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**Factoring the intestinal microbiome into the pathogenesis of autoimmune hepatitis**

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**Abstract**

The intestinal microbiome is a reservoir of microbial antigens and activated immune cells. The aims of this study were to describe the role of the intestinal microbiome in generating innate and adaptive immune responses, indicate how these responses contribute to the development of systemic immune-mediated diseases, and encourage investigations that improve the understanding and management of autoimmune hepatitis. Alterations in the composition of the intestinal microflora (dysbiosis) can disrupt intestinal and systemic immune tolerances for commensal bacteria. Toll-like receptors within the intestine can recognize microbe-associated molecular patterns and shape subsets of T helper lymphocytes that may cross-react with host antigens (molecular mimicry). Activated gut-derived lymphocytes can migrate to lymph nodes, and gut-derived microbial antigens can translocate to extra-intestinal sites. Inflammasomes can form within hepatocytes and hepatic stellate cells, and they can drive the pro-inflammatory, immune-mediated, and fibrotic responses. Diet, designer probiotics, vitamin supplements, re-colonization methods, antibiotics, drugs that decrease intestinal permeability, and molecular interventions that block signaling pathways may emerge as adjunctive regimens that complement conventional immunosuppressive management. In conclusion, investigations of the intestinal microbiome are warranted in autoimmune hepatitis and promise to clarify pathogenic mechanisms and suggest alternative management strategies.

**Key words:** Intestinal microbiome; Inflammasomes; Autoimmune hepatitis; Dysbiosis; toll-like receptors

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**Core tip:**  The intestinal microbiome is a reservoir of microbial antigens and activated immune cells that have been implicated in the pathogenesis of diverse systemic immune-mediated diseases. Dysbiosis, increased intestinal permeability, and molecular mimicry between microbial and self-antigens may initiate or sustain autoimmune hepatitis. Multiple drug, molecular, dietary, and probiotic interventions can modify the intestinal microbiome and attenuate the immune response. The role of the intestinal microbiome in autoimmune hepatitis warrants rigorous study, and new therapies may emerge that strengthen current treatment regimens.

Czaja AJ. Factoring the intestinal microbiome into the pathogenesis of autoimmune hepatitis. *World J Gastroenterol* 2016; In press

**INTRODUCTION**

Autoimmune hepatitis is a chronic immune-mediated inflammatory liver disease of uncertain cause[[1](#_ENREF_1),[2](#_ENREF_2)]. Population-based epidemiological studies have indicated that it is a rare chronic liver disease with an annual incidence of 0.67-1.9 cases per 100000 persons and a point prevalence of 4-42.9 cases per 100000 persons[[3-8](#_ENREF_3)]. Prevalence varies widely by geographical region (Singapore, 4 per 100000[[4](#_ENREF_4)]; Sweden, 10.7 per 100000[[9](#_ENREF_9)]; southern Israel, 11 per 100000[[8](#_ENREF_8)]; Spain, 11.6 per 100000[[6](#_ENREF_6)]; Norway, 16.9 per 100000[[3](#_ENREF_3)]; Netherlands, 18.3 per 100000[[10](#_ENREF_10)]; Denmark, 23.9 per 100000[[11](#_ENREF_11)]; New Zealand, 24.5 per 100000[[7](#_ENREF_7)]; and Alaska, 42.9 per 100000[[5](#_ENREF_5)]), and the incidence has been increasing in Denmark [[11](#_ENREF_11)] and the Netherlands[[10](#_ENREF_10)]. Rigorous epidemiological studies have not been performed in the adult population of the United States, but studies in the children of Utah have indicated an overall incidence of 0.4 cases per 100,000 and a prevalence of 3 cases per 100000 [[12](#_ENREF_12)]. The wide variability in the annual incidence and point prevalence of autoimmune hepatitis in different geographical regions and ethnic groups suggest that genetic and environmental factors contribute to its occurrence[[2](#_ENREF_2),[13](#_ENREF_13),[14](#_ENREF_14)].

Genetic factors within[[15-17](#_ENREF_15)] and outside[[18-22](#_ENREF_18)] the major histocompatibility complex (MHC) have been implicated as susceptibility factors for autoimmune hepatitis. Cytochrome P450 2D6 (CYP2D6) and formiminotransferase cylcodeaminase have been proposed as key antigenic targets in some patients[[23-25](#_ENREF_23)], and homologies between peptide sequences in CYP2D6 and hepatitis C virus (HCV), herpes simplex virus (HSV), and cytomegalovirus (CMV) have suggested that molecular mimicry between foreign and self-antigens initiates and sustains the disease[[2](#_ENREF_2),[23](#_ENREF_23),[26-28](#_ENREF_26)].

