Inflammatory Bowel Disease: Autoimmune or Immune-mediated Pathogenesis?

ZHONGHUI WEN and CLAUDIO FIOCCHI*

Division of Gastroenterology, University Hospitals of Cleveland, Case Western Reserve University School of Medicine, Cleveland, OH 44106-4952, USA

The pathogenesis of Crohn’s disease (CD) and ulcerative colitis (UC), the two main forms of inflammatory bowel disease (IBD), is still unclear, but both autoimmune and immune-mediated phenomena are involved. Autoimmune phenomena include the presence of serum and mucosal autoantibodies against intestinal epithelial cells in either form of IBD, and against human tropomyosin fraction five selectively in UC. In addition, perinuclear antineutrophil cytoplasmic antibodies (pANCA) are common in UC, whereas antibodies against *Saccharomyces cerevisiae* (ASCA) are frequently found in CD. Immune-mediated phenomena include a variety of abnormalities of humoral and cell-mediated immunity, and a generalized enhanced reactivity against intestinal bacterial antigens in both CD and UC. It is currently believed that loss of tolerance against the indigenous enteric flora is the central event in IBD pathogenesis. Various complementary factors probably contribute to the loss of tolerance to commensal bacteria in IBD. They include defects in regulatory T-cell function, excessive stimulation of mucosal dendritic cells, infections or variants of proteins critically involved in bacterial antigen recognition, such as the products of CD-associated *NOD2/CARD15* mutations.

**Keywords:** Inflammatory bowel disease; Crohn’s disease; Ulcerative colitis; Intestinal flora; Tolerance

INTRODUCTION

Chronic inflammatory diseases where an external infectious or noxious agent is not readily identified as the direct cause of the associated pathology are usually categorized as autoimmune or immune-mediated conditions. This implies a dominant role of the immune system in triggering and maintaining an inflammatory response against known or unknown antigens that are part of the host or intimately associated with it. In reality, chronic disabling inflammatory diseases of uncertain etiology are the result of highly complex and intimately integrated biological networks that also include genetic, environmental and neuroendocrine components (Straub and Besedovsky, 2003). This concept also applies to Crohn’s disease (CD) and ulcerative colitis (UC), two chronic inflammatory conditions of the gastrointestinal tract that are collectively known as inflammatory bowel diseases (IBD). There is considerable evidence demonstrating that immunopathogenic events are distinct in CD and UC (Fiocchi, 1998; Podolsky, 2002; Bouma and Strober, 2003), but these entities also share plausible pathogenic factors such as dietary antigens and the enteric commensal flora. The latter, in particular, has received a great deal of attention in the last decade due to mounting evidence that it may function as a “self-antigen” in IBD, a notion that will be discussed later on. Still in regard to the role of the enteric flora, when discussing organ-specific autoimmune or immune-mediated pathogenesis, a fundamental concept that cannot be overemphasized is that both CD and UC occur in a non sterile, “dirty” tissue environment, unlike any other type of autoimmune or immune-mediated disorder that develops in sterile, “clean” tissues, such as the joint in rheumatoid arthritis, the nervous system in multiple sclerosis, the thyroid in autoimmune thyroiditis, the bile ducts in primary biliary cirrhosis, the microcirculation in systemic lupus erythematosus, and so on. This crucial difference between IBD and other immunological conditions is at the core of the present review, and will allow us to consider the intriguing idea of the normal enteric flora not as an external environmental factor but an intrinsic, “self-component” of the intestine-specific immune response occurring in CD and UC.

AUTOIMMUNE EVENTS IN IBD

Classical criteria defining an autoimmune disease include the demonstration of B-cell clones producing polyreactive...
antibodies, antibodies specific for autoantigens, and pathogenic autoantibodies, T-cell clones that are specific for autoantigens and can transfer autoimmune disease, the precise identification of organ-specific autoantigens, and the reproduction of autoimmune diseases in experimental animal models (Rose and Bona, 1993). In addition, we know that autoimmune diseases are conditioned by genetic and environmental factors (Marrack et al., 2001), and among the latter a variety of infectious agents have been demonstrated to possess the ability to induce or activate autoreactive immune cells (Wercherfennig, 2001). Molecular mimicry has been invoked as one of the mechanisms responsible for the activation of autoreactive cells by microbial peptides that have structural similarities to self-peptides (Wercherfennig, 2001), but there is also evidence that antigenically unrelated infections or specific inflammatory signals can result in autoaggressiveness and induction of organ-specific autoimmunity, including in the gut (Vezy and Lefranc¸ois, 2002). Neither CD nor UC fulfill all or most of the criteria for classical autoimmunity. On the other hand, there is evidence that autoimmune reactivity does occur in IBD.

