

**Received:** 2005.09.21  
**Accepted:** 2005.12.16  
**Published:** 2006.02.01

**Authors' Contribution:**

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

# Integrating theories of the etiology of Crohn's Disease

## On the etiology of Crohn's Disease: Questioning the Hypotheses

**William M. Chamberlin<sup>1</sup>, Saleh A. Naser<sup>2</sup>**

<sup>1</sup> Department of Medicine, Texas Tech Medical Center, El Paso, TX, U.S.A.

<sup>2</sup> Departments of Molecular Biology and Microbiology and Biomolecular Science Center. Burnett College of Biomedical Sciences, University of Central Florida, Orlando, FL, U.S.A.

**Source of support:** Self financing

RA

### Summary

The most prominent theory describes the Crohn's Syndrome as a dysregulated, inappropriate immune response to otherwise innocuous bowel flora in a genetically susceptible host. The autoimmune theory assumes that a specific infectious agent does not exist. Data from mouse models, impairment of the mucosal epithelial barrier and the influence of gut flora are used to support the autoimmune theory. Critics claim that the dysregulated immune responses are not the primary disorder but secondary to an underlying infection. Two other theories are also consistent with the same data. The immunodeficiency theory hypothesizes that defects in innate immunity leading to compensatory immune processes underlie the Crohn's phenotype and suggests that therapy should stimulate immunity rather than suppress it. The mycobacterial theory proposes that *Mycobacterium avium* subspecies *paratuberculosis* is one of the causes of the Crohn's Disease syndrome. Mycobacterial molecules dysregulate immune signaling pathways as part of the organisms' evolved survival strategy. If MAP were to initiate the dysregulated immune response then the necessity to hypothesize that commensal gut flora provide the antigenic stimulus would be moot. Commensal bacteria would be relegated to a secondary role of modifying the immune response rather than occupying the central role of providing the initiating antigens. Critics claim that MAP is merely a secondary invader. The three theories differ primarily by emphasizing different aspects of the same overall process.

**key words:**

**Crohn's Disease • etiology • mycobacteria**

**Full-text PDF:**

<http://www.medscimonit.com/fulltxt.php?IDMAN=8181>

**Word count:**

4853

**Tables:**

–

**Figures:**

–

**References:**

42

**Author's address:**

William Chamberlin M.D., Department of Medicine, Texas Tech Medical Center, 4800 Alberta Ave., El Paso, Texas 79905, U.S.A., e-mail: William.Chamberlin@ttuhsc.edu or wchambrlin@aol.com

## BACKGROUND

Many articles address the treatment of Crohn's Disease (CD) but much less is known about its etiology which is contentious [1]. It is vital to examine and question the concepts that support the competing etiologic theories of the syndrome. There are three main theories for the etiology of CD: the autoimmune theory, the immune deficiency theory and the mycobacterial theory. At present most published material refers to autoimmune mechanisms as the most likely cause. The medical community is largely unaware of the mounting evidence supporting the mycobacterial and immune deficiency theories. We suggest that rather than competing with one another the theories may actually be complementary. Implications for prevention and treatment, however, are vastly different.

Crohn's Disease is a syndrome defined by clinical, endoscopic, radiologic and histologic criteria. As such there is probably more than one cause. The three leading theories have many aspects in common and all incorporate the same basic facts and observations but differ in how the data is interpreted.

## AUTOIMMUNE (IMMUNE DYSREGULATION) THEORY

Autoimmune disorders imply immune activity directed against self-antigens. Gut bacteria are not self-antigens but since we all contain commensal bacteria and our mucosal immune systems are normally in homeostatic balance with our flora, some argue that a disease caused by an imbalance of T-regulatory/T-effector function incited by normal gut flora should be considered an autoimmune disease. Purists take issue with this definition, but Crohn's Disease has been categorized as an autoimmune disorder for years. An alternative would be to call it an 'immune dysregulation disease'.

The autoimmune theory suggests that Inflammatory Bowel Disease results from inappropriate ongoing activation of the mucosal immune system driven by the presence of normal luminal flora.

"This aberrant response is most likely facilitated by defects in both the barrier function of the intestinal epithelium and the mucosal immune system" [2].

"The current leading hypotheses for the etiology of the Inflammatory Bowel Diseases (IBD) emphasize genetic predispositions to dysregulation of the gastrointestinal immune system" [3].

"Crohn's Disease is a dysregulated, inappropriate response of the intestinal immune system to otherwise innocuous luminal antigens in a genetically susceptible host" [4].

Depending on one's interpretation, the above quotations are definitions, statements about the pathogenesis or statements about the etiology of CD. The autoimmune theory implies that flaws exist in immune regulatory mechanisms that spawn an excessive Th1-type immune response directed at antigens from normal gut bacteria. Implicit in this theory is the belief that a specific microbial pathogen that initiates the dysregulated immune cascades does not exist. If

such a pathogen were to chronically persist, then the disorder would be regarded as an infectious disease and not an autoimmune process.