The principal target antigen in most white North American and northern European adults with autoimmune hepatitis is unknown, and as yet unrecognized self-antigens or foreign antigens that resemble self-antigens may trigger the disease or increase susceptibility to it, possibly by skewing components of the innate and adaptive immune responses toward a pro-inflammatory, autoreactive profile[[2](#_ENREF_2),[29](#_ENREF_29)]. The commensal bacteria of the intestine and their metabolic by-products constitute a reservoir of foreign antigens that can interact with mucosal immune cells and influence systemic immune responses[[30-34](#_ENREF_30)]. The human microbiome has already been implicated in the occurrence of multiple systemic immune-mediated diseases, including type 1 diabetes[[35-37](#_ENREF_35)], rheumatoid arthritis[[38-41](#_ENREF_38)], multiple sclerosis[[42](#_ENREF_42)], and inflammatory bowel disease[[43-45](#_ENREF_43)], and its role in the inflammatory liver diseases is also being scrutinized[[46-48](#_ENREF_46)].

Non-alcoholic steatohepatitis (NASH) may progress because of an influx of microbial products in the portal circulation, activation of toll-like receptors (TLRs) 4 and 9, and subsequent release of pro-inflammatory cytokines, including tumor necrosis factor-alfa (TNF-α) and interleukin (IL)-1β[[49-51](#_ENREF_49)]. Primary sclerosing cholangitis (PSC) expresses high levels of TLR4 and TRL9 in biliary epithelial cells (BEC), produces IL-1β, IL-8, and interferon (IFN)-γ in response to lipopolysaccharide (LPS), and commonly manifests atypical perinuclear anti-neutrophil cytoplasmic antibodies (pANCA)[[52-54](#_ENREF_52)]. These antibodies are directed against β-tubulin which cross-reacts with an antigen (FtsZ) present in all intestinal bacteria[[53](#_ENREF_53)]. Germ-free mice develop histologically more severe PSC than conventionally housed animals, and these findings suggest that commensal bacteria have a protective role against PSC[[55](#_ENREF_55)].

Similarly, primary biliary cholangitis (PBC) expresses TLR4 in BEC and periportal hepatocytes, produces pro-inflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α) in response to intestinal bacterial components (LPS, flagellin, and cytosine-phosphorothioate-guanine oligonucleotide [CpG]), manifests BEC-destructive LPS-stimulated natural killer (NK) cells, and produces antimitochondrial antibodies (AMA) that target an antigen (pyruvate dehydrogenase complex-E2) which shares sequence homologies with intestinal *Escherichia coli*[[56-62](#_ENREF_56)].

Autoimmune hepatitis may also be influenced by the intestinal microbiome. Concurrent features of PBC or PSC occur in 7%-18% of patients (“overlap syndromes”)[[63](#_ENREF_63),[64](#_ENREF_64)]; atypical pANCA are present in 49%-92% of individuals with autoimmune hepatitis[[65-68](#_ENREF_65)]; and alterations in the composition of the intestinal microbiota (dysbiosis) have been found in experimental autoimmune hepatitis[[69](#_ENREF_69)]. The structural proteins binding intestinal epithelial cells (zona occludens 1 and occludin) are reduced in patients with autoimmune hepatitis compared to healthy volunteers; plasma LPS levels are increased; and the numbers of intestinal anaerobes (*Bifidobacterium* and *Lactobacillus*) are decreased[[70](#_ENREF_70)]. These findings support the concept that autoimmune hepatitis is associated with dysbiosis, reduced permeability of the gastrointestinal mucosal barrier, and translocation of gut-derived microbial products into the systemic circulation.

The goals of this review are to describe the role of the intestinal microbiome in generating innate and adaptive immune responses, indicate how these responses may contribute to the development or maintenance of systemic autoimmune responses, and encourage investigations in autoimmune hepatitis that might improve understanding of its pathogenesis and results of its management.

**LITERATURE SEARCH**

Abstracts cited in PubMed were identified using the search words “intestinal microbiome”, “intestinal microbiome and autoimmunity”, and “intestinal microbiome and autoimmune hepatitis”. Key aspects of the abstracts judged pertinent to the review were noted, and full-length articles were selected from the abstracts. A secondary bibliography was developed from the references cited in the selected full-length articles, and additional PubMed searches were performed to expand the concepts developed in these articles. The discovery process involving abstract review and the acquisition of full-length articles was repeated, and a tertiary bibliography was developed after reviewing these selected articles. The number of abstracts cited by PubMed and reviewed for pertinence to this topic during the primary, secondary and tertiary searches exceeded 3800. Those judged most pertinent to the topic exceeded 200, and the number of full-length articles reviewed was 66.