Autoantibodies

The first reasonable indication that the immune system was involved in the pathogenesis of IBD emerged in the late 1950s and early 1960s with the demonstration of autoantibodies and cytotoxic leukocytes for colonic epithelial cells in UC patients (Broberger and Perlmann, 1959; Perlmann and Broberger, 1963). Soon after, serum antibodies against colonic epithelium were detected in the circulation of UC patients that were cross-reactive with Escherichia coli antigens, and the hypothesis was proposed that such immune cross-reactivity could represent a form of autoimmunity relevant to IBD pathogenesis (Perlmann et al., 1967). These initial reports were followed by a long series of studies demonstrating that both CD and UC patients possess antibodies against a range of potential autoantigens, including lymphocyte antigens (Korsmeyer et al., 1974), cytoskeletal proteins (Mayet et al., 1990), cardiolipin (Aichbiclier et al., 1999) and pancreatic proteins (Fricke et al., 1999).

Additional evidence for the possible involvement of antibody-dependent pathophysiology in IBD was introduced by the demonstration of alterations of serum immunoglobulins and the presence of rheumatoid factor and anti-F(ab')2 autoantibodies (MacDermott et al., 1981; Pallone et al., 1986), as well as circulating complement-fixing immune complexes in the serum of CD and UC patients (Doe et al., 1973; Jewell and McLennan, 1973). Moreover, activated complement was located in the microvessels of IBD-involved mucosa (Halstensen et al., 1989), and also in the gut epithelium in association with IgG1 antibodies (Halstensen et al., 1993). Based on these observations, the question of whether IBD could be a true, autoantibody-dependent autoimmune disorder was raised (Thayer, 1976; Snook, 1990), even though convincing verification for the existence of organ-specific pathogenic autoantibodies had not been obtained.

Autoantibodies to Intestinal Epithelial Antigens

The search for gut-specific autoantibodies has been relatively limited in IBD, and only two lines of investigation have yielded reasonable evidence suggesting that true autoantibodies may contribute to IBD pathogenesis.

Goblet cell glycoproteins, termed epithelial cell-associated components (ECAC), were initially identified in the rat intestine and later in the human intestine (Roche et al., 1981; Aronson et al., 1983), and the detection of ECAC-specific reactivity by circulating mononuclear cells and sera from CD and UC patients suggested that autosensitization to these protein had occurred in subjects with IBD (Aronson et al., 1983). Antigen-specific reactivity against ECAC was later demonstrated using mononuclear cells isolated from CD and UC mucosa (Roche et al., 1985). These mucosal cells were shown to be CD3+ lymphocytes, which induced antibody-mediated cytotoxicity for small or large bowel-derived ECAC but not control antigens (Roche et al., 1985). These findings, including the localization of the antigen(s) to goblet cells and apparent antigen-specific immune reactivity, were recapitulated in the cotton top tamarin, a spontaneous model of IBD resembling UC (Winter et al., 1989). These observations have not been followed by further biochemical characterization of the putative autoantigens or the mechanisms of autosensitization, and the importance of ECAC as a pathogenic autoantigen in IBD remains undefined.