Bouma describes experimental evidence that supports the autoimmune theory [3]. The mouse models fit into four main groups: spontaneous colitis from natural occurring genetic defects, colitis that occurs in mice by knocking out a regulatory gene or introducing a pro-inflammatory transgene, colitis resulting from rectal instillation of exogenous compounds (TNBS or oxalazone), and colitis resulting from transferring T-regulatory deficient mixed lymphocytes into lymphopenic hosts. Seven principles were deduced from these experiments. First, different genetic defects may manifest as bowel inflammation. Second, multiple genetic variables play a role in the susceptibility to bowel inflammation. Third, the gut flora plays a critical role in initiating, maintaining and regulating mucosal immune responses. Fourth, inflammation occurs from either excessive T-effector cell activity or deficient regulatory T-cell function. Fifth, gut inflammation involves either Th1 or Th2 cytokine patterns. Excessive Th1 cytokines are associated with CD. Sixth, the intestinal epithelial barrier is important in separating the immunogenic gut flora from the immune cells of the lamina propria. Seventh, mucosal inflammation can also be due to genetic abnormalities of innate immunity involving antigen processing cells, macrophages and NK cells. Critics claim that although these brilliant experiments are valuable in understanding mucosal immunology, the principles apply more to the physiology of intestinal inflammation in general and do not necessarily illuminate the cause of Crohn's Disease in the natural setting. Harsher critics claim that these mouse models are not models for human Crohn's Disease at all.

The discovery of mutant *NOD2* genes provides an example of the genetic correlates of CD [5,6]. *NOD2* proteins recognize intracellular bacteria, specifically the muramyl dipeptide component of peptidoglycan. These cytoplasmic proteins are expressed in intestinal epithelial cells, dendritic cells, macrophages and other immune processing cells. *NOD2* proteins recognize intracellular pathogens and initiate an appropriate immune response to kill them. Functional *NOD2* activates NF $\kappa$ B, a transcription factor associated with immune response genes whereas mutant variants do not. Mutant genes correlate with CD but CD occurs in patients without the mutant gene and the mutant gene can be found in people without the disease. Podalsky [2] opines that paradoxically these *NOD2* variants appear to result in reduced macrophage activation of NF $\kappa$ B in response to lipopolysaccharide. Paradoxes may be beneficial in that they suggest weaknesses in our theories. Reduced transcription of immune related genes in macrophages appears paradoxical only if CD is assumed to be caused by a primary immune regulatory disorder with excessive production of pro-inflammatory cytokines. It would not be paradoxical to observe reduced macrophage pro-inflammatory cytokines associated with mutant *NOD2* proteins if macrophages were infected with bacteria but could not recognize them to respond appropriately. A failure to recognize invasive pathogens and mobilize a coordinated cytokine response would result in a failure to clear the pathogen resulting in a chronic infection. Persistent antigenic stimulation via other antigen processing pathways might result in a dysregulated, excessive release of cytokines associated with an

inability to effectively eradicate the pathogen. This scenario is consistent with the immune-deficiency theory and the related mycobacterial theory of CD. It would also explain the excessive, dysregulated inflammatory response cited as evidence for an autoimmune process.

Bouma writes, "In light of the *NOD2* data, three possible hypotheses exist. The main hypothesis, at present, is that in the absence of *NOD2* activity there is defective activation of macrophages that leads to a persistent infection of macrophages owing to a marked *NOD2* independent effector T-cell response. However, persistent intracellular infection of macrophages has not been detected in CD, and other possibilities need to be considered" [3]. (Note: this assumption lies as the very heart of the autoimmune theory and is refuted by evidence supporting the mycobacteria theory.) "A second hypothesis is that in the absence of *NOD2* expression by epithelial cells microbial products that normally activate epithelial cells to secrete chemokines and defensins fail to do so, leading to first, the proliferation of bacteria in crypts and second, loss of barrier function allowing marked stimulation of mucosal cells by mucosal antigens. A third hypothesis is that recognition of microbial peptides by *NOD2* normally conditions APCs in a way that leads to their induction of regulatory and effector T-cell responses, and so failure of this mechanism disrupts mucosal homeostasis" [3]. The second and third hypotheses form the basis of the autoimmune theory. The first hypothesis supports the immune-deficiency and mycobacterial theories.

The belief that Crohn's Disease results from a primary dysregulated immune response has undergone modifications over the years. Initially CD was hypothesized to be an autoimmune disorder whereby the immune system lost tolerance to self-antigens and attacked the host. A modified hypothesis suggested that rather than self-antigens, the disease occurred because of an imbalance of immune regulatory mechanisms that was driven by antigens from normal gut bacteria. The association of *NOD2* mutant proteins with CD was incorporated into the theory by hypothesizing that a failure to recognize intracellular commensal bacteria by enterocytes and Paneth cells leads to decreased secretion of lysozyme, defensins and other antibacterial molecules so that gut flora survive in the mucosal crypts enabling them to successfully penetrate the mucosal barrier. An excessive response to these organisms by the adaptive immune system leads to chronic inflammation.