**INTESTINAL MICROBIOME AND IMMUNE RESPONSES**

The human intestinal tract contains 10-11 trillion bacteria comprising 500-1500 different species[[47](#_ENREF_47),[71-76](#_ENREF_71)]. *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* are the four phyla that predominate[[77-79](#_ENREF_77)], and a phylogenetic core of microbial species has been defined that is composed of 66 Operational Taxonomic Units (OTUs) present in most individuals[[80](#_ENREF_80)]. Luminal microbiota have greater diversity, and they are more tightly clustered than mucosal microbiota[[81](#_ENREF_81)]. *Firmicutes* and *Actinobacteria* are more abundant in the luminal populations, and *Proteobacteria* are more abundant in the mucosal populations[[81](#_ENREF_81)].

The composition of the microbiome is influenced by diverse environmental factors, including community sanitation levels and vaccination programs, and by host-related variables, including method of obstetrical delivery, age, genetic predisposition, dietary habits, personal hygiene, and antibiotic exposures. Changes in the intestinal microbiome tend to be slow from late childhood through adulthood with marked changes occurring mainly with advanced age[[74](#_ENREF_74),[82-85](#_ENREF_82)]. The microbiome becomes less diverse and more variable over short intervals with aging, and the species of *Bacteroides*, *Clostridium* and *Escherichia coli* constitute a greater proportion of the microflora in individuals aged ≥ 65 years[[82-84](#_ENREF_82)].

The intestinal microbiome varies in diverse ethnic groups[[75](#_ENREF_75),[86-89](#_ENREF_86)], and this diversity may reflect genetic factors, demographic issues (age, gender, socioeconomic status), lifestyle features (alcohol use, smoking, adiposity), and long-term diet[[90-92](#_ENREF_90)]. Disparities in the intestinal microbiota have been recognized between ethnic groups in the same country (rural versus urbanized)[[88](#_ENREF_88),[89](#_ENREF_89),[93](#_ENREF_93),[94](#_ENREF_94)] and between countries (Africa versus Europe, cross-Europe, and cross-Asia)[[87](#_ENREF_87),[88](#_ENREF_88),[91](#_ENREF_91)], and socioeconomic variations at individual and neighborhood levels have been associated with many of these differences[[88](#_ENREF_88),[95](#_ENREF_95)]. The nature of the long-term diet may be the critical element affected by the socioeconomic status[[95](#_ENREF_95)].

Amongst the diversity within the intestinal microbiota, common functional and phylogenetic elements have also been described[[89](#_ENREF_89),[96-99](#_ENREF_96)]. These common elements may be indispensable for the well-being of the individual as they can produce short-chain fatty acids, synthesize vitamins, and aid in digestion, metabolism and immune defense[[89](#_ENREF_89)]. The functional and phylogenetic core components have been shared across heterogeneous healthy human populations, and they tend to co-exist[[89](#_ENREF_89)].

The intestinal microbiome is essential for development of the intestinal immune responses which in turn maintain tolerance of the microflora[[41](#_ENREF_41),[100](#_ENREF_100),[101](#_ENREF_101)]. Germ-free mice have fewer CD4+ T lymphocytes in the lamina propria of the intestine, hypoplastic Peyer’s patches, less immunoglobulin A (IgA) production, and disorganized zones of T and B lymphocytes in the spleen and lymph nodes compared to wild-type mice[[102](#_ENREF_102),[103](#_ENREF_103)]. These immune deficiencies are corrected by the introduction of *Bacteroides fragilis*[[104](#_ENREF_104)]. Colonization also induces the production by IL-10-secreting, regulatory T cells (Tregs), possibly in response to the secretion of polysaccharide A by the bacteria and direct activation of TLR2 on the Foxp3+ Tregs[[105-108](#_ENREF_105)]. The introduction of *Clostridium* species induces similar changes[[109](#_ENREF_109),[110](#_ENREF_110)].