Another series of studies has pursued a different potential autoantigen which, unlike ECAC, appears to be large bowel- and disease-specific. IgG antibodies eluted from the colonic mucosa of UC patients, but not from patients with CD or other colonic inflammatory conditions, were found to recognize a 40kDa protein present exclusively in large bowel tissue, suggesting the possibility that such protein could represent an autoantigen mediating an antibody-mediated response in UC patients (Takahashi and Das, 1985). The generation of monoclonal antibodies against the 40kDa protein permitted to define its exclusive localization to epithelial cells of the human large bowel, and not upper or small bowel, liver, pancreas or non gastrointestinal tissues (Das et al., 1987). Interestingly, IgG1 and activated complement were found to colocalize with the 40kDa on colonoocytes of UC but not CD patients (Halstensen et al., 1993). Follow up studies showed that the epitope recognized by the monoclonal antibodies in human colon was selectively present in skin, eye, joint and biliary epithelium (Das et al., 1990; Bhagat and Das, 1994; Mandal et al., 1994). This localization is extremely intriguing because those tissues match exactly the sites where the major extra-intestinal manifestations of IBD
occur, and led to the speculation that autoantibodies or immune cells sensitized to the 40 kDa colonic protein could cross-react with the same epitope in other locations, trigger local pathology and explain the extraintestinal manifestations of IBD (Das, 1999). Further investigation revealed that lamina propria mononuclear cells isolated from UC mucosa spontaneously produced IgG1 against the putative autoantigen (Biancone et al., 1995), that has now been identified as a cytoskeletal protein of the tropomyosin family, specifically human tropomyosin fraction 5 (huTM5) (Das et al., 1993; Geng et al., 1998). Studies in animal models of IBD have yet to be performed to further substantiate the disease-causing potential of huTM5, and studies are under way to understand how this intracellular protein may be transported to the colonocyte surface to act as a target self-antigen. Thus, as is the case of ECAC, the true significance of huTM5 as a pathogenic autoantigen and the related mechanisms of colon inflammation are still uncertain.

pANCA and ASCA

In addition to epithelial-cell autoantibodies, two other types of antibodies were commonly detected in IBD patients: antineutrophil cytoplasmic antibodies (ANCA) and anti-Saccharomyces cerevisiae antibodies (ASCA), which are relatively specific for UC and CD, respectively.

In the original report, sera from the majority of patients with UC were found to contain antibodies recognizing an antigen(s) with a nongranular perinuclear (p) distribution in neutrophils (Saxon et al., 1990). The UC-associated pANCA were distinct from the ANCA found in Wegener’s granulomatosis or crescent glomerulonephritis, and did not react with myeloperoxidase, suggesting that they may “UC-specific”. In subsequent studies, the levels of pANCA in UC were consistently found to be significantly higher than those of patients with CD, infectious colitides, irritable bowel syndrome and miscellaneous diarrheal diseases, with a sensitivity of pANCA for UC of around 60% with a specificity of 80–90% (Duerr et al., 1991). These values were based on mostly white North American patients with UC, but are lower in other European or Asian populations (Sugi et al., 1999). Of interest, pANCA are present in the circulation of some animal models of experimental colitis, such as the interleukin (IL)-10-deficient and the T-cell receptor (TCR) α-deficient mice (Mizoguchi et al., 1997; Seibold et al., 1998). The neutrophil antigen(s) recognized by pANCA is still a matter of dispute, but it is clearly different from that associated with vasculitis. However, the answer to the more fundamental question of whether these autoantibodies are pathogenic and can induce gut inflammation appears to be a negative one. In fact, levels of pANCA in UC are not correlated to disease activity or extent, do not affect neutrophil function, patients can have UC without developing pANCA, and pANCA also occur in unaffected relatives of UC patients (Shanahan, 1994). However, pANCA may still be useful as serological markers of disease, possible markers of genetic susceptibility, or markers for disease heterogeneity.

