

Robin G. Lorenz · Vance J. McCracken · Charles O. Elson

## Animal models of intestinal inflammation: ineffective communication between coalition members

Received: 20 March 2005 / Accepted: 20 April 2005 / Published online: 19 July 2005  
© Springer-Verlag 2005

**Abstract.** The microbiota, epithelial cells, and mucosal immune cells in the intestine comprise an important gastrointestinal coalition. The intestinal microbiota can exert both beneficial as well as deleterious effects on their animal hosts. They interact with the innate defenses provided by epithelial cells through microbial recognition receptors. This communication, under normal conditions, results in a state of controlled inflammation. This article will focus on several animal models of intestinal inflammation, in which spontaneous or induced mutations or other genetic manipulations result in severe alterations in one of the members of the gastrointestinal coalition. These animal models of colitis have shown that alterations in communication between members of this coalition ultimately lead to gastrointestinal disease.

---

### Introduction

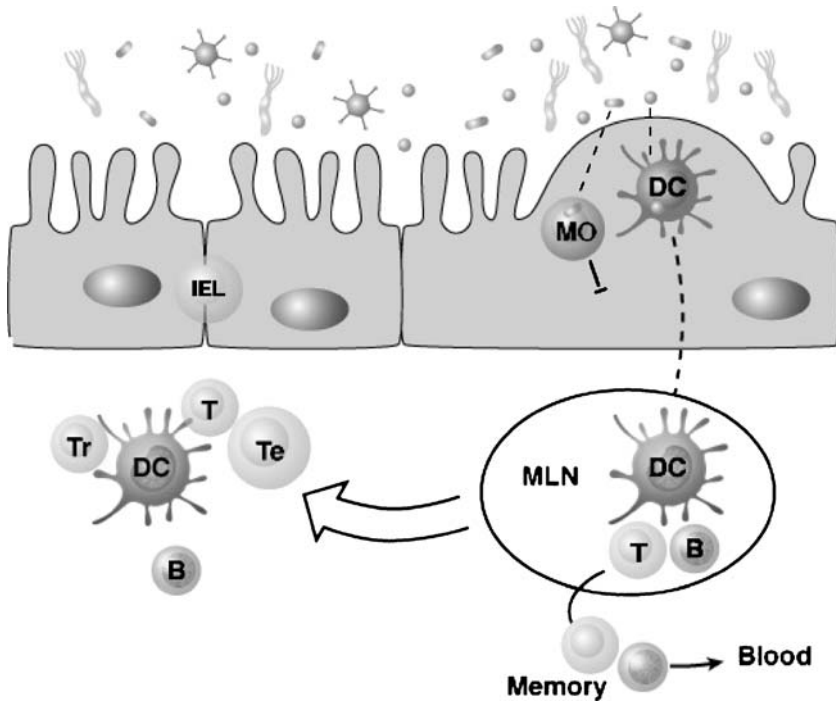
The adult gastrointestinal (GI) tract comprises a stable coalition between the resident microbiota, the epithelial barrier, and immune cells (Fig. 1). All three components are essential for functional and developmental maturity of the GI system. The mammalian GI tract harbors a dense and diverse community of bacteria, containing up to  $10^{14}$  CFU of bacteria, some ten times the number of host cells [1]. The majority of these microorganisms cannot be

---

R. G. Lorenz · V. J. McCracken  
Department of Pathology, The University of Alabama at Birmingham, Birmingham, AL 35294, USA

C. O. Elson  
Department of Medicine, The University of Alabama at Birmingham, Birmingham, AL 35294, USA

C. O. Elson (✉)  
Division of Gastroenterology and Hepatology, The University of Alabama at Birmingham,  
633 Zeigler Research Building, 703 S. 19th Street, Birmingham, AL 35294-0007, USA  
e-mail: coelson@uab.edu · Tel.: +1-205-9346060 · Fax: +1-205-9348493



**Fig. 1** Microbiota–epithelial–immune coalition. Enteric bacteria and their products can act on epithelial cells to alter epithelial gene expression and function. The epithelial cells in turn interact with immune cells within the epithelial layer and in the lamina propria. These epithelial and immune cells can influence the microbiota through the production of cytokines and immunoglobulins such as IgA. The mucosal immune response to the enteric bacteria is comprised of both regulatory T cells (*Tr*) and effector T cells (*Te*). In health, the *Tr* population is dominant, and thus the intestine is in a state of controlled inflammation. Abnormal epithelial function, abnormal innate immune cell function, deficient *Tr* function, or excessive *Te* function can all result in chronic intestinal inflammation

conventionally cultured and are only identifiable by molecular methods [2, 3]. The resident microbiota have been described as a virtual organ with a metabolic activity in excess of the liver and a collective genome (microbiome) much larger than its host [4]. The host is protected from the resident intestinal microbiota by the physical and functional barriers formed by the GI epithelium [5, 6]. These barriers are reinforced by the innate and adaptive mucosal immune system. If any component of the coalition is missing, or genetically or environmentally altered, the precarious coalition is broken and intestinal inflammation ensues.

It is apparent that bacteria exert beneficial as well as deleterious effects on their animal hosts. Recent studies on germfree mice and zebrafish have shown that the intestinal microbiota modulate a wide range of gastrointestinal functions, such as nutrient processing and absorption, maturation of the intestine, and stimulation of the mucosal immune system [7, 8]. The physical barrier is initially established in humans within 48 h of birth through membrane closure, which limits systemic exposure to antigens [9]. Luminal IgA also helps to protect

against mucosal penetration by commensals [10]. Innate defenses produced by intestinal epithelial cells include mucins and a variety of antimicrobial compounds, such as lysozyme and defensins. These molecules directly inhibit bacterial growth and protect the epithelium by preventing microbial adhesion and by forming a layer of antimicrobial peptides and secretory antibody close to the epithelial surface [11, 12]. Additionally, epithelial cells secrete several cytokines, including interleukin-1 $\alpha$  (IL-1 $\alpha$ ), IL-1 $\beta$ , IL-6, IL-8, IL-10, monocyte chemoattractant protein-1 (MCP-1), granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and transforming growth factor- $\beta$  (TGF- $\beta$ ) [13]. Epithelial cells are also able to sense the intestinal microbiota due to their expression of several microbial sensors including toll-like receptor (TLR)2, TLR4, TLR5, and TLR9 [14, 15].