Emerging evidence suggests that the intestinal microbiome can influence systemic immune responses by activating TLRs[[34](#_ENREF_34),[47](#_ENREF_47),[48](#_ENREF_48)] and promoting the formation of inflammasomes within the liver[[51](#_ENREF_51),[111-113](#_ENREF_111)] (Figure 1). Changes in the composition of the intestinal microbiome induced by antibiotics, genetic factors, or the disease (dysbiosis) may sustain or enhance the innate and adaptive immune responses by overcoming or circumventing normal tolerogenic responses to the commensal bacteria[[32](#_ENREF_32),[114-116](#_ENREF_114)]. Bacterial components may act as antigens that stimulate the systemic immune response[[30](#_ENREF_30),[31](#_ENREF_31),[34](#_ENREF_34),[47](#_ENREF_47),[48](#_ENREF_48)] or that prime immune cells within the intestine that subsequently access peripheral lymphoid tissue[[39](#_ENREF_39),[117](#_ENREF_117),[118](#_ENREF_118)]. Molecular mimicry between microbial and host-derived antigens and promiscuous targeting by antigen-sensitized lymphocytes may then initiate or strengthen the autoreactive response in genetically-predisposed individuals[[33](#_ENREF_33),[57](#_ENREF_57),[115](#_ENREF_115)]. Investigations in cell cultures, animal models, and patients with diverse systemic immune-mediated diseases have justified these hypotheses and warranted their consideration in autoimmune hepatitis[[46-48](#_ENREF_46)].

**KEY REQUIREMENTS FOR A MICROBIOME-DERIVED SYSTEMIC IMMUNE RESPONSE**

***Activation of toll-like receptors***

Activation of toll-like receptors (TLRs) are the key receptors within the intestine that recognize microbe-associated molecular patterns (MAMPS), pathogen-associated molecular patterns (PAMPs), and damage-associated molecular patterns (DAMPs)[[34](#_ENREF_34),[47](#_ENREF_47),[48](#_ENREF_48),[119](#_ENREF_119),[120](#_ENREF_120)] (Table 1). They are instrumental in generating an innate immune response to pathogens and cellular distress signals, and they can shape subsets of T helper lymphocytes that recognize microbial components and have the potential to cross-react with host antigens[[41](#_ENREF_41),[121](#_ENREF_121),[122](#_ENREF_122)]. Ten TLRs have been described in humans[[123](#_ENREF_123)], and each responds preferentially to specific ligands which may be viral and bacterial proteins or endogenous ligands in the absence of infection[[34](#_ENREF_34),[120](#_ENREF_120)]. All stimulated TLRs except TLR3 activate a signaling pathway that is dependent on the myeloid differentiation factor 88 (MyD88)[[124](#_ENREF_124),[125](#_ENREF_125)]. Signaling through the MyD88 pathway in turn activates nuclear factor-kappa B (NF-κB) and promotes the transcription of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6)[[123](#_ENREF_123),[125](#_ENREF_125),[126](#_ENREF_126)].

TLRs can also influence the adaptive immune response[[41](#_ENREF_41)] (Figure 1). TLRs expressed by dendritic cells and macrophages can upregulate class II molecules of the MHC and enhance antigen presentation to CD4+ helper T lymphocytes[[127](#_ENREF_127)] (Table 1). TLRs can also increase the expression of the co-stimulatory molecules, CD80, CD86 and CD40, on the antigen presenting cells and thereby favor T lymphocyte activation and differentiation[[41](#_ENREF_41),[127](#_ENREF_127)]. Kupffer cells express all TLRs except TLR5[[128](#_ENREF_128)], and they are the primary cells within the liver that respond to TLR ligands. The production of pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS) by the Kupffer cells promotes liver inflammation[[129](#_ENREF_129)] and the innate and adaptive immune responses[[41](#_ENREF_41),[127](#_ENREF_127)]. Hepatocytes, BEC, hepatic stellate cells (HSCs), and sinusoidal epithelial cells also express TLRs, but only HSCs express TLR1 through TLR9[[48](#_ENREF_48),[130](#_ENREF_130)].

The cytokine profile shapes the subsets of T lymphocytes that constitute the immune-mediated response, and it is influenced by the particular TLRs that are activated by ligands within the microenvironment (Table 1). Activation of TLR4 and TLR9 promotes the release of IL-12 and favors a type 1 cytokine pathway that is pro-inflammatory[[131](#_ENREF_131)]. TLR4 also induces the secretion of IL-23 and promotes the expansion and survival of pro-inflammatory T helper (Th) 17 lymphocytes[[132](#_ENREF_132)]. In contrast, activation of TLR2 favors the production of IL-10 and IL-13 which promotes an anti-inflammatory type 2 cytokine response[[133](#_ENREF_133)].

