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# A Multihit Model: Colitis Lessons from the Interleukin-10-deficient Mouse

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**Abstract:** Complex mechanisms are pulling the strings to initiate the development of inflammatory bowel disease. Current evidence indicates that an interaction of genetic susceptibilities (polymorphisms), environmental factors, and the host microbiota leads to a dysregulation of the mucosal immune system. In the past decades, the interleukin-10-deficient mouse has served as an excellent model to mirror the multifactorial nature of this disease. Here, we want to review in detail the interplay of the genetic factors, immune aspects, and especially summarize and discuss the role of the microbiota contributing to colitis development in the interleukin-10-deficient mouse model of inflammatory bowel disease as a multihit model.

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**Key Words:** Colitis, disease susceptibility, IBD, interleukin-10-deficient mouse, microbiota, microflora

Homeostasis in the gut relies on a fine-tuned interplay of the host's protective immunity and the microbiota that generate the intestinal environment.<sup>1</sup> Disruption of this homeostasis can lead to intestinal disorders such as inflammatory bowel disease (IBD). IBD is a chronic inflammatory disease of the gastrointestinal tract that encompasses Crohn's disease (CD) and ulcerative colitis. Epidemiological studies indicate that IBD affects more than 3.6 million people and that the number of cases has increased over recent years.<sup>2–4</sup> Symptoms of IBD include primarily diarrhea, abdominal pain, and rectal bleeding. However, there are frequent extraintestinal manifestations that may affect the skin, joints, eyes, bones, and the hepatobiliary tract.<sup>5</sup> The etiology of IBD still remains unknown although the current perception is that a genetically determined abnormal response of the mucosal immune system against the microorganisms of the normal intestinal flora leads to inflammation.<sup>6</sup> However, although a genetic susceptibility seems to be the root cause for IBD development, genetic factors seem to explain only a certain percentage of the disease outcome. A total of 163 susceptibility loci have been identified so far, but even this huge quantity of loci has been

postulated to explain only a minority of the disease risk variance suggesting that other factors contribute substantially to IBD pathogenesis.<sup>7</sup> These factors may include innate, adaptive, and environmental mechanisms that add on a large, yet to be defined, scale to the development and course of IBD. Recent efforts to understand the complex nature of IBD development focused especially on the contribution of the presence and composition of the host microbiota.<sup>8</sup> In this context, animal models of IBD have served as valuable tools to systematically determine and analyze the components pivotal for the development of this complex multifactorial disease.

## INTERLEUKIN-10-DEFICIENT MOUSE MODEL OF IBD

A genetically engineered model that has been extensively used to dissect IBD etiology is the interleukin-10 (IL-10)-deficient mouse, which was generated in 1993 by Kühn et al.<sup>9</sup> The generation was performed by use of targeted mutation disrupting the IL-10 gene by replacing a 500 base pair fragment of exon 1 with a termination codon and a neo expression cassette and by introducing a termination codon into exon 3 (*IL10<sup>tm1Cgn</sup>, IL10<sup>-/-</sup>*). The spontaneous onset of gut inflammation after weaning in mice with this mutation may be reversed by treatment with recombinant IL-10.<sup>10</sup> Colitis in IL-10-deficient mice is characterized by histological findings that are similar to those of human IBD.<sup>9,11</sup> In detail, the discontinuous and transmural inflammatory lesions are characterized by inflammatory cell infiltrates into the lamina propria and submucosa, epithelial hyperplasia, mucin depletion, crypt abscesses, ulcers, and thickening of the intestinal wall.<sup>11,12</sup> Colitis development in mutated mice develops shortly after weaning and originates from the fact that IL-10 is a key mediator of gut homeostasis.

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## MECHANISM OF IL-10 DEFICIENCY

IL-10 is an immunoregulatory cytokine that is essential for the maintenance of intestinal homeostasis and that can be secreted by a wide range of immune cells such as macrophages, dendritic cells, and T cells (Fig. 1). It suppresses effector functions of Th1/Th17 cells as well as NK cells and macrophages, thereby modulating the cellular immune response.<sup>13–15</sup> Subsequently, colitis in IL-10-deficient mice stems from an aberrant response of CD4<sup>+</sup> Th1-like T cells and an excessive secretion of the proinflammatory cytokines IL-12, IL-17, and IFN $\gamma$ .<sup>12,16</sup> Although originally believed to be a purely Th1-mediated inflammation, Th17 cells were later on shown to participate in the inflammatory immune response.<sup>17</sup> IL-12 was believed to be crucial for colitis induction as mice deficient for both IL-10 and IL-12p40 did not develop intestinal pathology and treatment of colitic IL-10-deficient mice with a monoclonal antibody directed against the IL-12p40 subunit's reduced intestinal inflammation.<sup>18</sup> However, the finding that the IL-12p40 subunit can also assemble to form the cytokine IL-23 has led to a reassessment regarding the contribution of IL-12 and IL-23 to inflammation development.<sup>19</sup> Several studies have now indicated that the T-cell-mediated colitis of IL-10-deficient mice also depends on IL-23 promoting inflammation through IL-17 and IL-6, underlining the fact that several mechanisms may