Although epithelial cells and innate immune cells have microbial recognition receptors and can respond vigorously to invasive bacteria, they exhibit little or no response to the abundant intestinal microbiota. This is despite the commensals expressing many of the same TLR ligands expressed by pathogenic bacteria. The cellular and molecular mechanisms responsible for this limited, noninflammatory host immune response to the microbiota are being identified by use of new genetic technologies to mutate or insert genes into mice. This technology has been used to generate large numbers of “knockout” and transgenic lines. Some of these mutants develop colitis as they age, indicating that the gene involved plays an important role in intestinal homeostasis. Mutations that affect either the epithelium or the innate or adaptive immune system are most common, supporting the importance of the microbial–epithelial–immune coalition in mucosal homeostasis. Most of these mutant mouse strains demonstrate pathogenic Th1 cell responses to the microbiota, which results in colonic inflammation and tissue damage. In virtually every instance, such colitis does not occur if the mice are raised under germfree conditions, but colitis returns if such mutant germfree mice are administered a normal commensal microbiota or given selected microbes. Transfer of colitogenic memory T cells from a conventional mouse into a germfree mouse does not result in colitis unless the germfree mouse is colonized with commensal bacteria [16].

This article will focus on multiple animal models of intestinal inflammation that have predominant alterations in one of the members of the gastrointestinal coalition: the intestinal microbiota, the epithelial cells, or the mucosal immune cells. These alterations in communication between members of this coalition ultimately lead to gastrointestinal disease.

---

## Microbes

One of the barriers to investigation of the interaction of the host with the microbiota is the latter’s sheer complexity, comprising an estimated 500–2,000 species. The aggregate number of genes associated with the microbiota, the “microbiome,” has been estimated at 2–4 million genes [4]. These estimates are based on newer molecular methods that can identify microbes without requiring their cultivation *in vitro* and on data from sequencing the entire genome of selected bacteria, including commensals. The most common approach to identification uses PCR amplification and then sequencing of the 16S ribosomal DNA that is present in all microbes. This technique was first used to identify microbes in soil and in the sea but is being applied also to analysis of the intestinal microbiota. The same types of bacteria are represented in the microbiota of both humans and mice. In both species, most of the bacteria are anaerobes and are numerically dominated by *Clostridia* spp., particularly

*Clostridia coccoides* group, and *Bacteroides* spp. [3, 17]. These bacteria almost certainly interact with one another in complex ways and form biofilms within the gut as do microbes in the mouth. Some reside in the lumen, others in mucus blanket over the epithelium, and yet others like *Helicobacter* spp. down in the crypts below the mucus layer [18]. Some species, such as segmented filamentous bacteria, are clearly more immunostimulatory than others, but the factors that determine this property are as yet unknown [19, 20]. The composition of the flora appears to change during active colitis, becoming less complex, with a smaller number of microbes becoming numerically dominant. Using fluorescent in situ hybridization (FISH) with 16S rDNA oligonucleotide probes, a complex microbial biofilm has been detected on the surface of the intestinal mucosa in patients with Crohn's disease, although biofilms are not seen in normal control intestine [97]. The biofilm occurred in areas where there was no underlying mucosal inflammation, and the role of this biofilm in stimulating pathogenic immune responses is unknown. There is a belief that microbes that reside closer to the mucosa are more likely to stimulate immune responses than are microbes that inhabit the lumen, but there are no data to support this notion at present. Clearly, bacteria that reside only in the lumen can have profound effects on the intestine [8]. It is fair to say that our ignorance about the microbial flora vastly exceeds our knowledge of it.

Interactions between the microbiota and the host are lifelong. The cellular and molecular basis of these interactions are being defined by experimental models in which there is some impairment of the host, usually by induced mutations of critical genes. Many microbes sit close to the epithelium and communicate with it. For example, some commensals can prevent inflammation by altering the NF- $\kappa$ B signaling pathway in epithelial cells [21]. Intestinal microbes likely translocate across the epithelial barrier at a continuous, but low, rate and interact with cells of the innate immune system. Several studies have shown uptake of bacteria by mouse dendritic cells (DC) in the lamina propria and Peyer's patches, a process that can result in activation of the IL-23 gene in DCs [10, 22, 23]. The innate immune system is capable of dealing with such translocating bacteria as demonstrated by the ability of *RAG*<sup>-/-</sup> mice, which lack adaptive immunity, to interact with the intestinal microbiota without overt inflammation. However, in mice with intact immune systems, commensal bacteria are coated with IgA, which represents another important focus of interaction of the host with the microbiota [24]. Intestinal IgA can alter the composition of the microbiota and can limit their translocation [25]. The IgA response to the microbiota in normal mice occurs in the absence of serum IgG responses and appears to be restricted to the mucosal compartment. This might be explained by the ability of bacteria-loaded mucosal DCs to interact directly with B cells to induce IgA responses independent of T cell help [10]. Thus the "normal" murine immune response to the microbiota is comprised of mucosal innate cell and IgA responses in the absence of systemic T cell or B cell responses.

Clearly, there is a great deal of cross-talk between the microbiota, epithelial cells, and immune cells: an ongoing "conversation" if you will. As will be discussed below, abnormalities of the latter two can disrupt this normal communication and result in inflammatory bowel disease. An important but unanswered question is whether an abnormal commensal microbiota, or "dysbiosis" of the microbiota, can induce inflammation in the absence of defects in either host epithelial or immune cells.

## Epithelial cells

A thin layer of epithelial cells covers the mucosal surface of the digestive system, which is in continuous contact with the enormous populations of intestinal microbiota. One major role of this gastrointestinal epithelium is barrier function, which includes both selective intestinal permeability and regulation of host defense mechanisms at the mucosal interface. A change in epithelial barrier permeability clearly allows for an increased exposure to microbial products, as has been demonstrated in both the murine IL-10<sup>-/-</sup> model of T regulatory cell (T<sub>r</sub>) dysfunction and in mice with an epithelial abnormality secondary to the forced expression of a dominant negative N-cadherin (NCADΔ) (Table 1) [26, 27]. Expression of NCADΔ along the entire crypt–villus axis causes a loss of endogenous E-cadherin on the cell surface and a disruption of cell–cell and cell–matrix adhesion. This loss produces an inflammatory disease characterized by a transmural infiltration of lymphocytes, histiocytes, and plasma cells. This transmural colitis develops only in areas adjacent to the chimeric epithelium which possesses defective barrier function due to lack of E-cadherin function, implying that the physical breakdown of the epithelial barrier is the primary mechanism for induction of IBD. Additional features of colitis in NCADΔ mice include increased intraepithelial lymphocytes (IELs), crypt abscesses characterized by neutrophil infiltrates, depletion of goblet cells, perturbed crypt–villus architecture, Paneth cell hyperplasia, and aphthoid ulcers. These features are similar to the histopathologic features of Crohn's disease. Intriguingly, disease does not develop when the dominant negative N-cadherin is expressed in the villus epithelium alone [27].