Lipopolysaccharide (LPS) from gram-negative bacteria is the principal ligand activating TLR4[[123](#_ENREF_123),[134](#_ENREF_134)], and un-methylated CpG sequences in bacterial and viral genomes activate TLR9[[135](#_ENREF_135)] (Table 1). The viral proteins of HCV, CMV and HSV are key ligands that activate TLR2[[34](#_ENREF_34),[136](#_ENREF_136),[137](#_ENREF_137)]. TLR2, TLR5, TLR7, and TLR8 are expressed by CD4+ T lymphocytes, and ligands that activate these TLRs (viral proteins[[136](#_ENREF_136)], flagellin [[138](#_ENREF_138)], and single-stranded ribonucleic acid[[139](#_ENREF_139)]) can directly activate memory lymphocytes and stimulate their proliferation[[140](#_ENREF_140)]. Naturally occurring Tregs express TLR2, TLR5 and TLR8, and they can also be activated directly by viral and bacterial components[[141](#_ENREF_141)]. TLRs can also block the suppressive effect of Tregs by the recognition of microbial products that induce secretion of IL-6[[142](#_ENREF_142)]. Pathogen-specific adaptive immune responses can be favored, and defense mechanisms can be strengthened. Microbial elements can thereby modulate the innate and adaptive immune responses through the TLRs and affect immune homeostasis indirectly by modulating the cytokine profile or directly by affecting immune cell proliferation.

TLR4 is a crucial signaling pathway by which HSCs increase the extracellular matrix[[46](#_ENREF_46)] (Table 1). The production of chemokines and adhesion molecules is mediated by activated TLR4 in HSCs[[46](#_ENREF_46)], and the chemo-attraction of inflammatory and immune cells to the liver stimulates the fibrotic process[[143](#_ENREF_143)]. TLR4 signaling also promotes activation of transforming growth factor-beta (TGF-β) by down-regulating production of an endogenous inhibitor of the TGF-β receptor[[144](#_ENREF_144)]. Furthermore, TLR4 signaling may down-regulate microRNA molecules that suppress the transcription of collagen[[46](#_ENREF_46),[145](#_ENREF_145)]. A polymorphism of the *TLR4 gene* may impair the response of TLR4 to LPS. The LPS-induced signaling pathway that activates NF-κB may thereby be disrupted and the production of the pro-inflammatory cytokines, TNF-α and interferon-beta (IFN-β), may be reduced[[146](#_ENREF_146)]. In this fashion, a genetic variation may affect the response of TLR4 to microbial ligands and the propensity for progressive hepatic fibrosis.

The signaling pathway involving TLR4, MyD88, and NF-κB has been implicated in the progression of multiple liver diseases[[54](#_ENREF_54),[58](#_ENREF_58),[144](#_ENREF_144),[147](#_ENREF_147),[148](#_ENREF_148)]. Concentrations of gut-derived endotoxins have been increased in animal models of hepatic fibrosis[[149](#_ENREF_149),[150](#_ENREF_150)] and in the systemic and portal circulation of patients with cirrhosis[[151](#_ENREF_151),[152](#_ENREF_152)]. Other TLRs may respond to different microbial ligands and influence the subsets of lymphocytes that orchestrate the autoreactive response. TLR signaling pathways have not been evaluated in autoimmune hepatitis.

***Stimulation of inflammasomes***

Inflammasomes are protein complexes that form within the cytoplasm of diverse cells, including macrophages, hepatocytes, and HSCs, in response to stimuli associated with cellular stress, damage or infection[[112](#_ENREF_112),[153](#_ENREF_153),[154](#_ENREF_154)] (Table 1). By releasing pro-inflammatory cytokines IL-1β and IL-18, they drive the inflammatory response to tissue injury and influence cell death, inflammatory activity, and fibrosis[[111](#_ENREF_111),[113](#_ENREF_113)] (Figure 1). TLRs and inflammasomes have separate routes of activation[[112](#_ENREF_112),[155](#_ENREF_155)], but cooperation between them is pivotal in promoting communication between the intestinal microbiota and the systemic immune response[[34](#_ENREF_34)]. Factors that increase the expression of inflammasomes, such as saturated fatty acids and bacterial endotoxin, may increase activation of TLR4 and promote hepatic fibrosis[[50](#_ENREF_50)].

Inflammasomes consist of a sensor protein that is within the family of non-obese diabetes (NOD)-like receptors (NLRs), an adaptor molecule (apoptosis-associated speck-like CARD-domain containing protein [ASC]), and pro-caspase 1[[156](#_ENREF_156)] (Table 1). The inflammasomes can sense microbial products[[157](#_ENREF_157)] and metabolic stress[[112](#_ENREF_112)], activate pro-caspase 1[[158](#_ENREF_158),[159](#_ENREF_159)], trigger the release of pro-inflammatory cytokines[[153](#_ENREF_153)], and shape the innate[[112](#_ENREF_112)] and adaptive immune responses[[160](#_ENREF_160)]. The expression of NLRP3 is upregulated in hepatocytes after stimulation with LPS, and Kupffer cells and sinusoidal epithelial cells also express high levels of NLRP1 and NLRP3[[113](#_ENREF_113)].