At the same time that pANCA emerged as UC-associated autoantibodies, an association of CD with ASCA was also being investigated. Levels of serum antibodies against multiple strains of S. cerevisiae (baker’s and brewer’s yeast) were found to be significantly elevated in the serum of CD patients, but not serum of UC patients (McKenzie et al., 1990; Giaffer et al., 1992). This was initially proposed as potentially indicating hypersensitivity to dietary antigens in CD. This hypothesis has not been pursued to any significant extent and is still a theoretical possibility, but it is well established that ASCA recognize mannose sequences in the cell wall mannan of S. cerevisiae, defining ASCA as a non autoantigen in IBD. Although not totally specific for CD, as ASCA can be detected in serum of some celiac disease patients (Giaffer et al., 1992), the lack of antibodies in the circulation of UC patients suggested that the combined measurement of pANCA and ASCA could help in the differential diagnosis of the two forms of IBD, a typically challenging situation for the practicing gastroenterologist. Two studies, one performed with adult and the other with pediatric subjects in which sera from UC and CD patients were assessed for both pANCA and ASCA, showed that the combination of a positive pANCA and a negative ASCA was highly specific (95–100%) for UC, whereas the combination of a negative pANCA and a positive ASCA was highly specific (95–100%) for CD (Ruemelle et al., 1998; Quinton et al., 1998). These results have been replicated in multiple reports, but unfortunately all studies agree that the sensitivity of the combined pANCA plus ASCA test is still too low (around 50–60%) to be useful as a general screening tool.

IMMUNE-MEDIATED EVENTS IN IBD

Immune Abnormalities in IBD

Taking into consideration all the information discussed so far, it is apparent that data favoring classical autoimmune pathogenic mechanisms, like antigen-specific autoreactive T- or B-cells, are scant and not necessarily robust in IBD. On the other hand, there is an overwhelming amount of data proving that abnormalities of the mucosal and systemic immune systems are intimately involved in the pathogenesis of both CD and UC. As indicated earlier, these abnormalities are almost certainly secondary, and occur in the context of other sine qua non conditioning factors, which include genetic predisposition, environmental changes, and the intestinal flora (Fiocchi, 1998; Podolsky, 2002). Most of the immune abnormalities described in the last two decades of investigation have been focused on mucosal immune events, particularly searching for imbalances or skewing of the Th1/Th2 paradigm (Neurath et al., 2002). Although the downstream
events of mucosal inflammation are nonspecific and predictably characterized by the production of high levels of cytokines, growth factors, free radicals and matrix-degrading enzymes, a clear dichotomy exists in regard to dysregulated upstream immune events in IBD (Monteleone et al., 2002). The transmural inflammation characteristic of CD is associated with a typical Th1 response dominated by high IL-12, IL-18 and interferon-γ production (Monteleone et al., 1997), whereas UC has been very recently defined as an atypical Th2 response mediated by CD1d-restricted NK T-cells that produce high levels of IL-13 (Fuss et al., 2004). Thus, the immune aberrations underlying the two main forms of IBD are clearly diverse. This conclusion, however, does not preclude the possibility that triggering phenomena or sensitizing agents are similar, if not the same. In fact, accumulating evidence derived from genetic, microbial and immunological observations strongly suggests that the normal indigenous flora of the intestine may be at the center of pathogenic events in IBD, a major point that will be the focus of the following discussion.

The Intestinal Flora as a Tolerizing “Self-antigen”

Bacteria are usually regarded as microorganisms derived from the surrounding environment that have the capacity to invade the host and eventually cause disease. In reality, the body harbors huge quantities of non-pathogenic “friendly” bacteria, the vast majority of which are located within the intestinal lumen. The gut contains at least 400 different species of mostly anaerobic bacteria, and the number of microbial cells in the lumen is approximately ten times greater than that of the eukaryotic cells in the human body. This enormous amount of enteric microbes constantly and intimately interacts with the host and confers benefits that are essential to health and survival (Guarnieri and Malagelada, 2003). DNA microarray analysis of the effect of colonization of germ-free mice with the Bacteroides thetaiotaomicron, a dominant component of normal human and murine flora, shows that this bacterium modulates expression of genes involved in intestinal nutrient absorption, mucosal barrier function, xenobiotics metabolism, angiogenesis and postnatal maturation (Hooper et al., 2001). Therefore, although the commensal gut flora technically is a foreign antigen, under physiological circumstances it behaves as a “self-antigen”, and is perceived as such by the host immune system. Based on this concept, if tolerance to the gut commensal flora is lost, this becomes a pathogenic event that may lead to a state of chronic intestinal inflammation.

Failure to Regulate Reactivity to the Gut Flora: A Central Event in IBD Pathogenesis?