Two alternative models of epithelial disruption due to loss of the expression of epithelial barrier proteins have also been described. The first is a model of colorectal hyperplasia and inflammation that develops after the targeted disruption of the keratin 8 gene (*mK8*<sup>-/-</sup>) [28]. Keratin 8 is a type II keratin which forms extended keratin filaments in simple epithelia. Villi in *mK8*<sup>-/-</sup> mice are elongated; however, no epithelial abnormalities are noted, as normal epithelial maturation is seen and production of mucin-producing goblet cells is maintained. The only intestinal abnormality associated with villi elongation is the presence of submucosal

**Table 1** Selected animal models of intestinal inflammation

Model	Defective coalition member	Bacterial flora driven	Strains examined; strain variation; genetic modifiers	Reference
C3H/HeJBir	Adaptive: effector	Yes	C3H/HeJ	[94]
SAMP/Yit	Adaptive: effector	Yes	SAMP1/Yit	[91]
STAT-4 Tg	Adaptive: effector	Probable	FVB/N	[90]
CD4 <sup>+</sup> , CD45RB <sup>hi</sup> transfer	Adaptive: regulation	Yes	BALB>B6	[74]
<i>IL-10</i> <sup>-/-</sup>	Epithelial and adaptive: regulation	Yes	C3H, BALB, 129>(129×B6)F1>B6	[86]
NCADΔ	Epithelial	Probable	C57BL/6	[27]
<i>mK8</i> <sup>-/-</sup>	Epithelial	Unknown	FVB/N	[28]
<i>ITF</i> <sup>-/-</sup>	Epithelial	Unknown	Undefined	[29]
<i>Mdr1a</i> <sup>-/-</sup>	Epithelial	Yes	FVB/N	[36]
DSS	Epithelial: chemical	Yes	Multiple	[44]

and mucosal inflammation; however, a coagulative liver necrosis and pericholangial fibrosis were also observed. A second model of impaired epithelial defense has been generated by the disruption of the gene encoding intestinal trefoil factor (*ITF*<sup>-/-</sup>) [29]. *ITF*<sup>-/-</sup> mice exhibit normal mucosal architecture but have an expanded proliferative compartment. There is no increase in intestinal inflammation at baseline, but *ITF*<sup>-/-</sup> mice are extremely sensitive to intestinal injury: *ITF*<sup>-/-</sup> mice exhibit impaired mucosal healing and extensive colitis and high mortality after oral administration of dextran sulfate sodium (DSS).

Although microbial products are largely prevented from gaining access to host tissues by the epithelial barrier, antigens do routinely enter by one of two pathways. In the first and better characterized scenario, the M cell, a specialized epithelial cell type, transports microbial products to an underlying aggregation of lymphoid cells within Peyer's patches, where antigen presentation and affinity maturation occur [30, 31]. The second route of entry involves transport via a transcellular epithelial pathway or less commonly via a paracellular route [32]. Microbial products entering by this route may gain access to basolateral epithelial microbial sensors, and may be taken up by antigen presenting cells in the lamina propria and presented to lamina propria T cells [31]. The physical epithelial barrier for transcellular and paracellular fluxes is tightly controlled by membrane pumps and tight junctions, respectively [33, 34]. One membrane pump that is found in very high levels in human and murine distal small intestine and colon is P-glycoprotein, the product of the multidrug-resistance gene 1a (*mdr1a*) [35]. One function of P-glycoprotein is to limit intestinal transcellular permeability of xenobiotics. Dysregulated expression of P-glycoprotein results in intestinal inflammation because P-glycoprotein-deficient mice (*mdr1a*<sup>-/-</sup>) develop spontaneous transmural colitis when maintained under specific pathogen-free conditions [36]. The inflammation is characterized by a thickening of the mucosa, crypt abscesses, and ulcerations extending to the muscular layer. The inflammation is composed of infiltrating T cells, B cells, and granulocytes. This inflammation has been described as resembling human ulcerative colitis [36]. Colitic *mdr1a*<sup>-/-</sup> mice also have elevated serum anti-flagellin responses and Th1 T cell responses to flagellin, and the colitis can be attenuated by the administration of antibiotics and accelerated by infection with *Helicobacter bilis*, illustrating the dependence on microbial factors in this murine model of IBD [37, 38]. Extensive studies of the function of P-glycoprotein as a drug transporter in the human intestine have shown that the apical surfaces of superficial columnar epithelial cells in the colon and distal small bowel express P-glycoprotein, which can pump drugs such as corticosteroids and cyclosporin [39]. Further interest in intestinal P-glycoprotein was raised when it was shown that human *MDR1* is present in a region of the human genome (7q21.1) that harbors an IBD disease susceptibility gene and that functional polymorphisms of human *MDR1* correlate with P-glycoprotein expression and activity [40]. A polymorphism recently discovered in human volunteers (C3435T) causes a significant decrease in the expression and function of P-glycoprotein, and humans bearing the C3435T polymorphism are at increased risk for developing ulcerative colitis [40, 41].

Disruption of epithelial barrier function can contribute to intestinal inflammation [42]. Barrier function includes both epithelial integrity, as well as secretion of immune modulators by intestinal epithelial cells. Disruption of the permeability barrier is common in IBD models that utilize physical agents such as DSS or trinitrobenzenesulphonic acid (TNBS), and colitis associated with IL-2 deficiency has also been shown to have abnormal epithelial barrier function [43]. The DSS colitis model is characterized by acute clinical symptoms

(diarrhea and/or grossly bloody stools) and the presence of erosions and inflammation [44]. These signs and symptoms are preceded by early crypt loss (day 3), which is not accompanied by inflammation, but is accompanied by a decrease in expression and function of P-glycoprotein and increased mucosal permeability [43, 45]. Therefore, in this model, inflammation is a secondary phenomenon. This concept is reinforced by the demonstration that acute DSS colitis does not require the presence of T cells or B cells [46]. The critical mediators of acute DSS colitis have not been completely elucidated, but it has been reported that IL-6, -12, -17, -18, TNF $\alpha$ , and the background strain of the mice all play modulating roles [47–51]. The main proposed mechanism of the action of DSS has been its toxicity for epithelial cells leading to increased colonic permeability [43].