The structural diversity of the ligands that activate NLRP3 is greater than the structural motifs that activate the TLRs, and the inflammasomes may be responsive to a broader range of activation signals than the TLRs[[161](#_ENREF_161)] (Table 1). Together the TLRs and NLRs provide receptors for signaling pathways that can respond to diverse endogenous and exogenous danger signals, including microbial components, and they each can generate pro-inflammatory responses that sustain and enhance the innate and adaptive immune responses to liver injury. TLRs may also have a counter-regulatory effect on the inflammasomes[[34](#_ENREF_34)]. Chronic stimulation of the TLRs by LPS induces the production of IL-10 and reduces the activation of NLRP3[[162](#_ENREF_162)]. Furthermore, activation of TLR2 or TLR4 can increase the autophagy of hepatocytes, the degradation of NLRP3, and the suppression of IL-1β production[[163](#_ENREF_163)]. Inflammasomes have not been characterized in autoimmune hepatitis, and their interactions with TLRs have not been defined in this disease.

***Emergence of immunogenic intestinal microbiota (dysbiosis)***

Systemic inflammatory and immune-mediated diseases have been associated with intestinal microbiomes that distinguish them from normal or other disease-specific populations[[116](#_ENREF_116)] (Table 1). The intestinal microbiota have differed in patients with rheumatoid arthritis compared to patients with fibromyalgia[[164](#_ENREF_164)]. Decreased diversity in the microbiome has been associated with an increased risk of type 1 diabetes[[165](#_ENREF_165)], the occurrence of atopic diseases, including asthma[[166-169](#_ENREF_166)], and the presence of Crohn’s disease[[170](#_ENREF_170)]. Patients with multiple sclerosis have reduced numbers of *Clostridia* and *Bacteroides* species compared to normal individuals[[42](#_ENREF_42)], and patients with type 1 diabetes have more colonies of *Bacteroides*[[37](#_ENREF_37),[171](#_ENREF_171)]. Compositional shifts in the intestinal microbiota, particularly the relative frequencies of certain bacterial taxa, have been associated with the phenotype and genotype of inflammatory bowel disease[[172](#_ENREF_172)].

These findings have suggested that alterations in the composition of the intestinal microflora (dysbiosis) may disrupt intestinal and systemic immune tolerances and contribute to immune-mediated diseases[[114](#_ENREF_114),[116](#_ENREF_116)]. Depletion of the commensal bacteria may allow intestinal populations of pathogenic or immunogenic organisms to proliferate and generate ligands that activate TLRs and NLRs[[116](#_ENREF_116),[173](#_ENREF_173)] (Figure 1). The major uncertainty has been whether the dysbiosis has been a cause or an effect of the disease.

Antibiotics are the principal agents promoting dysbiosis, and their use has been implicated in creating the dysbiosis associated with the occurrence of atopic diseases[[167](#_ENREF_167),[168](#_ENREF_168),[174](#_ENREF_174)], asthma[[166](#_ENREF_166)], type 1 diabetes[[165](#_ENREF_165)], and celiac disease[[175](#_ENREF_175)]. Twin studies have also indicated that genetic factors can shape the intestinal microbiome[[176](#_ENREF_176),[177](#_ENREF_177)], and immune-mediated diseases with genetic predispositions have manifested dysbiosis[[30](#_ENREF_30),[47](#_ENREF_47),[116](#_ENREF_116)]. Importantly, the contribution of the 8.1 ancestral haplotype, which includes the DRB1 alleles commonly associated with systemic autoimmune diseases including autoimmune hepatitis, is probably small[[178](#_ENREF_178)], and dysbiosis rather than genetic factors has been implicated in the occurrence of experimental NASH[[51](#_ENREF_51)].

The composition of the intestinal microflora may also influence the gender bias for autoimmune disease[[179-181](#_ENREF_179)] (Table 1). Colonization by commensal microbes early in the life of NOD mice raises serum testosterone levels and protects male mice from developing type 1 diabetes[[179](#_ENREF_179)]. Furthermore, the transfer of the intestinal microbiota from mature male NOD mice to immature female NOD mice alters the intestinal microbiome of the females and protects them from developing diabetes[[179](#_ENREF_179)]. Blockade of the androgen receptor attenuates the microbiome-specific changes in the female mice and supports the concept that the commensal bacteria of the intestine can affect the propensity for autoimmune disease in genetically-susceptible animals by altering sex hormone levels or receptor sensitivities[[179](#_ENREF_179)].