Alternatively, Watanabe et al. suggest that functional *NOD2* activation normally inhibits signaling from Toll-Like Receptor 2 pathways. "A loss-of-function mutation on *NOD2* together with TLR2 signals delivered by other bacterial products will result in enhanced cytokine responses by macrophages (or dendritic cells) to commensal bacteria and result in inflammation" [7]. This hypothesis is challenged by the Ferwerda et al. who show that wild-type *NOD2* and Toll-Like Receptors (TLR-2 and TLR-4) are non-redundant recognition systems that act synergistically in response to mycobacterial antigens [8]. Sechi found that over 70% of CD patients with a *NOD2* mutation had PCR evidence of a MAP infection. This findings suggests that a specific pathogen may be involved [9].

Clearly CD involves dysregulated mucosal immunity. Enteric bacteria both shape and are shaped by the mucosal immune

system and exert a strong effect on the disease. Disease activity is associated with an aberrant and destructive Th1 inflammatory response with multiple factors playing a role in a complex process that eventually results in dysregulated immunity and a destructive inflammatory process. The key question is whether there is, or is not, a specific microbe whose continued presence is necessary for the disease to manifest. The only difference between the autoimmune theory and the infectious theory for the etiology of Crohn's Disease lies in the belief that there exists, or does not exist, a specific infectious agent whose chronic presence and persistent antigenic stimulus alters immune homeostasis.

## MYCOBACTERIAL THEORY

Microbes and immune systems co-evolved. By definition, a successful pathogen must possess mechanisms that enable it to survive at least until it can reproduce. It no longer makes sense to discuss an infectious disease without addressing how the immune system responds to the microbe and how microbial molecules in turn modulate the immune response. Mycobacteria have co-evolved with vertebrates and have speciated to fill various niches. Some species, specifically *Mycobacterium tuberculosis*, are among the most successful human pathogens known. It infects two billion people worldwide, almost one third of humanity, but only around 10% ever develop significant disease. Clearly other variables involving the immune system play a critical role in who manifests disease and who does not. It is the interplay of the host's molecular pathways with the molecular systems of the microbe that determine the clinical manifestations.

*M. tuberculosis* possesses molecules that protect it from destruction and dysregulate host immune responses. Its tough lipid-laden cell wall offers physical protection and also contains virulence factors. *M. tuberculosis* is also able to prevent macrophage phagosomes-lysosome fusion, thus enhancing its survival inside of macrophages. Interference with correct cytokine signaling is a known microbial survival strategy [10–12]. An imbalance of Th1-associated cytokines result in an excessive but nevertheless ineffective cell-mediated immune attack. Chronic, persistent antigenic stimulation leads to a chronic destructive disease. Luckily, nearly 90% of the time the immune system contains the TB infection and returns to a homeostatic balance consistent with a healthy state. Other factors may disrupt this balance resulting in reactivation of the disease. If the technology were lacking to demonstrate the presence of *M. tuberculosis* in intestinal tuberculosis, current theorists might observe the excessive, destructive immune response seen in tuberculosis and suggest that they were dealing with an autoimmune disease caused by dysfunctional immune regulatory mechanisms. The mycobacterial theory suggests similar processes are involved in the pathogenesis of CD.

Interest in a possible mycobacterial role in the etiology of Crohn's disease is not new [13–21]. Dalziel, in 1913, reported the histopathologic and clinical similarities of animal paratuberculosis, intestinal tuberculosis, and human chronic granulomatous enteritis that later became known as Crohn's disease (CD) [22,23]. Johne's disease is the name given to a chronic enteritis that affects cattle and other species that is caused by *Mycobacterium avium* subspecies paratubercu-

RA

losis (MAP), a member of the *Mycobacterium avium* complex. MAP possesses many of the same virulence associated molecules as *M. tuberculosis*. Naser's group recently demonstrated impaired phagosome-lysosome fusion in MAP infected phagocytes from CD patients (unpublished). It is probable that MAP interacts with the human immune system in ways that are similar to other known pathogenic mycobacteria resulting in a spectrum of disease manifestations ranging from simple colonization and a healthy phenotype to severe inflammatory bowel disease. The complex interactions of the many variables that influence the clinical phenotype remain to be determined. Animal models of MAP related diseases are abundant. MAP is a well-recognized pathogen that causes inflammatory bowel disease in a wide range of mammals. Johne's Disease is a major concern for the dairy and sheep industries. The spectrum of clinical disease in different mammals varies considerably and overlaps with that found in CD in humans. (Comparative pictures may be viewed online) [21].

Supporting evidence for the involvement of MAP in CD is grouped into several categories: PCR identification of MAP DNA; serum MAP specific antibodies; imaging of MAP DNA by in-situ hybridization; and cultures of MAP from tissue, milk and blood.

### PCR

Map's fastidious and slow growing characteristics led investigators to rely heavily on the use of PCR for accurate identification of MAP [24–27]. Bull and Hermon-Taylor identified MAP DNA in 92% of CD tissue versus 26% in controls [25]. However, other laboratories have not been as successful likely due to differences in methodology.