Changes in the epithelial barrier clearly lead to an increased exposure of the mucosal immune system to intestinal microbiota, and many studies have now shown the importance of this microbiota to the initiation of murine inflammatory bowel disease [42]. However, it is now also clear that these intestinal microbiota can be recognized by the healthy gastrointestinal tract, and that this recognition is actually critical for normal steady-state conditions. Intestinal epithelial cells express several microbial sensors *in vivo* and *in vitro*, such as TLR2, -4, -5, and -9, which may play a role in the epithelial response to intestinal microbiota [14, 15, 52–54]. In the normal intestine, expression of these microbial sensors appears to be restricted to niches where exposure to microbiota would be limited. TLR4 expression is restricted to epithelial cells at the bottom of crypts, and TLR5 expression may be limited to the basolateral surface of intestinal epithelia [14, 55]. In addition, it has been reported that human intestinal epithelial cells are unresponsive to TLR2 ligands [56]. However, it has recently been reported that mice deficient in MyD88, an adaptor molecule essential for TLR-mediated signals, were extremely sensitive to the administration of DSS [57]. This same increased sensitivity to DSS colitis was also seen if wild-type animals were treated with a combination of antibiotics, indicating that the intestinal microbiota were actually protecting against colonic injury. The mechanisms by which intestinal microbiota could play a protective role are not clear; however, enterocyte function can clearly be modulated by both gram-positive and gram-negative microorganisms [58].

In the inflamed intestine, the levels and pattern of expression of TLR4 have clearly been demonstrated to change dramatically, as TLR4 RNA levels are increased and expression is no longer limited to crypt cells, but extend throughout the crypt–villus axis [14, 53, 59]. The mechanisms that drive these changes in the expression and response of epithelial microbial sensors are not clear. The importance of internalized LPS has been recently reported in intestinal epithelial cells, as prevention of LPS internalization significantly impairs recognition by epithelial cells and TLR4 clearly traffics to cytoplasmic compartments in polarized intestinal epithelium [54, 55]. In addition, the recent identification of epithelial NOD2, an intracellular protein that recognizes muramyl dipeptide, further implicates intracellular recognition of microbial products by sensors in epithelial cells as critical in IBD [60–64]. Therefore, the epithelial cell appears to play a role of interpreter in the gastrointestinal tract's coalition, listening to the signals from the microbiota and turning them into mediators that the mucosal immune system can understand.

## Immune cells

---

### Innate immunity

As mentioned previously, innate immune cells of the intestine play a major role in host interactions with the microbiota, particularly with those that translocate across the epithelium. The first encounter of such bacteria is likely to be with resident macrophages, which are abundant in the intestinal lamina propria. A recent important study of human intestinal macrophages has discovered that they are intensely phagocytic and bacteriocidal, but do not produce or release cytokines, nor do they express many cell-surface receptors and costimulatory molecules that would lead to immunity or inflammation [65]. This phenotype has been termed “inflammatory anergy.” Intestinal macrophages eat and destroy translocating bacteria in silence without issuing alarm or danger signals to the rest of the immune system in the form of inflammatory cytokines or chemokines. Interestingly, exposure of blood monocytes to the intestinal stroma can convert them to the intestinal macrophage phenotype, an effect blocked by antibody to TGF $\beta$  [65]. The benefits of inflammatory anergy in regard to translocating commensal bacteria are obvious. The macrophages could also deal with small inocula of invasive pathogens, although in this case, the epithelium would likely be sounding the alarm by releasing chemokines and cytokines. Dendritic cells are also present in the intestine, although at lower numbers. Whether intestinal dendritic cells have some form of inflammatory anergy is, at present, unknown. However, dendritic cells in the intestine or draining lymph nodes are likely to play a pivotal role in determining whether a given microbe, commensal or otherwise, results in a benign immune response or in inflammation. Mesenteric lymph node dendritic cells bearing the CD134L marker have been shown to be required to sustain pathogenic CD4<sup>+</sup> T cells that mediate chronic intestinal inflammation [66]. Thus, DCs are important not only in initiating inflammation, but also in maintaining it.

Severe impairment of innate immune function can result in intestinal inflammation, even in the absence of adaptive immunity. This has been shown in mice with deficient myeloid cell STAT3 expression and in mice that are deficient in the molecule A20, which provides important feedback signals to toll-like receptors [67–70]. In both instances, *RAG*<sup>-/-</sup> mice bearing mutations in these genes have intestinal inflammation in the absence of B cells or T cells. There are likely subtle defects in innate immunity in many other models. For example, in *IL-10*<sup>-/-</sup> mice, dendritic cells exhibit an excessive IL-12 response to TLR4 signaling [71]. We have previously shown that there are genetic modifiers in different inbred strains that can greatly affect the phenotype exhibited by *IL-10*<sup>-/-</sup> mice [72]. One of those modifiers, a colitis susceptibility gene locus on chromosome 3 (termed *Cdcs1*), has recently been found to regulate the innate immune response to TLR ligands, as well as the CD4<sup>+</sup> T cell response to microbial antigens [73]. How exactly an impaired innate immune response to microbes translates into inflammatory bowel disease is as yet unknown, but deficiency in innate immune function has been found in autoimmune diseases as well. The CARD15 mutation conferring susceptibility to Crohn’s disease in humans may be yet another instance of deficient innate responses resulting in chronic intestinal inflammation.

## Adaptive immunity: regulation

As mentioned above, local mucosal regulatory mechanisms limit the immune response to antigens of the bacterial flora. Impairment of these mechanisms results in an unrestrained effector response to intestinal bacteria and chronic intestinal inflammation. Multiple cells contribute, but CD4<sup>+</sup> T regulatory cells appear to play the predominant role in mucosal regulation. The induced mutations that result in IBD in mice are defining the critical, non-redundant pathways of mucosal immune regulation.

Subacute combined immunodeficient (SCID) mice have intact innate immunity but lack B cells and T cells. The adoptive transfer of normal CD4<sup>+</sup>, CD45RB<sup>hi</sup> T cells into SCID mice results in colitis and wasting, whereas transfer of the reciprocal CD4<sup>+</sup>, CD45RB<sup>lo</sup> does not (Table 1) [74]. Disease can be prevented by treatment with anti-IFN $\gamma$ , anti-TNF $\alpha$ , or murine IL-10 but not by administration of IL-4, consistent with the colitis being mediated by Th1 effector cells [75]. One of the most important aspects of the CD45RB transfer model is that colitis is abrogated by co-transfer of the CD4<sup>+</sup>, CD45RB<sup>lo</sup> T cell subset or of whole CD4<sup>+</sup> T cells along with the colitogenic CD4<sup>+</sup> CD45RB<sup>hi</sup> T cell subset. Prevention of colitis by this subset can be abrogated by administration of either anti-TGF $\beta$  or anti-IL10R [76, 77]. These results are consistent with the presence in the CD4<sup>+</sup>, CD45RB<sup>lo</sup> subset of regulatory population(s) producing IL-10 and TGF $\beta$ 1. Some data suggest that this regulatory population may be derived from the CD4<sup>+</sup>, CD25<sup>+</sup> “natural Treg” subset. Transfer of natural Tregs can prevent and treat colitis in this model [78]. Exogenously generated Tr1 cells producing high levels of IL-10 are also capable of inhibiting the induction of colitis in this model *in vivo* [79].

