Genotype and Phenotype

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Objectives: The Vienna classification divides Crohn's disease (CD) into 3 behavior groups, inflammatory, stricturing, and penetrating types. The aim of our study was to evaluate the effect of genotype and phenotype on disease behavior in pediatric CD.

Study design: Evaluation of 128 pediatric onset CD was followed by analysis of 235 pediatric and adult onset CD patients, all with at least two years of follow-up (mean 4.9 and 6.4 years respectively). Phenotype, ethnicity, and disease duration were recorded. Patients were genotyped for polymorphisms in the NOD2/CARD15 gene.

Results: Patients under the age of 9 had more colonic involvement. Pediatric disease at end of follow-up was classified as inflammatory (78%), penetrating (6%) and stricturing (17%), while 31% had perianal disease. Duration of follow-up was associated with more stricturing and penetrating disease. NOD2 was associated with ileal disease. There was no association between mean age of onset and NOD2/CARD15, or one of these with disease behavior. These observations were identical in the final mixed adult pediatric cohort. Sephardic Jewish origin was inversely correlated with inflammatory behaviour (p=0.006), independent of NOD2 genotype.

Conclusions: Duration of disease and ethnicity, irrespective of NOD2/CARD15 genotype, were the only predictors for penetrating or stricturing disease.

Natural History of Crohn's Disease in Children: A Population-based Cohort Study in Northern France

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Introduction: The natural history of Crohn's disease (CD) is poorly described and a limited number of population-based studies is available. The aim of this study was to describe the clinical course and morbidity of this childhood cohort.

Method: In a geographically derived incidence cohort diagnosed from 1988 to 2002 (Registre Epimad) we identified all patients with onset of CD before the age of 17 years. During this period 480 cases of childhood CD were recorded (8 % of all CD cases in Northern France) with mean standardized incidence of $2.5/10^5$ (1).

Results: 430 had a follow-up including 386 with a follow-up time equal or higher than 24 months. 207 M and 179 F with a median age of 14 years [11-15] were followed for a median of 83.5 mos [52-122]. CD location at diagnosis and last follow up was both small bowel and colon (62% and 82% respectively), colon only (23% and 10%), small bowel only (15% and 8%). Upper gastrointestinal tract lesions were present in 31% of patients at diagnosis and in 47% at follow-up. Anoperineal lesions (fistulae and/or abcesses) were present in 9% of patients at diagnosis and in 24% at follow-up. Among children, 87.3% required treatment with corticosteroid. Corticosteroid resistance occurred within 1 year in 6% and corticosteroid dependence in 24%. 239 children (62%) were started on immunomodulators (azathioprine and/or methotrexate) with a response within 1 year in 72%, intolerance in 14% and failure in 27%. Ninety-six children (25%) received infliximab with a response in 79%. The cumulative colectomy probability rate was 8% after 1 year, 20% after 3 years and 32% at 5 years; these probabilities were not modified by immunomodulator therapy.

Conclusions: This is one of the largest population-based studies of CD in children. Childhood-onset CD is characterized by a widespread location, high percentage of patients requiring early corticosteroid therapy with high occurrence of dependence. Despite common use of immunomodulators, one third of children require surgery 5 years after diagnosis. These data could justify resort to early aggressive medical therapy in this subset of patients.

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High Circulating Glucocorticoid Bioactivity Predicts a Steroid-dependent Disease in Children with IBD

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Prescribing glucocorticoids to paediatric patients is challenging, as there are no means to define at the introduction of the therapy those who respond well to steroids or to identify the ones prone to severe side-effects. The steroid dosage is commonly based on body weight, and at present the adjustments to treatment are based on clinical evaluation and not on specifically monitored steroid response.

In the current work we used a recently developed recombinant cell bioassay (Raivio et al, *J Clin Endocrinol Metab* 87:3740) to examine the contribution of peroral corticosteroid treatment to serum glucocorticoid bioactivity (GBA) in 22 paediatric patients with inflammatory bowel disease (IBD), introduced to steroid therapy due to an exacerbation of the disease. The aim was to trace out a possible link between GBA and steroid responsiveness. We also looked for an association between GBA and the development of glucocorticoid related side-effects. The reference range (± 2 SD) for serum GBA (13-116 nM cortisol equivalents) was defined in 101 paediatric patients not on steroid therapy. Serum GBA did not correlate with age, sex or weight of the patients, or with the time of drawing the sample.

In the 22 IBD patients on steroids, glucocorticoids were introduced as a single daily dose of either prednisolone (n=19) or budesonide (n=3), and the patients were followed at 2-4 week intervals. The pretreatment serum cortisol and GBA correlated linearly ($r = 0.953$, $P < 0.001$). Peroral prednisolone treatment brought about a fourfold increase in the mean serum GBA from 84 ± 127 to 311 ± 344 nM cortisol equivalents at 2 weeks of therapy ($P<0.001$). Two patients that were switched from budesonide to prednisolone showed an increase in their serum GBA at that point ($P<0.05$). Importantly, the mean serum GBA during the first two months of the steroid therapy correlated with steroid dependency in later face of the treatment ($P<0.05$). This correlation was not attributable to the corticosteroid dose. The level of GBA did not, however, reflect the development of acute glucocorticoid related side-effects.

In conclusion, 1) high serum GBA level at an early phase of the steroid treatment anticipates steroid dependent disease, 2) GBA measurements can be used to assess relative biopotencies of exogenous glucocorticoids and 3) assessing circulating GBA provides a novel means to evaluate the glucocorticoid dose individually.

Contribution of the Complex NOD1/CARD4 Insertion/Deletion Polymorphism +32656 to IBD in Scottish Children: Effects on Susceptibility, Phenotype and Interaction with NOD2/CARD15

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Background/aims: NOD1 and NOD2 are both intracellular pattern-recognition receptors. NOD2 is a well established determinant of disease susceptibility and phenotype in Crohn's Disease (CD). Recently, an association was found between the NOD1+32656*1 deletion variant of a complex deletion*1/insertion*2 polymorphism and inflammatory bowel disease (IBD). The NOD1 gene lies within a previously described IBD locus (7p14). Our aim was to assess the influence of NOD1+32656 on disease susceptibility and phenotype in the Scottish early onset IBD population.

Methods: 2207 individuals (313 IBD patients (205 CD, 81 UC, 27 IBDU) (median age at diagnosis 11.1 years (8.6-12.9)), 522 parents, 1372 healthy controls) were genotyped for NOD1+32656 A/C by TaqMan and direct sequencing. Transmission Disequilibrium Testing, case-control and detailed genotype-phenotype analyses were then performed.

Results: TDT analysis in the Scottish childhood onset IBD cohort did not show any distortion of transmission of NOD1+32656 variants. In case-control analysis, none of the genotypes studied in IBD, CD, UC or IBDU differed significantly from controls. In childhood onset CD, multifactorial genotype-phenotype analyses (also controlling for NOD2 variant carriage) confirmed the influence of carriage of the NOD1+32656 insertion*2 allele on gastric body disease (p=0.01 OR 4.77 CI 1.32-17.27), ileal disease (p=0.03 OR 3.92 CI 1.11-13.89), perianal disease (p=0.008 OR 6.76 CI 1.63-28.00) and CD surgery (p=0.04 OR 0.15 CI 0.02-0.92).

Conclusions: Variant alleles of NOD1+32656 are not associated with IBD in the Scottish early onset IBD population. However, we have provided evidence for novel genotype-phenotype associations in childhood onset CD.

IBD Genes: The Old and New Candidates

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Inflammatory Bowel Diseases (IBD) are complex genetic disorders resulting of the interplay between genetic and environmental risk factors. Until today, few of these risk factors have been firmly

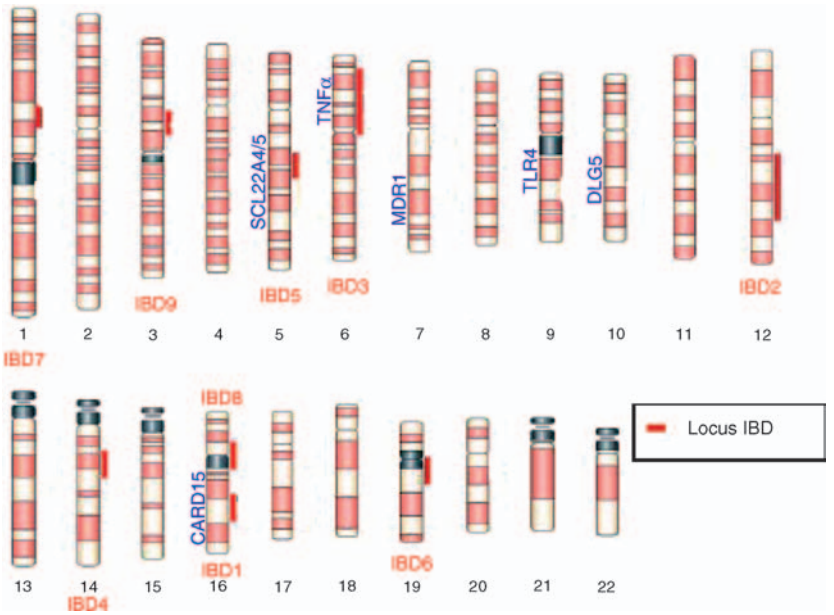

FIG. 1. IBD susceptibility loci. Anonymous validated loci are indicated in light gray with their corresponding location. The susceptibility genes proposed in the literature are noted in dark gray.

established as aetiological. For example, the only environmental risk factor known until today is tobacco with a protective role in Ulcerative Colitis (UC) and a negative effect in Crohn's Disease (CD). The genetic component of UC predisposition seems to be less important than for CD with a lower rate of familial cases and a lower concordance rate in monozygotic twins.

Several groups have reported genome scans looking for IBD genes and nine IBD loci have been retained (OMIM266600, Fig 1). None of these loci confers a relative risk higher than 2, illustrating the fact that IBD are true complex genetic disorders. Most of these loci are associated with CD (e.g. IBD1, IBD5, DLG5.) or IBD in general (e.g. IBD3) while few are involved in UC only (e.g. IBD2).

Some of the IBD susceptibility loci are still anonymous (e.g. IBD2, IBD4, IBD6, IBD7, IBD8, IBD9) and additional work is required to find the genes. For a limited number of loci, the positional cloning approach was able to propose strong candidate genes (CARD15, SCL22A4/5, TNF-α, DLG5). Finally, other candidates have been tested because of functional reasons with some evidences of association with IBD (e.g. MDR1 and TLR4) (for review see 1).

The best studied gene is Caspase Recruitment Domain 15 also known as Nucleotide Oligomerisation Domain 2 (CARD15/NOD2) (2,3). This gene is involved in CD predisposition, especially in case of paediatric age of onset, ileal involvement and local complications. People mutated on their two chromosomes are at the higher risk to develop the disease. CARD15/NOD2 belongs to a family of genes involved in innate immunity from plants to Human. It codes for an intracellular protein able to activate the NF-κB pro-inflammatory pathway. Components of the bacterial cell wall are able to activate Card15/Nod2. This response seems to be defective for CD associated mutations and many authors consider CD as a new form of immune deficiency. Unfortunately, despite of many works during these last five years, it is not clearly understood how Card15/Nod2 may induce CD lesions.

Because Card15/Nod2 is involved in innate immunity, genes playing a role in bacterial recognition by the host have also been studied including Toll Like Receptor 4 (TLR4) and CARD4/NOD1. For these genes, associations have been reported in some studies but not in all and these candidates are still under investigation.

A linkage on chromosome 6p overlapping with the Major Histocompatibility Complex region has been widely replicated (IBD3). In this region, associations have been inconstantly showed between the *HLADRB1∗0103* allele and severe UC and colonic CD in one hand and between TNF-α promoter polymorphisms and IBD on the other hand.

A CD susceptibility gene has been firmly established on chromosome 5q with many replication studies (4). It is located in a region containing several cytokine genes. However, CD has not been associated with some genetic variants of these obvious candidate genes but rather with polymorphisms within genes coding for organic cathionic transporters (OCTN1 and 2). These genes are not involved in immune processes but in the transport of carnitine and xenobiotics.

Multi-drug Resistance 1 (MDR1), a gene coding for an efflux transporter pump involved in xenobiotic protection was also investigated. It is expressed in epithelial cells and MDR1 knock out mice develop colitis spontaneously. MDR1 gene polymorphisms have been associated with IBD in several (but not all) studies. Interestingly, MDR1 also makes a link with xenobiotics suggesting a relationship between these environmental factors and CD.

Finally, drosophila discs large homologue 5 (DLG5), a gene expected to be important in epithelial functions, has also been proposed to have a role in IBD predisposition in males. Unfortunately, most of the replication studies failed to replicate the initial observation.

All these studies demonstrate that the genetic predisposition to IBD is important but difficult to catch and IBD are sometimes cited as an example for complex genetic disorders. Despite of these difficulties, gene discovery is expected to help resolving the disease mechanisms and developing new specific therapeutic approaches.

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Is There a Pediatric Genotype or Phenotype of IBD?

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The clinical manifestations of Crohn's disease (CD) are highly variable, with significant diversity in age of onset, disease location, and disease behavior (disease phenotype). Advances in our current understanding of the processes involved in Crohn's disease have shed light on both on the possible pathogenesis and on the sources of clinical diversity.

The first disease susceptibility gene identified was the NOD2/CARD15 gene on chromosome 16, which encodes a pattern recognition receptor. Three loss of function mutations have been associated with CD. A loss of function polymorphism in another pattern recognition receptor, Toll like receptor 4 appears to be associated with susceptibility in European populations. A locus on chromosome 5 (OCTN1, OCTN2) has been found to be associated with susceptibility in Caucasians, while conflicting results have been reported for the DLG gene on Chromosome 10, and the TNF alpha promoter on Chromosome 3.Genetic diversity for disease association has been documented over the last few years, and may contribute to discrepancies between studies from different populations.

A significant association has been found between the disease phenotypes and disease genotype. Patients who carry a NOD2/CARD15 mutation are significantly more likely to have ileal disease, and NOD2/CARD15 – variants are less likely to have ileal and more likely to have colonic involvement. Carriage of NOD2/CARD15 is also associated with stricturing disease. There have been previous reports associating disease severity with polymorphisms in the TNF alpha promoter. Theoretically, an effect of genotype on phenotype may not have to be mediated by susceptibility genes. Polymorphisms in genes that increase or decrease inflammation or specific inflammatory mediators, may also affect disease phenotype and/or behavior.

If patients with different phenotypes have different genotypes, the question arises if pediatric CD is different from adult onset disease in phenotype or genotype. Pediatric onset CD is a distinct phenotype based on age, and several recent studies indicate that beyond age of onset (AOO), pediatric AOO might have other characteristics that are different from adult AOO. Male gender is also more frequent in early AOO. Patients with AOO in the first decade tend to have more colonic and less ileal involvement than adult patients or adolescents. These differences in pediatric phenotype might suggest that genes other than NOD2/CARD15 might play a more significant role in pediatric AOO, or that ileal susceptibility and gender susceptibility might be due to environmental or developmental causes.

Earlier studies in mostly adult populations consistently found that homozygotes and compound heterozygotes for NOD2/CARD15 mutations had an earlier mean age of onset, but there may be multiple inherent methodological flaws in many of these studies. Problems include lack of inclusion of a proportion of first decade onset children in cohorts, using means for age and not distributions or correction for skewing, use of multiple family members in the same cohort which is associated with earlier AOO, and lack of stratification for genotype by age. In addition, homozygosity or compound heterozygosity for NOD2/CARD15 is an uncommon event and thus does not offer a good explanation for AOO in the majority of pediatric AOO.

Recent studies that have compared AOO between NOD2/CARD15 in Italy, Scotland and Israel have not found a difference in mean age of onset between carriers of a mutation and wild type. Studies from Israel comparing pediatric onset to adult onset found that NOD2/CARD15 carriage may be less prevalent in early onset disease, suggesting that genes other than NOD2/CARD15 may be more prevalent in early onset disease, and explain early age of onset and colonic phenotype. Studies from Europe and the US have not found significant correlations between the IBD 5(OCTN1/2 haplotypes), and DLG polymorphisms, with phenotype or age of onset in pediatric disease. A polymorphism in the TNF alpha promoter was found to be correlated with pediatric onset (versus adult AOO) and colonic location in a single study in the Israeli population, this study needs to be reproduced in other populations before conclusions can be drawn about an association between a specific genotype and pediatric AOO can be drawn.

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The Role of Aluminum in Bacterial-induced Colitis in Young IL-10-deficient Mice

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Background: Aluminum (AL) is a common environmental compound with immune adjuvant activity and granulomatous inflammation inducer. AL exposure in food additives, air and water pollution is ubiquitous in western cultures. Since loss of tolerance to commensal intestinal bacteria is involved in the pathogenesis of IBD, we postulate that dietary AL increases luminal bacterial virulence and/or enhances mucosal immune responses to enteric bacteria. Therefore, AL effects on bacterial growth, the ability of intestinal bacterial lysate to stimulate effector immune responses, the capacity of AL preloaded pathogenic bacteria to enhance immune responses in vitro and the in vivo effect of dietary AL on immune- mediated young murine colitis, were explored.

Methods: Growth of murine intestinal bacterial on increasing AL concentration (AL 0-662 μM) was assessed by spectrophotometry. Immune responses were studied by

$[H]^3$ Thymidine incorporation, gamma-interferon by Elisa on IL-10 KO colitic mouse splenocytes stimulated by murine E. coli grown in increasing AL concentrations. Young 15 IL-10 KO germ free mice were colonized with specific pathogen free enteric microbiota, fed a low AL, and exposed to 3 different AL concentrations: low (0.03 μM), middle (5 μM) and high (500 μM) AL L-lactate added to their drinking water. Colitis was measured by blinded histologic scores (0-4+) and spontaneous IL-12 secretion by cultured colonic fragments.

Results: Bacterial growth was suppressed slightly by high AL concentrations in the media. Lower AL concentrations (150-200 μM) stimulated, while higher concentrations inhibited in vitro T cell proliferation and IFN secretion by splenocytes. In vivo AL feeding worsened colitis, with increased cecal histological scores accompanying higher AL intake (2.0 ± 0.2 low vs. 3.8 ± 0.2 high, $p<0.05$). Colonic IL-12 secretion by colonic strip cultures increased with higher AL intake (3.5 ± 1.6 vs. 4.9 ± 0.4 ng/ml). Dietary AL affected luminal bacterial metabolism by inducing pink E. coli colonies on MacConkey agar plates.

Conclusions: Environmental AL stimulates immune responses to enteric bacteria in vitro and enhances bacterial- induced experimental colitis in Young IL-10 KO mice. Dietary exposure to AL may in part

explain the increased incidence of pediatric IBD in Western countries adopting Western culture.

Contribution of Various Susceptibility Genes to Pediatric-onset IBD

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Background/aims: Heritability appears greater in early- versus late-onset inflammatory bowel disease (IBD). We evaluated the contribution and phenotypic correlations of a confirmed locus (IBD5) and confirmed (CARD15) or suggested (OCTN1, OCTN2, MDR1, DLG5, TLR4 and NOD1/CARD4) susceptibility genes in a single center population of patients diagnosed at age $\leq$ 17 years.

Methods: 380 probands (60% Male) with IBD (total 251 CD, 107 UC, indeterminate colitis 22), their parents, and 42 affected sibs were genotyped for the IBD5 markers: IGR 2096, IGR 2198, IGR 2230, OCTN1, and OCTN2; the 3 risk alleles of CARD15 (R702W, G908R and 1007fsinsC); MDR1 (rs1045642); NOD1/CARD4 (rs2075820); TLR4 (rs4986790) and DLG5 (1248696, 2289310). Evidence of susceptibility was sought using FBAT (family-based association test); phenotypic correlations were sought using multivariate logistic modeling (MLM).