Researchers in Heidelberg performed the largest MAP PCR study. Resected bowel from 300 patients with Crohn's Disease, Ulcerative Colitis and non-IBD controls were assayed for MAP DNA. MAP-DNA was identified in 52/100 CD patients, 2/100 UC patients and 5/100 controls [28].

PCR data can be criticized because the technique assays DNA which could either be from live bacteria or merely the scattered debris from killed organisms and therefore of questionable biologic consequence. To address this question investigators isolated granulomas from CD tissue using laser capture micro-dissection. MAP DNA associated with granulomas would not be viewed as scattered debris. Ryan identified MAP DNA in 40% of granulomas from CD patients versus 0% from granulomas taken from controls [29]. The importance of this finding was diminished by the subsequent finding by the same authors of *E. coli* DNA in CD granulomas. Perhaps of greater significance is the finding by Mishina identifying MAP RNA by RT-PCR in 8/8 CD patients versus 2/4 controls, a finding that implies active metabolic processes at the time of tissue fixation [30].

### Serum antibodies to p35, p36

Assays for serum antibodies against MAP specific proteins were performed. Defining positives as the presence of IgG to both antigens, 77% of 61 patients were positive versus 0% of 35 normal control patients, and 8% of 12 patients with ulcerative colitis.<sup>31</sup>

### Imaging by in-situ hybridization

Using IS900 as a MAP specific gene probe, El-Zaatari identified MAP DNA in CD intestinal tissue in 40% of 15 CD patients with granulomas versus 0% of 22 controls [32]. Sechi imaged MAP DNA inside of granulomas in 83% of 33 CD patients whose specimens were reviewed (pictures may be reviewed on-line) [33].

### Culture evidence

Over the last century it was the failure to grow or image mycobacteria from CD tissue that caused researchers to abandon the mycobacterial theory of Crohns. Advances in culture technique and more specific PCR assays allowed the successful culture and detection of Map in Mycobacterial Growth Indicator Tube (MGIT, Becton Dickinson) culture media following 10–12 weeks of incubation from 7 of 8 CD tissue and none from 3 controls [34]. Advances in culture methods now allow researchers to grow MAP from various sources.

### Cultures from Intestinal tissue

Hermon-Taylor cultured MAP from 42% (14/33) CD bowel pinch biopsies versus 9% (3/33) of controls after 14–88 weeks incubation time. Sixty percent of the specimens incubated for over 60 weeks were positive [27].

Cultures from Lymph Nodes: Naser cultured Map from two out of three mesenteric lymph nodes.

### Milk cultures

MAP was cultured from 2 of 2 breast milk samples from lactating CD patients versus 0 of 5 normal controls [35].

### Blood cultures

Naser cultured MAP from the buffy coats of blood taken from 50% (14/28) CD patients versus 0% of 15 healthy controls. Interestingly, 2/9 patients with Ulcerative Colitis grew MAP from their blood. Current immunosuppressive medication did not correlate with positive blood cultures [36].

If MAP causes CD the inclination is to treat patients with antibiotics and monitor for clinical improvement. The problem is that atypical mycobacterial diseases are often very difficult to treat. For example, successful eradication of *M. avium* pulmonary disease in HIV negative patients requires multiple antibiotics and is often unsuccessful. How much of this resistance to therapy is due to qualities inherent in mycobacteria or to factors that predisposed the patients to the infection in the first place is unclear. The same uncertainties may apply to *M. avium* paratuberculosis intestinal infections.

For MAP, the optimum antibiotic regimen is unknown. Experience with *M. avium* suggests that in general most antibiotics, including most of the older tuberculosis drugs, are not effective. Because of the tendency for mycobacteria to develop resistance when just one drug is used, any therapeutic regimen that includes fewer than two effective drugs can be expected to fail. Also, any antibiotic regimen

less than 6 months (or possibly 12 or 24?) would predictably fail in the long run. Any meaningful interpretation of data from past drug trials would have to take these caveats into consideration.

With these limitations in mind, a summary of several recent antibiotic trials may still be helpful. However, given the problems of drug resistance, only those studies that treated patients for at least six months and included at least two drugs thought to be effective against *M. avium* should be included. These constraints narrow the field to studies that used at least two of the following: clarythromycin, azithromycin, rifabutin or rifampin.

Studies that included the caveats mentioned above were reported to have a 60–80% response rate whereas other drug regimens were less effective or had no effect [37]. It is interesting to note that the trials with a positive result used antibiotics effective against *M. avium* whereas the negative trials used other antibiotics. All groups would be expected to alter enteric flora. The differences in efficacy suggest activity against a specific organism rather than a generalized alteration of the bowel flora.

The imposed restrictions cited above seem valid but using them may incur accusations that the constraints select out the positive trials from all the others. Single antibiotic regimens with metronidazole and ciprofloxacin (both effective against MAP) have been used for decades without knowing how they worked. They seem to be beneficial but their use does not support or refute any theory on the etiology of Crohn's Disease. Until more definitive proof is provided, it is probably judicious to consider anti-mycobacterial therapy in a similar manner: it seems to be beneficial for many patients but does not, by itself, prove or disprove anything.