After transfer to SCID mice, both CD4<sup>+</sup>, CD45RB<sup>hi</sup> and CD4<sup>+</sup>, CD45RB<sup>lo</sup> T subsets traffic to the intestine and populate both lamina propria and intraepithelial compartments [80]. The cell surface markers that they express are typical of mucosal lymphocytes, namely,  $\alpha_E\beta_7$ <sup>hi</sup>, CD69<sup>hi</sup>, L-selectin<sup>lo</sup>, and CD45RB<sup>lo</sup>. Inhibition of cell migration to the intestine with anti- $\beta$ 7 integrin or anti-MAcCAM-1 attenuates disease [81]. IL-12 is required for disease initiation and possibly perpetuation; antibody blockade of CD40L, which is required for sustained IL-12 production, prevents colitis and ameliorates established disease [82]. When colitogenic CD4<sup>+</sup>, CD45RB<sup>hi</sup> T cells are transferred to SCID recipients with a reduced flora, or to recipients that are treated with antibiotics, the colitis is attenuated [80, 83]. These results indicate that the bacterial flora is driving the colitis. Indeed, the transferred T cells demonstrate reactivity to antigens of the bacterial flora and become oligoclonal [84, 85]. This model illustrates two important concepts, namely, that normal T cells can cause intestinal inflammation and, secondly, that these effector cells are prevented in normal mice from doing so by the presence of regulatory cells.

IL-10 deficient mice (*IL-10*<sup>-/-</sup>) develop normally; however, with age, the animals develop anemia, growth retardation, and chronic IBD [86]. The lamina propria and submucosa of affected areas is infiltrated with T cells, macrophages, neutrophils, B cells, plasma cells, and occasional multinucleated giant cells. There is an increased and aberrant expression of MHC class II on the epithelium of both small intestine and colon. Germfree *IL-10*<sup>-/-</sup> mice do not develop IBD unless they are reconstituted with a bacterial flora [87]. Anti-IFN- $\gamma$  given to young *IL-10*<sup>-/-</sup> mice attenuates their colitis, as does the administration of IL-10 or anti-IL-12p40 [88, 89]. Anti-IL-12p40 can also treat established disease in adult *IL-10*<sup>-/-</sup> mice [89]. These data are consistent with the pathogenic mechanism being an enhanced Th1 response in the mucosa due to a lack of inhibition by IL-10. This leads to macrophage activation and

overproduction of inflammatory cytokines such as IL-1, IL-6, and TNF- $\alpha$ , all of which have been demonstrated in the lesions.

#### Adaptive immunity: effector cells

Normal mucosal homeostasis is maintained by a balance between regulatory and effector cells; thus, disease also could result from excessive effector cell function that overcomes normal regulatory mechanisms. Indeed, there are several models where this appears to be the case. The clearest example of IBD due to excessive effector cell function is the STAT-4 transgenic mouse (STAT-4 tg) [90]. STAT-4 is phosphorylated (along with STAT-3) following IL-12 binding to its receptor on the surface of CD4<sup>+</sup> T cells. The phosphorylated STAT-3/STAT-4 complex translocates to the nucleus of the cell, activating the expression of genes such as IFN $\gamma$ . If T cells overexpress STAT-4, they should be more sensitive to IL-12 signaling and should have enhanced Th1 responses. To test this idea, FVB mice transgenic for STAT-4 under control of the CMV promoter were generated. STAT-4 mRNA was not increased in cells from unperturbed transgenic mice nor did such mice develop IBD. However, when the mice were immunized with DNP-KLH in CFA, transgene expression was increased in both spleen and colon, and the transgenic mice developed an unremitting colitis manifested by diarrhea, weight loss, and severe transmural inflammation with dense infiltrates of CD4<sup>+</sup> T cells expressing nuclear STAT-4 and producing IFN $\gamma$  and TNF $\alpha$ . CD4<sup>+</sup> T cells isolated from colitic STAT-4 transgenic mice proliferated and produced large amounts of IFN $\gamma$  when stimulated with lysates of intestinal bacteria and were able to transfer colitis to SCID mice. Transfer of similarly treated wild-type, nontransgenic CD4<sup>+</sup> T cells did not cause disease. STAT-4 transgenic mice should have a normal regulatory cell activity; thus, this model is the clearest example of excessive effector cell activity overwhelming endogenous regulatory mechanisms to cause disease.

Two spontaneous models of colitis have been described which have been informative. These may be due to a breakdown in communication of the coalition, but the exact mechanisms are as yet undefined. The first is the SAMP1/Yit model, which develops ileitis spontaneously at about 20 weeks of age with transmural involvement, granulomas, alterations in epithelial morphology, and in some instances perirectal fistulas [91]. The role of intestinal flora in this model has been demonstrated, as antibiotic treatment results in an inhibition of Th1 cytokine production [92]. The cytokines TNF $\alpha$  and IL-12 also appear to be involved [93]. The C3H/HeJBir strain develops colitis which is localized to the cecum and right colon of young mice [94]. This colitis is usually mild and resolves by 10–12 weeks of age. These mice exhibit an increased production of secretory IgA and have increased B cell and T cell responses to commensal bacterial antigens [95]. Adoptive transfer of bacterial antigen-activated CD4<sup>+</sup> T cells from C3H/HeJBir mice into histocompatible C3H/HeSnJ SCID recipients induces a focal colitis similar to that observed spontaneously [96]. Commensal bacterial flagellins have been recently identified as immunodominant antigens driving the pathogenic response [38]. Interestingly, about half of patients with Crohn's disease respond to these same flagellins. Further identification of the antigens and mechanisms that drive the pathogenic T cell responses to the microbiota should lead to a better understanding of approaches to treat patients with intestinal inflammation.

---

## Conclusions

The normal intestine is in a state of controlled inflammation. All three components of the GI coalition—the intestinal microbiota, the intestinal epithelium, and the immune system—are critical in this control. The epithelium forms both a physical and a functional barrier to protect the host from the resident intestinal microbiota. However, the presence of this microbiota is also critical for normal immune homeostasis to occur because the microbiota interacts with both the epithelium and innate cells of the mucosal immune system. This innate response is reinforced by the mucosal adaptive immune response to intestinal microbiota, a response that is normally dominated by regulatory T cells. The animal models discussed in this article reveal that when any member of this coalition is missing or defective, the result is intestinal inflammation.

**Acknowledgements.** This work was supported by NIH grants DK59911, DK44240, and DK60132, by RPG-99-086 from the American Cancer Society, and by the University of Alabama at Birmingham Digestive Diseases Research Development Center Grant DK064400.