Results: 66% patients were non-Jewish Caucasian; 23% Jewish Caucasian. 24% of CD and 14% of UC probands had affected first degree relative(s). UC was extensive in 77% and 21% had undergone colectomy. CD macroscopically involved small bowel (sb) only in 54% and colon only in 14%. At most recent follow-up, CD was inflammatory in 74% and stricturing in 17%. 30% had undergone resection. FBAT demonstrated no associations with pediatric onset UC. A very strong association was evident between sb CD and 1007fsinsC ($p = 0.000001$). IBD5 markers demonstrated significant association to colonic CD in non-Jewish families (p=0.004). MDR1 demonstrated significant association with CD (p=0.03) primarily in Jewish patients (p=0.02). A similar association was seen for TLR4 (CD: p=0.06; Jewish: p=0.04). No association was found between CD and the NOD1/CARD4 or DLG5 polymorphisms. MLM confirmed the association between 1007fsincC and isolated sb CD (OR: 5.4, p=0.001). Whilst the risk of stenotic disease was increased in isolated sb disease (OR: 8.2, p=0.001) and Jewish heritage (OR: 3.4, p=0.008), no independent effect of 1007fsinsC was evident (OR: 0.7, p=0.6). TLR4 was strongly associated with isolated colonic disease in both CD (OR: 3.6, p=0.02) and IBD overall (OR: 2.5, p=0.04).

Conclusions: In this cohort of exclusively early onset IBD, we confirm the association of CARD15, IBD5 and TLR4 with CD susceptibility and document a novel association with MDR1. Previously described associations with DLG5 are not replicated. We demonstrate that variation in TLR4 predisposes to isolated colonic disease. Our findings reinforce the importance of ethnic stratification in genetic studies, and support the role of barrier function and innate immunity in CD pathogenesis.

Expression of mRNA and Protein of Toll-like Receptors 2 and 4 Are Upregulated in Children with IBD

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Background/aim: Chronic inflammation in inflammatory bowel disease (IBD) may result from exaggerated stimulation of the mucosal immune system by the endogenous luminal bacterial flora. Bacterial products are recognized by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), which are key regulators of the innate immune system. Therefore, the expression of TLR2, TLR3 and TLR4 in colonic biopsy samples taken from children with active IBD were studied and compared to controls.

Methods: Colonic biopsy specimens were collected from 12 children [median (range): 13 (6-18) yr] with newly diagnosed IBD (n-IBD) and 23 children [15 (8-18) yr] with relapsed IBD on treatment (r-IBD) from macroscopically involved and noninvolved mucosa. Specimens were also obtained from 8 controls [median (range): 14 (6-16) yr]. TLR2, TLR3 and TLR4 mRNA, and - related protein expression were determined by real-time reverse transcription polymerase chain reaction (RT-PCR) and Western blot, respectively.

Results: The TLR2 and TLR4 mRNA expression were significantly increased in the involved colonic mucosa of children with n-IBD and r-IBD compared to controls, respectively ($p<0.05$). We found higher TLR2 and TLR4 mRNA expression in the involved vs. noninvolved colonic mucosa of children with n-IBD and r-IBD, respectively ($p<0.05$). TLR2 protein expression in the involved mucosa of patients with n-IBD and r-IBD were 8.9 and 8.5 times higher than controls, respectively ($p<0.001$ and $p<0.0001$). Involved mucosa in children with n-IBD and r-IBD depicted 5-fold and 4.5-fold elevation of TLR4 protein expression in comparison to controls, respectively ($p<0.001$ and $p<0.0001$). In the noninvolved mucosa of children with n-IBD and r-IBD, TLR2 and TLR4 mRNA and protein expression were similar to controls. The TLR3 mRNA and protein expression were similar in all groups studied.

Summary/conclusions: Our results of increased expression of TLR2 and TLR4 in the inflamed colonic mucosa of children with IBD confirm the hypothesis that innate immunity has an important role in the pathogenesis of this disease. Nevertheless, the similar expression of these PRRs in the noninvolved colonic mucosa in children with IBD and in controls does not support their primary role in the development of IBD.

Paneth Cells: A Source of the Proinflammatory Cytokine TNF-α in IBD

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Background: Traditionally, aberrant adaptive mucosal immune responses by T and B cells have been regarded as the main contributors to the pathogenesis of Inflammatory Bowel Disease (IBD). IBD is possibly associated with the absence of anti-inflammatory cytokines such as IL-10 and/or overproduction of inflammatory mediators such as tumor necrosis factor-α (TNF-α). Paneth cells are located in the crypts of the small intestine and play a pivotal role in small intestinal microbial homeostasis. Defects in these cells have been implicated in the pathogenesis of IBD. It has been proposed that intestinal inflammation may result from a loss of function in these cells that is associated with a failure of microbial eradication at the mucosal surfaces of the small intestine. Alternatively, we hypothesize that Paneth cells actively contribute to inflammation through the production of inflammatory cytokines.

Methods: Jejunal segments were collected from IL-10 KO mice about 50 days prior to the expected onset of colitis for histological analysis. Results were compared with tissue from wild type littermates (WT). Human ileal and colonic biopsies from two patients with Ulcerative Colitis were stained for TNF-α.

Results: Prior to lesion development, IL-10 KO mice expressed high levels of TNF-α mainly within the granules of the Paneth cells. This was not seen in the small intestine of WT mice. Preliminarily, we have been able to extend this paradigm to the human setting as metaplastic Paneth cells located in the colon appeared to be a major source of TNF-α in human ulcerative colitis.

Conclusions: We hypothesize that TNF-α production by Paneth cells is triggered by commensal bacteria. Paneth cell-derived TNF-α may be regarded as an important player in chronic inflammation of the intestine.

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OPENING LECTURES

The Drugs Work . . . But Do the Patients?

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The World Health Organisation has defined health as "a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity". Response to drug therapy is usually assessed by disease activity indices such as the PCDAI. These indices include data about symptoms, physical signs, growth and laboratory tests of inflammation but there is usually minimal or no input from the patient. Health related quality of life (HRQoL) has been defined as "the functional effect of an illness and its treatment on a patient as perceived by the patient". Measures of HRQoL need to emphasise the patients own assessment which may differ substantially from that of healthcare professionals. There are two main types of instrument to measure HRQoL: 1) generic instruments can be used in healthy children or children with other chronic illnesses as well as IBD. Such instruments can be useful to compare the impact of different diseases on HRQoL but they may be too imprecise to reflect impaired function in specific illnesses such as IBD or to assess response to therapy. 2) Disease specific instruments are more sensitive to the problems of children with specific illnesses. An ideal HRQoL instrument would be: simple and quick to administer, reproducible, valid, sensitive, easily interpreted and widely accepted.

In a meta-analytic review of chronic diseases inflammatory bowel disease had the most profound effect on mental health. In one study 56% of children with IBD compared to 18% of controls had a psychiatric disorder and these were almost exclusively emotional disorders. A further comparison of children with chronic illnesses reported psychiatric disorders in 60% of children with IBD, 30% with tension headaches, 20% with diabetes and 15% of healthy controls. An Italian study into the long term effects 10 years after surgery in 21 children with ulcerative colitis showed that 15 had normal emotional status and 14 had normal social life. The first published study on HRQoL in children with IBD by Rabbett et al reported that many of the affected children had problems with school attendance, sports, holidays and staying at friend's houses. Children on steroids had more depressive symptoms. A recent study in the Netherlands showed that younger children (aged 8-12 years) with IBD had comparable HRQoL and better cognitive functioning than a reference population whereas adolescents had a significantly impaired HRQoL in four domains (body complaints, motor functioning, autonomy and negative emotions).

An instrument to measure HRQoL in children with IBD (IMPACT) was developed in Canada and has recently been validated. The original instrument has been simplified and validated in the Netherlands and has also been translated into French. A cross-cultural comparison has shown that the concerns of children with IBD in the United Kingdom are broadly similar to those of the Canadian children. Further validation is underway in the United Kingdom and a computerised touch-screen version has been developed. In a study of children with active Crohn's disease treated with exclusive enteral nutrition 23 of 26 achieved clinical remission at 8 weeks, with improvement in IMPACT scores. The change in IMPACT score was predictive of achieving a clinical remission but not of histological improvement. A prospective study of children with IBD in the USA attending a one week camp showed a significant improvement in total IMPACT score and several domains.

Adolescents with IBD have been shown to have higher HRQoL when they have a closer social support network. Those with recent onset IBD relied more on family members than peers for emotional support and depended more on their parents coping skills than their own. There was a significant agreement between adolescent and parental HRQoL score and stressful event ratings. Parents were also found to be adequate raters of objective components of the child's HRQoL but not of more subjective components. Paediatricians were found to overestimate physical symptoms. In a recent study in the Netherlands adolescents with IBD used more avoidant coping styles than healthy peers. Use of a predictive coping style and less use of a depressive reaction pattern was associated with better HRQoL in 3 out of 6 domains. Adolescents with IBD (especially boys) had worse HRQoL and showed more internalising problem behaviour compared with healthy peers, an important predictor of HRQoL was self-esteem. In a similar study in the United Kingdom we have shown that children with IBD used cognitive restructuring as the principle way of coping. Other popular coping strategies included wishful thinking, distraction and seeking social support. Adolescents were more likely to accept their disease with 88% adopting resignation compared to only 23% of younger patients.

With the development of new drugs we can expect to see improvements in the management of children with IBD. It is important that the response to these new drugs is considered not just in the conventional way by measuring disease activity but also from the patients' perspective. The IMPACT questionnaire is a valid, reliable and sensitive instrument to measure HRQoL in children with IBD and should be included in the assessment of all new therapies. In view of the considerable psychiatric/psychological morbidity associated with IBD and its treatment it is important that we consider other ways to improve the HRQoL and coping strategies of affected children. These may include: development of support networks, counseling, psychiatric/psychological support and/or education.

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CHALLENGES IN PAEDIATRIC IBD

Common Errors in the Management of Children with IBD

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Children with inflammatory bowel disease are medically complex patients requiring an experienced multidisciplinary team of health care providers. In these children, therapy must avoid both errors of commission (misdiagnosing and overtreating) and errors of omission (undertreating and undermonitoring). The first errors to be avoided are misdiagnosis of self-limited colitis as inflammatory bowel disease, and misdiagnosis of ulcerative colitis as Crohn's disease or indeterminate colitis. Children with suspected IBD should undergo diagnostic evaluation with contrast radiography, colonoscopy, and upper endoscopy, with the performance of endoscopic biopsies. Therapy should ideally be initiated after a definitive endoscopic diagnosis. The currently available ESPGHAN Porto document should be utilized as an aid for accurate diagnosis and classification. [1]

After diagnosis, the treating physician must aim for a full clinical remission, rather than simply accepting a partial clinical response. It is important not to simply rely on clinical symptoms and how the patient feels in assessing remission. The optimal remission includes: no abdominal pain or diarrhea, normal hematocrit, normal albumin, normal acute phase reactants, normal growth, normal bone density, normal psychological functioning, and (ideally) normal mucosa. In a patient who "feels well" but whose laboratory status suggests ongoing inflammation, endoscopic evaluation and assessment of the intestinal mucosa is an important guide to therapy. For most children with moderate to severe Crohn's disease, achieving such a remission without prolonged

use of corticosteroids requires early introduction of an immunomodulator (6-mecaptopurine, azathioprine, or methotrexate). [2] Safety of 6MP/AZA use can be improved by obtaining thiopurine methyltransferase (TPMT) genotyping or assessing TPMT activity at the start of therapy.

For patients who have persistently active disease despite 6MP, AZA, or methotrexate, a wide variety of other agents, including infliximab, adalimumab, cyclosporine, tacrolimus, and thalidomide are available. While many of these drugs are helpful in treating severe and diffuse disease (especially involving the colon), surgery is a very effective modality that will improve quality of life in many patients with ulcerative colitis and Crohn's disease. In certain patients, overusing biologics or immunosuppressives instead of surgery does not improve the illness, results in poorer quality of life, and places the patient at increased risk of infection and lymphoma. Surgery is the treatment of choice for abdominal abscess or obstructive stricture, and should be considered in any patient with a limited region of medically refractory Crohn's disease. [3] The principal complication of Crohn's disease surgery is postoperative recurrence, which occurs in up to 50% of children by 3 years. Given the high likelihood of such recurrence, monitoring for recurrence is encouraged, though the optimal regimen to prevent and treat postoperative recurrence has yet to be determined. In patients with severe ulcerative colitis treated with cyclosporine, tacrolimus, or infliximab, there remains a high likelihood of surgery even if there is a good initial response to therapy.

Even ''well'' children and adolescents with inflammatory bowel disease require frequent visits during their periods of remission. At our inflammatory bowel disease center, we recommend patients be seen in clinic at a minimum of every 3 months. At these visits, we assess compliance and also monitor growth parameters and (if appropriate) micronutrient deficiencies. Growth should be monitored every three months using a stadiometer, and sexual maturity status should be documented at least twice a year. Most patients with CD will grow if their disease is well controlled, corticosteroid use is kept to a minimum, and nutrition is optimized. Those that do not grow should undergo an endocrinologic evaluation for growth hormone deficiency. Patients with active IBD are at risk for zinc deficiency and hypovitaminosis (especially of vitamins A, D, and E). The hypovitaminosis D may be a contributing factor to osteopenia, which is present in approximately 30% of children with IBD. [4] While the optimal diagnostic and treatment regimen for osteopenia in children with IBD has yet to be determined, the identification of osteopenia should at least prompt consideration of treatment with recommended doses of vitamin D and calcium. Patient with ileal resections from Crohn's disease should undergo monitoring of vitamin B12 levels and vitamin B12 repletion if necessary.

In addition to medical and nutritional complications of this disease, the physician caring for patients with inflammatory bowel disease must be aware of psychological symptoms, including chronic fatigue, depression, anxiety, and noncompliance. Approximately 25% of children and adolescents with IBD have depressive symptoms, which may be unappreciated by the patient's physicians. Such psychosocial issues affect quality of life, and may also contribute to noncompliance with medical regimens. A multidisciplinary medical treatment program involving cognitive behavioral therapy, antidepressant medications, and group support (such as IBD camp) will often result in improvement in symptoms. [5] Adolescents are notoriously noncompliant with medical regimens, and may stop their medications without their parent's knowledge. This is particularly true of college students, who are on their own without parental supervision for the first time in their lives. Kane et al have demonstrated that noncompliance correlates strongly with relapse probability in adults with ulcerative colitis. [6] Therefore, the physician visit is an important time to constantly remind the patient that noncompliance may result in worse disease and increased risk of bowel cancer.

In summary, the essential ingredient in avoiding errors in inflammatory bowel disease is knowledge of not only the patient's bowel disease, but the patient as a whole. Frequent follow up visits, medical assessment, nutritional assessment, and psychosocial assessment will aid the trained clinician to identify and prevent complications of both IBD and the therapies used to treat it. The following list summarises the common errors:

1. Making the wrong diagnosis
2. Aiming for clinical response rather than clinical remission
3. Overrelying on clinical parameters in assessing response
4. Overuse of corticosteroids
5. Not determining thiopurine methyltransferase activity or genotype prior to the use of 6-mercaptopurine or azathioprine
6. Overtreating with immunosuppression when surgery is needed
7. Failing to monitor for postoperative recurrence in patients who have surgery
8. Not carefully monitoring growth parameters and micronutrient status
9. Not screening for anxiety or depression
10. Failing to monitor compliance or adherence

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Mucosal Healing in Crohn's Disease: The Main Goal or Medical Overkill?

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Crohn's disease (CD) is a chronic recurrent intestinal mucosal inflammatory process leading to a continuous transmural reaction, potentially complicated by fistula formation or fibrostenosing processes. The precise etiology of Crohn's disease is unknown. However, recent data point out to an altered immune homeostasis within the intestinal mucosa in genetically predisposed individuals. This may result in an exaggerated and uncontrolled inflammatory response probably triggered by the intestinal flora and/or other environmental factors. Some experimental and *in vivo* animal data indicate regulatory defects within the innate immune system probably as causal in the development of IBD. Activated macrophages and T lymphocytes seem to be responsible for the amplification of the inflammatory reaction leading to macroscopic lesions, such as erosion and ulcerations of the intestinal mucosa.

Unfortunately, until today CD can not be cured and its course is chronic with recurrent relapses. Several - mainly empiric - therapeutic concepts were developed to control and down-regulate the inflammatory reaction in CD. However, the ideal treatment of CD should not only suppress all clinical symptoms – without any side effects -, but also allow to completely heal the inflammatory lesions, thereby protecting from any further relapses. One outcome measure for this could be macroscopic and histological healing of all mucosal lesions, as recently proposed by Dr. D'Haems and colleagues (1).

Anti-inflammatory drugs, such as steroids have a well established place in the treatment of CD, and most often steroids allow to induce complete remission. However, despite its efficacy to induce remission, mucosal lesions persist under steroid medication, as analyzed by the French GETAID group (2). Thus, it is interesting to note, that clinical symptoms and biological inflammatory abnormalities can be perfectly controlled by steroids, without inducing mucosal healing. This absence

of complete mucosal healing might explain why a large number of patients relapse within 12 months after stopping steroids. On the other hand, treatment of active CD with exclusive enteral nutrition – with a comparable efficacy to steroids – was reported to induce mucosal healing (3). Inflammatory and clinical parameters are equally normalized as under steroid medication, and the patients are doing extremely well. If mucosal healing is a predictor of long term remission, one might expect that induction of remission by enteral nutrition is followed by a prolonged relapse free interval, contrary to patients treated with steroids. This might be true, however, only one published study (4) analyzed in 19 patients the outcome after steroid or enteral nutrition as induction therapy, confirming a somewhat lower relapse rate after therapy with enteral nutrition.

Immunosuppressors, such as azathioprine (AZA), 6-mercaptopurine (6-MP) or methotrexate (MTX) have a well established place in the treatment of adult and pediatric Crohn's disease. These drugs are directed against T lymphocytes by inducing apoptosis of activated T cells. After a lag-interval of 3 to 6 months, immunosuppressive therapy allows to control adaptive immune responses in CD and to maintain remission. The recent study of Dr. Markowitz and co-workers (5) clearly showed that the relapse rate of children under 6-MP (9%) was significantly lower compared to children on placebo (47%) while in both groups initial remission was induced by steroids. It is interesting to note that prolonged medication with AZA/6-MP was reported to induce mucosal healing. Unfortunately, no control endoscopies were performed in the pediatric multicenter 6-MP-study of Dr. Markowitz, therefore, the rate of mucosal healing under 6-MP versus placebo is unknown. It is very intriguing that half of the patients on placebo remained in remission until the end of the observation interval of 548 days. A priori, after an induction therapy with steroids, no mucosal healing occurred, and placebo was without any effect. Nevertheless these patients were able to remain in longterm remission without any immunosuppressive treatment. One might say, that these children are ''relapsers'' in the near future, however, objective data are completely missing. These clear cut prospective data clearly challenge the concept of mucosal healing as predictor of longterm remission.