Preliminary results have been published by Selby et al as proceedings in the Gastroenterology Society of Queensland update, June 2005. Selby presented data from the Australian anti-paratuberculosis trial which used sub-therapeutic doses of Rifabutin (450 mg) Clarithromycin (750 mg) and Clofazimine (50 mg) per day. In spite of the sub therapeutic doses which normally would not be used in any MAC trials, highly significant improvement over placebo group was achieved with a  $P < 0.0187$  at sixteen weeks. Although maintenance of remission was similar for standard treatment and anti-MAP therapy treatment for the next 12 months, this highly significant improvement at sixteen weeks is equivalent to that which is achieved by Remicade in its original studies. Furthermore Selby presented additional data showing that high-dose Metronidazole and Metronidazole plus Ciprofloxacin also achieved remission in Crohn's Disease. Both Metronidazole and Ciprofloxacin are known to be active against atypical mycobacteria.

Skeptics challenge the mycobacterial theory on several points. First, immune suppression with corticosteroids or anti-TNF antibodies is associated with recurrences of latent tuberculosis. If mycobacteria are involved in CD, why does the clinical picture improve with immune suppression? The answer may lie in the fact that clinical manifestations reflect the interplay of the immune response with bacterial antigens. Short term symptomatic improvement may be achieved at the cost of long term deterioration in the course

of the disease. For example, corticosteroids are often given in severe cases of pulmonary tuberculosis to suppress inflammation and improve oxygenation while the antibiotic cocktails kill the infection. Inflammation diminishes, x-rays improve and patients 'feel better' when given corticosteroids, but steroids interfere with eradication of the organism and have an overall detrimental effect on the course of the disease. Steroids are also used in the acute treatment of leprosy to reduce swelling and consequent neuronal damage, but no one doubts that antibiotics are the mainstay of leprosy therapy. The same is probably true of MAP infections. Importantly, since MAP is a much slower growing species than *M. tuberculosis*, it might be much harder to detect an overall deterioration in the course of the disease. Nevertheless, many physicians currently suspect that steroids do adversely affect the course of CD. This issue has not yet been addressed for anti-TNF therapies.

On the other hand, as an unproven hypothesis, anti-TNF therapy may conceivably have a bactericidal effect on MAP that could account for its clinical benefit. At present nobody truly understands how anti-TNF works but the working theory is that antibodies bind to membrane-bound TNF on activated immune cells and initiate apoptosis. Immune initiated apoptosis of infected cells is a well-recognized immune strategy used to eradicate intracellular infections. Apoptosis of MAP infected macrophages could result in the death of the microbe [38]. MAP differs from *M. tuberculosis* in that in humans MAP assumes an obligatory intracellular spheroplast form that does not thrive extracellularly whereas *M. tuberculosis* exists as an acid-fast, cell wall intact form that can survive extracellularly. Destruction of infected macrophages would allow immune mechanisms to kill MAP but not *M. tuberculosis* in the extracellular spaces. Apoptosis of infected macrophages and effector Th1-cells would quickly dampen the inflammatory cytokine stream that drives the disease process. This explanation is offered as a hypothetical scenario.

Skeptics also question other evidence that supports an etiologic role for mycobacteria. There are both positive and negative PCR studies involving MAP-DNA assays from CD tissue. These conflicting results confuse the picture and prevent any definite conclusions from being drawn. Although MAP-DNA was identified by in-situ hybridization in granulomas from 83% of CD patients [39], other studies identifying *E. coli* DNA in CD granulomas have challenged or negated the importance of this finding [39]. Live cultures of MAP from CD tissues could be innocent colonizing bacteria attracted to diseased tissue, and the discovery of live Map cultured from the breast milk of 2/2 (100%) women is dismissed as not meeting statistical significance. The finding of Map in blood cultures from 14/28 CD patients and not in any healthy controls requires confirmation from another laboratory. The importance of this discovery has already been challenged by the argument that positive MAP blood cultures merely reflect the compromised intestinal mucosal barrier found in inflammatory bowel diseases that would allow intestinal bacteria access to the blood stream. The lack of bacteremia with other colonic bacteria suggests otherwise.

#### IMMUNE-DEFICIENCY THEORY

Doctors Korzenik and Dieckgraefe in St. Louis postulate that dysfunctional neutrophils play a central role in an in-

flammatory immune process that results in the Crohn's Disease phenotype. They suggest that defects of innate immunity compromise neutrophil function thus enabling microbes to survive long enough so that a compensatory, excessive Th1 immune response is activated resulting in the CD phenotype. Neutrophil dysfunction is hypothesized to result from an interplay of genetic factors, environmental factors, or possibly exotoxins associated with subsets of the gut flora [40]. The central concept is that defects of innate immunity result in an excessive, compensatory immune response to as yet unidentified microbes producing the Crohn's disease phenotype.