contribute to colitis development.<sup>17,20,21</sup> In this context, it has recently been demonstrated that colitis and intestinal inflammation in IL-10-deficient mice also depend on an IL-13R $\alpha$ 2-mediated attenuation of IL-13 activity, highlighting a role for IL-13 and its decoy receptor in the regulation of the Th1/Th17-mediated inflammation in IL-10-deficient mice.<sup>22</sup> Interestingly, a recent study found evidence that the critical balance of the IL-10- and IL-12/23-seesaw is governed by the PI3K subunit p110 $\delta$ , which controls the homeostatic interaction of antigen-presenting cells and T cells, and p110 $\delta$ -deficient mice have also been shown to be prone to spontaneously occurring Th1- and Th17-mediated colitis.<sup>23,24</sup> Therefore, it has been speculated that one mechanism responsible for the excessive inflammatory responses in IL-10 deficiency may be due to loss of induction of p110 $\delta$ .<sup>23</sup>

## REGULATORY INFLUENCE OF IL-10 IN THE INTESTINAL INNATE AND ADAPTIVE IMMUNE RESPONSE

Immunologic tolerance to bacterial antigens in the intestine is primarily mediated by IL-10 and TGF $\beta$  produced by regulatory T cells.<sup>8,25</sup> This is demonstrated by the finding that monoassociation of IL-10-deficient mice with *Enterococcus faecalis* led to chronic colitis and induced persistent NF- $\kappa$ B activation as well as

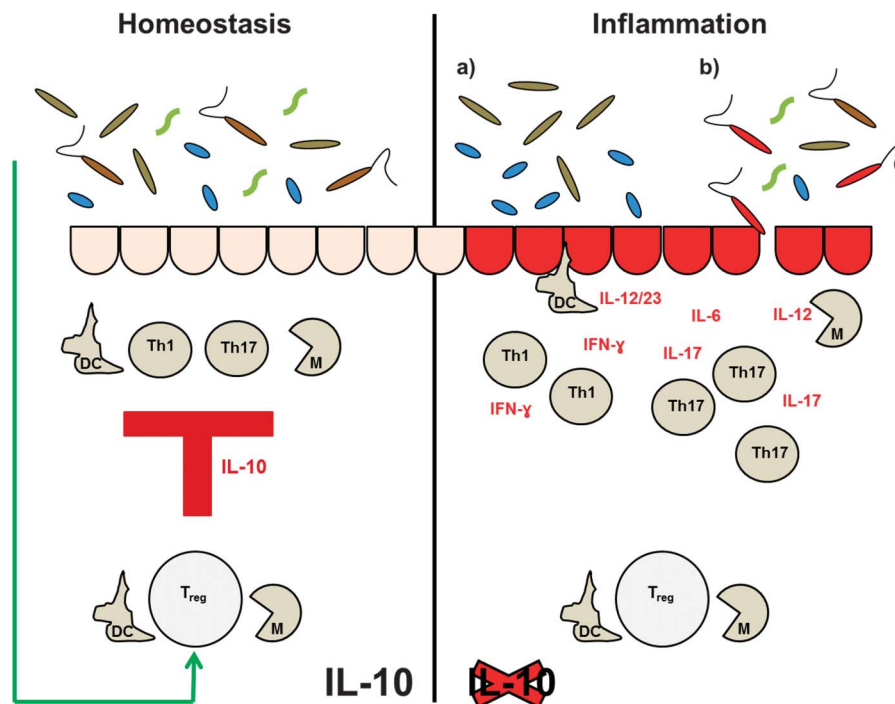


FIGURE 1. IL-10 maintains intestinal homeostasis (left side). It is produced by dendritic cells (DC) and macrophages (M), but the main source of IL-10 are T<sub>reg</sub> cells. Effector functions of the innate immune response (DC and M) and of the adaptive immune response (Th1/Th17 cells) are sufficiently suppressed by these cells through IL-10. Interestingly, the intestinal microbiota has been shown to induce IL-10-producing T<sub>reg</sub> cells in an effort to establish a mutually beneficial commensalism. In IL-10 deficiency (right side), the response of Th1/Th17 cells is not counterbalanced anymore and colitis stems from an excessive secretion of proinflammatory cytokines. However, several hits have to find their target before colitis occurs. Either a misbalanced microbiota (a) dysbiosis contributes to colitis development or the presence of a specific pathogen initiates inflammation (b).