In fact, there are scant data available which support the concept of mucosal healing as ultimate goal in the treatment of CD. Dr D'Haens and collaborators recently suggested that induction of remission by the anti-TNF-directed antibody, infliximab, is correlated to mucosal healing. If mucosal healing means the absence of any inflammatory lesions all along the GI-tract, the evaluation is relatively simple; however, details were not presented in the substudy of the ACCENT 1 study, evaluating mucosal healing in 74 and 58 patients at 10 and 54 weeks of scheduled versus on demand infliximab perfusions (6). However, in a previous report (7) the potential of mucosal healing after one perfusion of infliximab clearly differed according to the location: disappearance of ulcerations was most pronounced in the right colon and rectum, but less in the ileum, transverse and descending-sigmoid colon. In addition in that study, in 10% of patients the ileocaecal valve could not be intubated, probably due to stenosis of the valve. Therefore, endoscopic analysis and interpretation of mucosal healing is not that easy. In addition, no good endoscopic scoring system is available, allowing accurate quantification all mucosal lesions.

I am wondering if complete mucosal healing is the appropriate parameter to look at, since the majority of our CD patients presents with marked lipomatosis of the involved bowel segments, as easily visualized by CT scan. This lipomatosis might reflect a transmural inflammatory reaction and most often it persists on follow-up exams, despite clinical and biological remission (unpublished personal data). It would be of interest to analyze if complete mucosal healing is correlated to the disappearance of mesenteric lipomatosis or not. Therefore, one might ask if the disappearance of transmural inflammation is not a better predictor for long term remission.

Finally, nobody will deny that healing of all inflammatory lesions is a desirable goal. Yet, does it justify to run the risk of a prolonged immunosuppressive or -modulatory therapy, especially for pediatric patients who might require medical treatment for several decades? To date, only three different therapies were shown to have the potential to produce mucosal healing: enteral nutrition, AZA/6-MP and infliximab. Whereas enteral nutrition is free from side-effects, AZA/6-MP and infliximab might be responsible for serious side-effects, such as infectious complications (varicella, tuberculosis) or malignancies (lymphomas). The prolonged and/or combined use of an immunsuppressor and biological drug puts especially the pediatric patient at an increased risk to develop a severe T-cell lymphoma (8). Given the fact that all of these drugs must be considered as symptomatic and not causal therapy, it is difficult to recommend their uncritical use based only on the potential to induce mucosal healing. Personally, I find it difficult to put a patient on heavy immunosuppression who is in full clinical and biological remission with a normal growth pattern and normal quality of life, but who still presents inflammatory lesions on endoscopic and histological follow-up analyses. However, it is important to learn if these patients have a higher risk to develop penetrating or structuring complications in the future which would justify a more aggressive therapy including an increased risk of side-effects. To this aim large multicenter long term follow-up studies are required to identify and hopefully validate markers for CD patients who have a stable course even on milder therapy.

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Intervention for Growth Failure in Children with IBD

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Impairment of linear growth and pubertal development is an important potential complication of inflammatory bowel disease (IBD), particularly Crohn disease, developing during childhood and early to mid-adolescence. Normal growth is a marker of success of therapy. ''Normal'' children, however, grow at very different rates. Patterns of growth and pubertal progression in young patients with IBD can only be accurately recognized as pathologic, if the variations in normal development of healthy children and adolescents are first appreciated.

Prompt recognition of IBD is important in avoiding a long period of growth retardation due to untreated inflammation. The greater the height deficit at diagnosis, the greater is the demand for catch-up growth. In caring for children with IBD, it is important to obtain pre-illness heights,

so that the impact of the chronic intestinal inflammation can be fully appreciated. Following diagnosis and institution of treatment, regular measurement and charting of height, together with calculation of height velocity, are central to management.

Increased understanding of the mechanisms of linear growth impairment associated with chronic inflammatory disease points the way toward better management. IGF-1, produced by the liver in response to growth hormone (GH) stimulation, is the key mediator of GH effects at the growth plate of bones. The role of genes in influencing the phenotypic expression of IBD, including its propensity for growth impairment, is an intriguing focus of research. Chronic undernutrition has long been implicated and remains an important and remediable contributing factor in the observed growth impairment. Inflammatory cytokines similarly inhibit linear growth through interference with hepatic release of IGF-1 and additionally via direct effects on growing bone. Cytokines also appear to impair end-organ responsiveness to circulating testosterone, thereby compounding the effects of undernutrition in delaying progression through puberty. Chronic daily corticosteroid administration in children augments the growth impairment associated with inflammatory disease. The growth suppressive effects of glucocorticoids are multifactorial, and include central suppression of GH release, decreased hepatic transcription of GH receptor, such that production of IGF-1 is decreased, and decreased IGF-1 binding in cartilage. Hence exogenous corticosteroids create a state of functional GH deficiency.

Current pediatric treatment regimens aimed at facilitating growth limit use of corticosteroids via optimization of immunomodulatory drugs, use of enteral nutrition in Crohn disease, and, if necessary, surgery for ulcerative colitis and for intestinal complications of localized Crohn disease. Biologic agents with the potential for mucosal healing hold promise of growth enhancement even among patients with otherwise refractory disease, whose growth was previously compromised. For all interventions, there is a window of opportunity, which must be taken advantage of before puberty is too advanced.

Few interventions, however, have been tested in the randomized controlled trial setting in children; the comparative effects of therapies on growth have seldom been rigorously assessed. Instead, growth outcomes have been reported mainly in observational studies. These include retrospective accounts of improved linear growth following intestinal resection, initiation of enteral nutrition, and during infliximab maintenance therapy.

Two long-term randomized, controlled maintenance trials have examined the effects of, respectively, cyclical enteral nutrition and 6-mercaptopurine on linear growth. In a multi-center Canadian study, children achieving clinical remission with either prednisone or enteral nutrition, were randomized to receive longterm low dose prednisone on alternate days or exclusive enteral nutrition one month out of four. In this eighteen month study, linear growth was better in the children receiving liquid diet therapy. In the well-known study of Markowitz and colleagues, children with moderate to severe Crohn disease treated with an initial course of prednisone were randomized to receive either concomitant 6-mercaptopurine or placebo. A beneficial effect of 6-mercaptopurine on linear growth was not clearly apparent in this study in spite of the steroid-sparing effect and improved control of intestinal inflammation, perhaps a function of sample size and difficulties inherent in comparing growth rates among patients of varying ages and pubertal stages.

Improvement in IGF-1 levels and height velocity have been observed following growth hormone therapy in small numbers of steroid-dependent children with Crohn's disease. Three to six months of testosterone therapy, carefully supervised by paediatric endocrinologists, has been used in boys with extreme delay of puberty and its associated growth spurt. Experience with both these adjunctive hormonal therapies is very limited. Treatment of intestinal inflammation and assurance of adequate nutrition remain of prime importance.

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Extraintestinal Manifestations in IBD: How Do They Correlate with the Gut?

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Both Crohn's disease (CD) and ulcerative colitis (UC) are often associated with extraintestinal manifestations, which occur in approximately one third of the patients. There is only scanty published data on the occurrence of these manifestations in children and adolescents. Therefore most of the reviewed data of this summary is taken from studies on adult patients.

Some of the extraintestinal disorders may be direct anatomical or metabolic complications of the intestinal inflammation (i.e. osteoporosis, growth retardation, cholelithiasis, nephrolithiasis, ureteral obstruction and fistulas, thromboembolic disease, amyloidosis). However, for many other extraintestinal manifestations, the pathogenesis is not well understood and the association with IBD is not clear. The organs most commonly involved are the skin, eyes, bone and joints and biliary tract, although nearly every organ may be tangled. Some of the manifestations are clearly related to intestinal disease activity (i.e. erythema nodosum, episcleritis, peripheral arthritis, orofacial lesions), whereas others occur independently (i.e. pyoderma gangraenosum, anterior uveitis/iritis, ankylosing spondylitis, sacroileitis, primary sclerosing colangitis). Most of these extraintestinal manifestations are probably immunologically mediated. Some of them carry the hallmarks of autoimmune disorders. Auto-antibodies against organ-specific antigens shared by the intestine and other involved organs may be important. Animal as well as human studies suggest a key role of the intestinal microbiota for the dysregulatory activation of the intestinal immune system and the induction of the chronic inflammatory process in IBD. It has been suggested that antigen cross-reactivity shared by extraintestinal organs may be responsible for some of the immune-related consequences. IBD as well as extraintestinal manifestations have a familial predisposition with a high concordance rate among twins. In IBD and some of the immunologically mediated extraintestinal manifestations such as uveitis/irits, erythema nodosum, primary sclerosing cholangitis, and ankylosing sponylitis, association with genes in the HLA region have been demonstrated, suggesting an important role for genetic factors.

Dermatologic manifestations: *Erythema nodosum*, which is seen in about 15% of CD patients and is slightly less common in UC, correlates well with intestinal disease activity Skin lesions usually develop after the onset of bowel symptoms, and patients frequently respond to the treatment of the underlying bowel disease. Local treatment with corticosteroids is an option. *Pyoderma gangraenosum*, however, is seen more often in UC (5%) than in CD, where it usually only develops in Crohn's colitis. There is no correlation to bowel disease activity. Pyoderma gangraenosum may occur years before IBD is diagnosed, and it also may develop years after colectomy in UC patients. Treatment may be difficult. There are reports on efficient treatment with corticosteroids, azathioprine, thalidomide, cyclosporine, and infliximab. *Orofacial lesions* are common in Crohn's disease, and rarely occur in ulcerative colitis. They include aphthous stomatitis, cobblestone lesions of the buccal mucosa, and swelling and induration of the lips. *Perianal skin tags* occur in 75-80% of patients with CD and are associated with colonic inflammation

Rheumatologic and skeletal manifestations: *Peripheral arthritis* occurs in up to 20% of CD patients and less often in UC. It correlates well with bowel disease activity, and is usually cured in UC patients after colectomy. Arthtitis is often migratory and asymmetrical and

Extraintestinal manifestations and complications of IBD

		Frequency in IBD	Correlation with intestinal disease activity
Dermatologic manifestations	Erythema nodosum	Up to15%, CD>UC	+
	Pyoderma gangraenosum	Up to 5%, CD<UC	-
	Perianal skin tags	Up to 50% in CD	+
	Orofacial lesions	10%, CD>>UC	+
Rheumatologic and skeletal manifestations	Peripheral arthritis	Up to 20%, CD>UC	+
	Ankylosing spondylitis	10%, CD>UC	-
	Sacroileitis	10-50%, CD=UC	-
	Osteoporosis	2-30%, CD>UC	+
Ocular manifestations	Episcleritis	3-4%, CD>UC	+
	Anterior uveitis/iritis	Up to10%, CD=UC	-
Hepatobiliary manifestations	Primary sclerosing cholangitis	1-5%, CD<UC	-
	Cholelithiasis	10%, CD>UC	+
Other complications	Growth retardation	Up to 30%, CD>UC	+
	Nephrolithiasis	2-6% in CD	+
	Ureteral obstruction and fistulas	2-8% in CD	+
	Thromboembolic disease	1-6%, CD=UC	+
	Amyloidosis	1% in CD	+
	Pancreatitis	5%, CD=UC	+

affects mainly the large joints of the lower limbs. Usually it is not deforming. *Ankylosing spondylitis*, however, is not related to intestinal activity and usually does not respond to medical or surgical treatment of the underlying intestinal inflammation. It occurs in about 10% of IBD patients and is also more common in CD. Most cases are HLA-B27 antigen positive. Symmetric often asymptomatic *sacroiliitis*, which occurs both in UC and CD, is not related to intestinal activity either. *Osteoporosis* is a disease complication resulting from calcium malabsorption, malnutrition, long term corticosteroid treatment, and vitamin D deficiency.

Ocular manifestations: These are commonly associated with rheumatologic manifestations. *Episcleritis* occurs in 3 to 4% of IBD patients, more commonly in CD. It is associated with intestinal disease activity. Usually it is a mild disorder and may be treated with topical corticosteroids. *Anterior uveitis/iritis* may be diagnosed in up to 10% of CD and UC patients. There is no association with intestinal disease activity and it may also develop during remission of IBD and after colectomy. Visual impairment and scarring of the iris are possible complications.

Hepatobiliary manifestations: *Primary sclerosing cholangitis* is characterized by multiple intrahepatic and extrahepatic bile duct strictures, frequently resulting in biliary cirrhosis within about five to ten years after diagnosis. The condition is independent of intestinal disease activity and can be detected before the diagnosis of IBD or even years after colectomy. 1-5% of IBD patients, more often UC patients, develop primary sclerosing cholangitis. However, 50 to 75% of patients with primary sclerosing cholangitis have IBD. Treatment is difficult. Ursodeoxycholic acid may improve liver function tests, however, long term benefit is controversial. *Cholelithiasis* is more common in CD than in UC. It is caused by malabsorption of bile acids in patients with ileitis or after ileal resection.

Other complications:

- *Growth retardation* in IBD is strongly associated with disease activity. Inflammatory mediators such as tumour necrosis-α play a crucial role. Impressive catch-up growth can be observed as soon as remission of intestinal inflammation is achieved. Malabsorption due to intestinal inflammation and malnutrition as well as side effects of corticosteroid treatment also contributes to growth retardation.
- *Nephrolithiasis* occurs in CD patients with severe chronic ileitis and after small intestinal resection. In these patients nonabsorbed fatty acids bind dietary calcium. Luminal oxalate, which normally binds to dietary calcium, is therefore absorbed in increased quantities in the colon, resulting in hyperoxaluria. As a consequence, calcium oxalate calculi develop causing nephrolitiasis.
- *Ureteral obstruction and fistulas* are a consequence of intestinal inflammatory tumours in CD.
- *Thromboembolic disease* may develop because of by thrombocytosis, increased serum concentrations of coagulation factors, and other coagulation disorders such as antithrombin III and protein S deficiency.
- *Amyloidosis* can be observed in patients with longstanding active CD. Amyloid is deposited in intestine and kidneys and can cause diarrhoea and renal failure. It can be treated with colchicine.
- *Pancreatitis* can be caused by cholelithiasis, duodenal fistula, mechanical obstruction of the pancreatic duct or it can be a side effect of azathioprine.

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A Prospective Multicenter Study: Development and Validation of a Pediatric Ulcerative Colitis Activity Index

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Background/aim: Existing measures of ulcerative colitis (UC) activity are generally not a product of rigorous development and were not developed for pediatric use. Our aim: to develop and validate a

non-invasive, multi-item measure of UC activity for use in pediatric clinical trials.

Methods: A judgmental approach was used for item generation using a Delphi group of 48 experts in pediatric IBD and a thorough literature review. Further item reduction and item weighting was performed by regression analysis using a prospective cohort of pediatric UC patients from 5 IBD centers. Physician global assessment of the disease activity (PGA) was used as the dependent variable and the PUCAI items as the predictors. The β estimates of the model served to guide the weighting, governed by maximizing R^2 and aided by a correlation matrix. Reliability was assessed by Intra-Class Correlation coefficient (ICC) using two way random ANOVA. Validation of the weighted PUCAI was performed on a separate prospective cohort of UC children undergoing colonoscopy. Colonoscopic appearance and PGA served as the constructs. PUCAI was also simultaneously compared with a common invasive adult index (UCDAI; mayo clinic score). Sample size calculation was based on 10 patients/df of the weighting model (n=160) and further 30 for the correlation validity.

Results: A list of 41 potential items was generated by the expert panel. Rank order was considered in item reduction, following 4 rounds of feedback to the group. Gradations for the 11 highest ranking items were selected by consensus following much iteration. The draft PUCAI was completed independently by two physicians assessing 157 children with UC (age 12.7 ± 3.8 yr, 52% males; 77% extensive; 34% moderate to severe; 19% mild and 47% quiescent). ROC curves confirmed the validity of the gradations. Reliability was excellent (ICC>0.9 for all included items). Eight items were most important in the regression analysis: stool number, consistency and blood, abdominal pain, activity level, nocturnal diarrhea, albumin and CRP. In the validation set, the weighted index was highly correlated with both PGA ($r=0.86$, $p<0.001$) and colonoscopic appearance ($r=0.77$, $p<0.001$), higher than two commonly used, non-invasive adult indices, calculated simultaneously (Lichtiger (r=0.65) and Seo index (r=0.66)). The PUCAI also showed excellent correlation ($r=0.92$, $p<0.001$) with the reference invasive index (mayo-score). The laboratory items, improved the PUCAI performance, but not significantly.

Conclusions: The rigorously developed PUCAI has excellent reliability and validity. It provides a non-invasive measure of disease activity for use in pediatric clinical trials.

Improvement in a Biomarker of Bone Formation during Infliximab Therapy in Pediatric Crohn's Disease: Results of the REACH Study

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Background: Childhood Crohn disease (CD) is associated with significant deficits in bone density and mass. In addition to its role in the pathogenesis of intestinal inflammation, TNF-α has direct, detrimental effects on osteoblast number and function. Prior studies have demonstrated decreased biomarkers of osteoblast bone formation in CD.

Aims: To examine short-term changes in biomarkers of bone formation [serum bone-specific alk phos (BSAP)] and resorption [urine C-telopeptide of collagen crosslinks (CTX)] following initiation of infliximab (IFX) and associations between bone biomarkers, linear growth, and disease activity following 54 weeks of IFX therapy in children in the REACH study.

Methods: 112 patients ages 6-17 yr with moderate to severe CD received IFX induction (5 mg/kg/dose) at weeks 0, 2, and 6; week 10 responders were then randomized to IFX q8 or q12 weekly maintenance therapy. Serum BSAP and urine CTX were collected at baseline and 10 weeks. Disease activity (PCDAI) and height z-scores were assessed at baseline and 54 weeks. Complete data were available in 82 patients. Changes in biomarkers were compared using a paired t-test. Correlations between biomarkers and other parameters were assessed using Pearson correlation coefficients.

Results: Baseline BSAP levels were significantly lower in patients with greater disease activity ($r = -0.26$, $p<0.02$). Serum BSAP increased significantly during the induction period (Table). Increases in BSAP were associated with greater increases in height z-score ($r=0.27$, $p<0.05$) and decreases in disease activity ($r = -0.40$, $p<0.001$) over the study period. CTX also increased significantly during induction (Table) and was associated with growth ($r=0.24$, $p<0.05$) but not disease activity.

Conclusions: IFX therapy is associated with a dramatic short-term increase in the bone formation biomarker BSAP, consistent with inhibition of adverse TNF-α effects on osteoblasts. The increase in CTX likely reflects coupling of bone formation and resorption. While improvement in BSAP was associated with improved linear growth, the impact on bone health is unknown. Prospective studies are needed to assess infliximab effects on bone density and structure.

Bone biomarkers

	Baseline∗	Week 10∗	% Change	p
BSAP (U/L)	48.1±26.2	91.0±47.0	107±97	<0.001
CTX(μg/μmol cr)	811.7±442.1	1035.0±622.7	43±77	<0.001

∗ Mean±SD

DIAGNOSTIC CONSIDERATIONS IN IBD

Audit of Gastroduodenal Mucosal Lesions: Do They Help in the Discrimination of Patients with IBD?

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Macro- and microscopic mucosal lesions in the upper gastrointestinal tract (UGT) may be identified in many patients with inflammatory bowel disease (IBD). Approximately two thirds of all patients with Crohn's disease (CD) and half of those with ulcerative colitis (UC) have microscopic abnormalities, irrespective of presence of symptoms (1-3). Therefore, routine use of esophago-gastro-duodenoscopy (EGD) in the initial evaluation of children suspected of IBD has been suggested. The ESPGHAN IBD Working Group recently published guidelines supporting this practice (4). However, this is considered controversial by some clinicians and clinical practice may vary among centres.