Acting on this theory, clinical trials were performed testing the effects of GM-CSF on CD patients. Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) stimulates neutrophil activity both qualitatively and quantitatively. As with most cytokines, GM-CSF is pleiotropic and has multiple effects on the immune system. The initial results were very promising and led to further testing. They report in the May 26, 2005 New England Journal of Medicine that after eight weeks of daily GM-CSF injections 48% of 81 patients receiving daily GM-CSF injections had a 100 point improvement on the Crohn's Disease Activity Index versus only 26% of the control group. Forty percent of the treated group went into complete remission versus 19% of controls [41].

The theory does not suggest a specific pathogen but uses *M. tuberculosis* as an example of how neutrophil dysfunction might allow the organism to successfully infect macrophages resulting in a pathologic process similar to Crohn's Disease. Clearly the immune-deficiency theory and the mycobacterial theory are closely related but emphasize different aspects of the infection/immune interaction. Interestingly, GM-CSF activates macrophages to kill intracellular microbes and pathogenic mycobacteria suppress GM-CSF in infected macrophages [38].

### Essential points

The three etiologic theories for CD have much in common. Their main differences are that they emphasize different aspects of the same complex disease process.

The autoimmune theory looks at the data, assumes that there is no specific infectious microbe and concentrates on the dysregulated destructive immune processes found in Crohn's Disease. Since antigenic stimulation must be involved and since the normal gut flora clearly influences the activity of the mucosal immune system, the autoimmune theory hypothesizes that the initiating antigens are from normal gut flora. Treatment strategies are aimed at suppressing inflammation and immunity.

The mycobacterial theory states that MAP is a specific pathogen that is a major etiologic agent in Crohns, perhaps not the only infectious agent but certainly the most prevalent. The MAP theory embraces all that is described about immune dysregulation, predisposing genetic factors, immune deficiency, the role of gut flora and a compromised mucosal epithelial barrier. MAP is believed to behave similarly to *M. tuberculosis* or *M. leprae* and is able to dysregulate immune signaling as one of its evolved survival strategies. Although there is considerable evidence for MAP infection in CD pa-

tients, critics argue that Map infection and CD co-exist but are not related. Marcel Behr responds, "Applying the principles of Ockham's razor, the most parsimonious explanation in a patient without other illness is that MAP in a genetically susceptible host results in the CD phenotype" [42].

The immunodeficiency theory suggests that disturbances of innate immunity play a central role in the pathogenesis of CD and therapy should be aimed at bolstering innate immunity rather than suppressing it. It implies that the excessive, dysregulated and destructive immune response represents a compensatory process due to the failure of innate mechanisms to clear invasive microbes. This theory suggests that subsets of gut flora may exert an immune suppressive effect on neutrophils presumably through exotoxins. A specific invasive organism is not incriminated.

If an invasive bacteria were involved in the CD process, the question arises whether an inherited immune deficiency predisposes the patient to the microbe. If so, should CD be classified as an immune disorder or as an infectious disease? The same questions can be asked about tuberculosis and leprosy. By no means all people exposed to these pathogens develop clinically significant disease. It appears that some combination of burden of infection along with susceptibility to infection, whether innate or circumstantial, is required to produce a mycobacterial illness.

The microbial world and the immune system co-evolved together and are so intimately intertwined that it is not helpful to separate the two. The important point is to understand the process and plan therapy accordingly.

### CONCLUSIONS

In summary, the three theories share many things in common but differ in how they interpret the data and in their emphasis on different aspects of the disease process. The immune deficiency theory emphasizes defects in innate immunity that predispose to infection and suggests that therapy should be directed at enhancing immunity rather than suppressing it. Although a specific pathogen is not identified, the pattern of the immune response suggests an intracellular organism that infects macrophages and dendritic cells. The mycobacterial theory emphasizes the role of *Mycobacterium avium paratuberculosis* as a specific pathogen that elicits an excessive and ultimately destructive inflammatory response producing the CD phenotype. The autoimmune theory assumes that a specific pathogen does not exist, emphasizes the dysregulated, excessive immune response and hypothesizes that immune regulatory dysfunction allows otherwise benign bacteria to drive the destructive disease process. The autoimmune theory hypothesizes that an unknown environmental agent may trigger the dysregulated immune responses in a genetically susceptible host.

The concepts associated with these three theories are intertwined. Not only would immune deficiency predispose to a mycobacterial infection, but pathogenic mycobacteria are known to further dysregulate and suppress immunity. The aberrant, immune responses that result may be approximated in mice bred to be deficient in homeostatic regulatory networks. These selectively bred animals respond excessively to normal enteric antigens thus lending support to

the autoimmune hypothesis. Although the mouse models approximate inflammatory bowel disease, the colitis seen in these animals does not necessarily represent Crohn's Disease occurring in the natural setting. Quite possibly *M. paratuberculosis* may be the unknown environmental factor mentioned in the autoimmune theory.

And, as a disclaimer, the different theories may each describe the etiopathogenesis of totally different diseases that all get lumped together under the Crohn's Disease label. Since Crohn's Disease is thought to be a syndrome consisting of different diseases, it could be that the three theories outlined above each describe a different disease that gets labeled as Crohn's Disease.