TLR2 and subsequent proinflammatory gene expression in intestinal epithelial cells. Therefore, IL-10 seems to play a substantial role in regulating bacterial-induced epithelial NF- $\kappa$ B activation.<sup>25</sup> Various studies have demonstrated that IL-10 operates on innate and on adaptive mechanisms to suppress inflammatory responses in the intestine, which we want to shortly highlight here. For a detailed overview on the control of intestinal inflammation by IL-10, see Kole and Maloy.<sup>26</sup> Briefly, IL-10 effects on innate immune responses can be attributed mainly to macrophages and dendritic cells. In the gut, macrophages and dendritic cells are a major source of IL-10, and its production is induced by means of TLR-dependent and TLR-independent pathways. IL-10 has been shown to interfere with the maturation process of dendritic cells and to block the function of mature dendritic cells. Furthermore, IL-10 terminated macrophage activation and proliferation and inhibited macrophage autophagy, thereby maintaining the anergic phenotype of macrophages and dendritic cells that is characteristic for the state of intestinal homeostasis.<sup>13,26</sup> In addition, IL-10 also seems to have protective effects on intestinal epithelial cells (IEC) as a proteome analysis of IEC derived from IL-10-deficient mice revealed defects in the response to endoplasmic reticulum (ER) stress, energy metabolism, and apoptosis.<sup>27</sup> Furthermore, IEC from IL-10-deficient mice and from patients with IBD displayed increased expression levels of the glucose-regulated ER stress protein 78, and IL-10 was shown to block inflammation-induced ER stress response mechanisms.<sup>28</sup> However, the most substantial impacts of IL-10 may be attributed to effects on the adaptive immune response especially on the control of T-cell expansion and T-cell-mediated cytokine production. The maintenance of small intestinal and colonic tolerance by IL-10-producing regulatory T ( $T_{reg}$ ) cell subsets has been nicely reviewed recently.<sup>29</sup> IL-10 may exert its effects on T cells directly by promoting the maintenance, expansion, and function of  $T_{reg}$  cells or indirectly by depriving naive T cells of their appropriate stimuli.<sup>13,26</sup> As mice with a  $T_{reg}$  cell-specific deletion of IL-10 develop spontaneous colitis,  $T_{reg}$  cells have been proposed as the critical source of IL-10 to maintain homeostasis at the environmental interface of the intestine.<sup>30</sup> However, interestingly, recent studies indicate that macrophages play a central role in gut homeostasis, and the prevention of colitis as IL-10 receptor signaling was shown to drive macrophages to express homeostatic tolerogenic functions.<sup>31–33</sup> Remarkably, protective effects of IL-10 in the gut seem to depend on microbial colonization.<sup>34</sup> The differentiation of CD4<sup>+</sup> Foxp3<sup>+</sup> IL-10-producing cells can be induced in the intestine by the microbial product of a commensal bacterium, *Bacteroides fragilis*, thereby actively inducing mucosal tolerance.<sup>35</sup> Recently, the induction of IL-10 production by indigenous *Clostridium* species and subsequent promotion of colonic  $T_{reg}$  cells was described.<sup>36</sup> Therefore, the induction of IL-10 by intestinal bacteria has been hypothesized as an important factor by which the intestinal microbiota establishes a mutually beneficial commensalism.<sup>26,37</sup> In summary, IL-10 is one of the main factors to maintain intestinal homeostasis.

Therefore, the IL-10-deficient mouse model of IBD has been used widely as a valuable tool to dissect the complex mechanisms of this disease. However, analogous to the human situation, disease development in the IL-10-deficient mouse relies on genetic and on environmental factors, eminently the composition of the microflora.

## GENETIC AND ENVIRONMENTAL FACTORS MODIFY DISEASE SUSCEPTIBILITY

A decade ago, Mähler and Leiter<sup>38</sup> elucidated that genetic and environmental factors determined the course of colitis development in the IL-10-deficient mouse. Since then, a vast number of studies were conducted that focused on the differential contribution and interplay of these factors in the IL-10-deficient mouse model of IBD. Here, we want to review these studies and our own experiments, focusing especially on the role of the microbiota contributing to colitis development.