Gender may also influence the composition of the intestinal microbiota and in turn the propensity to develop autoimmune disease[[180](#_ENREF_180)] (Table 1). The intestinal microbiota differ in male and female NOD mice, and this difference disappears after male castration. Furthermore, the greater frequency of female NOD mice to develop type 1 diabetes compared to male NOD mice is lost in germ-free animals[[180](#_ENREF_180)]. These findings suggest that the intestinal microbiome can influence sex hormone levels [[179](#_ENREF_179)] and also be influenced by them[[180](#_ENREF_180)] , possibly in a self-amplification loop.

The increased female propensity for autoimmune disease may relate to estrogenic effects that enhance the autoreactive response directly by promoting a pro-inflammatory (type 1) cytokine pathway of lymphocyte differentiation[[182](#_ENREF_182)] and indirectly by altering the intestinal microbiome to favor the translocation of sensitizing microbial antigens[[180](#_ENREF_180)]. Imbalances between blood estrogen and progesterone levels have affected the immune response during and immediately after pregnancy[[182](#_ENREF_182)], and the treatment of peripheral blood mononuclear cells with 17-β estradiol has increased their response to immunogens and the expression of TLR8[[183](#_ENREF_183)]. The relationship between sex hormone levels and the intestinal microbiome during pregnancy, menses, and menopause remains uncertain and important to clarify.

Multiple factors can promote dysbiosis, and a pathogenic or circumstantial relationship with autoimmune disease has not been established. Nevertheless, the association of dysbiosis with diverse systemic immune-mediated diseases [[116](#_ENREF_116)], its recognition in autoimmune hepatitis[[69](#_ENREF_69),[70](#_ENREF_70)], its possible genetic associations [[176](#_ENREF_176), [177](#_ENREF_177)], and its gender bias[[179-181](#_ENREF_179)] suggest that dysbiosis may constitute an important antigenic or hormonal reservoir that can promote the autoreactive response in diverse systemic immune diseases, including autoimmune hepatitis.

***Molecular homologies between microbial and self-antigens***

Epitopes can be shared between microbial components and self-antigens, and this molecular mimicry can result in cross-reacting antibodies or the activation of T lymphocytes in genetically-predisposed individuals[[33](#_ENREF_33),[184](#_ENREF_184),[185](#_ENREF_185)] (Figure 1). The reactive T lymphocytes can in turn exhibit promiscuous activity[[186](#_ENREF_186)] and target epitopes that are distant homologues to the initial antigenic trigger (epitope spread)[[187](#_ENREF_187)] (Table 1). Bacterial components have generated autoantibodies found in systemic autoimmune diseases, such as systemic lupus erythematosus (SLE)[[188](#_ENREF_188)] and the antiphospholipid syndrome[[189](#_ENREF_189)], and they have been implicated in the progression of SLE[[190](#_ENREF_190)] and the exacerbation of Sjogren’s syndrome, possibly through the activation of memory lymphocytes[[33](#_ENREF_33),[188](#_ENREF_188),[191](#_ENREF_191)]. The atypical pANCA found in PSC and autoimmune hepatitis target an antigen (β-tubulin) that cross-reacts with a bacterial antigen[[53](#_ENREF_53)], and the antibodies to pyruvate dehydrogenase complex-E2 found in PBC cross-react with intestinal *Escherichia coli*[[56](#_ENREF_56),[57](#_ENREF_57)]. The principal mechanism by which the intestinal microbiota may sustain or extend the autoreactive response is molecular mimicry, and experimental animal models of autoimmune hepatitis should evaluate this hypothesis.

***Breech of the intestinal mucosal barrier***

Interactions between the intestinal microenvironment and the systemic immune response imply that the natural barrier between the intestinal and systemic domains can be breeched (Table 2). There is ample evidence to justify this supposition, but the actual mechanisms are uncertain. Reactive T lymphocytes expressing intestinal receptors can be found in the pancreatic islets and lymph nodes of patients and mice with type 1 diabetes[[117](#_ENREF_117),[118](#_ENREF_118),[192](#_ENREF_192),[193](#_ENREF_193)], and lymphocytes originating in the gut mucosa have been implicated in the autoreactive response in experimental autoimmune encephalomyelitis[[194](#_ENREF_194)]. Furthermore, microbial components have been detected in the plasma of patients with cirrhosis[[151](#_ENREF_151),[152](#_ENREF_152)] and portal circulation of animals with non-alcoholic fatty liver disease[[51](#_ENREF_51)].