There is mounting evidence that IBD represents a condition where the normal homeostatic balance between the enteric flora and mucosal immunity is lost, resulting in the chronic inflammatory response that typifies CD and UC. Three scenarios can be envisioned that might explain this outcome: first, the quantitative or qualitative composition of the flora has changed; second, the immune response towards the normal gut flora is abnormal; third, the regulatory mechanisms that control flora-host interaction have gone away.

The Intestinal Flora in IBD

The complexity of the human gut flora has long represented a major obstacle to the full characterization of the innumerable microorganisms scattered all along the gastrointestinal tract. Nevertheless, the major groups of aerobic and anaerobic bacteria are known, and their progressive numerical increase from the upper to the lower
bowel is well-known (Guarnier and Malagelada, 2003). The complexity of the gut flora rapidly increases after birth, and by the age of one year its composition resembles that of an adult person (Favier et al., 2002). There are also observations indicating that, even though gut bacteria are relatively stable throughout the life of an individual, each person has a customized flora and that variations in bacterial composition occur depending on feeding (breast milk vs. bottle), different environments, and use of antibiotics, prebiotics and probiotics.

Recent reports have indicated that a relationship exists between the composition of the intestinal microflora and the propensity to suffer from allergic inflammation (Kirjavainen et al., 2002), and there are differences in gut microbiota between infants who will and those who will not develop allergies even before the appearance of any clinical manifestations of atopy (Bjorksten et al., 2001). The same may be true for IBD, as suggested by prospective studies showing that children with an abnormal flora have a greater chance of developing CD than children with a normal flora (VandeMerwe et al., 1988). These results are compatible with two important concepts previously introduced: that the gut flora participates of the education of systemic and mucosal immunity, and that an altered flora may lead to an aberrant immune response. There are few studies that have analyzed the microflora of adults with established IBD, but it appears that both quantitative and qualitative differences are present in both CD and UC patients. In patients with UC there is a significant decrease in the number of anaerobic bacteria, anaerobic gram-negatives and lactobacilli (Fabia et al., 1993), while in CD patients there is a significant increase in enterobacteria independent of the clinical activity of disease (Seksik et al., 2003).

In addition, the total number of bacteria found in the colonic mucus of IBD patients is significantly increased compared to controls (Schultsz et al., 1999), and this number increases with the severity of mucosal inflammation (Swidsinski et al., 2002). Together, these observations support the notion that alteration of the gut commensal flora may change its immune recognition from a “self-antigen” to a new or foreign antigen towards which the local immune system may mount a response which is manifested as IBD. 

Enhanced Reactivity to Bacterial Antigens in IBD

The possibility that patients with IBD develop an aberrant immune response against components of the gut flora has been under consideration for a long time. A number of reports show that CD and UC patients have increased titers of antibodies against *E. coli*, aerobes, anaerobes and even enteric bacterial pathogens, and that these antibodies are of both systemic and mucosal origin (Monteiro et al., 1971; Tabaqchali et al., 1978; Blaser et al., 1984; Macpherson et al., 1996). The previously discussed ASCA in CD patients probably reflect the same type of broad anti-microbial reactivity associated with IBD (McKenzie et al., 1990; Giaffer et al., 1992). More recently, novel gut bacterial antigens have been reported in association with IBD, including the *Pseudomonas fluorescens*-associated sequence I2, the outer membrane porin C of *E. coli* (OmpC), and bacterial flagellins, towards which CD patients develop significantly higher antibody titers than UC or control subjects (Sutton et al., 2000; Landers et al., 2002; Lodes et al., 2004). Interestingly, it has been suggested that the higher the antibody responses against bacteria and more numerous the antigens against which they react, the more likely it is that CD patients will experience a more severe clinical course, as indicated by a greater frequency of strictures, perforations, and surgical interventions (Mow et al., 2004). If confirmed, this observation would indicate that the greater the level of sensitization to enteric bacteria, the stronger and more damaging the ensuing mucosal immune response might be.