## CONTRIBUTION OF THE GENETIC BACKGROUND TO COLITIS DEVELOPMENT IN THE IL-10-DEFICIENT MOUSE

Early on, it has been demonstrated that the genetic background of IL-10-deficient mice determines colitis susceptibility and severity. Several inbred mouse strains are highly susceptible to disease development (e.g., C3H/HeJBir and 129/SvEv), whereas others are relatively resistant (e.g., C57BL/6J and C57BL/10) indicating a significant contribution from genetic background modifiers.<sup>12,38–40</sup> C3H/HeJBir (C3Bir-*IL10*<sup>-/-</sup>) susceptibility and C57BL/6J (B6-*IL10*<sup>-/-</sup>) resistance were analyzed by the use of segregating F2 and backcross populations derived from these 2 strains laying the foundation to further dissect IBD etiology.<sup>38,40–42</sup> From an F2 analysis performed at the Jackson Laboratory and the reciprocal N2 crosses to the respective C3Bir-*IL10*<sup>-/-</sup> and B6-*IL10*<sup>-/-</sup> parental strains performed at the Hannover Medical School colitogenic quantitative trait loci (QTL) were identified and designated as *Cdcs1-10* (*Cdcs* for Cytokine deficiency-induced colitis susceptibility).<sup>41,42</sup> A major C3Bir-derived QTL, *Cdcs1*, was detected on chromosome 3 contributing to lesions in both cecum and colon and interacting epistatically or additively with multiple loci on other chromosomes.<sup>38</sup> Furthermore, *Cdcs1* was confirmed as a large effect QTL by using an F2 intercross of C3Bir-*IL10*<sup>-/-</sup> and B6-*IL10*<sup>-/-</sup> mice and lymphatic vascular changes as a trait.<sup>43</sup> As peak linkage analyses indicated the presence of more than 1 genetic modifier in this region, reciprocal *Cdcs1*-congenic strains were generated to further characterize the *Cdcs1* region.<sup>44</sup> The transfer of *Cdcs1* from the C3Bir to the B6 background rendered IL-10-deficient B6. C3Bir-*Cdcs1*-congenic mice susceptible and vice versa. Fine mapping of *Cdcs1* using a total of 15 reciprocal subcongenic lines demonstrated that this QTL contains at least 3 subintervals (*Cdcs1.1–Cdcs1.3*) with independent genetic factors that confer innate hyporesponsiveness and adaptive hyperresponsiveness.<sup>44–46</sup> Interestingly, the *Cdcs1* region has independently been identified in

several studies by the use of different colitis models and genetic backgrounds as a major locus regulating intestinal inflammation by both innate and adaptive immune mechanisms.<sup>47</sup> To assess in detail the influence of this congenic element on the adaptive immune response, functional studies are currently performed by our group.<sup>48</sup> In addition, the IL-10 model has demonstrated that even resistant backgrounds can contribute susceptibility genes once they are unmasked by segregation from major resistance loci. An example is *Cdcs6* with the candidate gene *Cd14*, whose expression level determines protection from disease.<sup>49,50</sup> Apart from the genetic background contributing to IBD development, it has early on been demonstrated that the presence and composition of the gut microbiota determines disease outcome as well. In this context, it is noteworthy that the overall histopathology scores for both the parental strain and their F1 hybrids were significantly lower in the Hannover Medical School facility than those determined for the Jackson Laboratory facility indicating that environmental factors, most likely differences in the composition of the microflora, modulated the genetic susceptibilities.<sup>38,51</sup> Notably, these differences were observed without even sanitizing these mice. Therefore, to determine environmental factors interacting with the *Cdcs1* locus, we performed embryo transfer-sanitization of C3Bir-*IL10*<sup>-/-</sup>, B6-*IL10*<sup>-/-</sup>, and the respective congenic mouse strains carrying the whole *Cdcs1* region to maintain animals in noncolitogenic specific pathogen-free and germ-free environments. Then, to dissect *Cdcs1* contribution to colitis susceptibility in interaction with environmental factors pivotal for colitis development, we used these sanitized mice for infection experiments. The outcome of these studies will be discussed in context with current literature and recent developments in the next section.

### CONTRIBUTION OF THE MICROBIOTA TO COLITIS DEVELOPMENT IN THE IL-10-DEFICIENT MOUSE

Sellon et al<sup>52</sup> demonstrated as early as 1998 that resident enteric bacteria are necessary for the development of spontaneous colitis in IL-10-deficient mice as colitis development was not observed under germ-free conditions. In addition, it was shown that the inflammatory response in both, adult and neonatal, germ-free IL-10-deficient mice is similar when colonized with a microbial flora indicating that the absence of IL-10 leads to a loss of tolerance towards bacterial antigens that is independent of the time at which microbial colonization occurs.<sup>53</sup> Furthermore, it was early on shown that antibiotic treatment may prevent or attenuate colitis development in several mouse models of IBD.<sup>54-57</sup> Treatment of neonatal IL-10-deficient mice with either neomycin/metronidazole or ciprofloxacin prevented the onset of colitis for up to 12 weeks indicating that an early alteration of the normal intestinal flora may affect colitis progression in later life.<sup>58,59</sup> The use of antibiotics with a selective aerobic or anaerobic spectrum had different therapeutic effects in various sections of the colon of IL-10-deficient mice indicating that subsets of aerobic or anaerobic bacteria show regional differences in their