EGD should be carried out in children with clinically suspected upper UGT IBD, but being an invasive procedure its additional use in all patients needs to be justified. It may be hypothesized that EGD could lead to a better discrimination between CD and UC. This would be especially useful in children with indeterminate colitis. A number of pathological macro- and microscopic features are used in the diagnosis of CD. However, systematic evaluation of such classic diagnostic criteria when applied in UGT lesions for discrimination between CD and UC has not been performed. When reviewing the literature granuloma appears to be the only pathological finding detected by EGD, that has a documented ability to discriminate between CD and UC *and* is frequently identified. Other characteristics of CD either have a low ability to discriminate or seldomly occur (1). Thus, at present only identification of granulomas can be used as an instrument to evaluate the usefulness of routine EGD.

Granulomas may be identified by EGD in one fourth to one third of all CD children (1-3,5). They are most often reported in the gastric antrum, followed by the duodenum and esophagus, but systematic evaluation of their distribution in the upper GIT is missing. Granulomas identified in the upper GIT only have been reported in 7-15% of CD children (Table 1). However, the frequency of children that can be given a diagnosis of CD solely based upon detection of upper GIT granulomas

TABLE 1. *Granulomas detected in EGD only*

	Granulomas in EGD only	Granulomas in EGD leading to CD diagnosis
Tobin et al. (1)	4/25 (16%)	0%
Abdullah et al. (2)	9/15 (8%)	?
Kundhal et al. *Am J Gastroenterol* 2003	6/39 (15%)	5/39 (13%)
Castellaneta et al. (5)	11/54 (20%)	7/54 (13%)
Shaoul et al *Inflamm Bowel Dis* 2004	1/7 (14%)	0%
Lemberg et al. (3)	4/55 (7%)	4/55 (7%)

(those who would not have been diagnosed as CD if ileocolonoscopy alone was carried out) seems to range between 0 and 13% in the published (so far sparse) literature (Table 1).

The use of routine EGD in the IBD work-up can be expected to increase the diagnostic yield with regard to discrimination between CD and UC. However, data are sparse and this issue should be further addressed in prospective studies.

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New and Old Serological Markers in IBD

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Many patients with inflammatory bowel disease, and more in particular Crohn's disease (CD) express antibody responses to a variety of microbial and autoantigens. By studying a panel of markers, up to 80% of CD patients will have antibodies to at least 1 antigen with almost 50% of patients responding to more than one antigen (1). The antibodies that have been described and studied most in Crohn's disease include the antibodies directed against mannose epitopes from the yeast *Saccharomyces cerevisiae* (ASCA), antibodies to the outer-membrane porin C of Escherichia coli (OmpC), antibodies directed against a Pseudomonas fluorescens-associated sequence (I2), and antibodies directed against flagellin (cBir1). More recently, a set of new antiglycan antibodies has been proposed in patients with CD. Atypical perinuclear Anti-neutrophil cytoplasmic antibodies (pANCA) are the antibodies typically associated with UC (in 40-60% of patients), but can be found in 10% of patients with colonic UC-like CD (2).

Although the pathogenesis of all these antibodies remains unclear, recent data suggests that patients with a genetic defect in the innate immune response, characterized by the presence of CARD15/NOD2 mutations, may be at greater risk to develop an exaggerated adaptive immune response to these bacterial sequences.

It is unclear when these antibodies appear during life although a recent study using a Israeli Defense Corps serum repository, showed that antibodies may preceed clinical onset of the disease (3). In this study, 32 CD patients were identified from whom serum was available before diagnosis and 10/32 patients were tested ASCA+ already before diagnosis.

Antibodies offer little value to the current diagnostic algorithm of CD or UC, partly because of their modest sensitivity (50-60% for ASCA, 20-30% for I2 and anti-OmpC). However, their high specificity (95-99%) may warrant their use in patients where distinction between CD or UC is not clear (4). In these patients with so-called colitis-type unclassified (IBD-U, previously called indeterminate colitis), a prospective multicenter study recently observed that almost half of patients tested negative for these markers, and that these patients have a greater chance of remaining unclassified over time (5).

More and more data suggest that both the presence as well as the magnitude of immune response to microbial antigens are associated with a more aggressive and complicated small bowel and/or fibrostenosing or internal perforating disease, with a greater risk for abdominal surgery (1). Whether these antibodies also predict more aggressive disease needs to be studied in a prospective way. A recent prospective study in children demonstrated that the time to develop a disease complication was significantly faster in the presence of immune reactivity, thereby predicting disease progression to more aggressive disease phenotypes among pediatric CD patients (6). In this way, these markers could indicate which patients may benefit from more aggressive earlier therapy.

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Imaging Diagnosis: Current Approach and New Acquisitions

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Imaging represents an important step in the diagnosis and follow-up of IBD. The importance of an accurate diagnostic imaging is especially true in the small bowel, where clinicians have no other tools than radiology to identify inflammatory lesions.

Several diagnostic tests are available to investigate the small bowel: conventional X-rays (small bowel follow-trough and small bowel

enteroclysis), sonography and cross-sectional imaging (Computed Tomography, CT and/or Magnetic Resonance, MR) (1). The choice of the single modality is in most of the cases related to local equipment availability and medical expertise. However, non-invasive imaging modalities should be preferred, and in particular those using no ionizing radiations (sonography, MR). This is particularly important in a paediatric environment where the young patients have an expectations to undergo numerous examinations in the following years.

Sonography needs special technique (oral administration of a water-soluble contrast agent, usually polyethileneglycol, PEG), technical expertise and long examination time (the operator has to follow the progression of the contrast agent through the bowel); and more importantly, sonography is not able to provide a panoramic view of the entire small intestine, making the communication between operator and clinician (especially a surgeon) more difficult (1).

Thanks to the progress of modern diagnostic equipments, cross-sectional imaging is becoming the primary modality for the evaluation of the bowel. In fact, both CT and MR are able to provide a panoramic evaluation of the small bowel, with MR preferred in a paediatric environment for issues related to radiation safety (2,3).

The challenge for an accurate evaluation of the small bowel for any cross-sectional imaging is to have an optimal luminal distension. Two are the approaches: the oral administration of a contrast agent (''MR enterography''), preferred in paediatric patients, and the administration of the contrast agent through a previously positioned naso-jejuneal tube (''MR enteroclysis'').

The major advantage of cross-sectional imaging compared with conventional x-ray is the assessment of the wall as well as of the surrounding fat tissue. It means that imaging moves from the evaluation of the lumen with an indirect analysis of the wall and mesentery, to a direct assessment of wall thickness and the involvement of the mesentery. Morphologic evaluation of inflammatory lesions is not only limited to the assessment of the presence of single/multiple strictures, and the length and severity of the strictures themselves, but it can also provide information about fibrotic changes of the wall which may alter patient management (surgery versus medical therapy).

Furthermore, quantitative information about disease activity may be obtained. Two are the approaches, both showing optimal correlation between MR data and clinical indexes: the former based on the acquisition of contrast-enhanced sequences (correlation between wall enhancement and disease activity) (3); the latter based on special fat-saturated T2 weighted images, able to detect inflammatory changes with great sensitivity (correlation between hyperintense signal of bowel wall and disease activity) (4).

Another important advantage of cross-sectional imaging is direct examination of the mesentery, showing chronic changes of the fat tissue (fibro-fatty proliferation). Additionally common complications, like abscesses and fistulas, can be easily detected. Two of the major areas of research development in MR imaging of the small bowel are new oral contrast agents and the assessment of disease activity.

The aim of the research of new contrast agents is focused on the development of more tolerable and palatable agents able to provide better luminal distension: the goal is to have a bowel distension using an oral approach as closer as possible to enteroclysis. It will enhance patient compliance and make the examination easier.

In the assessment of disease activity, the goal is to move from qualitative analysis (visual evaluation of contrast enhancement or hyperintense signal of the bowel wall) to quantitative analysis (perfusion imaging), offering information about regional blood flow, blood volume and mean transit time; these parameters should correlate with disease activity and might be helpful in the assessment of the response to therapy.

Diagnostic imaging of the colon is another area under investigation. Today, the most important innovation is represented by CT-colonography (CTC); at the moment this test is accurate for the evaluation of polyps and colonic carcinoma, but only few experiences are available on IBD patients. Moreover, the relatively high dose of ionizing radiations delivered to patients using CTC is not adequate for IBD patients, usually young subjects requiring frequent investigations in the follow-up.

Although still under research investigation, MR-colonography might represent the imaging of the colon for the next future and preliminary experiences seem to confirm this assumption (5). MR colonography is based on a volumetric acquisition of a cleansed and distended colon; the distension is obtained by a water enema.

Preliminary results are very promising: colonic wall is clearly depicted in normal cases; wall thickening together with pathologic enhancement secondary to inflammatory lesions is also easily appreciated. Morphologic alterations of the colonic wall together with simultaneous involvement of the distal ileal loop may help in the differential diagnosis between ulcerative colitis and Crohn's disease, not always easy in the paediatric population.

The trend of the research in MR-colonography is to try to reduce invasiveness and patient discomfort avoiding bowel preparation (''Prepless'' MR-colonography). It can be obtained by administering to patients an oral contrast agent during the days before the examination: the goal of the contrast agent is to tag the stools (''fecal tagging''). Once the stools are tagged they become virtually undistinguishable by the water enema used for colonic distension.

Finally, in rectum and anal canal diagnostic imaging provides excellent information about the site and extension of abscesses and fistulas with respect to the anal sphincter complex and may guide patient management and treatment. The challenge of any imaging modality in the management of fistulas is to define the course of the track between these openings so that the appropriate surgical option can be used. Transrectal sonography is the easiest approach, although difficult due to poor patient compliance; in most of the patients with active anal fistulas the examination cannot be completed due to patient intolerance. MR is the imaging modality of choice: it is performed using external coils without any preliminary patient preparation. Thanks to the high contrast resolution MR is able to depict tortuous and deep fistulous tracts and to distinguish between inter-sphincteric and trans-sphincteric fistulas. MR can also differentiate between an active and a fibrotic fistulous track. Abscesses in the ischio-anal fossa can also easily detected.

In conclusion, imaging diagnosis in IBD has sustained a real revolution in the past few years and it is now able to support clinical decisions more accurately. New challenges are open and they are waiting for a reply from the radiological community in the next future.

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Wireless Capsule Endoscopy in Inflammatory Bowel Disease: State of the Art and Image of the Future

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Despite notable advancements in gastrointestinal endoscopy, assessment of the small bowel has remained relatively inaccessible. Recently, the development of wireless capsule endoscopy (CE) has revolutionized enteroscopy, providing for the first time a non-invasive method for the complete endoscopic evaluation of the small bowel mucosa. The Pillcam SB (Given Imaging, Yoqneam, Israel) was approved by the FDA

in the USA and HPB Canada for use in adults and children 10 years of age and older.

As in adults, barium small bowel follow through (SBFT) and colonoscopy with ileoscopy (CI) have been the traditional methods for evaluating known or suspected small bowel CD in the pediatric age group [1]. However, SBFT has low sensitivity for early or subtle inflammatory lesions of CD, and CI can at best only evaluate the terminal ileum.

A recent consensus statement suggested that capsule endoscopy is a far more promising tool for the evaluation of the small bowel in IBD [2]. A meta-analysis examined 11 prospective comparative studies for the diagnosis of CD using CE versus other imaging modalities [3]. The yield for CE was significantly higher compared to SBFT (63% and 23%, respectively). Similarly, the yield for CE versus CI was 61% and 46%, while that for CE versus CT was 69% and 30%, respectively. CE thus appears to be more sensitive for small bowel CD than other traditional imaging modalities. Moreover, a normal CE examination has a very high negative predictive value, essentially ruling out small bowel CD.

Capsule endoscopy has been shown to be safe and highly useful in pediatric patients, as in adults [4]. For children too young or incapable of swallowing the capsule, direct insertion into the duodenum using a front-loaded gastroscope has proven to be highly efficient [4]. Limited data exist to date on the use of CE in the diagnosis of IBD in children. In the one comparative and prospective, self-controlled pediatric trial reported to date [5], lesions consistent with a diagnosis of small bowel CD was found only by CE in 10/20 (50%) patients suspected of CD, while this diagnosis was ruled out in 8 patients. Two remaining cases were found by CE to have eosinophilic gastroenteropathy. CE has also been reported to be useful in the evaluation of possible CD among young patients presenting with a protein-losing enteropathy and/or growth failure when other studies are negative. An economic analysis comparing CE to other traditional modalities for diagnosing CD suggested that CE may also be less costly as a first-line test in this situation. However, further studies on this subject are needed to confirm this approach from a cost-effectiveness outcomes point of view.

Capsule endoscopy may also be useful in several other important clinical situations in IBD [2,4] including: a) the non-invasive assessment of the small bowel for active disease in patients known to have CD who present with unexplained symptoms or signs (eg anemia), where the cause may be functional or dietary; b) determining the presence of post-operative recurrence of CD; c) discriminating between ulcerative colitis and Crohn's disease in patients with indeterminate colitis, and d) assessment of small bowel mucosal healing after treatment. The limited data to date, all in adult cases, suggest that while CE was able to identify more proximal small bowel disease, IC was as or more sensitive overall for CD recurrence. A few small pilot studies also found that CE led to a change in diagnosis in up to 40% of patients with IC, and that CE outperformed IBD serological markers in identifying small bowel CD in IC patients. However, larger prospective studies are needed to confirm the usefulness of CE in IC and post-operative recurrence.

As there is no gold standard test for CD, the diagnosis is generally based on a constellation of clinical, endoscopic, histological, radiological and biochemical findings. One might thus justifiably question whether a diagnosis of CD may be made on the basis of CE findings. Moreover, one has to consider whether CE findings are specific for CD. There is evidence that up to 13% of normal, asymptomatic adults have small bowel mucosal breaks and other minor lesions detected by CE. Thus CE findings are alone not sufficient for a diagnosis of CD. Other potential causes include gluten sensitive or allergic enteropathies, infectious, ischemic, rheumatic, autoimmune as well as immunodeficiency related disorders and especially drug-induced etiologies. Non-steroidal anti-inflammatory drug (NSAID) induced enteropathy is particularly common and should be considered in all cases. At the present time, it remains unclear as to whether the lesions detected by CE in NSAID enteropathy can be reliably distinguished from those due to CD. A standard CE terminology system has been developed along with a capsule endoscopic CD scoring index. However, these are not yet fully validated and hence not yet universally adopted.

The major contraindication to CE is a known or suspected gastrointestinal tract obstruction and/or small bowel stricture, because of the increased risk of capsule retention. Overall, the incidence of capsule retention varies from 0.1 to 5% of cases. Most of the cases of capsule retention in the pediatric age group are due to small bowel CD, although eosinophilic, drug induced (NSAID), and radiation enteropathy are other possible causes [2,4]. The vast majority of cases of small bowel capsule retention are asymptomatic. To minimize the risk of capsule retention, a careful history should be taken regarding the presence of any obstructive symptoms. Nevertheless, most CE studies reported to date that resulted in capsule retention ''excluded'' patients at risk for small bowel strictures by requiring a normal SBFT prior to capsule ingestion. Therefore, a normal SBFT does not eliminate all risk of retention, particularly in patients with known CD. The rate of capsule retention in adult series examining patients with suspected Crohn's disease is quite low [2]. However, in the only prospective pediatric trial of CE for suspected CD, capsule retention was seen in 10% (2/20) of cases, despite a negative SBFT [5]. In both cases, the capsule passed the unsuspected inflammatory stenosis after treatment with oral corticosteroids.

Recent studies have shown that the ''patency'' capsule is beneficial in screening patients suspected of having a stricture or obstruction [2,6]. Its body, comprised of lactose with barium, is identical in size to the regular imaging capsule. It incorporates a tiny radiofrequency identification (RFID) tag, facilitating its detection using an RFID scanner or plain abdominal x-rays. The patency capsule remains intact for a minimum of 30 hours and then begins to disintegrate. If excretion of the patency capsule intact is witnessed or the RFID tag is undetectable by 30 hours, it is safe to proceed with capsule endoscopy.

In summary, CE has revolutionized the field of small bowel enteroscopy, leading to improvements in the diagnosis and evaluation of small bowel diseases, including IBD. As we look to the future, newer technology may allow the capsule to have other diagnostic and therapeutic capabilities.

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THERAPEUTIC DILEMMAS IN PEDIATRIC IBD

Are Children With IBD Receiving the Optimal Care?

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No worldwide consensus exists regarding the optimal treatment of IBD in children. Evidence based, clinical guidelines are only beginning to be developed, and are hampered by the lack of controlled clinical trials in children. Furthermore, determining what constitutes optimal care requires more than completing controlled clinical trials. It also requires assessing whether treatments that appear beneficial in clinical trials result in improved outcomes for children in routine clinical

practice. Outcomes data from large population-based cohorts are required to make these assessments. Unfortunately, these kinds of data are also limited.

However, different methods of investigation including published surveys of pediatric gastroenterologists' therapeutic practices, regional observational data registries and national quality improvement initiatives are starting to define clinical practices and patient outcomes. The results of these investigations highlight similarities and differences in the worldwide clinical practices of pediatric gastroenterologists.

Virtually no population-based data regarding therapy for children with ulcerative colitis (UC) have been published, but consensus regarding optimal induction therapy appears to exist. Most children with UC respond well to initially selected treatment (5-aminosalicylate or rectal therapy for mild UC, corticosteroids ± 5-ASA for moderate-severe UC) such that only 12% require the addition of more intensive therapy within 30 days of diagnosis (1). Still, 4% require colectomy by 1 year after diagnosis, and disagreement remains about the risk/benefit ratio of immunomodulatory and biologic therapy for maintenance versus colectomy in children with moderate-severe UC (2).

Similarly, there is no worldwide consensus regarding induction therapy for children with Crohn disease (CD), especially in terms of the role of nutritional interventions versus corticosteroids. A meta-analysis of pediatric studies finds the two approaches equally efficacious, although nutritional therapies may lead to fewer complications and improved growth (3). Despite this, clinicians disagree about which approach is optimal. As highlighted in a recent international survey of pediatric gastroenterologists, for children with mild to moderate CD failing first line therapy, 57% of European physicians select sustained enteral nutrition but 71% of US physicians select corticosteroids as their next treatment of choice (4). While 79% of European survey respondents believe that nutritional therapy is efficacious and 62% use it frequently, only 46% of US physicians agree about the efficacy of nutritional therapies and only 4% use them regularly.

Clinical practice outcomes from large pediatric cohorts can provide data to judge whether specific treatments provide optimal care. A multicenter, observational study of routine clinical practice by a U.S./Canadian consortium revealed that 84% of children with moderate-severe CD at diagnosis receive corticosteroids within 30 days of diagnosis (5). A complete or partial response by 3 months is seen in 84% of those treated. However, 31% are corticosteroid dependent and 8% require surgery by 1 year. With such therapy, growth velocity improves significantly compared to the year prior to diagnosis, but remains subnormal. How these response and growth rates compare to a group of children whose treatment is based on a sustained nutritional intervention is unknown, as comparable large scale studies describing the results of nutritional therapy in clinical practice have not been published.

There seems to be agreement, based on both open label experiences and a placebo controlled trial, that thiopurines represent optimal maintenance therapy for children with CD. Sixty six percent of US physicians and 54% of European physicians prescribe thiopurines for $\geq$50% of their CD patients (4). The regional US/Canadian observational registry confirms the widespread early use of immunomodulators in clinical practice, with 80% of children presenting with moderate-severe CD receiving 6-MP or azathioprine by one year after diagnosis (5). Early thiopurine use decreases corticosteroid exposure and hospitalization rate, but not infliximab use or surgery (6).