---

## References

1. Berg RD (1996) The indigenous gastrointestinal microflora. *Trends Microbiol* 4:430
2. McCracken VJ, Lorenz RG (2001) The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. *Cell Microbiol* 3:1
3. Wang X, Heazlewood SP, Krause DO, Florin TH (2003) Molecular characterization of the microbial species that colonize human ileal and colonic mucosa by using 16S rDNA sequence analysis. *J Appl Microbiol* 95:508
4. Shanahan F (2002) The host–microbe interface within the gut. *Best Pract Res Clin Gastroenterol* 16:915
5. Bevins CL, Martin-Porter E, Ganz T (1999) Defensins and innate host defence of the gastrointestinal tract. *Gut* 45:911
6. Kagnoff MF, Eckmann L (1997) Epithelial cells as sensors for microbial infection. *J Clin Invest* 100:6
7. Rawls JF, Samuel BS, Gordon JI (2004) Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proc Natl Acad Sci U S A* 101:4596
8. Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI (2001) Molecular analysis of commensal host–microbial relationships in the intestine. *Science* 291:881
9. Bines JE, Walker WA (1991) Growth factors and the development of neonatal host defense. *Adv Exp Med Biol* 310:31
10. Macpherson AJ, Uhr T (2004) Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* 303:1662
11. Ouellette AJ (1999) IV. Paneth cell antimicrobial peptides and the biology of the mucosal barrier. *Am J Physiol* 277:G257
12. Magnusson KE, Stjernstrom I (1982) Mucosal barrier mechanisms. Interplay between secretory IgA (SIgA), IgG and mucins on the surface properties and association of salmonellae with intestine and granulocytes. *Immunology* 45:239
13. McCracken VJ, Gaskins HR (1999) Probiotics and the immune system. In: GW Tannock (ed) *Probiotics a critical review*. Horizon Scientific Press, Wymondham, UK, p 85
14. Ortega-Cava CF, Ishihara S, Rumi MA, Kawashima K, Ishimura N, Kazumori H, Udagawa J, Kadowaki Y, Kinoshita Y (2003) Strategic compartmentalization of Toll-like receptor 4 in the mouse gut. *J Immunol* 170:3977
15. Gewirtz AT, Navas TA, Lyons S, Godowski PJ, Madara JL (2001) Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. *J Immunol* 167:1882

16. Veltkamp C, Tonkonogy SL, De Jong YP, Albright C, Grenther WB, Balish E, Terhorst C, Sartor RB (2001) Continuous stimulation by normal luminal bacteria is essential for the development and perpetuation of colitis in Tg(epsilon26) mice. *Gastroenterology* 120:900
17. Kibe R, Sakamoto M, Hayashi H, Yokota H, Benno Y (2004) Maturation of the murine cecal microbiota as revealed by terminal restriction fragment length polymorphism and 16S rRNA gene clone libraries. *FEMS Microbiol Lett* 235:139
18. Swidinski A, Loening-Baucke V, Lochs H, Hale LP (2005) Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. *World J Gastroenterol* 11:1131
19. Klaasen HL, Van der Heijden PJ, Stok W, Poelma FG, Koopman JP, Van den Brink ME, Bakker MH, Eling WM, Beynen AC (1993) Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. *Infect Immun* 61:303
20. Umesaki Y, Okada Y, Matsumoto S, Imaoka A, Setoyama H (1995) Segmented filamentous bacteria are indigenous intestinal bacteria that activate intraepithelial lymphocytes and induce MHC class II molecules and fucosyl asialo GM1 glycolipids on the small intestinal epithelial cells in the ex-germ-free mouse. *Microbiol Immunol* 39:555
21. Neish AS (2004) Bacterial inhibition of eukaryotic pro-inflammatory pathways. *Immunol Res* 29:175
22. Niess JH, Brand S, Gu X, Landsman L, Jung S, McCormick BA, Vyas JM, Boes M, Ploegh HL, Fox JG, Littman DR, Reinecker HC (2005) CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 307:254
23. Becker C, Wirtz S, Blessing M, Pirhonen J, Strand D, Bechthold O, Frick J, Galle PR, Autenrieth I, Neurath MF (2003) Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells. *J Clin Invest* 112:693
24. van der Waaij LA, Kroese FG, Visser A, Nelis GF, Westerveld BD, Jansen PL, Hunter JO (2004) Immunoglobulin coating of faecal bacteria in inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 16:669
25. Fagarasan S, Muramatsu M, Suzuki K, Nagaoka H, Hiai H, Honjo T (2002) Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. *Science* 298:1424
26. Madsen KL, Malfair D, Gray D, Doyle JS, Jewell LD, Fedorak RN (1999) Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. *Inflamm Bowel Dis* 5:262
27. Hermiston ML, Gordon JI (1995) Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. *Science* 270:1203
28. Baribault H, Penner J, Iozzo RV, Wilson-Heiner M (1994) Colorectal hyperplasia and inflammation in keratin 8-deficient FVB/N mice. *Genes Dev* 8:2964
29. Mashimo H, Wu DC, Podolsky DK, Fishman MC (1996) Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. *Science* 274:262
30. Owen RL (1999) Uptake and transport of intestinal macromolecules and microorganisms by M cells in Peyer's patches—a personal and historical perspective. *Semin Immunol* 11:157
31. McGhee JR, Lamm ME, Strober W (1999) Mucosal immune responses: an overview. In: Ogra PL (ed) *Mucosal immunology*. Academic, San Diego, p 485
32. Perdue MH (1999) Mucosal immunity and inflammation. III. The mucosal antigen barrier: cross talk with mucosal cytokines. *Am J Physiol* 277:G1
33. Baumgart DC, Dignass AU (2002) Intestinal barrier function. *Curr Opin Clin Nutr Metab Care* 5:685
34. Berkes J, Viswanathan VK, Savkovic SD, Hecht G (2003) Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation. *Gut* 52:439
35. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A* 84:7735
36. Panwala CM, Jones JC, Viney JL (1998) A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, *mdr1a*, spontaneously develop colitis. *J Immunol* 161:5733
37. Maggio-Price L, Shows D, Waggle K, Burich A, Zeng W, Escobar S, Morrissey P, Viney JL (2002) *Helicobacter bilis* infection accelerates and H. hepaticus infection delays the development of colitis in multiple drug resistance-deficient (*mdr1a*<sup>-/-</sup>) mice. *Am J Pathol* 160:739
38. Lodes MJ, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, Fort M, Hershberg RM (2004) Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest* 113:1296