capacity to mediate IL-10-deficient colitis.<sup>54</sup> That bacterial sensing by intestinal immune cells, which is essential for inflammation development in IL-10-deficient mice has also been indicated by the finding that colitis development involves TLR-induced NF- $\kappa$ B activation derived mostly from mucosal immune cells.<sup>60</sup> Furthermore, spontaneous colitis in IL-10-deficient mice requires MyD88 signaling, and IL-10<sup>-/-</sup> MyD88<sup>-/-</sup> double knockout mice do not develop colitis.<sup>61,62</sup> Interestingly, it has been demonstrated that immunostimulatory DNA sequences derived from bacterial DNA ameliorated colonic inflammation in IL-10-deficient mice, suggesting that these potent activators of innate immune responses may have a physiologic anti-inflammatory role in the intestine.<sup>63</sup> In addition, CpG motifs of bacterial DNA have been shown to exert a protective effect in mouse models of IBD by inducing an antigen-independent tolerance as the presence of a bacterial flora was not required.<sup>64</sup> However, in IL-10-deficient mice raised under germ-free or specific pathogen-free conditions the introduction of specific bacteria either exerted a protective effect or promoted inflammation depending on the bacterial species.<sup>51</sup> Briefly, members of the *Lactobacillus* and *Bifidobacterium* species attenuated colitis whereas *E. faecalis* and, in most studies, *Helicobacter* spp. exerted

**TABLE 1. Microbiota Effects in the IL-10-deficient Mouse Model of IBD**

Status	Pathogen	Effect	Reference
GF		No colitis	52
Antibiotic treatment		Prevention of colitis	58,59
	<i>Lactobacillus</i> spp	Attenuation of colitis	65,69
	<i>Bifidobacterium</i> spp	Attenuation of colitis	69
	<i>E. faecalis</i>	Proinflammatory	68,70-73
	<i>E. coli</i>	Proinflammatory	70,71
	<i>H. hepaticus</i>	No colitis induction	74,75
SPF		Proinflammatory	66,67,76
GF	<i>H. hepaticus</i>	No colitis	77
GF	<i>H. hepaticus</i> + <i>L. reuteri</i>	Proinflammatory	75
	<i>B. wadsworthia</i>	Proinflammatory	78
	<i>L. reuteri</i>	Proinflammatory	75
GF	Murine norovirus	No colitis induction	77
SPF	Murine Norovirus	Proinflammatory	77
ASF	Murine Norovirus	Proinflammatory	77
	<i>B. fragilis</i>	Proinflammatory	79
SIHUMI		Proinflammatory	80

ASF, altered Schaedler flora; GF, germ-free; SIHUMI, simplified human microbiota consortium; SPF, specific pathogen-free.



a proinflammatory effect.<sup>65–69</sup> See Table 1 for an overview on microbiota effects in IL-10-deficient mice.

However, it still remains controversial whether a specific individual pathogen induces inflammation, whether a disruption of the intestinal microbiota is the decisive factor leading to inflammation or whether these factors have to act in concert. Therefore, in general, the contribution of the microbiota towards colitis development in IL-10-deficient mice may be divided into effects due to specific (pathogenic) agents that may serve as colitogenic triggers and into effects that occur due to the general composition of the microbiota in terms of an imbalance of the commensal flora (dysbiosis).

### CONTRIBUTION OF INFECTIOUS AGENTS (OR TRADITIONAL PATHOGENS) TO COLITIS DEVELOPMENT IN THE IL-10-DEFICIENT MOUSE

Various members of the microbiota including bacteria, viruses, yeasts and fungi have been speculated as potential IBD pathogens but their specific role and the respective contexts in which they may trigger inflammation have still to be defined. It has early on been demonstrated that the (mono-) association of a susceptible mouse strain like the IL-10-deficient mouse with a single commensal bacterial species, e.g., *E. faecalis* or *Escherichia coli*, can trigger inflammation (depending as well on a susceptible genetic background). Here, however, monoassociation selectively initiated immune-mediated intestinal inflammation with different colitis phenotypes and dual association was shown to have additive effects as this induced severe, early-onset pancolitis.<sup>68,70,71</sup> Inoculation of IL-10-deficient mice with different *Enterococcus* species resulted in colonic inflammation and colonic gene expression changes similar to those detected in human IBD.<sup>72</sup> The development of chronic colitis in IL-10-deficient mice mono-colonized with *E. faecalis* has in part been attributed to the interaction of this enteric bacterium with the IEC inducing protein changes that are involved in ER stress.<sup>27</sup> In a recent study, germ-free IL-10-deficient mice were monoassociated with a probiotic *E. faecalis* and a colitogenic control strain. Proinflammatory responses were observed in both set-ups underlining the notion that even probiotic bacteria may trigger inflammation in an IBD-prone host like the IL-10-deficient mouse.<sup>73</sup> On the other hand, probiotic bacteria have also been shown to enhance the epithelial barrier function in IL-10-deficient mice when administered for 4 weeks leading to a complete normalization of intestinal barrier integrity.<sup>81</sup> Recently, another colitogenic bacterium, *Bilophila wadsworthia*, was identified in IL-10-deficient mice that were fed a diet high in saturated fat. Furthermore, the monoassociation of germ-free IL-10-deficient mice with *B. wadsworthia* also led to a proinflammatory Th1 cell-mediated colitis.<sup>78</sup> In contrast, the monoassociation of germ-free IL-10-deficient mice with *Helicobacter hepaticus*, a classical colitogenic trigger to induce colitis, does not necessarily lead to inflammation.<sup>74</sup> However, when maintained under specific pathogen-free conditions, *H.*