Pediatric gastroenterologists also appear to agree that infliximab represents optimal treatment for children with moderate-severe, corticosteroid dependent and fistulizing CD, unresponsive to immunomodulators. By contrast, whether the use of infliximab in UC represents optimal care remains unanswered given the paucity of pediatric data for this indication.

It appears, therefore, that significant work needs to be done to determine whether children with IBD are receiving optimal care. Both controlled trials and population-based outcome analyses are required before consensus is reached regarding what constitutes optimal care and whether children around the world are receiving it.

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Immunomodulation with AZA/6-MP/MTX: Current Use in IBD

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Azathioprine (AZA) and 6-mercaptopurine (6-MP) are potent immunomodulators that have been used in the management of IBD for more than 30 years. Methotrexate (MTX) is less widely used, partly based on custom but also because of concerns regarding toxicity. This brief review will highlight some of the key issues arising from the numerous studies published since 2000, most involving adult patients.

AZA/6-MP are now much more widely used than in the past. Markowitz et al (2001) surveyed the membership of NASPGHAN and compared the results with those of an identical survey in 1990. Significantly more now used these agents for Crohn's disease (CD) in children with growth failure, perianal and non-perianal fistulas, and for post-operative prophylaxis. Early use (following diagnosis) has probably increased – partly due to the publication of a frequently cited pediatric randomised controlled trial (RCT) (Markowitz et al 2000) showing that this practice reduced relapse frequency. This was hardly surprising given the known effects of AZA in adults, but of course that trial did not address the complex risk / benefit issues for routine use.

Does immunomodulator therapy alter the natural history of IBD? In a recent retrospective study of 2573 adults with CD seen over 25 years during which AZA and MTX usage increased progressively (initially unused and in the final phase used in 56%) the risk of resection, strictures and penetrating disease was unaltered.(1)

Can the use of these agents reduce the risk of osteoporosis? Osteoporosis is a special concern in young people given that bone demineralisation early in life increases the risk of osteoporosis in later years. In a DXA scan study of adults with CD and UC, remission for >3 years and AZA usage were associated with significantly increased bone mineral density. (2)

In my Department we undertook a retrospective cohort study to explore the possibility that patient characteristics (clinical, laboratory, radiological and endoscopic findings) at the time of first presentation with IBD might identify those who would subsequently receive AZA. The most significant finding was that those requiring IV corticosteroids at presentation usually ran a difficult course afterwards requiring AZA treatment. This supports the view that such patients should be considered for AZA therapy from the outset. No other patient characteristic usefully predicted the need for AZA.

In an important 30 year retrospective review the efficacy of AZA in 622 recipients among 2205 IBD patients was examined (3). This study provides information that could not easily be obtained from prospective studies. The RCT evidence in support of immunomodulator therapy is stronger for CD than UC. Here, however, AZA was even more likely to achieve remission in UC than in CD. Given the concerns about toxicity with AZA/6-MP there is a long-standing question regarding optimal treatment duration. This study provides compelling evidence that the

effectiveness of AZA does not wane over time, and when discontinued relapse rates at 1, 3 and 5 yr were increased. Importantly, the risk of relapse was no less in those who had taken AZA for longer periods. Consistent with this study, two recent randomised placebo controlled withdrawal studies have shown that discontinuation after 2 and 3.5 years of treatment respectively was associated with an increased risk of relapse. It appears that the traditional practice of withdrawing treatment after a few years may not be well founded.

Given their proven efficacy AZA/6-MP might be used in all patients as maintenance therapy were it not for the risk of side-effects. Most published series suggest that some form of adverse effect occurs in 10-15% of patients. These drugs are subject complex metabolism, and thiopurine methyltransferase (TPMT) is a key enzyme. Enzyme activity in Caucasian populations has a tri-modal distribution, being high (89%), intermediate (11%), or low (0.3%). Those with low levels have high levels of 6-thioguanine nucleotides (6-TGN). 6-TGN levels are important, being associated with both immunosuppression and myelosuppression. Measurement of TPMT activity (phenotype) or genotyping may identify those at high risk of myelosuppression with standard doses of AZA/6-MP. However, there is much debate about the merits of measuring TPMT. This is one of the first examples of pharmacogenetic research potentially assisting clinical practice. Unfortunately, myelosuppression may occur even in those with normal enzyme activity, and so blood count monitoring is still necessary. Some therefore argue that TPMT measurement it not justified. A recent economic cost analysis supported its routine measurement, but as so often the generalisability of the study's findings may be argued. There are other potentially important reports in relation to TPMT. It is inhibited in vitro by aminosalicylates, raising concerns about co-treatment with such agents. Low TPMT activity might have a potential for causing long-term toxicity. Children with acute lymphoblastic leukaemia may be at increased risk of later developing secondary treatment-related malignancy (acute myelogenous leukaemia) if they have a low TPMT level and have received thiopurines. Of course they have additional risk factors, being treated with various other anti-neoplastic drugs, and these concerns may not be relevant to IBD patients.

There are several contradictory studies (underpowered) examining the correlation between RBC 6-TGN levels and induction of remission. A recent meta-analysis has now reported that higher levels are indeed associated with remission (4). The authors suggest that 6-TGN level measurement might be useful in specific circumstances - identifying those who unlikely to responder (6-TGN level greater than the effective threshold), those who are non-compliant (very low 6-TGN levels). There has been discussion for many years about the possibility that AZA/6-MP might be associated with an increased risk of lymphoma, as is reported in transplant patients. A recent meta-analysis of cohort studies suggested that there was a four-fold increased incidence of lymphoma in patients with IBD patients treated with a thiopurine (5). There was considerable heterogeneity between the studies which included patients from specialist centres. Large population based studies have failed to identify this association. The increased risk could be due to treatment or disease severity, association not necessarily indicating causation. Generally it is agreed that the risk must be small and that the risk / benefit ratio firmly supports the use of these drugs in IBD.

Although there is convincing RCT evidence that MTX is effective in CD it is much less widely used than the thiopurines. A recent survey of Canadian gastroenterologists indicated that 33% never used it, and 61% considered it a second-line therapy. Of the paediatric gastroenterologists surveyed, 17.6% never used it. This reluctance is perhaps related to perhaps unjustified concerns about potential side effects. A recent Cochrane review (CD003459) concluded that MTX (25 mg/wk, IM) is an effective treatment in adults with chronically active steroid resistant CD. In those who respond MTX also appears effective in maintaining remission. However, unlike rheumatoid arthritis in which low dose oral MTX has a central role in disease management, there is a lack of evidence for this strategy in IBD. Two studies compared MTX with AZA and 6-MP and found no difference, but they lacked statistical power. MTX metabolism is complex, and a subject of pharmacogenetic research. A recent study examined the frequency of various potentially relevant polymorphisms in patients with IBD. An association was reported between a mutation in the methylenetetrahydrofolate reductase enzyme gene and the risk of side effects (6).

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Nutritional Therapy in Crohn's Disease: Is It Truly Useful for Inducing Remission?

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The aim of therapy in Crohn's disease is to achieve clinical remission with minimal side effects. Since the inflammatory process is focussed on the intestinal mucosa, ideally clinical remission should be associated with a reduction in mucosal inflammation and mucosal healing. Enteral nutritional therapy can induce clinical remission, and reduce systemic and mucosal inflammation with minimal side effects. Thus despite the availability of more potent treatments for Crohn's disease it remains a widely used therapy for active disease, particularly in the paediatric population.

Clinical efficacy: Over the last twenty years there have been numerous studies which have shown that feeding patients with a variety of different liquid diets: elemental, semi elemental and polymeric, as the sole source of nutrition (enteral nutritional therapy), is effective in achieving remission in Crohn's disease. From meta-analysis of adult studies the efficacy appears however to be less than that of corticosteroids (1), although if only paediatric trials are analysed the two therapies appear to be equally effective (2). In recent years it is in this paediatric group of patients that enteral therapy has been most widely used (and studied). This has been partly due to concerns regarding potentially deleterious effects on linear growth of the standard alternative therapy of corticosteroids, as well as unanswered questions as to the long term safety of other therapies such as infliximab.

The type of feed: polymeric/semi-elemental/elemental (whole protein/peptide/amino acid) does not appear to influence efficacy (1), but alterations in other specific components of the liquid diet (glutamine/lipid profile) have been shown to alter clinical outcome. Polymeric feeds do however have a significant advantage over many elemental/semi-elemental feeds with regards palatability, allowing oral ingestion, and thus potentially improving compliance.

Traditionally enteral nutritional therapy has been administered as 100% of the nutritional requirements of the patient, for several weeks. This approach has recently been validated. Johnson et al (3) compared a less ''severe'' regimen (just 50% of requirements met by elemental liquid diet, the rest by normal food) with 100% elemental diet and found a higher remission rate in the 100% elemental diet group (42% versus 15%). Supplementation with enteral nutrition rather than 100% nutritional therapy may still however have a role in maintaining remission over longer periods of time where complete food exclusion becomes impracticable.

Enteral nutritional therapy does appear to have varying success in treating Crohn's disease at different intestinal sites. Anecdotally

nutritional therapy is particularly effective in the management of oral disease. Reviewing the remission rates of 62 children treated with enteral therapy (not selected by disease site) Afzal et al (4) found significantly higher remission rates for children with small bowel and ileo-colonic disease when compared to children with isolated colonic disease (90%, 85%, 47%). This finding confirms the clinical impression of many gastroenterologist that enteral nutrition is less effective for colonic disease, but since the study was not comparative the relative efficacy of enteral nutrition and corticosteroids was not studied, and indeed colonic disease may well be more resistant to other medical therapies.

Enteral nutrition–systemic inflammation: Numerous studies have reported a reduction in systemic inflammation (C-reactive protein, and erythrocyte sedimentation rate) in association with the clinical response to enteral nutrition. More specifically levels of serum tumor necrosis factor α (TNFα), and interleukin-6 (IL-6) decline in response to treatment (5,6). The IL-6 response was evident within just a few days of starting therapy.

Enteral nutrition and mucosal healing: The most compelling evidence that enteral nutrition can influence the disease process of Crohn's disease, comes from those studies that have directly assessed the effects of this therapy on the inflamed intestinal mucosal. The most detailed study in this field was by Fell et al (7). In a cohort of 29 children treated with a polymeric feed for eight weeks, they demonstrated that together with the clinical response there was macroscopic and histological evidence of mucosal healing. Furthermore these mucosal changes were associated with a fall in interleukin-1β mRNA in the mucosal biopsies of both the colon and terminal ileum. There were also changes in response to treatment in three of the other cytokines measured, although there were differences in these effects between small and large bowel. In the terminal ileum there was a fall in interferon γ mRNA, with a concurrent rise in transforming growth factor β (TGFβ) mRNA. In the colon these two cytokines did not change significantly, but there was a fall in interleukin-8 mRNA.

Other studies which have assessed the effect on enteral nutrition on the intestinal inflammation adopting different methodologies have produced similar results: a fall in luminal interleukin-1β assessed by whole gut lavage, and a fall in interferon γ and interleulin-2 producing cells in mucosal biopsies.

Discussion: The fall in mucosal pro-inflammatory cytokines and the documented mucosal healing in response to enteral nutrition strongly suggest that this therapy is acting directly on the disease process itself rather than exerting its clinical effect indirectly by some nutritional means. How enteral nutrition is able to reduce mucosal inflammation has been the cause of much speculation. A likely mechanism is the potential effect of enteral nutrition on the intestinal micro-flora. Other potential mechanisms have however been explored, ranging from the influence of the lipid profile of the liquid diet, to a putative action of other components such as TGFβ which is present in some feeds.

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Use of Methotrexate to Induce and Maintain Remission in Crohn's Disease: A Regional Cohort Study

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Introduction/aim: There is limited data on the use of Methotrexate (MTX) for induction and maintenance of remission in children with histologically confirmed, chronic and non-remitting Crohn's Disease (CD). We therefore aimed to review our patients treated with MTX, in terms of both induction and maintenance of remission.

Methods: A retrospective and prospective cohort study of patients with CD, resistant to or intolerant of Azathioprine (AZA), who were treated with MTX (16 week induction course of s/c MTX (15mg/m^2) followed by the equivalent oral dose) from 1/8/97 to 31/12/05 at our regional centre. Paediatric Crohn's Disease Activity Index (PCDAI) was used as a well-validated score to objectively measure disease activity during treatment; PCDAI $\leq$10 indicated full remission, $\leq$15 indicated partial remission and a score $>$30 indicated severe disease.

Results: 27 patients were treated with an induction course of s/c MTX; 25 (93%) patients entered remission. These 25 patients had CD diagnosed at a mean (range) age of 11.4yrs (4.0-15.6) and received MTX at a mean (range) age of 13.5yrs (9-18.0). The median PCDAI at start of treatment was 35 (IQR 22.5-40). Median time to partial/complete remission was 15 weeks (IQR 8-16), with a median duration of follow-up of 114 weeks (IQR 85-179). 8 (32%) entered clinical remission with PCDAI$\leq$15 and maintained long-term remission (6 full remission, 2 partial remission). 17 (68%) of patients relapsed, 5 (20%) having only a single relapse and all re-entering remission (1 requiring surgery and 1 requiring a further s/c course of MTX). 12 (48%) patients had $\geq$2 relapses, with 7 of these patients receiving Infliximab, 1 receiving Adalimubab, 4 requiring surgery and 2 further patients undergoing assessment for surgery; 5 (20%) of these have still not entered remission. On MTX treatment, 9 (36%) had mild nausea/vomiting and 13 (52%) had raised ALT (2 subsequently undergoing liver biopsy, with treatment then continued).

Summary/conclusions: MTX appears to be effective in inducing (93%) and maintaining (52% $\leq$ 1 relapse in median of $>$2 years follow-up) remission in patients who have failed to remit on AZA and also appears to be well tolerated and safe. Those who have had multiple relapses have all had complicated clinical courses and severe intractable disease. However, a stronger evidence base is needed from well-designed RCTs for both induction and maintenance of remission of CD by MTX.

Videocapsule Endoscopy in Pediatric Crohn's Disease: A 4-year Experience

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Background/aim: We report our experience in 80 children who underwent videocapsule endoscopy (VCE).

Patients/methods: Prospective, single center study of consecutive cases (2001-2005) in individuals 18 years or less, referred for possible

Crohn's disease (CD), gastrointestinal bleeding, polyposis or protein losing enteropathy/malabsorption. Patients referred for suspected CD had chronic abdominal pain accompanied by at least 2 of the following inclusion criteria: chronic diarrhea, anorexia and weight loss, anemia, hypoalbuminemia, positive ASCA serology. Cases for identification of a source of intestinal bleeding had negative initial workup including a colonoscopy and esophagogastroduodenoscopy at minimum. VCE was performed after an overnight fast using the Given Imaging M2A capsule. Small bowel preps and prokinetics were not employed.

Results: Complete data was available for 80 of the 85 patients studied (58% female). Mean age of those studied was 14.1 ± 2.7 years (range 4-18). Weight ranged from 16-104 kg. In 2 patients (2.5%), age 4 and 17 yr, the capsule placed endoscopically in the duodenum. Mean gastric emptying and small bowel transit time was 37.7 and 212.8 minutes, respectively. The cecum was not visualized in 7.5% of cases. Overall, 21 (26.2%) had a new diagnosis of CD established by VCE, while 6 others (7.5%) had possible CD (lesions non-diagnostic). Other diagnoses were small bowel polyposis in 6 cases (7.5%), 7 (8.8%) with vascular lesions, 15 (18.5%) with non-specific changes of small bowel erosions or erythema, 2 had eosinophilic enteritis, and 1 case each with small intestinal lymphangiectasia and celiac disease. The VCE study was normal in 17 (21%) patients. Overall, the leading indication (61.2%) was suspected Crohn's disease. A.diagnosis of CD with typical lesions in the small bowel was made by VCE alone in 42.2% of the 49 patients referred for CD. 12.2% had an alternate diagnosis, and the VCE was normal in the remainder. Capsules were temporarily retained in the small bowel in 2 cases (2.5%) due to an inflammatory stricture despite recent normal small bowel follow through x-rays. In both cases, corticosteroid therapy resulted in the capsule being passed without symptoms. No other adverse events were encountered.

Conclusions: VCE is a highly effective modality for diagnosing patients with symptomatic ''occult'' CD of the small bowel, undetected by conventional diagnostic methods, and is the leading reason for referral for a VCE in pediatrics. The overall diagnostic yield (specific positive findings) was 55%. VCE also excluded CD with precision. Clinical follow-up of the non-diagnostic studies (26% overall) is ongoing.

Upper Gastrointestinal Endoscopy Is Valuable in the Diagnosis of Crohn's Disease

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Aim: To determine whether routine UGIE should be done in addition to colonoscopy and small bowel contrast radiology to help establish the diagnosis of Crohn's disease.

Methods: In the 18 month period to Nov 2005 children referred to four Departments of Paediatric Gastroenterology with suspected IBD were routinely evaluated using the recently published 'Porto Criteria' (UGIE, ileocolonoscopy and small bowel contrast radiology; *JPGN* 2005;41:1-7). We reviewed the investigative findings in 106 with Crohn's disease. The following were recorded: (1) macroscopic ulceration, cobblestoning, strictures, skip lesions, loop separation (based on endoscopy or small bowel contrast radiology); (2) histological evidence of severe inflammation, ulceration, crypt abscesses, and architecture changes, and granulomas.

Results: UGIE revealed macroscopic and histological abnormalities highly suggestive of Crohn's disease in 26% (28/106) and 38% (40/106) of cases respectively. The macroscopic abnormalities were confined to the upper gastrointestinal tract (i.e., small bowel contrast radiology and colonoscopy normal) in 5% (5/106) and the histological abnormalities in 2% (2/106) (i.e., colonic biopsies normal). Overall, the combination of UGIE and colonoscopy revealed the presence of granulomas in 55% of cases (58/106). Granulomas were present in both upper gastrointestinal tract and colon in 20% (21/106), in the colon alone in 26% (27/106), and confined to the upper gastrointestinal tract in 9% of cases (10/106)

Summary: UGIE contributes usefully to the diagnosis of Crohn's disease. In a substantial minority of cases it reveals characteristic macroscopic and histological abnormalities that would be missed with colonoscopy and barium contrast alone.

Conclusions: This study supports adherence to the Porto Criteria.

HOT TOPICS IN IBD

Controversial Surgical Issues in Paediatric IBD

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Ulcerative Colitis

In children ulcerative colitis is more aggressive than in adults. Children present more often with widespread disease and develop pancolitis more often than adults. Therefore, children require more aggressive medical treatment than adults; corticosteroids are usually needed to control the initial disease. The side effects of systemic high dose corticosteroid treatment on a growing and developing body are significant and often an indication for surgical treatment.

Between 40-70% of children with ulcerative colitis undergo surgical treatment. As most patients can be stabilised by medical treatment, emergency operations for toxic megacolon, unremitting bleeding or refractory fulminant colitis are not common today. The typical indications for surgery of ulcerative colitis are poor response to optimal medical treatment, dependence on high dose corticosteroids with significant side effects, delay in growth and maturation and severe extra-intestinal manifestations of the disease.

The actual timing of surgery is a controversial issue. Some physicians tend to wait until significant side-effects of the management develop, others advocate earlier surgery because of the more severe initial presentation in children. Surgery should not be considered as a primary or early treatment of ulcerative colitis. A significant proportion of patients achieve long-term symptomatic relief with conservative treatment and may remain in remission with minimal or no medication. Moreover, the functional outcome following restorative proctocolectomy is not comparable with normal bowel function. Before proctocolectomy is undertaken Crohn's disease should be ruled out with every possible measures. Crohn's disease patients should not undergo restorative proctocolectomy.