Fewer data are available on the cell-mediated immune response to bacterial antigens in IBD. The existing reports also indicate that there is an enhanced T-cell reactivity against microbial antigens of enteric and non enteric origin in IBD, particularly in CD patients, at both the systemic and intestinal mucosa level (Bull and Ignaczak, 1973; Pirzer et al., 1991; Young et al., 1994; Duchmann et al., 1999). Supporting evidence for sensitization to bacterial antigens in IBD can also be found in animal models of experimental colitis, where CD4+ T-cells reactive against enteric flora are detected in both the spleen and the colon, and they can transfer IBD in adoptive transfer experiments in SCID mice (Cong et al., 1998; Wirtz et al., 1999). In addition, it is worth noticing that some enteric antigens like I2, to which enhanced humoral immune responses are found in IBD, also function as superantigens (Dalwadi et al., 2001), and non enteric superantigens like *Staphylococcus aureus* enterotoxin B, or enteric superantigens like *Yersinia pseudotuberculosis* can activate T-cells and elicit or aggravate gut inflammation of which they are not the causative agents (Lu et al., 2003).

Abnormal Regulation of Anti-bacterial Reactivity in IBD: Loss of Tolerance to the Enteric Flora

While the above-mentioned studies certainly indicate that abnormal immune reactivity to bacteria is intimately associated with IBD pathogenesis, the essential role of the luminal microbiota in triggering or maintaining IBD was only confirmed with the use of animal models in which the presence, quantity and type of the flora could be experimentally controlled and manipulated. The report that a germ-free condition prevents development of gut inflammation in HLA-B27 transgenic rats was the first to bring attention to the fact that experimental IBD is dependent on the presence of intestinal bacteria (Taurog et al., 1994). This seminal observation was followed by others showing that the luminal bacterial load and its composition together determine the degree of
inflammation, and different strains display intrinsically
dissimilar proinflammatory capacities, that vary from
potent to moderate to absent depending on the genetic
background of the host (Rath et al., 1999a,b). The
observation that IBD fails to develop in the absence of
tenteric flora has now been confirmed in numerous animal
models of IBD, and has lead to the widely accepted
paradigm “no bacteria, no colitis”.

If the commensal gut flora is required to develop IBD,
and an inappropriately strong immune response against it
is a key mechanism responsible for the development of
chronic intestinal inflammation, how does such damaging
immune response develop? One possibility, already
discussed, is that the antigenic properties of the flora
have changed and an immune response is mounted to what
previously was a “self-antigen” towards which tolerance
had been established. The reverse possibility is that the
flora is not significantly altered in IBD, but the normal
state of tolerance to luminal bacterial antigens has been
lost (Khoo et al., 1997). In regard to the second
possibility, a classical study revealed that human
peripheral blood and mucosal mononuclear cells fail to
proliferate when exposed to bacteria from autologous
intestine, but do so after exposure to bacteria from
heterologous intestine, indicating that tolerance to the
autologous gut microbiota normally exists (Duchmann
et al., 1995). In contrast, mucosal immune cells from
active CD or UC patients vigorously proliferate after
co-culture with bacteria from the autologous intestine,
indicating that tolerance is lost in IBD. Similar findings
were seen in a hapten-induced model of experimental IBD
(Duchmann et al., 1996). Whether loss of tolerance in
IBD is broadly directed to the whole flora or only some
components of it is not clear yet, but the presence of
quantitatively and qualitatively different antibodies to
multiple microbial antigens in various patients subsets
suggests that a selected, rather than a global, loss of
tolerance occurs in IBD (Landers et al., 2002). The extent
and severity of this loss of tolerance is still being defined,
as it has been recently demonstrated that loss of tolerance
in IBD patients is not exclusive for bacterial antigens and
occurs also to orally administered soluble proteins (Kraus
et al., 2004).

Potential Mechanisms Contributing to the Loss of
Tolerance in IBD

The exact cause for the loss of tolerance in IBD is not
known. This is an extremely active area of investigation
and specific mechanisms are under consideration, some of
which will be briefly discussed.

Loss of tolerance can be viewed as an inadequate function
of immunoregulatory cells. This rather traditional view has
gained considerable strength with the identification of
specific subsets of immunoregulatory cells, among which
inducible type 1 T regulatory cells (Tr1) and spontane-
ously occurring FOXP3+CD4+CD25+ regulatory cells
figure prominently (Roncarolo et al., 2001; Bach, 2003).