*hepaticus* was shown to trigger colitis development in IL-10-deficient mice.<sup>66,67</sup> On contrary, other studies could not report induction of colitis by *H. hepaticus*.<sup>74,75</sup> Mähler and Leiter already proposed in 2002 that the divergent results of *H. hepaticus* ability to induce colitis in IL-10-deficient mice may depend on the interactions of this bacterium with other members of the microbiota.<sup>38</sup> This is corroborated by the finding that, as mentioned above, germ-free IL-10-deficient mice did not develop colitis when infected with *H. hepaticus*. And, it was recently shown that after cocolonization of mice with *Lactobacillus reuteri*, *H. hepaticus* again served as a colitogenic trigger-inducing thyphlocolitis.<sup>75</sup> Furthermore, this notion was recently underlined by a study demonstrating that the susceptibility of IL-10-deficient mice to *H. hepaticus*-induced intestinal pathology was correlated with significant differences in the composition of the intestinal microbiota.<sup>82</sup> Interestingly, the microbiota composition of susceptible mice included the presence of *B. wadsworthia* and *L. reuteri*, both of which have been shown to have colitogenic potential in IL-10-deficient mice.<sup>75,78,82</sup> Furthermore, it has been speculated that *H. hepaticus*-induced inflammation may rely on the presence of bacteria capable of inducing lymphoid structures as infection of IL-10-deficient mice with *H. hepaticus* leads to a strong adaptive immune response.<sup>18,26,67</sup> Another recent study confirmed that an intestinal microbiota is needed for the development of colitis in IL-10-deficient mice infected with *H. hepaticus* and demonstrated that alteration of the microbiota by antibiotic treatment can modulate the disease phenotype. However, colitis initiation and progression was possible in the presence of several different microbial communities.<sup>83</sup> It also has to be considered that *H. hepaticus*-induced inflammation in itself will affect and modulate the microbiota composition of the host, which in turn may depend both on the host immune response and the characteristics of the microbiota and which both might vary in a mouse strain-specific manner.<sup>76</sup>

As described earlier, a major colitogenic QTL, *Cdcs1*, was detected by using the IL-10-deficient mouse model of IBD, and this locus has been hypothesized to contribute to colitis development in this model not only by conferring innate hyporesponsiveness and adaptive hyperresponsiveness but also by interacting specifically with the intestinal microbiota. Next, we want to dissect *Cdcs1* contribution to colitis susceptibility in interaction with environmental factors pivotal for colitis development.

### GENETIC × MICROBIAL INTERACTIONS: ELUCIDATING *CDCS1* CONTRIBUTION TO COLITIS DEVELOPMENT IN INTERACTION WITH THE MICROBIOTA

As mentioned above, we used sanitized C3Bir-*IL10*<sup>-/-</sup>, B6-*IL10*<sup>-/-</sup>, and the respective *Cdcs1*-congenic IL-10-deficient mice for infection experiments with *H. hepaticus* and treatment with DSS and murine norovirus (MNV). After infection with *H. hepaticus*, the C3Bir haplotype, as expected, conferred a more