The gold standard of surgery for ulcerative colitis has been proctocolectomy and permanent ileostomy. Limited colonic resections, and colectomy and ileorectal anastomosis have been abandoned, as these have been associated with high incidence of complications and recurrence of the disease. Proctocolectomy and permanent ileostomy gives excellent control of ulcerative colitis and related symptoms but is not very well tolerated by children and adolescents because of related social restrictions and permanently altered body image. Since late 1970's restorative proctocolectomy with ileoanal anastomosis has gained overall acceptance as the standard operative procedure for adult and also paediatric ulcerative colitis. In early stages of the development of restorative ileoanal pull-through for ulcerative colitis straight pull-through without reservoir formation was used by many paediatric surgeons. Today most surgeons advocate the use of ileal reservoir because of better functional outcome. The most popular and easiest to construct is the J-pouch. More complex pouches such as S- and W-pouches are used by some surgeons. Some surgeons advocate one-stage operations but most use staged surgery due to safety issues in these often significantly immunosuppressed patients.

Restorative proctocolectomy is a major operation with significant incidence of postoperative complications. Septic complications are common as most patients with refractory ulcerative colitis are immunosuppressed because of high-dose corticosteroid treatment. The nutritional status of many patients is often not very good due to long-term diarrhoea and poor nutrient intake. To avoid septic complications it is imperative that systemic corticosteroids are tapered to as low level as possible or preferably changed to locally acting budesonide that has less systemic immunosuppressive effects. The nutritional status should also be improved. It is usually possible to do this by dietary measures.

Crohn's Disease

Like ulcerative colitis Crohn's disease is more aggressive in children than in adults. Moreover, the presentation of Crohn's disease in children is usually less localised than in adults. There is no definitive curative treatment for Crohn's disease. As Crohn's disease is an affliction of the whole gastrointestinal tract surgery does not offer any permanent cure. Unlike in ulcerative colitis surgical treatment is palliative in nature and aimed to treat the complications of the disease. It is evident that the development of novel drug therapies has reduced the need for surgical therapy for Crohn's disease. The indications for surgery are limited to cases that are refractory to medical therapy or medical therapy is poorly tolerated. Acute indications include medically unmanageable toxic megacolon or acute bleeding, both of which are rare. Subacute or chronic conditions that may require surgery include refractory strictures, internal or external fistula and intra-abdominal abscesses.

The main principle in the surgical treatment of Crohn's disease is to save bowel length. Radical resections are not indicated; resection should be limited to the segment of bowel, which is causing symptoms. Isolated skip lesions are left alone if they do not cause obstruction. In adults strictureplasty has been shown to be an effective bowel saving surgical method for multiple fibrotic stenoses. The long-term outcome in terms of disease activity, risk of recurrence and quality of life has been very similar than following resectional surgery. There are only a few reports of strictureplasty for Crohn's disease in children but the preliminary results are similar as in adults. Although controversial, strictureplasty probably should be favoured also in children because it saves bowel length in patients who have early onset of the disease and are likely to be candidates for further surgery during their long life span.

Major bowel resections are sometimes required in association with severe and widespread colonic disease. In these cases rectum should be spared. Ileoanal anastomosis with or without a pouch is not indicated for Crohn's disease; long-term outcome is very unpredictable and often poor, moreover, pouch complications are very common.

Perianal manifestations of Crohn's disease are very common in children. These include skin tags, fissures and fistulas. In most cases perianal manifestations cause mild symptoms or are asymptomatic. Conservative approach is warranted and surgical treatment should be considered only in severely symptomatic high rectoperineal or rectovaginal fistulas that do not respond to increased immunosuppressive therapy. If surgery is required for a high perianal fistula resection of the mostly diseased usually left colonic segment and temporary bowel diversion may increase the success rate of the fistula repair. In very severe perianal disease, especially if it is associated with severe rectal manifestation, proctectomy may be the only possibility to guarantee a reasonable quality of life.

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Management of Perianal and Fistulizing Crohn's Disease

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Over the course of the disease, between 13 and 35% of Crohn's disease (CD) patients will develop fistulizing complications (1). These fistulas occur predominantly in the perianal region (2/3 of fistulas) and most patients only develop one fistula. However, perianal and abdominal fistulizing disease is usually very debilitating to the patient and often refractory to treatment. Even if most adult and pediatric gastroenterologists rely on expert advice from colorectal surgeons or proctologists to guide their therapy, an insight in accurate diagnosis and disease assessment, medical and surgical treatment options and in expected success and recurrence rates with treatment will help to improve the management of patients with fistulizing disease. We will focus on several aspects of clinical management in perianal Crohn's disease based on current evidence from clinical trials and international guidelines.

Outcome Parameters in Fistulizing Crohn's Disease: Clinical versus Imaging-based Assessment

The etiology of penetrating fistulas is still a matter of debate but areas of slow transit appear to be prime locations for internal orifices. Abdominal fistula penetration through the bowel wall occurs predominantly in the ileum more particularly proximal to stenosed bowel segments. Perianal perforating disease can develop in an anal crypt as is the case for most sporadic fistulas or through deep rectal fissures and ulcers. Perianal fistulas occur in 12-15% of patients with ileal or ileocecal disease, in 42% with left sided disease and rectal sparing, and in 92% of patients with rectal involvement, which underscores the role of rectal involvement as the cradle of perianal disease (1). Perianal disease can be classified as primary or secondary. Primary fistulas occur on the background of proximal disease activity in the bowel and probably reflect extension of the inflammatory reaction through anatomical gateways. Secondary lesions are formed by the perforation of deep ulcerations contaminated by fecal contents and gradually migrating towards the skin or the vagina. A more relevant classification developed by St Mark's hospital is based on anatomical criteria (Parks classification (2)). This classification differentiates between simple and complex fistulas and scores extension in the supralevatoric tissue and in the ischiorectal fossae. As the management of enterocutaneous fistulizing CD depends heavily on an accurate anatomical description and assessment of intestinal disease activity, imaging tools are indispensable. The choice of clinical endpoints is extremely important in trials specifically designed to evaluate perianal disease (45). For the assessment of clinical severity the Perianal Disease Activity Index (PDAI) which incorporates criteria of fistula discharge, pain, restriction of sexual activity, type of perianal disease and degree of induration, has been used in most recent randomized controlled trials. However, the trials with infliximab have introduced a more empirical endpoint easily assessable by inspection and external examination. Cessation of drainage from cutaneous orifices despite gentle compression has now become a reference endpoint. This outcome parameter is relevant to the patient's quality of life, but may not adequately reflect the changes in the internal fistula tracks as is discussed below. For abdominal fistulas spiral CT and barium meals/enteroclysis have been the standard imaging techniques. Increasingly CT and MRI enteroclysis are being used for this purpose since they allow the visualization of both fistula tracks and diseased bowel segments. Also for perianal fistulizing disease accurate staging of the local extension is key to successful management. Schwartz et al. showed that combination of examination under anesthesia (EUA) combined with either MRI or endoscopic rectal ultrasound (EUS) provides superior preoperative staging and will change surgical management decisions in up to 15% of cases(3). More particulary in patients with local or systemic signs of abscess and in whom surgery will be the initial treatment, MRI appears to be indicated. Evidence supporting the use of MRI to assess improvement of fistulizing disease with medical treatment is accumulating.

Quality of the Evidence Supporting Medical Treatment Options Is Highly Variable

Evidence-based medical treatment options are clearly more limited as compared to the treatment of luminal disease. Antibiotics, immunosuppressives, topical treatments and biological agents have all been studied for their efficacy to improve the condition of patients with fistulizing disease. The levels of evidence underlying these various

treatment strategies range from data obtained in a randomized controlled trial (level Ib) in the case of infliximab to case reports or expert opinion (level III and IV) for methotrexate or topical agents.

The use of the antibiotics, metronidazole and quinolones, is based on evidence from case series only (level of evidence III). In clinical practice 3-4 month courses of antibiotics (metronidazole 750-1500 mg/d, ciprofloxacine 1000 mg/d) are administered. Bernstein et al showed in an uncontrolled trial that drainage from fistulas improves with metronidazole but tapering of antibiotics resulted in high recurrence rates (6). Long term use of nitroimidazoles is hindered by side effects such as metallic taste, paresthesias and nausea. Topical metronidazole (2% to 10%) cream is used to ameliorate anal Crohn's disease but again not based on controlled trials.

Systemic corticosteroids are not indicated for the treatment of fistulizing CD and may worsen sepsis. In recent placebo controlled trials a proportion of patients was taking systemic corticosteroids at baseline, but this merely reflects disease activity in proximal bowel segments.

There are no controlled trials with improvement of perianal disease as a specific endpoint evaluating the role of purine analogues (azathioprine/6-mercaptopurine) in the treatment of perianal Crohn's disease. However, in current clinical practice these agents are used based on combined evidence from 5 controlled trials with improvement of perianal disease as a secondary endpoint and from uncontrolled case series (4). The doses used are identical to those for luminal disease. The benefit of methotrexate (MTX) in the management of perianal fistulizing disease also stems from recent uncontrolled trials suggesting that MTX alone or in combination with infliximab ameliorates fistulas (7-8).

The evidence to support the use of the calcineurin inhibitors, cyclosporine and tacrolimus in this indication is also limited. The use of cyclosporine IV as an induction regimen followed by oral cyclosporine is based on uncontrolled case series showing clinical improvement. A small placebo controlled trial demonstrated that Tacrolimus 0.2 mg/kg daily improved fistula drainage but did not result in closure of cutaneous orifices. Moreover, renal toxicity was considerable in this trial (9). Topical therapy with tacrolimus has also been reported but only in uncontrolled series.

Evidence to use the chimeric anti-TNF antibody infliximab stems from two randomized controlled trials (evidence level Ib) and from numerous uncontrolled case series. In a pivotal trial Present et al. showed that 5 mg/kg and 10 mg/kg infliximab given at weeks 0, 2, 6 result in a rapid closure of at least 50% of cutaneous orifices (primary endpoint) at two consecutive timepoints in 68% of patients treated with 5 mg/kg IV and 55% of patients treated with this dose achieved cessation of drainage from all cutaneous orifices (versus 13% of placebo treated patients) (10). The fistula improvement was associated with an amelioration in quality of life scores, patient global assessment and in PDAI. The efficacy of infliximab in fistulizing disease short term led to a larger placebo controlled maintenance trial (ACCENT 2) (11), which again demonstrated that patients treated with 3 initial infusions followed by every 8 week maintenance had a median time to loss of response after achieving remission of more than 40 weeks as compared to 14 weeks ($P<0.001$) in patients treated with only one infusion of infliximab. More placebo than infliximab maintained patients (62% versus 42%) had a loss of response over 54 weeks. However, this illustrates that even in an era of biologicals refractory perianal disease is prone to disease recurrence. Specific trials evaluating the role of infliximab in the treatment of abdominal perforating disease are not available, but a recent small cohort study suggested a lack of long term efficacy (12).

Most Patients with Fistulizing Disease Benefit from a Combined Surgical-Medical Approach

For most patients optimal management of perianal and abdominal fistulizing disease will consist of combined medical and surgical treatment (see treatment algorithm, Fig. 1). First, abscesses and local sepsis always necessitate incision and drainage. Simple and low (not sphincter perforating) fistulas can further be managed medically. For more extensive and complex perianal fistulas drainage of abscesses followed by Seton placement and immunosuppressives plus infliximab appears to be the best clinical practice. In patients responding to initial surgical/medical treatment, maintenance immunosuppressives and infliximab is warranted. In patients with debilitating symptoms failing the initial treatment temporary or definitive fecal diversion should be considered. Rectovaginal fistulas are a particular phenotype in perianal

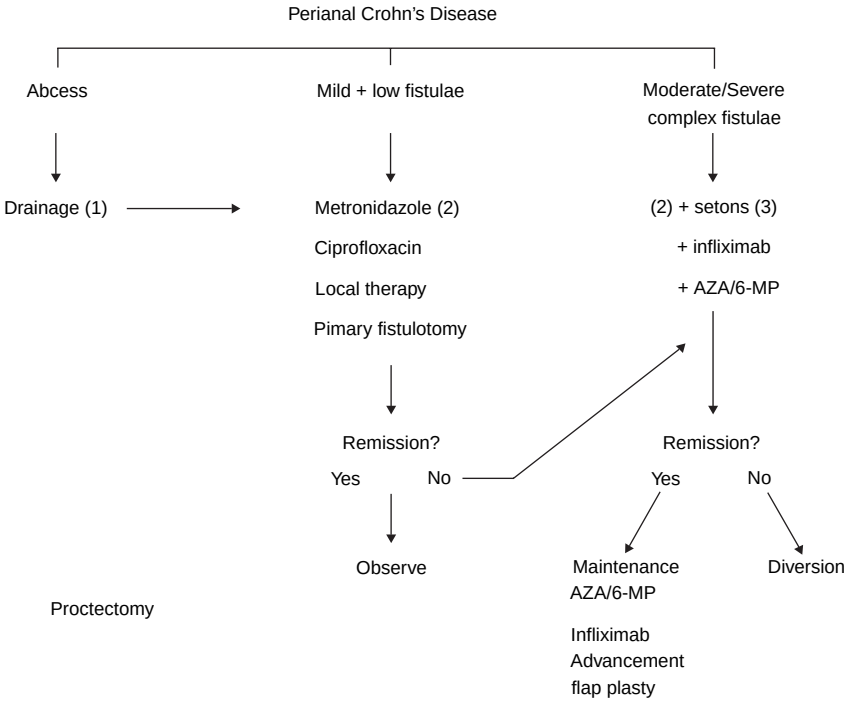

FIG. 1. Treatment algorithm for perianal fistulizing Crohn's disease (18).

Crohn's diseas since the short track between rectum and vagina appears to limit the benefit from medical treatment. Open label data suggest that they are also refractory to inflximab treatment (13). Surgery with advancement flap plasty is still the mainstay of management although this procedure has also a high recurrence rate. Infliximab and immunosuppressives may have an adjuvant effect in ameliorating the outcome through healing of rectal disease, although controlled data are lacking.

Therapy for Fistulizing CD Is Always Based on a Medium to Long Term Maintenance Approach

Generally, complex perianal CD is characterized by extensive local inflammation and this necessitates long term approaches to optimize management. Treatment with immunosuppressive is charactezized by delayed onset of action. Also, although infliximab appears to act rapidly to improve perianal disease recent evidence inidicates that most patients will also need maintenance infliximab. Both MRI, EUS and transcutaneous ultrasound imaging of internal fistula tracks has demonstrated that after 3 infusions of infliximab inflammatory lesions persist in virtually all patients and that only after one year of maintenance treatment with infliximab local inflammation is suppressed, although complete closure of fistula tracks is observed in a small minority (14-15-16). Optimal treatment strategies with infliximab for perianal disease are summarized in Table 1.

TABLE 1. *Optimizing infliximab treatment in perianal Crohn's disease (17)*

	Role of imaging
Assessment before initiating infliximab	
Assess fistula complexity and presence of abscesses	
drainage of sepsis if needed	EUA + MRI or EUS
Exclude rectal stenosis and dilate if needed	Flex. sigmoidoscopy
Ensure temporary drainage of cavities with Seton's	
Optimizing infliximab treatment	Rationale
0-2-6 induction regimen	Optimal response in RCT
8 week maintenance in patients responding	Decreased ATI formation
	Improvement of internal inflammatory lesions
Concomitant immunosuppressives	Decreased ATI formation

EUA: examination under anesthesia, EUS: endoscopic ultrasound, RCT: randomized controlled trial, ATI: antibodies to infliximab.

Conclusions

For most patients with refractory fistulizing Crohn's disease isolated medical treatment is not the optimal management. Patients will benefit most from a collaborative approach by radiologists, gastroenterologist and colorectal surgeons. Accurate staging of perianal disease before treatment initiation, surgical drainage of sepsis, and a combined medical/surgical management are key to optimize the chances of long term fistula closure. Depending on the complexity and location of fistula tracks antibiotics, immunesuppressives and maintenance infliximab can be used in medical therapy although evidence from specific placebo controlled trials has only been obtained for infliximab. During the course of maintenance treatment reassessment of internal fistula tracks using EUA and/or MRI, may serve to prevent fistula reccurrence due to unrecognized local inflammation.

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Diagnosis, Risk Factors and Prevention in Post-operative Crohn's Disease

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Surgery remains unavoïdable in about 80% of patients with Crohn's disease (CD) without any definitive effect in most of the cases despite new medical therapies (1). Data about pediatric CD and recurrence after surgery does not appeared different than from adult's CD although pediatric data are rare or concerned small population and retrospective data (2-5).

Theoretically recurrence after surgery (no CD lesion left) should be differentiated from a relapse after surgery which leaves identified area of CD. In fact the high rate of recurrence after surgery make this difference with no real signification and suggests that surgery should be limited to the surgical complication and not try to clear out all the CD lesions. The post operative recurrence rate varies with its definition: endoscopic, clinical or surgical. Data from endoscopic follow up show an 80 to 100% of recurrence at three years and clinical recurrence without therapy of 40% at two years.

Risk factors of recurrence have been mainly studied in adults and are similar in pediatric studies. Colonic location, extension of the disease (>100cm) and the absence of prophylactic treatment are recognized risks in all studies as well as recurrence of endoscopic lesions one year after surgery. Perforating CD should not be considered as a risk factor.

Prophylaxis of post operative recurrence has been also studied essentially in adults. Prevention treatment should be done systematically within one month after surgery when risk factors are present. Endoscopy might be indicated at 2 years for those without treatment and clinically asymptomatic. The first line drug recommended is mesalazine at a dose of 40-50mg/kg/day. Several studies and a meta-analysis showed a significative but modest reduction of recurrence by 15%. Metronidazole delayed recurrence as mesalazine but is rarely used because of side effect during long term treatment. Azathioprine or 6 mercaptopurine (6MP) is recommended in patients with high risks of recurrence or a complex CD. In a multicentric study Hanauer SB et al (2004) demonstrated a lower rate of clinical (50% vs 78%) or endoscopic (43% vs 64%) relapse with 6 MP as compare to placebo over 2 years follow up. No data exits as it concerns the potential effect of anti TNF alpha antibodies.

In conclusion surgery remain very frequent in CD with a high risk of recurrence notably when risk factors are present. Prevention of recurrence by medical therapies notably mesalazine and 6MP should be used in patient with high risk of recurrence and complex CD. Their efficacy although significantly better than placebo, is unfortunatly relative (50% of cases).

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Probiotics in IBD: Facts and Fantasies

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If you look at the past experiences in the US and Europe, the interest for a possible role of probiotics in IBD is to say the least quite ''surprising''. While in the US the culture of probiotics is still in an early stage in terms of market opportunities and usage by physicians and patients, in Europe the situation is totally different. In many countries, namely Italy, France, Scandinavian countries and to some extent Germany, the Netherlands and Spain, many probiotic preparations have been on the market for almost 30-40 years. These preparations are considered now as food supplements but initially were registered as drugs or OTCs. Doctors were prone to prescribe them ''as useful placebo'' and patients had the perception that they were ''good'' for the gut health. In addition, in the above cited countries, many foods that are part of the normal diet are dairy foods or fermented with probiotics. So we can say that contrary to the US population, a large portion of the European population has been and is continuously exposed to probiotics. Now, new data seem to suggest a role for probiotics in preventing and/or treating IBD.