There is substantial evidence, primarily from animal
models of IBD, that regulatory T cell are involved in the
control of intestinal inflammation (Toms and Powrie, 2001).
CD4+, IL-10-secreting Tr1 cells suppress antigen-specific
immune responses and actively prevents experimental
colitis (Groux et al., 1997), and CD4+CD25+ regulatory
T-cells can cure established murine colitis through
mechanisms involving cell-to-cell contact as well as IL-10
and transforming growth factor β1 (Mottet et al., 2003).
Several laboratories are presently investigating the function
of immunoregulatory T-cells in humans with IBD,
and defects in the number or activity of these cells are
likely to emerge in CD or UC patients. However, even after
detection, the true significance of immunoregulatory defects
in IBD will be difficult to interpret and fit into IBD
pathogenesis because of the intricate and mutual regulatory
networks between regulatory T-cells and other immune
cells, and the complex nature of bacterial antigen
recognition. For instance, microbial activation of TLR on
dendritic cells (DC) can block the suppressor effect of
CD4+CD25+ regulatory cells and allow a pathogen-
specific immune response to occur (Casare and Medzhitov,
2003). On other hand, CD4+CD25+ T-cells can restrain the maturation and antigen-presenting function of
DC (Misra et al., 2004), which, as mentioned below,
will affect their capacity of recognize and respond to
bacterial stimuli.

Dendritic cells are a heterogeneous population of bone
marrow-derived antigen-presenting cells that influence
essentially all aspects of innate and acquired immunity
(Liu, 2001). They sense the surrounding microbial
environment through TLR, and signaling through
different TLR generates distinct biological responses,
which vary from excitatory to suppressive. In the intestine
DC are found scattered in all lymphoid compartments,
displaying distinct properties and functions (Staag et al.,
2003), and can penetrate the space in between epithelial
cells and sample luminal bacteria that they subsequently
present to immune cells in the mucosa (Rescigno et al.,
2001). It is now generally believed that DC are critical to
the balance between tolerance and active immunity and,
because intestinal DC appear to be excessively activated
in IBD (Staag et al., 2003), it is possible that their function is skewed towards active immunity rather than
tolerance.

Due to the persistent and massive presence of the
commensal flora, intestinal epithelial cells may have
adapted by developing mechanisms that avoid activation
by TLR-dependent microbial signals with a pro-
inflammatory potential. In support of this hypothesis
recent reports show that human intestinal epithelial cells
are broadly unresponsive to ligation of TLR2, which
recognizes Gram-positive cell wall components such as
peptidoglycan and certain lipoproteins (Melmed et al.,
2003). In addition, while short term stimulation with
bacterial lipopolysaccharide or lipoteichoic acid activates
pro-inflammatory cascades in epithelial cells, prolonged
stimulation—mimicking what occurs in the intestinal
milleu—results in state of prolonged hyporesponsiveness associated with downregulation of TLR surface expression (Otte et al., 2004). Changes in bacterial flora composition or alteration of epithelial cell surface receptor expression could bypass these control mechanisms that prevent excessive activation by bacteria, and result in sustained pro-inflammatory signals blocking the development of tolerance.