severe inflammation in the cecum upon infection and BCR3 mice (originally resistant but now carrying the *Cdcs1* haplotype of the C3Bir mice) showed severe inflammation in the cecum as well. However, unexpectedly, C3Bir mice demonstrated a milder form of inflammation in the colon than B6 mice, and *H. hepaticus* infection alone was not sufficient to restore the original more severe phenotypes. Therefore, *Cdcs1* modified *H. hepaticus*-induced inflammation, but the original strain-specific response seemed to be driven by the composition of the microbiota. Subsequently, microbiota composition was determined by T-RFLP demonstrating that *H. hepaticus*-induced inflammation led to a strain-dependent decrease in bacterial richness and diversity (with C3Bir showing less richness and diversity than B6) and to strain-specific blooms of specific bacterial families correlating with colitis susceptibility.<sup>76</sup> Therefore, the influence of the mouse strain-dependent, and especially *Cdcs1*-dependent, differences of the microbiota composition on colitis susceptibility should be the focus of future studies. To further dissect *Cdcs1* contribution to colitis development, DSS treatment was used for colitis induction in sanitized IL-10-deficient and congenic mice. Again, the discrepancy to the original phenotype was detected with B6 mice displaying a more severe inflammation in the colon. However, BCR3 and BCR2 mice (both carrying the *Cdcs1* haplotype of C3Bir mice but with differential interval lengths) were most susceptible and demonstrated the highest scores for inflammation (own observation). These findings again underline that the microflora not only modifies the expression and degree of inflammation in the IL-10-deficient mouse model of IBD but also modifies mouse strain-specific susceptibilities. This, however, is at least partly caused by genetic modifiers of the *Cdcs1* region as they interact with environmental factors. Interestingly, this is corroborated by several QTL studies where the *Cdcs1* region was independently replicated by use of different models, most of which were depending on microbial factors.<sup>47</sup> Finally, we were recently able to demonstrate that an MNV infection can provide a colitogenic stimulus in the IL-10-deficient mouse that depends on the presence of the commensal microflora.<sup>77</sup> Here, MNV infection of germ-free IL-10-deficient mice did not trigger inflammation, but colonization of germ-free mice with an altered Schaedler flora before MNV infection was sufficient to induce colitis. That non-bacterial members of the microbiota such as noroviruses may contribute to or initiate the development of IBD has been widely discussed, especially because it was recently shown that norovirus infection leads to the development of colitis in mice with a mutation in the CD susceptibility gene *Atg16L1*.<sup>84–86</sup> Remarkably, norovirus infection has been demonstrated to aggravate IBD symptoms in human patients as well.<sup>87</sup> However, so far, no evidence has been found for a viral infection to be the sole causative factor for the development of IBD but rather the combination of specific genetic susceptibilities and the presence of viral factors, probably in interaction with the endogenous microflora, contribute to IBD development. Our own observations regarding *Cdcs1* contribution to colitis development in interaction with the microbiota underline this notion as the genetic susceptibility may be

abolished or fundamentally reversed when the composition of the microbiota changes.

## CONTRIBUTION OF AN INTESTINAL DYSBIOSIS TO COLITIS DEVELOPMENT IN THE IL-10-DEFICIENT MOUSE

It has early on been postulated that the development of IBD does not stem from a single causative factor but rather that an interaction of genetic susceptibilities, environmental factors, and the host microbiota leads to a dysregulation of the mucosal immune system. However, only recently, the contribution of a misbalanced microbiota has become the focus of attention in this complex disease. It can even be speculated that the effect of microbiota composition and diversity may have similar effects or may even be a more important contributor to colitis development than the effect of pathogen colonization.<sup>88</sup> However, it is currently accepted that an imbalance between putative harmful versus protective bacterial species may promote intestinal inflammation.<sup>89,90</sup> In this context, the reduction of anaerobic bacteria such as *Bacteroides* species, *Eubacterium* species, and *Lactobacillus* species has been reported in patients with IBD.<sup>91</sup> Furthermore, fewer *Firmicutes* bacteria were found in IBD samples with a simultaneous increase in *Bacteroidetes* and, in CD samples only, *Enterobacteriaceae*.<sup>92</sup> Other metagenomic studies have shown a decrease especially in the *Firmicutes* and the *Bacteroidetes* phyla in patients with CD.<sup>93–95</sup> Interestingly, *B. fragilis* has been shown to exhibit a protective effect on colitis development in mice infected with *H. hepaticus*. As studies with IL-10-deficient mice indicated, the protective effect was demonstrated to be mediated through a functional requirement for IL-10-producing T cells.<sup>96</sup> Therefore, the decrease in the richness of *Bacteroidetes* species may contribute to inflammation. However, commensal *Bacteroides* species have also been demonstrated to induce colitis in mice deficient for both, IL-10 and TGF $\beta$ .<sup>79</sup> In addition, several other bacteria have been shown to be relevant to the dysbiosis in IBD as they independently induced intestinal inflammation in IL-10-deficient mice, including certain *E. coli* and *E. faecalis* strains.<sup>70,71</sup> It has early on been shown that IL-10-deficient mice, similar to patients with CD, exhibit alterations in the species and levels of bacteria colonizing the colon compared with controls. Furthermore, reduced levels of probiotic bacteria were detected and, when administered early on, demonstrated to exert a protective effect.<sup>59,97</sup> Also, the diversity of cecal bacteria was found to be altered before and after the onset of colitis in IL-10-deficient mice.<sup>98</sup> Another study also reported changes in the diversity and composition of the intestinal microbiota with a progressive decrease in diversity and richness and an increase of *Proteobacteria* and *E. coli* during the onset of inflammation in IL-10-deficient mice.<sup>99</sup> A recent study focusing on the changes in the intestinal steroid profile of IL-10-deficient mice during the onset of colitis detected a reduced microbial diversity compared with wild-type controls, which was accompanied by a proliferation of *E. coli* and *Enterococcus gallinarum*.<sup>100</sup> Therefore, the substantial