So a ''cruel'' question comes to my mind: if this is true, why do we have IBD in Europe? Since we were and still are exposed to probiotics (thanks to our eating habits and doctors' prescription), we should be less affected by IBD. On the contrary newly diagnosed patients are there and in some countries their number is steadily increasing. Even though, better diagnosis could be a relevant factor for that, my answer is: *the probiotics do not work for IBD!* As a matter of fact, preparations containing only one or two strains and at a concentration of a few billion per gram (that I would define as ''first generation probiotics'') not only did not reduce the incidence of IBD in the European Countries where they were prescribed massively, but failed when tested in double-blind placebo-controlled studies. A number of trials have been recently completed and have clearly shown that these preparations have no major role in the prevention/treatment of IBD (see Table 1).

So what is wrong? I believe that the origin of our mistake is the intrinsic characteristics of the products employed and at the same time a certain degree of ''Ignorance'' or just ''too much love for money''. Let me make an example regarding the ''Ignorance'', paradigmatic but not restricted to *Lactobacillus* GG. This product was administered for the prevention of NEC (Necrotizing Enterocolitis) at a dosage of a few billions per gram ($6x10^9$ Colony Forming Units/g) to premature babies whose weight was under 1.5 kg. Almost the double dosage ($12x10^9$CFU/g) was administered to adults with Crohn's disease. Does it make sense that a premature baby weighing 1.5 kg or less ingests $6x10^9$ bacteria and an adult about 70 kg ingest only twice as much? Do we have similar cases for other dietary or pharmaceutical preparations? Assuming that the dosage administered to kids for trivial gut problems (between 1 and $10x10^9$) is adequate, why isn't the amount of the bacteria increased proportionally for the adults? And then, we come to the second factor: ''Money''. As today's prices, big pharmaceutical or nutrition Companies sell probiotic bacteria at a cost ranging between 10 and 20 € per bottle. Each bottle contains in the best scenario some 60 capsules, each capsule usually containing at best 10 billion bacteria in one or two strains. So the cheapest product may cost 10 to 20 € for 600 billion bacteria. This is a price the customer will accept to pay, dreaming of a possible efficacy in IBD or other more or less serious disorders. In my opinion the efficacy is not there because the bacteria are not enough, and the strains are too few. Indeed, the only product that up to now showed data endorsed by the American Society of Gastroenterology, by the British and also by the German Society of Gastroenterology is the preparation called VSL#3.

VSL#3 is a mixture of various strains of lactic acid bacteria and bifidobacteria at a concentration of 450 billion microorganisms per sachet and has been proven effective in several IBD trials (1). Studies are in progress to evaluate its efficacy in pediatric IBD, IBS and rotavirus diarrhea. Now, if VSL#3 seems to be effective, how come no major pharmaceutical or nutrition Company ever showed an interest to market a similar product? Let's do some calculations: if 600 billion bacteria are sold at 10 € as illustrated above, one sachet of VSL#3 should be sold at 8 €, whereas current price ranges between 1.5 and 2 €. VSL#3 will never bring the rich revenues that attract the big pharmaceutical and nutrition Companies! Huge investments are necessary to fully investigate the role of probiotics in IBD, but it seems that in this scenario, the research activities will rely on the good will of scientists.

TABLE 1.

Author	Title and reference	Design	Number of patients	Treatment, dosage and duration	Results
Prantera	Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG. *Gut* 51:405-9 (2002)	Double blind, randomized, placebo controlled	45	*L. rhamnosus* GG $1,2x10^{10}$ CFU /day *vs* placebo for 12 months	Recurrence rate in the treated group not significantly different than placebo group
Kuisma	Effect of Lactobacillus rhamnosus GG on ileal pouch inflammation and microbial flora. *Aliment Pharmacol Ther* 17:509-15 (2003)	Double blind, randomized, placebo controlled	20	*L. rhamnosus* GG $4x10^{10}$ CFU/day *vs* placebo for 3 months	None of the patients in the treated and placebo groups experienced an amelioration of pouchitis symptoms
Laake	Assessment of mucosal inflammation and circulation in response to probiotics in patients operated with ileal pouch anal anastomosis for ulcerative colitis. *Scand J Gastroenterol* 38:409-14 (2003)	Open study	10	Cultura® (*L. acidophilus B. lactis*) $5x10^{10}$ CFU/day for 1 month	Partial endoscopic improvement, no istologic improvement
Schultz	*Lactobacillus* GG in inducing and maintaining remission of Crohn's disease. *BMC Gastroenterol* 4:5 (2004)	Double blind, randomized, placebo controlled	11	*L. rhamnosus* GG $2x10^{9}$ CFU/day *vs* placebo for 6 months	Relapse rate in the treated group not significantly different than placebo group
Bousvaros	A randomized, double blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. *Inflamm Bowel Dis* 11:833-9 (2005)	Randomized double blind placebo controlled	65	*L. rhamnosus* GG 1×10^{10} CFU/day *vs* placebo for 24 months	Relapse rate in the treated group not significantly different than placebo group
Marteau	Ineffectiveness of *Lactobacillus johnsonii* LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomized, double blind, placebo controlled GETAID trial. *Gut* 55:842-847 (2006)	Randomized double blind placebo controlled	98	*L. johnsonii* LA1 $4x10^{9}$ CFU/day *vs* placebo for 6 months	Relapse rate in the treated group not significantly different than placebo group

Reference
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SATURDAY, NOVEMBER 25, 2006
OPENING LECTURE

5-ASA in the Era of Biologics

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Large clinical trials have shown that sulphasalazine (SASP), a compound of 5-aminosalicylic acid (5-ASA, mesalamine) and sulfapyridine (SP), was more effective than placebo in the treatment of patients with active Crohn's disease (CD) and ulcerative colitis (UC). The uncertain efficacy of this drug when the disease is limited to the small intestine and the appearance of side effects mainly connected with SP, have led to the elaboration of new products made up exclusively from 5-ASA. Several oral preparations of 5-ASA are available with different delivery systems which allow the release of the active drug in different intestinal tracts. The efficacy of 5-ASA compounds is conditioned by the local concentration of the drug and by the amount of active substance which reaches the diseased site.

Till today the 5-ASA preparations remain the mainstay of treatment for induction of remission in mild to moderately active CD and UC, and to prevent relapse of quiescent disease.

Treatment of Active Disease

Crohn's Disease

Different 5-ASA compounds have been evaluated in many controlled trials to determine their efficacy in treating CD acute flares, generally showing a therapeutic advantage over placebo, but inferior to steroids. The largest controlled study on 5-ASA in active disease compared three different doses of Pentasa (1, 2, and 4 g/day) versus placebo. The lower dosages were ineffective, but 43% of patients on 4 g achieved remission at 16 weeks in comparison with 18% on placebo. In a subsequent trial

from the Mayo Clinic, 3.2 g/day of 5-ASA (Asacol) given for 16 weeks were superior to placebo in achieving remission in patients with mild to moderate CD. Mesalamine (Salofalk) at a dosage of 4.5 g/day for 8 weeks was shown to be as effective as 6-methylprednisolone 48 mg/day in inducing remission in 34 patients with ileocolonic involvement. In a multicenter Italian study, Asacol 4 g daily was employed in two different formulations, tablets and a new microgranular preparation, compared to 6-methylprednisolone 40 mg daily in 94 patients with mild to moderately active CD localised to the terminal ileum (1). The percentage of remission observed at 12 weeks was 79% with the new formulation of 5-ASA in comparison with 61% obtained with steroid and 60% with mesalamine tablets. A recent meta-analysis of the three placebo controlled studies of Pentasa employed at a dose of 4 g/day for 16 weeks in a total of 615 patients with active CD, showed a mean reduction of the Crohn's Disease Activity Index (CDAI) from baseline of -63 points, compared with – 45 points for placebo (p=0.04) (2). These results confirm that Pentasa 4 g daily is superior to placebo in reducing CDAI, but the clinical significance is not clear. Subgroup analysis does not provide sufficiently answers to whether one group of patients benefits more than another.

Mesalamine slight efficacy could be explained by several aspects related to the drug: the fairly low dosage of 5-ASA administered in the first studies, since only in the most recent trials doses of 4 g and over have been employed; the use of 5-ASAs with different delivery systems; the probable topical action of mesalamine; the eventual faecal loss of the drug, following the accelerated transit or a wide bowel resection.

Some aspects that may explain the slight efficacy could be related to the disease: CD affects different locations of the intestine with different disease behaviour; CD often involves the deep layers of the gut, and 5-ASA, because of its topical action, should be more efficacious when the lesions are localised in the superficial layer.

Ulcerative Colitis

A Cochrane systematic review on oral 5-ASA for active UC included 19 trials involving 2032 patients (3). Nine studies were placebo controlled and 10 compared mesalamine with SASP. The results showed that mesalamine was more than twice as effective as placebo, but not significantly better than SASP and exhibited a dose-response relationship up to dosages of 4.0-4.8 g/day. This is equivalent to SASP doses of 10.0 to 12.0 g/day, which are not typically tolerated. In clinical trials with oral mesalamine compounds, patients with pancolitis and left-sided colitis responded similarly. However, rectal 5-ASA preparations are more effective than oral mesalamine for treatment of acute distal colon disease. In three randomized controlled studies which compared an oral 5-ASA compound with local 5-ASA, symptomatic improvement and remission was in favour of topical administration. Unfortunately, therapy with enema is not well accepted by patients. In a recently published exploratory study on 79 patients with left-sided colitis, a formulation of mesalamine with a new oral delivery system, 5-ASA Multi-Matrix (MMx), was comparable to 5-ASA enema in inducing remission after 8 weeks of treatment (4). The tablets of 5-ASA MMx (1.2 g) are characterized by the presence of a double matrix: the core of the tablet consists of mesalamine incorporated in microparticles of lipophilic matrix, dispersed in turn within a hydrophilic matrix. The core is then coated with a gastro-resistant polymer film with pH-dependent dissolution. The coating begins to dissolve only in the final tract of the ileum; at this point, the hydrophilic matrix starts to erode and the drug diffuses out of the lipophilic matrix. In this way the release of 5-ASA is slow and gradual, with an homogeneous distribution along the colon. The results of the trial showed that 5-ASA MMx 3.6 g/day was effective as 5-ASA enema 4 g/day for inducing remission in active left-sided UC.

Maintenance of Remission

Crohn's Disease

Given the high safety profile of 5-ASAs, these compounds are widely employed in maintenance of CD remission. A large number of studies have been conducted on mesalamine in prevention of symptoms relapse after a medically induced remission and in prevention of endoscopic lesions or symptoms after surgery, unfortunately with contradictory results. In order to clarify this controversy, three meta-analysis have analysed the results of all the trials on 5-ASA employed in CD maintenance.

In the first metanalysis, published in 1994, 6 out of 8 randomized trials concerned the prevention of symptoms relapse after a medically induced remission, showing that mesalamine significantly reduced the relapse frequency. The other two trials were studies on recurrence prevention after surgery. One of them has given negative results and the other one did not include a control group. A second metanalysis published few months later showed a benefit of 5-ASA in maintaining medically induced remission. A third metanalysis was published later in 1997 (5). More trials were included and subsequently a more severe method of selection was employed. The analysis concluded that the effectiveness of mesalamine was statistically significant in the prevention of recurrence in surgically induced remission group, but not in the patients who were in remission because of medical therapy. After this last metanalysis, the largest trial on 5-ASA prophylaxis of postoperative CD in a population of 318 patients was published. The difference between placebo and active drug, Pentasa, 4 g daily was not statistically significant, but a post hoc analysis showed a significant difference in favour of the treatment when the disease was localized in the small bowel, without colon involvement.

These trials show problems of heterogenous study design. Difference in dosage of mesalamine employed; difference in 5-ASA compounds used with different delivery systems; difference in inclusion of trials, since were involved patients with medically induced remission together with patients who were in post-operative remission; difference in drugs employed for inducing remission of the acute phase.

Ulcerative Colitis

In UC the main role for oral 5-ASA is long-term maintenance treatment. All 5-ASA drugs show superiority to placebo and comparable efficacy to SASP in maintaining remission, but in a meta-analysis the parent compound had a modest therapeutic advantage (6). However, mesalamine is better tolerated than SASP, so that the choice can be influenced by tolerability (5-ASA is tolerated by 80% of patients who are unable to tolerate SASP). Maintenance therapy with 5-ASA preparations may reduce the risk of colorectal cancer. This favours long term treatment for patients with extensive UC.

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EVOLVING BIOLOGICAL THERAPIES FOR IBD

An Historical Overview of the Treatment of IBD: Why Do We Need Biological Therapies?

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A sea change has occurred in the management of inflammatory bowel diseases (IBD) over the last decade with the introduction of "biologic" disease-modifying drugs. Derived from biologic sources, these

medications target specific molecules in the inflammatory cascade (chiefly tumor necrosis factor (TNF-alpha) and interleukins (IL)). Colloquially known as ''biologicals'' (Table 1), they have become an integral part of IBD management, altering the natural history of this condition.

The most successful approach so far has been anti-TNF strategies. Thus infliximab, an anti-TNF antibody has set a new standard against which future therapies will have to be measured, being a disease modifying drug, achieving not only significant long term clinical remission but also thorough mucosal healing (resulting in longer duration of remission and avoidance of disease complications). Other advantages are the steroid sparing effect and restoration of normal growth in pediatric patients. To justify the significant costs associated with anti-TNF-alpha strategies, the compounds should fulfill these criteria. However, infliximab has not been able to completely silence the inflammatory response. Moreover, adverse events are present in a selection of patients receiving long-term therapy. For this reason, efforts to develop alternative pharmacological strategies targeting TNF-alpha continue, and most likely, further unraveling of pathogenetic mechanisms will facilitate future developments of anti-TNF-alpha therapies.

Important advances in therapy of IBD have occurred in the past 20 years. Conventional treatment of IBD includes nutritional therapy (particularly in children), aminosalicylates, corticosteroids, immunsuppressive drugs (azathioprine, its metabolite 6-mecaptopurine, methotrexate, cyclosporin), and antibiotics. The introduction of immunosuppression therapy in the 1990s by the Mount Sinai group was the first attempt to really change the natural evolution of Crohn's disease (CD) and less so in ulcerative colitis (UC). These disease course modifying drugs have limitations. Their onset of action is delayed, they have potentially serious side effect profiles and need to be given for many years. Moreover, up to 60% of patients do not respond to these drugs long-term, even with optimal dosage [1]. Refractory IBD impairs quality of life and eventually results in debilitating complications resulting in the need for (re)/surgery. In addition long-term corticosteroid therapy carries a heavy burden of serious and irreversible side effects including growth impairment and osteoporosis that often offset their therapeutic benefit in the long term. Fortunately, dissection of the immune/ inflammatory cascade in the early 1990's has lead to a huge proliferation of drugs targeting specific components of the cascade and to the development of new biologic therapies.

Biologic treatments can be divided into three categories: 1) inflammatory cytokine inhibitors; 2) the anti-inflammatory cytokines; and 3) selective inhibition of adhesion molecules. Only one of more than 75 biologics that have been evaluated to date has received FDA approval for the treatment of CD. In1998, approval was received for infliximab in the treatment of luminal CD not responsive to conventional treatment. Subsequently it has been shown to be effective in fistulizing disease as well as on associated manifestations of CD, including pyoderma gangrenosum, arthritis and sacroiliitis, episcleritis and uveitis, upper gastrointestinal involvement with CD and metastatic CD. Recent findings (ACT1 and ACT2 trials) [2] demonstrating the benefit of infliximab in UC has ushered in the age of biologicals in this disease.

Current practice is to commence biologic therapy once a patient has failed to respond to conventional therapy. Evidence from rheumatoid arthritis studies supports early intervention with biological therapies. The benefits of a ''top-down'' approach to the treatment of CD and UC, beginning with biologic therapy, versus our current practice, the ''step-up'' algorithms (gradually progressing through treatments of increasing potency), seems to be compelling. Early stages of the disease may be more susceptible to immunomodulation. However our evidence-based data for this are still in its infancy, and are mainly obtained from pediatric studies. [3]

Furthermore there is a need to provide evidence of real ''disease modification''. [4] As this observation could be explained by the predominant inflammatory component in ''recent'' disease versus less inflammation and more fibrosis in long standing disease [3]. This therapy is, however, associated with problems of immunogenicity (i.e development of antibodies). Several treatment strategies, such as systemic maintenance therapy, concomitant immunosuppression, and prophylactic systemic steroids, decrease the formation of antibodies to Infliximab. [5] Nevertheless, a proportion of patients lose the option of continued infliximab therapy due to unmanageable infusion reactions or loss of response. Adalimumab, the all-human monoclonal antibody to TNF-alpha promises to be less immunogenic and as effective as Infliximab.

Initial clinical trials of anti-TNF-alpha agents showed few serious adverse events but post marketing surveillance revealed several important complications. Adverse effects, such as infection (mainly linked to concomitant use of systemic steroids) [6], tuberculosis, lymphoma and other malignancies, are related to the intrinsic

TABLE 1. *Biological therapies (8)*

For Crohn's disease		
Proinflammatory inhibitors	Infliximab	Approved for therapeutic use by FDA
	CDP571	Failed phase 3 and 4
	CDP870	Phase 3
	Etanercept	Failed phase 2
	Adalimumab	Phase 3
Anti-inflammatory cytokines	Interleukin 10	Failed phase 2 and 3
	Interleukin 11	Failed phase 2
Anti-leukocyte adhesion therapies	Natalizumab	Phase 3 marketing suspended
	Antegrem MLN-02, 2DP-02	Phase 2
	Alicaforsen, Isis 2302	Failed phase 3
Th1 polarization inhibitors	Anti-interleukin 12 antibody	Phase 2
	Fontolizumab	Phase 2
Scarring and epithelial replacement	Somatropin	Phase 2
Immunostimulation	Figrastim	Phase 2a
	Sargamostrim	Phase 3
For ulcerative colitis		
Anti-TNF therapies	Infliximab	Phase 3
	CDP571	Failed phase 4
Anti-inflammatory cytokines	Interleukin 10	Failed phase 2
Anti-leukocyte adhesion therapies	Natalizumab	Phase 3, marketing suspended
	Antegrem MLN-02, 2DP-02	Phase 2
	Alicaforsen, Isis 2302	Phase 3
T-cell proliferation inhibitors	Humanized daclizumab	Phase 2
	Basiliximab	Phase 2a
Anti-CD3 therapies	Visilizumab	Phase 2
Epithelial replacement and repair	Epidermal growth factor	Phase 2
	Keratinocyte growth factor	Failed phase 2

mechanism of action and are likely to be common to all anti-TNF-alpha agents with its prolonged use. At this time, the safety data accumulated with the use of infliximab in clinical trials and from cases reported in commercial use indicate that there is no clear increase over the background incidence of malignancies in general. For the other biologicals the exposure has been too limited to detect increases in the malignancy rate. [7] Furthermore, biologicals are contraindicated in patients with active infections, history of TB, heart failure, demyelinating illness and malignancy. Therefore, bioloigical therapies based on alternative pathways in the inflammatory cascade would be valuable in the future.

Many other appealing therapeutic agents, for example small molecules that inhibit the key cytokines (based on expanding knowledge of the components of the inflammatory cascade) are currently in development and hold great promise. The most promising newer agents anticipated to become part of the medical armamentarium in the future include: cytokine and anticytokine therapies ranging from antigen presentation and T-cell activation, the most centrally located steps in the cascade, over the production of proinflammatory cytokines, migration of inflammatory cells (blocking leukocyte migration) to blockade of effector signals. Other promising strategies are the induction of apoptosis of T-cells or a particular subset of these cells. This is reassuring, as a significant proportion of patients do not respond to the currently licensed agents, as for long- term fistula healing in CD, and for the management of refractory moderate and severe UC.