A genetic defect could be at the root of the issue of broken tolerance in IBD. This, until recently, far-fetched conjecture has suddenly become a realistic possibility with the discovery of the association of CD with mutations of the NOD2/CARD15 gene on chromosome 16, the so-called IBD1 locus (Hugot et al., 2001; Ogura et al., 2001). The gene product of NOD2/CARD15 is a cytosolic protein that activates NF-κB following intracellular stimulation by bacterial products, and NOD2/CARD15 normally functions as a general sensor of peptidoglycan through the recognition of muramyl dipeptide, the minimal bioactive peptidoglycan motif common to all bacteria (Girardin et al., 2003). In view of the well established connection between recognition of bacterial products and CD pathogenesis, variants of the NOD2/CARD15 protein with impaired recognition capacity and altered down-stream signaling acquire critical importance in the development and control of gut inflammation. At first sight, given its NF-κB activating function, it is difficult to reconcile the paradox that an apparent “loss of function” of NOD2/CARD15 results in inflammation. One possibility is that the host compensates this loss of function with an excessive or protracted activation of adaptive immunity, and the other possibility is that the NOD2 protein does not necessarily behave a pro-inflammatory molecule (Girardin et al., 2003). Two recent reports lend support to the second possibility. Normal NOD2-expressing epithelial cells are resistant to invasion by Salmonella typhimurium, while those carrying the CD mutations of NOD2 are unable to constrain bacterial growth, suggesting that NOD2/CARD15 is a component of innate immunity responses to luminal bacteria acting as an anti-bacterial factor (Hisamatsu et al., 2003). Moreover, NOD2-deficient mice or carrying a CD-like CARD15 mutation exhibit increased TLR2-mediated activation of NF-κB and excessive Th1 responses which may trigger inflammation (Watanabe et al., 2004).

Finally, infections can break T-cell tolerance (Rocken et al., 1992), although epidemiological or clinical evidence that infections precede the appearance of IBD is largely anecdotal. Nevertheless, the enormous bacterial load of the intestinal lumen may itself play an important role in breaking tolerance. In fact, recent studies show that sustained exposure to bacterial antigen induces down-regulation of the TCR ζ chain and impaired T-cell function (Bronstein-Sitton et al., 2003), and activation of autoantibodies.

FIGURE 1  Schematic diagram of autoimmune and immune-mediated events in IBD pathogenesis. **Left:** Self-antigens derived from intestinal epithelial cells (hexagons), neutrophils (diamonds), and other host cells are internalized and processed by antigen-presenting cells (APC), and presented to B-cells which produce autoantibodies such as epithelial cell-associated components (ECAC), pANCA, lymphocytoxic antibodies, anti-pancreas antibodies, etc. **Right:** Loss of tolerance to the commensal autologous flora results in an enhanced reactivity against gut bacterial antigens and the inappropriate activation of effector CD4+ helper T-cells which induce macrophage activation and production of pro-inflammatory cytokines. Possible causes for the loss of tolerance include infections, excessive dendritic cell (DC) stimulation by the gut flora, inadequate regulatory T-cell function, or genetic factors, such as the CD-associated NOD2/CARD15 variants, that affect both epithelial and immune cell function.
antigen-presenting cells by microbial products via TLR breaks self tolerance and can induce autoimmune disease (Waldner et al., 2004).

CONCLUSIONS

Based on the available information, it seems fair to conclude that immune-mediated appear to be more important than autoimmune phenomena as overall pathogenic mechanisms of IBD. It seems also fair to conclude that some difference probably exists in regard to the relative contribution of autoimmune vs. immune-mediated phenomena in each form of IBD, since there is reasonable evidence for a role of autoreactivity against colonic epithelial cells in UC, whereas immune reactivity against intestinal flora is the prominent feature of CD (Fig. 1). However, simply addressing the contribution of the immune system to IBD pathogenesis, no matter in how many exquisite details, is unlikely to provide answers to the fundamental question of why these chronic inflammatory disorders have appeared in the last century and their prevalence and incidence continue to rise worldwide, affecting now populations where CD and UC were essentially unknown until a few decades ago. All chronic inflammatory diseases of unknown origin incorporate, in addition to immune dysregulation, genetic predisposition and environmental factors in their mechanisms of emergence (Ermann and Fatham, 2001). Unfortunately, the contribution of genes to disease development is still not amenable to therapeutic intervention, and returning our increasingly “clean” environment back to a “dirty” one, as proposed by the hygiene hypothesis (Bach, 2002), is not practically feasible. Thus, even though an immunotherapeutic approach to IBD addresses the mechanisms rather the cause of disease, the continued investigation of autoimmune and immune-mediated phenomena in IBD is the best hope to gain a therapeutic handling on these devastating conditions, but this must be done in parallel with a better understanding of the interactions, both symbiotic and pathologic, that occur between mucosal immunity and the commensal enteric flora.

References


Submit your manuscripts at http://www.hindawi.com