39. Ho GT, Moodie FM, Satsangi J (2003) Multidrug resistance 1 gene (P-glycoprotein 170): an important determinant in gastrointestinal disease? *Gut* 52:759
40. Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, John A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, Brinkmann U (2000) Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci U S A* 97:3473
41. Schwab M, Schaeffeler E, Marx C, Fromm MF, Kaskas B, Metzler J, Stange E, Herfarth H, Schoelmerich J, Gregor M, Walker S, Cascorbi I, Roots I, Brinkmann U, Zanger UM, Eichelbaum M (2003) Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. *Gastroenterology* 124:26
42. Strober W, Fuss IJ, Blumberg RS (2002) The immunology of mucosal models of inflammation. *Annu Rev Immunol* 20:495
43. Kitajima S, Takuma S, Morimoto M (1999) Changes in colonic mucosal permeability in mouse colitis induced with dextran sulfate sodium. *Exp Anim* 48:137
44. Cooper HS, Murthy SN, Shah RS, Sedergran DJ (1993) Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* 69:238
45. Iizasa H, Genda N, Kitano T, Tomita M, Nishihara K, Hayashi M, Nakamura K, Kobayashi S, Nakashima E (2003) Altered expression and function of P-glycoprotein in dextran sodium sulfate-induced colitis in mice. *J Pharm Sci* 92:569
46. Dieleman LA, Ridwan BU, Tennyson GS, Beagley KW, Bucy RP, Elson CO (1994) Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. *Gastroenterology* 107:1643
47. Mahler M, Bristol IJ, Leiter EH, Workman AE, Birkenmeier EH, Elson CO, Sundberg JP (1998) Differential susceptibility of inbred mouse strains to dextran sulfate sodium-induced colitis. *Am J Physiol* 274:G544
48. Takagi H, Kanai T, Okazawa A, Kishi Y, Sato T, Takaishi H, Inoue N, Ogata H, Iwao Y, Hoshino K, Takeda K, Akira S, Watanabe M, Ishii H, Hibi T (2003) Contrasting action of IL-12 and IL-18 in the development of dextran sodium sulphate colitis in mice. *Scand J Gastroenterol* 38:837
49. Naito Y, Takagi T, Handa O, Ishikawa T, Nakagawa S, Yamaguchi T, Yoshida N, Minami M, Kita M, Imanishi J, Yoshikawa T (2003) Enhanced intestinal inflammation induced by dextran sulfate sodium in tumor necrosis factor-alpha deficient mice. *J Gastroenterol Hepatol* 18:560
50. Naito Y, Takagi T, Uchiyama K, Kuroda M, Kokura S, Ichikawa H, Yanagisawa R, Inoue K, Takano H, Satoh M, Yoshida N, Okanoue T, Yoshikawa T (2004) Reduced intestinal inflammation induced by dextran sodium sulfate in interleukin-6-deficient mice. *Int J Mol Med* 14:191
51. Ogawa A, Andoh A, Araki Y, Bamba T, Fujiyama Y (2004) Neutralization of interleukin-17 aggravates dextran sulfate sodium-induced colitis in mice. *Clin Immunol* 110:55
52. Akhtar M, Watson JL, Nazli A, McKay DM (2003) Bacterial DNA evokes epithelial IL-8 production by a MAPK-dependent, NF-kappaB-independent pathway. *FASEB J* 17:1319
53. Cario E, Podolsky DK (2000) Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun* 68:7010
54. Cario E, Brown D, McKee M, Lynch-Devaney K, Gerken G, Podolsky DK (2002) Commensal-associated molecular patterns induce selective toll-like receptor-trafficking from apical membrane to cytoplasmic compartments in polarized intestinal epithelium. *Am J Pathol* 160:165
55. Hornef MW, Normark BH, Vandewalle A, Normark S (2003) Intracellular recognition of lipopolysaccharide by toll-like receptor 4 in intestinal epithelial cells. *J Exp Med* 198:1225
56. Melmed G, Thomas LS, Lee N, Tesfay SY, Lukasek K, Michelsen KS, Zhou Y, Hu B, Arditi M, Abreu MT (2003) Human intestinal epithelial cells are broadly unresponsive to Toll-like receptor 2-dependent bacterial ligands: implications for host-microbial interactions in the gut. *J Immunol* 170:1406
57. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 118:229
58. Otte JM, Podolsky DK (2004) Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. *Am J Physiol Gastrointest Liver Physiol* 286:G613
59. Cario E, Rosenberg IM, Brandwein SL, Beck PL, Reinecker HC, Podolsky DK (2000) Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. *J Immunol* 164:966
60. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nunez G, Cho JH (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 411:603

61. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 411:599
62. Hampe J, Cuthbert A, Croucher PJ, Mirza MM, Mascheretti S, Fisher S, Frenzel H, King K, Hasselmeier A, MacPherson AJ, Bridger S, van Deventer S, Forbes A, Nikolaus S, Lennard-Jones JE, Foelsch UR, Krawczak M, Lewis C, Schreiber S, Mathew CG (2001) Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. *Lancet* 357:1925
63. Ogura Y, Saab L, Chen FF, Benito A, Inohara N, Nunez G (2003) Genetic variation and activity of mouse Nod2, a susceptibility gene for Crohn's disease. *Genomics* 81:369
64. Ogura Y, Lala S, Xin W, Smith E, Dowds TA, Chen FF, Zimmermann E, Tretiakova M, Cho JH, Hart J, Greenson JK, Keshav S, Nunez G (2003) Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis. *Gut* 52:1591
65. Smythies LE, Sellers M, Clements RH, Mosteller-Barnum M, Meng G, Benjamin WH, Orenstein JM, Smith PD (2005) Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. *J Clin Invest* 115:66
66. Malmstrom V, Shipton D, Singh B, Al-Shamkhani A, Puklavec MJ, Barclay AN, Powrie F (2001) CD134L expression on dendritic cells in the mesenteric lymph nodes drives colitis in T cell-restored SCID mice. *J Immunol* 166:6972
67. Welte T, Zhang SS, Wang T, Zhang Z, Hesslein DG, Yin Z, Kano A, Iwamoto Y, Li E, Craft JE, Bothwell AL, Fikrig E, Koni PA, Flavell RA, Fu XY (2003) STAT3 deletion during hematopoiesis causes Crohn's disease-like pathogenesis and lethality: a critical role of STAT3 in innate immunity. *Proc Natl Acad Sci U S A* 100:1879
68. Alonzi T, Newton IP, Bryce PJ, Di Carlo E, Lattanzio G, Tripodi M, Musiani P, Poli V (2004) Induced somatic inactivation of STAT3 in mice triggers the development of a fulminant form of enterocolitis. *Cytokine* 26:45
69. Boone DL, Turer EE, Lee EG, Ahmad RC, Wheeler MT, Tsui C, Hurley P, Chien M, Chai S, Hitotsumatsu O, McNally E, Pickart C, Ma A (2004) The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. *Nat Immunol* 5:1052
70. Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A (2000) Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* 289:2350
71. Ruiz PA, Shkoda A, Kim SC, Sartor RB, Haller D (2005) IL-10 gene-deficient mice lack TGF-beta/Smad signaling and fail to inhibit proinflammatory gene expression in intestinal epithelial cells after the colonization with colitogenic *Enterococcus faecalis*. *J Immunol* 174:2990
72. Farmer MA, Sundberg JP, Bristol IJ, Churchill GA, Li R, Elson CO, Leiter EH (2001) A major quantitative trait locus on chromosome 3 controls colitis severity in IL-10-deficient mice. *Proc Natl Acad Sci U S A* 98:13820
73. Beckwith J, Cong Y, Sundberg J, Elson CO, Leiter EH (2005) A colitis susceptibility gene locus regulates the CD4<sup>+</sup> T cell response to bacterial antigens. *Gastroenterology* 126:A45
74. Morrissey PJ, Charrier K, Braddy S, Liggitt D, Watson JD (1993) CD4<sup>+</sup>T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified CD4<sup>+</sup> T cells. *J Exp Med* 178:237
75. Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL (1994) Inhibition of Th1 responses prevents inflammatory bowel disease in *scid* mice reconstituted with CD45RB. *Immunity* 1:553
76. Powrie F, Carlino J, Leach MW, Mauze S, Coffman RL (1996) A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) CD4<sup>+</sup> T cells. *J Exp Med* 183:2669
77. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F (1999) An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med* 190:995
78. Asseman C, Read S, Powrie F (2003) Colitogenic Th1 cells are present in the antigen-experienced T cell pool in normal mice: control by CD4<sup>+</sup> regulatory T cells and IL-10. *J Immunol* 171:971
79. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG (1997) A CD4<sup>+</sup> T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389:737
80. Aranda R, Sydora BC, McAllister PL, Binder SW, Yang HY, Targan SR, Kronenberg M (1997) Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4<sup>+</sup>, CD45RBhigh T cells to SCID recipients. *J Immunol* 158:3464