In summary, biologics have revolutionized the treatment of IBD. The biological era has now expanded past anti-TNF therapy following positive results with agents that inhibit leucocyte recruitment and block IL-12 (as well as granulocyte-macrophage colony stimulating factor, daclizumab, visulizumab and epidermal growth factor). Increasing evidence suggests that early intervention is the key to combating IBD. The future challenge is to find the optimal time point to introduce biologics into the treatment paradigm and the best combination therapy. Further challenge is to tailor the therapy according to the specific genetic, clinical, biochemical, and serologic profile of the patient thus the specific subtypes of IBD syndrome disease. However unanswered questions remain regarding the long-term safety of biologicals with the need for further prospective RCTs to evaluate the issue of oncogenicity and complications associated with long-term treatment in IBD.

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Optimizing Anti-TNF-α Therapy in IBD

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The introduction of infliximab (Remicade) as the first monoclonal antibody to TNFalpha has changed the management of Crohn's disease (CD) and recently also of ulcerative colitis (UC) dramatically. Infliximab is indicated in refractory luminal and fistulizing CD and in patients with refractory moderate to severe UC. Infliximab is also indicated to treat corticosteroid dependent CD and UC and extraintestinal manifestations of the disease. There are insufficient data yet that support use of anti-TNF agents as first line therapy in all patients, although this may be an option in selected patients with a more aggressive disease course. However, at this moment no clear predictors of such an aggressive and complicated disease course exist.

The preferred way of administration of infliximab is induction therapy using an loading dose of three IV infusions of 5 mg/kg at weeks 0-2-6. The response rates are better when the patients are treated with a correct dose of azathioprine or methotrexate at start of infliximab treatment. Since the majority of patients will relapse if not retreated a long term strategy is necessary and the optimal long term approach is systematic retreatment with 5 mg/kg 8-weekly. The main safety problems with infliximab mainly concern immunogenicity leading to infusion reactions, loss of response and serum sickness-like delayed infusion reactions. Concomitant immunosuppressive therapy has shown to reduce antibody to infliximab (ATI) formation, as is prophylaxis with hydrocortisone prior to each infusion of infliximab. Episodic therapy on relapse is less efficacious because it is frequently associated with ATIs. Systematic maintenance with 8 weekly infliximab decreases the rate of complications, hospitalisations and surgeries. These effects are probably achieved thanks to thorough healing of the bowel. For fistulizing Crohn's disease systematic 8 weekly retreatment with 5 mg/kg after a loading dose at 0, 2 and 6 weeks is needed as fistula healing is slow and often incomplete as witnessed by MRI monitoring studies. Fistulizing disease especially perianal disease necessitates a medical surgical approach. Many patients will need drainage of abscesses, placement of setons, fistulotomy, advancement flap plasty, sphincter repair. If setons are in place they should be kept until the first infusions have been administered and can then be removed when maintenance in therapy is carried out.

When the quality or the duration of response to infliximab decrease this will be most likely due to high titers of ATIs with formation of complexes and early elimination of the drug. If patients relapse earlier than 8 weeks, it is often recommended to shorten the interval and it is our practice to do this on an individual basis. However, infusing shorter than 4 weekly does probably not make sense and alternative treatment strategies, including more humanized or human anti-TNF agents should be seeked. Increase of the dose to 10 mg/kg is also an option as it may restore the efficacy of the drug, but this is more costly.

After a drug free interval of more than 14 weeks' prophylaxis with hydrocortisone prior to infliximab infusion is recommended to avoid infusion reactions and one should be prepared to treat a delayed hypersensitivity reaction with high dose 40-60 mg/day of oral prednisone. Patients who have already suffered such reactions should receive prophylaxis and prolonged glucocorticosteroid treatment, but again, should probably be safer switched to more human antibodies.

The humanized anti-TNF antibody CDP-870 (Cimzia, UCB), a Pegylated anti-TNF antibody fragment or the fully human antibody adalimumab (Humira, Abbott) have shown to be more effective than placebo in inducing and maintaining remisison and are good alternatives for patients losing response to infliximab. The advantages of these antibodies include a reduced immunogenicity profile and the advantage of subcutaneous administration. However, although the amount of murin protein present in monoclonal antibodies is related to the immunogenicity of the drug, more humanized antibodies and even fully human antibodies are not free of immunogenicity at all.

For anti-TNF naïve patients, it is difficult to position the different anti-TNF agents, since all have similar efficacy in inducing and maintaining remission in CD patients. The choice will therefore depend on ease of administration (intravenous versus subcutaneous), frequency of dosing, immunogenicity profile and costs.

Although the safety profile of anti-TNF agents is overall good, the rate of opportunistic infections is increased mainly in patients treated concomitantly with immunosuppression. Adverse events associated with anti-TNF strategies include also tuberculosis, demyelinating disease and worsening of congestive heart failure. Malignancy rates in patients treated with anti-TNF strategies do not seem to be increased, although caution is needed for the risk of lymphomas (especially

hepatosplenic T-cell lymphomas), especially in young patients on concomitant azathioprine therapy. Safety aspects of these drugs need to be further followed up.

Changing the Therapeutic Pyramid: Can We Alter the Natural Course of Pediatric IBD?

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How successful is the present approach for the treatment of Pediatric Inflammatory Bowel Disease? At our institution we have completed a prospective cohort study of newly diagnosed pediatric Crohn's disease patients compared with healthy controls over a 12 month interval. Children received ''standard therapy.'' Standard therapy consisted of initial induction with corticosteroids and early induction of 6-mercatopurine/azathiopurine. Infliximab was given if this therapy was unsuccessful in producing a remission off steroids. Outcome measures included linear growth, body composition, muscle strength and bone density. Our study revealed that height, bone density and lean body mass did not improve over this period of time. Cross section moment of inertia (bone strength) was worse at the end of the study. Our patients exhibited significant deficits in muscle mass with preserved fat mass (Inflammatory Cachexia). Cosnes et al (1) showed that the rate of surgery since 1981 has remained steady despite recent widespread use of immunomodulators. These studies suggest that we need to improve our present ''standard therapy'' for the treatment of pediatric IBD. Clinical studies have shown that Crohn's disease can be effectively treated using monoclonal antibodies and other biologic agents that block the action of certain proinflammatory cytokines, including tumor necrosis factor (TNFα) and interferon-gamma (IFN-γ), or that interfere with the activation of T cells and their directed migration to the intestinal mucosa. Can we change our current standard ''Step-Up'' approach to a ''Top-Down'' approach using these new therapies at the time of diagnosis? Risk versus benefit must always be considered when one begins a new therapeutic approach.

Recent concerns about the development of hepatosplenic T-cell lymphoma in adolescents and young adults being treated with azathioprine (AZA) and infliximab must be considered. Also, we need to remember that the treatment with corticosteroids leads only to a 1-year remission rate $\leq$26%, corticosteroid dependency, limited mucosal healing and significant toxicity.

The use of biologic therapies for newly diagnosed patients with IBD would hopefully result in corticosteroid avoidance, superior mucosal healing, improved linear growth, lean body mass and bone strength. At present, the therapies that are available and could be considered for ''Top-Down'' therapy include: Infliximab (chimeric anti-TNFα IgG_1 monoclonal antibody), Adalimumab (human anti-TNFα monoclonal IgG_1 antibody), Certolizumab (humanized PEGylated Fab′ fragment of an anti-TNFα IgG_4 monoclonal antibody), Sargramostim (GM-CSF) and Natalizumab (humanized anti-α4 integrin IgG_4 monoclonal antibody). Romeo et al. (1) studied Infliximab as a first choice therapy with newly diagnosed pediatric Crohn's disease patients. It was a retrospective study comparing the clinical outcome, at 12 months, of 13 patients receiving infliximab versus 19 patients receiving ''standard induction therapy.'' At the end of the study, the rate of relapse, the PCDAI and the endoscopic scores were significantly lower in the group receiving infliximab as initial therapy. In the standard therapy group, 4 patients required surgery for intestinal resections.

Hommes et al (1) studied ''Step-Up'' (traditional therapy) versus ''Top-Down'' therapy (induction therapy with Infliximab) for adult patients with moderate to severe CD in a large (n=129) multi-center open label randomization study. Their co-primary endpoints (remission, off steroids and no surgery) were determined at 6 and 12 months. Top-Down therapy consisted of 3 infusions of Infliximab (weeks 0, 2, and 6) plus 2.0 to 2.5 mg/kg/d of AZA. Patients who relapsed on this therapy were given repeated Infliximab (episodic therapy for flares, not maintenance therapy); corticosteroid therapy could be added if patients failed to respond to Infliximab plus AZA. Step-Up therapy consisted of topical budesonide 9 mg/d; patients who failed to respond to topical therapy were stepped up to systemic prednisone 40 mg/d and corticosteroid therapy could be repeated as necessary. For patients who required repeated steroid therapy or who were steroid-dependent, AZA was added. Finally, Infliximab was given in cases in which AZA alone failed to achieve remission. This study showed that the Top-Down therapy was significantly more likely to result in remission at 6 months ($p<0.01$) and 12 months ($p<0.05$) than the Step-Up therapy. Significantly more relapses occurred in the Step-Up group over the 24 month period of the study ($p=0.018$). Significantly more steroids were used in the Step-Up group versus the Top-Down group at one year (70.2 days vs. 0.54 days; $p<0.001$) and at 2 years (79.7 days vs. 5.6 days; $p<0.001$). Also there was a trend for more surgeries to be performed in the Step-Up group. The proportion of patients on immunomodulators was 77% in the Step-Up group compared to 94% in the Top-Down group at the end of 2 years. Approximately 15% to 20% of patients in either group required the use of Infliximab one year after initiation of the study. At the end of 2 years the occurrence of adverse events and serious adverse events were similar in both treatment groups. In a sub-study of the above trial, D'Haens et al. (1) compared the rate of mucosal healing between the Top-Down therapy versus Step-Up therapy. Forty-four patients had repeat colonoscopies at the end of 24 months of the study. Of these forty-four patients, twenty-four patients were in the Top-Down group versus 20 patients in the Step-Up group. Complete ulcer disappearance was seen in 73% of the Top-Down group and 30% of the Step-Up group ($p<0.001$). Mucosal healing was significantly more pronounced in the Top-Down group (ulcer reduction 88% versus 47%, $p<0.001$). It was concluded that the Top-Down strategy is superior to induce mucosal healing two years after the start of therapy. The superiority of Top-Down versus classic Step-Up therapy is maintained even in the Step-Up patients who receive Infliximab plus immunomodulators later on.

The conclusions from these trials were that induction therapy with Infliximab plus AZA was superior to sequential Step-Up therapy in achieving remission, steroid sparing, and mucosal healing. It is suggested that the Top-Down approach will result in better long-term outcomes such as a decreased need for intestinal resections and hospitalizations, and an improvement in quality of life. However, one must consider that Top-Down therapy, though more robust, could expose some patients unnecessarily to the potential adverse effects of this combined therapy of Infliximab plus immunomodulators.

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Biological Therapy in Ulcerative Colitis

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Despite therapy with corticosteroids and immunomodulators some patients with ulcerative colitis still require colectomy. For children presenting with moderate to severe disease the likelihood of colectomy is 5–10% at one year and up to 26% by 5 years (1). In adults, approximately 30% of patients started on corticosteroids fail therapy and require colectomy within a year (2). In children, corticosteroid dependency is seen in 45% of patients started on these medications. These sobering observations have prompted the development of biological agents to treat this disorder. Table 1 shows the biological agents for which published data are available.

Initial studies of infliximab for the treatment of ulcerative colitis were conflicting. In 2005, two randomized, double-blind, placebo controlled studies, ACT 1 and ACT 2, were published in a single paper evaluating the efficacy of infliximab for induction and maintenance in 728 adults with active ulcerative colitis (Mayo score 6–12) (3). Clinical response at 8 weeks (decrease in Mayo score by 3 points) was observed

in approximately 65% of subjects receiving a 3 dose induction of infliximab (either 5 mg/kg/dose or 10 mg/kg/dose) compared to approximately 33% of placebo patients. Clinical remission at week 8 (Mayo score of 2 or lower, no item more than 1) was observed in approximately 33% of infliximab treated patients versus 10% in the placebo group. Mucosal healing at week 8 was seen in approximately 60% of infliximab treated patients versus 30% of placebo treated patients. Week 54 data were available for 364 ACT 1 patients; 42% of infliximab treated patients were in remission compared to 20% of those treated with placebo. Clinical remission without corticosteroids was seen in 9% of placebo treated patients, 26% of those receiving 5 mg/kg maintenance doses of infliximab every 8 weeks and 16% of those receiving 10 mg/kg doses. At the start of ACT 1 and ACT 2 approximately 30% of patients were felt to have corticosteroid refractory disease, 50-60% were taking corticosteroids at the time infliximab was initiated, and 70% were receiving 5-aminosalicylate preparations and 40-50% immunomodulators. Average disease duration was approximately 6 years.

In one study of hospitalized adults with severe ulcerative colitis refractory to corticosteroid therapy, the addition of infliximab was superior to placebo in preventing colectomy (Odds ratio 4.9, range 1.4-17) (4). No placebo controlled data are available for pediatric ulcerative colitis. Cumulatively approximately 50 pediatric patients have been reported with short-term improvement noted in 75-80% of subjects and longer term improvement in 60% (5). It has been suggested that patients with chronic corticosteroid dependent disease are less likely to respond to infliximab than those with short-term disease. However, these observations were based on very small patient numbers.

MLN02 is a humanized monoclonal antibody that specifically recognizes the $\alpha_4\beta_7$ heterodimer and selectively blockades the interaction between leukocytes and vascular endothelium in inflamed gut. 181 adult patients with active ulcerative colitis, not receiving corticosteroids or immunomodulators, received either placebo or one of two doses of MLN02 (Day 1, 29). Clinical remission at week 6 was 14% in the placebo group and 32% in the treated groups. In the lower dose group (0.5mg/kg) mucosal healing was noted in 28% vs 12% in the higher dose group (2 mg/kg) and 8% of those treated with placebo (6).

Visilizumab is a humanized anti-CD3 antibody that selectively induces apoptosis in activated T cells. Phase I/II studies showed efficacy in corticosteroid resistant UC. Cytokine release syndrome and elevations in EBV DNA levels observed.

Daclizumab, a recombinant humanized IgG1 monoclonal antibody to IL-2R (CD 25), and basilixumab, a chimeric monoclonal IL-2R antibody, have been used in open label trials in ulcerative colitis. Data on efficacy are conflicting.

Epidermal growth factor is a mitogenic peptide that stimulates cell proliferation in the GI tract. A phase II study of recombinant EGF enemas showed efficacy in patients with active distal ulcerative colitis (83%) versus placebo (8%). Concern has been raised about the potential of EGF to upregulate proto-oncogenes in a population with known increased cancer risk.

RDP58 is a decapeptide that blocks TNF production post-transcriptionally, and also inhibits production of IFNγ, IL-2, and IL-12. In one Phase II study RDP 58 which shown to induce remission better than placebo in mild to moderate ulcerative colitis.

Alicaforsen (ISIS 2302), a 20-base phosphorothioate antisense oligodeoxynucleotide, hybridizes to an untranslated region of the human ICAN-1 messenger RNA. There is eventual reduction of ICAM-1 expression. Limited clinical studies have shown efficacy in distal ulcerative colitis when administered by enema.

TABLE 1. *Biological agents used to treat ulcerative colitis*

Agent	Compound	Target
Infliximab	Antibody	TNF-α
MLN-02	Antibody	$\alpha_4\beta_7$
Visilizumab	Antibody	CD3
Daclizumab	Antibody	CD25 (IL-2R)
Basilixumab	Antibody	CD25 (IL-2R)
Epidermal growth factor	Protein	EGF-R
RDP58	Peptide	MAP/JNK Kinase
Alicaforsen	Antisense oligopeptide	ICAM-1

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Is Stem Cell Transplantation the Way Forward for Refractory Crohn's Disease?

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Since both genetic and milieu factors are presented in the etiology of Crohn's disease (CD), a re-setting of the entire immune system by treatment with hematopoietic stem cell transplantation (SCT) is a novel treatment to restore normal immunity without attacking self-epitopes. The genetic determinants of immune-mediated diseases usually reside both within and without the major histocompatibility complex (MHC) genes, with the non-MHC determinants usually linked to mechanisms of immune regulation. Impairment in the control of intestinal T-cell function and turn-over, most likely caused by defects in normal apoptotic mechanisms closely linked to TNF-α activity, appears to be of significant importance for this dysregulation and for the perpetuation of the pathological inflammatory response in CD (1).

Autologous SCT would demand eradication of environmental factor(s), for example, an infectious organism, and then autoreactive activated clones of effector cells followed by recovery of patient's own naïve T cells that will recognize other epitopes. Allogeneic SCT from an HLA-matched donor offers the potential to completely replace a genetically autoimmune disease-prone lymphohematopoietic cells by donor lymphocytes inducing graft-versus-autoimmunity (GVA) in line with graft-versus-leukemia (GVL) in patients with hematological malignancies. Given the possible heterogeneous pathogenesis of CD, one cannot yet assume that autologous HSCT should be preferred before an allogeneic procedure. Experience from these both procedures involving CD patients has been limited but intriguing.

A patient with CD who received an autologous bone marrow transplantation due to haematopoietic malignancy and subsequently had a resolution of the inflammatory bowel disease was reported more than a decade ago. The first series with autologous SCT in patients suffering from severe chronic active CD was reported from the group at Northwestern University Medical Center in Chicago (2). An extended phase I study from the same centre presented lately 12 patients with chronic active, refractory CD. (3)

Peripheral blood stem cells were used after T-cell depletion (*ex vivo* CD34+ cell selection) to achieve quicker engraftment and less toxicity, as well as to deplete autoreactive, activated T-cells. For the latter purpose also equine antithymocyte globulin (eATG) was included in

the conditioning regimen (*in vivo* T-cell depletion), together with high-dose cyclophosphamide. The autologous SCT was well tolerated, with neutrophil and platelet engraftment as expected after high-dose conditioning. No infectious complications or transplantation-related-mortality (TRM) was reported during a median follow-up of 18.5 (range 7-37) months. However, only 3 patients exceeded recurrence-free survival of >3 years.

Experience on allogeneic SCT in CD is generated from patients with an autoimmune disorder (AID) and a concomitant hematological disorder (24 malignancies, 23 aplastic anemias) (4). Encouraging, freedom of AID relapse was superior after allogeneic SCT compared to autologous SCT (89% at 18 years vs. 38% at 5 years; log rank: p=0.0002). Specifically, four out of five patients with CD and leukaemia, who received standard ablative induction therapy followed by allogeneic SCT (marrow) from healthy donors, were reported to be in clinical remission (three without immunosuppressive therapy) after a median of 8 years of follow-up (5). The patient that had a relapse displayed a mixed donor-host haematopoietic chimerism, suggesting that a complete ablation of the former immune system is essential for long-term success. A series from Germany, describing the outcome after allogeneic SCT in 12 patients with malignant disorders and concomitant CD, also showed that the bowel disorder did not re-appear but in one patient with mild persistent symptoms, but the length of follow-up was shorter (median 34 months) than in the American study (6).

A new venue for allogeneic SCT has opened by reduced conditioning (induction) regimens decreasing the toxicity. Outside the context of coincidental disease, the hypothesis for GVA has been strengthened by the clear therapeutic effects of donor lymphocyte infusions for relapses in patients transplanted for autoimmune blood diseases. Also adoptive transfer of allogeneic mesenchymal stem cells might carry a potential for healing of CD as demonstrated for severe bowel damage of graft-vs-host disease after allogeneic SCT for acute leukaemia (7).

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