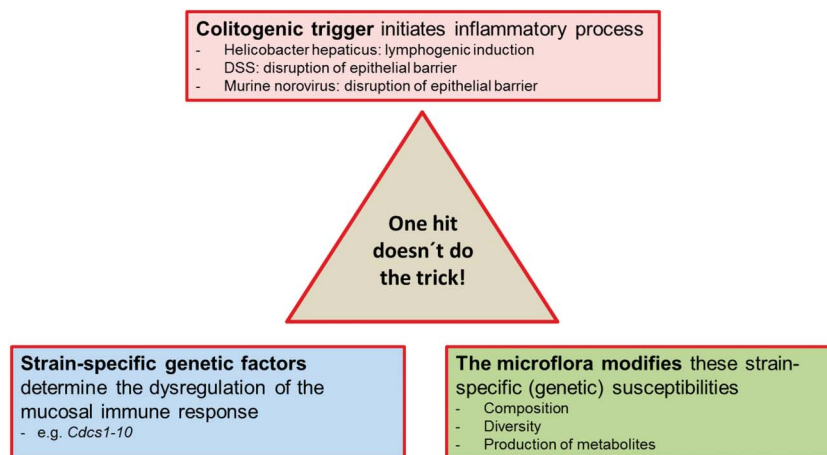


FIGURE 2. A multi-hit model: the IL-10-deficient mouse.

loss of bacterial diversity is believed to be not only a contributing factor for colitis development in the IL-10-deficient mouse but also to be a dominant factor in IBD pathogenesis.<sup>101</sup> Microbiome studies with the largest pediatric cohort to date revealed an axis defined by an increased abundance of *Enterobacteriaceae*, *Pasteurellaceae*, *Veillonellaceae*, and *Fusobacteriaceae* and a decreased abundance of *Erysipelotrichales*, *Bacteroidales*, and *Clostridiales* correlating strongly with the disease status.<sup>102</sup> In this context, studies elucidating the impact of introducing a limited flora into germ-free and/or IBD susceptible mice such as the IL-10-deficient mouse are mandatory to systematically analyze microbiota contribution to IBD development. An elegant study determining microbiota–host interactions at a complex yet defined level used germ-free IL-10-deficient and wild-type mice to demonstrate that a simplified human microbiota consortium induced colitis in these mice.<sup>80</sup> Furthermore, immunodominant bacterial species were identified in these “humanized” mice that induced Th1 and Th17 immune responses independent of luminal and mucosal bacterial concentrations. Also, a recent study described the influence of an altered diet on the microbiota and subsequent colitis development in IL-10-deficient but not wild-type mice. Here, it was shown that dietary fat-induced tauracholic acid promotes the expansion of the sulfate-reducing pathobiont *B. wadsworthia* driving colitis in IL-10-deficient mice.<sup>78</sup> In summary, although indications for a correlation between the composition of the microbiota and the development of IBD are piling up, final evidence for a causative role of the host microbiota in IBD pathogenesis has yet to be provided.<sup>85</sup> In this context, using the IL-10-deficient mouse model of IBD will help to complete our understanding of the dynamic role of the microbiota in this complex disease.

### IL-10-DEFICIENT MOUSE SERVES AS A MULTIHIT MODEL

We have elaborated in detail on the interplay of genetic factors, immune aspects, and the role of the microbiota

contributing to colitis development in the IL-10-deficient mouse, working out the facets of this excellent mirror of the multifactorial disease of IBD. The IL-10-deficient mouse serves as a multihit model where a colitogenic trigger initiates the inflammatory process, strain-specific genetic factors determine the dysregulation of the mucosal immune response, and where the microflora modifies these susceptibilities and responses (Fig. 2). The IL-10-deficient mouse is an excellent model to further define the hypothesis elaborated by Maloy and Powrie<sup>37</sup> that the induction and perpetuation of intestinal inflammation may require the convergence of several abnormalities affecting intestinal homeostasis and that only when a specific threshold is reached inflammation manifests. As the entirety of recent studies indicate that IL-10 is a crucial regulatory cytokine suppressing inflammation because of the dysregulation of the microbial homeostasis,<sup>26</sup> further utilization of this model will help to provide much-needed insights not only into IBD-related microbiota–host interactions but also into the primary versus secondary role of dysbiosis in this disease.

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