81. Picarella D, Hurlbut P, Rottman J, Shi X, Butcher E, Ringler DJ (1997) Monoclonal antibodies specific for beta 7 integrin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) reduce inflammation in the colon of scid mice reconstituted with CD45RBhigh CD4<sup>+</sup> T cells. *J Immunol* 158:2099
82. De Jong YP, Comiskey M, Kalled SL, Mizoguchi E, Flavell RA, Bhan AK, Terhorst C (2000) Chronic murine colitis is dependent on the CD154/CD40 pathway and can be attenuated by anti-CD154 administration. *Gastroenterology* 119:715
83. Morrissey PJ, Charrier K (1994) Induction of wasting disease in SCID mice by the transfer of normal CD4<sup>+</sup>/CD45RBhi T cells and the regulation of this autoreactivity by CD4<sup>+</sup>/CD45RBlo T cells. *Res Immunol* 145:357
84. Matsuda JL, Gapin L, Sydora BC, Byrne F, Binder S, Kronenberg M, Aranda R (2000) Systemic activation and antigen-driven oligoclonal expansion of T cells in a mouse model of colitis. *J Immunol* 164:2797
85. Brimnes J, Reimann J, Nissen M, Claesson M (2001) Enteric bacterial antigens activate CD4<sup>(+)</sup> T cells from scid mice with inflammatory bowel disease. *Eur J Immunol* 31:23
86. Lohler J, Kuhn R, Rennick D, Rajewsky K, Muller W (1995) Interleukin-10-deficient mice: a model of chronic mucosal inflammation. In: Tytgat G, Bartelsman J, van Deventer S (eds) *Inflammatory bowel disease*, vol. Falk Symposium 85. Kluwer Academic Publishers, Boston, p 410
87. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB (1998) Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 66:5224
88. Berg DJ, Davidson N, Kühn R, Müller W, Menon S, Holland G, Thompson-Snipes L (1996) Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4<sup>+</sup> TH1-like responses. *J Clin Invest* 98:1010
89. Davidson NJ, Hudak SA, Lesley RE, Menon S, Leach MW, Rennick DM (1998) IL-12, but not IFN-gamma, plays a major role in sustaining the chronic phase of colitis in IL-10-deficient mice. *J Immunol* 161:3143
90. Wirtz S, Finotto S, Kanzler S, Lohse AW, Blessing M, Lehr HA, Galle PR, Neurath MF (1999) Cutting edge: chronic intestinal inflammation in STAT-4 transgenic mice: characterization of disease and adoptive transfer by TNF- plus IFN-gamma-producing CD4<sup>+</sup> T cells that respond to bacterial antigens. *J Immunol* 162:1884
91. Matsumoto S, Okabe Y, Setoyama H, Takayama K, Ohtsuka J, Funahashi H, Imaoka A, Okada Y, Umesaki Y (1998) Inflammatory bowel disease-like enteritis and caecitis in a senescence accelerated mouse P1/Yit strain. *Gut* 43:71
92. Bamas G, Marini M, Moskaluk CA, Odashima M, Ross WG, Rivera-Nieves J, Cominelli F (2002) Down-regulation of intestinal lymphocyte activation and Th1 cytokine production by antibiotic therapy in a murine model of Crohn's disease. *J Immunol* 169:5308
93. Pizarro TT, Arseneau KO, Bamas G, Cominelli F (2003) Mouse models for the study of Crohn's disease. *Trends Mol Med* 9:218
94. Sundberg JP, Elson CO, Bedigian H, Birkenmeier EH (1994) Spontaneous, heritable colitis in a new substrain of C3H/HeJ mice. *Gastroenterology* 107:1726
95. Brandwein SL, McCabe RP, Cong Y, Waites KB, Ridwan BU, Dean PA, Ohkusa T, Birkenmeier EH, Sundberg JP, Elson CO (1997) Spontaneously colitic C3H/HeJBir mice demonstrate selective antibody reactivity to antigens of the enteric bacterial flora. *J Immunol* 159:44
96. Cong Y, Brandwein SL, McCabe RP, Lazenby A, Birkenmeier EH, Sundberg JP, Elson CO (1998) CD4<sup>+</sup> T cells reactive to enteric bacterial antigens in spontaneously colitic C3H/HeJBir mice: increased T helper cell type 1 response and ability to transfer disease. *J Exp Med* 187:855
97. Swidsinski A, Ladhoff A, Perntaler A, Swidsinski S, Loening-Baucke V, Ortner M, Weber J, Hoffmann U, Schreiber S, Dietel M, Lochs H (2002) Mucosal flora in inflammatory bowel disease. *Gastroenterology* 122:44