## REFERENCES:

- Gastroenterology – Special issue; 2004, vol. 126, number 6
- Podalsky DK: Inflammatory bowel disease. *N Eng J Med*, 2002; 347: 417–29
- Bouma G, Strober W: The immunological and genetic basis of inflammatory bowel disease. *Nature Review: Immunology*, 2003; 3: 521–33
- Lim W, Hanauer S: Emerging biologic therapies in inflammatory bowel disease. *Reviews in Gastroenterological Disorders*, 2004; 4: 66–85
- Ogura Y, Bonen DK, Inohara N et al: A frame shift mutation in *NOD2* associated with susceptibility to Crohn's disease. *Nature*, 2001; 411: 603–6
- Hugot JP, Chamaillard M, Zouali H et al: Association of *NOD2* leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature*, 2001; 411: 599–603
- Kelsall B: Getting to the Guts of *NOD2*. *Nature Medicine*, 2005; 11(4): 383–84
- Ferwerda G, Girardin S, Kullberg B: *NOD2* and Toll-Like Receptors are Nonredundant Recognition Systems of *Mycobacterium tuberculosis*. *PLOS Pathogens*, 2005; Vol. 1: issue3: e34. (may be viewed online)
- Geijtenbeek T, Van Vliet SJ, Koppel EA et al: *Mycobacteria* target DC-SIGN to suppress Dendritic cell function. *J Exper Medicine*, 2003; 197(1): 7–17
- Sechi L, Gazouli M, Ikonomopoulos J et al: *Mycobacterium avium* subsp. *Paratuberculosis*, Genetic Susceptibility to Crohn's Disease, and Sardinians: the Way Ahead. *Journal of Clinical Microbiology*, 2005; 5275–77
- Karakousis PC, Bishai WR, Dorman SE: *Mycobacterium tuberculosis* cell envelope lipids and the host immune response. *Cellular Microbiology*, 2004; 6(2), 105–16
- Nagabhushanam V, Solache A, Ernst J et al: Innate inhibition of adaptive immunity: *Mycobacterium tuberculosis*-induced IL-6 inhibits macrophage responses to IFN-gamma. *J of Immunology*, 2003; 171: 4750–57
- Chiodini RJ: Crohn's disease and the mycobacterioses: a review and comparison of two disease entities. *Clin Microbiol Rev*, 1989; 2: 90–117
- Chiodini RJ, Van Kruiningen HJ, Thayer WR, Coutru JA: Spheroplastic phase of mycobacteria isolated from patients with Crohn's disease. *J Clin Microbiol*, 1986; 27: 357–63
- McFadden JJ, Butcher PD, Chiodini R, Hermon-Taylor J: Crohn's disease-isolated mycobacteria are identical to *M. para*, as determined by DNA probes that distinguish between mycobacterial species. *J Clin Microbiol*, 1987; 25: 796–801
- Markesich DC, Graham DY, Yoshimura HH: Progress in culture and subculture of spheroplasts and fastidious acid bacilli isolated from intestinal tissues. *J Clin Microbiol*, 1988; 26: 1600–3
- Haagsma J, Mulder CJJ, Eger A et al: *M. para* isolated from patients with Crohn's disease in The Netherlands. *Scand J Gastroenterol*, 1989; 24: S158–68
- Gitnick G, Collins J, Beaman B et al: Preliminary report on isolation of mycobacteria from patients with Crohn's disease. *Dig Dis Sci*, 1989; 34: 925–32
- Moss M, Sanderson J, Tizard M et al: PCR detection of *M. paratuberculosis* and *M. avium* subsp. *silvaticum* from long term cultures of Crohn's disease and control tissues. *Gut*, 1992; 33: 1209–13
- Chamberlin W, Graham DY, El-Zaatari F et al: Review article: *Mycobacterium avium* subsp *paratuberculosis* as one cause of Crohn's disease. *Alim Pharmacol Ther*, 2001; 337–46
- Greenstein RJ: Is Crohn's Disease caused by a mycobacterium: Comparisons with leprosy, tuberculosis and Johne's Disease. *The Lancet Infectious Diseases*, 2003; 3: 507–14
- Dalziel TK: Chronic interstitial enteritis. *Br Med J*, 1913; 2: 1068–70
- Crohn BB, Ginzburg K, Oppenheimer GD: Regional ileitis: a pathological and clinical entity. *J Am Med Assoc*, 1932; 99: 1323–28
- Moss M, Sanderson J, Tizard M et al: PCR detection of *M. paratuberculosis* and *M. avium* subsp. *silvaticum* from long term cultures of Crohn's disease and control tissues. *Gut*, 1992; 33: 1209–13
- Dell'Isola B, Poyart C, Goulet O et al: Detection of *M. paratuberculosis* by polymerase chain reaction in children with Crohn's disease. *J Infect Dis*, 1994; 169: 449–51
- Lisby G, Andersen J, Engbaek K, Binder V: *Mycobacterium paratuberculosis* in intestinal tissue from patients with Crohn's disease demonstrated by a nested primer polymerase chain reaction. *Scand J Gastroenterol*, 1994; 29: 923–29
- Bull TJ, McMinn EJ, Sidi-Boumedine K et al: Detection and verification of *Mycobacterium avium* ssp. *paratuberculosis* in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease. *J Clin Microbiol*, 2003; 41: 2915–23
- Autschbach F, Eisold S, Hinz U et al: High Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* IS900 DNA in gut tissues from individuals with Crohn's disease. *Gut*, 2005; 54: 944–49
- Ryan P, Bennett MW, Aarons S, Shanahan F: PCR detection of *Mycobacterium paratuberculosis* in Crohn's disease granulomas isolated by laser capture microdissection. *Gut*, 2002; 51: 665–70
- Mishina D, Katsel P, Brown ST et al: On the etiology of Crohn's Disease. *Proc Natl Acad Sci USA*, 1996; 93: 9816–20
- Naser S, Shafran I, El-Zaatari FAK: *Mycobacterium avium* subsp *paratuberculosis* in Crohn's disease is serologically positive. *Clin Diag Lab Immunol*, 1999; 6: 282
- Hulten K, El-Zimaity HMT, El-Zaatari FAK et al: Association between *Mycobacterium paratuberculosis* and granulomatous Crohn's disease by in situ hybridization. *ASM*, 199; 640: U-31
- Sechi LA, Mura M, Tanda F et al: Identification of *Mycobacterium avium* subsp *paratuberculosis* in biopsy specimens from patients with Crohn's disease identified by in situ hybridization. *J Clin Microbiology*, 2001; 39: 4514–17
- Schwartz D, Shafran I, Romero C et al: Use of short-term culture for identification of *Mycobacterium avium* subsp. *paratuberculosis* in tissue from Crohn's disease patients. *Clin Microbiol Infect*, 2000; 6: 303–7
- Naser S: Isolation of *Mycobacterium avium* subsp *paratuberculosis* from breast milk of Crohn's disease patients. *American Journal of Gastroenterology*, 2000; 95(4): 1094–95
- Naser S, Ghobrial G, Romero C, Valentine J: Culture of *Mycobacterium avium* subspecies *paratuberculosis* from the blood of patients with Crohn's Disease. *The Lancet*, 2004; 364: 1039–44
- Sartour RB: Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology*, 2004; 126: 1620–33
- Tesema MZ, Koets AP, Rutten V, Gruys E: How does *Mycobacterium avium* subsp. *paratuberculosis* resist intracellular degradation? *The Veterinary Quarterly*, 2001; 23(4): 153–62
- Korzenik JR, Dieckgraefe BK: Is Crohn's Disease an immunodeficiency? A hypothesis suggesting possible early events in the pathogenesis of Crohn's Disease. *Dig Dis and Sciences*, 2000; 45(6): 1121–29
- Ryan P, Kelly R, Lee G et al: Bacterial DNA within Granulomas of Patients with Crohn's Disease- Detection by Laser Capture Microdissection and PCR. *Am J Gastroenterol*, 2004; 99: 1539–43
- Korzenik JR, Dieckgraefe BK, Valentine JF et al. and the Leukine in Crohn's disease study group. *Sagramostim* (Granulocyte-macrophage colony stimulating factor) for active Crohn's disease. *New England Journal of Medicine*, 2005; 352(21): 31–39
- Behr M, Semret M, Poon A, Schurr E: Crohn's Disease, *Mycobacteria* and *NOD2*. *The Lancet Inf Dis*, 2004; 4: 136–37

RA

# Index Copernicus

Global Scientific Information Systems  
for Scientists by Scientists

[www.IndexCopernicus.com](http://www.IndexCopernicus.com)



TM

**INDEX**  
**COPERNICUS**  
**INTERNATIONAL**



**EVALUATION & BENCHMARKING**

**PROFILED INFORMATION**

**NETWORKING & COOPERATION**

**VIRTUAL RESEARCH GROUPS**

**GRANTS**

**PATENTS**

**CLINICAL TRIALS**

**JOBS**

**STRATEGIC & FINANCIAL DECISIONS**

## Index Copernicus integrates

### IC Scientists

Effective search tool for collaborators worldwide. Provides easy global networking for scientists. C.V.'s and dossiers on selected scientists available. Increase your professional visibility.

### IC Virtual Research Groups [VRG]

Web-based complete research environment which enables researchers to work on one project from distant locations. VRG provides:

- ⊗ customizable and individually self-tailored electronic research protocols and data capture tools,
- ⊗ statistical analysis and report creation tools,
- ⊗ profiled information on literature, publications, grants and patents related to the research project,
- ⊗ administration tools.

### IC Journal Master List

Scientific literature database, including abstracts, full text, and journal ranking. Instructions for authors available from selected journals.

### IC Patents

Provides information on patent registration process, patent offices and other legal issues. Provides links to companies that may want to license or purchase a patent.

### IC Conferences

Effective search tool for worldwide medical conferences and local meetings.

### IC Grant Awareness

Need grant assistance? Step-by-step information on how to apply for a grant. Provides a list of grant institutions and their requirements.

### IC Lab & Clinical Trial Register

Provides list of on-going laboratory or clinical trials, including research summaries and calls for co-investigators.