The migration of gut-derived bacteria and bacterial products from the intestinal lumen to the liver, mesenteric lymph nodes, and other extra-intestinal sites may occur by translocation[[195](#_ENREF_195)] (Figure 1). Translocation implies that intestinal permeability has been increased, possibly because tight junctions within the intestinal mucosa have been weakened or the intestinal barrier has been overwhelmed by bacterial overgrowth[[46](#_ENREF_46),[195-197](#_ENREF_195)]. The translocated bacterial products, including LPS and unmethylated CpG, can then be delivered to the liver via the portal vein and activate TLRs and NLRs[[123](#_ENREF_123),[130](#_ENREF_130)]. Dysbiosis and a “leaky gut” have been implicated in the development of NASH, diabetes, and the metabolic syndrome[[197](#_ENREF_197),[198](#_ENREF_198)].

Bacteria produce short chain fatty acids (acetic acid, butyric acid, and propionic acid) which can affect the tight junctions within the intestinal mucosa[[197](#_ENREF_197),[199](#_ENREF_199),[200](#_ENREF_200)] (Table 2). Butyrate, which is the conjugate base of butyric acid, induces mucin synthesis in the intestinal mucosa, strengthens tight junctions, and reduces bacterial transport across the stressed epithelium[[201-204](#_ENREF_201)]. Butyrate may also have an anti-inflammatory effect by promoting the extra-thymic differentiation of peripheral Tregs[[205](#_ENREF_205),[206](#_ENREF_206)] and inhibiting NF-κB and the transcription of pro-inflammatory cytokines[[207](#_ENREF_207)]. Other short chain fatty acids (propionate) and bacterial by-products (succinate and acetate) do not induce the production of mucin and may increase gut permeability[[37](#_ENREF_37),[202](#_ENREF_202)].

Sodium butyrate acts in part by modulating the beta-catenin-dependent Wnt signaling pathway within cells [[208](#_ENREF_208)]. This pathway affects the transcription of genes that influence cell proliferation and differentiation. In colon carcinoma cell lines, the levels of beta-catenin transcriptional complexes within the cell influences its physiological response to butyrate. High levels of transcriptional complexes result in apoptosis of the cell and low levels result in the reversible limitation of cell growth after exposure to butyrate[[208](#_ENREF_208)]. The ability of butyrate to modulate cell proliferation and apoptosis may in turn influence cell viability and function, and these effects may help maintain the integrity of the gastrointestinal mucosal barrier.

Butyrate can also modulate cell responses to stress of the endoplasmic reticulum by promoting the apoptosis of the cell or its preservation through autophagy [[209-211](#_ENREF_209)]. Butyrate enhances the expression of peroxisome proliferator-activated receptor-gamma (PPARγ) and the activation of caspases (especially caspase 3) that induce apoptosis in colorectal cell lines [[212](#_ENREF_212)]. It is also one of the short chain fatty acids, including propionate, that can induce autophagy in distressed cells and preserve their survival by generating energy and retarding the intrinsic (mitochondrial) pathway of apoptosis [[213](#_ENREF_213), [214](#_ENREF_214)]. Gut-derived short chain fatty acids, such as butyrate and propionate, may be important moderators of intestinal mucosal cell proliferation and function, and they may contribute to the prevention of systemic autoimmune responses and progressive colorectal cancer[[208](#_ENREF_208)].

Lactate, which is a bacterial byproduct of carbohydrate fermentation, also reduces intestinal permeability[[203](#_ENREF_203)] (Table 2). Lactate is fermented mainly to butyrate by intestinal microflora[[215](#_ENREF_215)]. The acetyl-coenzyme A pathway is the major route of butyrate production from lactate, and the intestinal microflora have considerable variability in lactate consumption[[215](#_ENREF_215)]. Furthermore, lactate-utilizing bacteria exhibit variable production of butyrate depending on the availability of other substrates [[216](#_ENREF_216)]. Patients with type 1 diabetes have a lower proportion of butyrate- and lactate-producing bacteria in their intestinal microbiome than case-control subjects, and the dysbiosis favoring increased intestinal permeability may contribute to the development of type 1 diabetes[[217](#_ENREF_217),[218](#_ENREF_218)].

Intestinal epithelial cells are bound together by structural proteins[[37](#_ENREF_37),[219-221](#_ENREF_219)] that are organized into a tripartite junctional complex consisting of a tight junction, adherens junction, and desmosome[[222](#_ENREF_222)]. Occludin is the only known transmembrane protein with a domain in the paracellular space, and it is the principal component of the tight junction. Zona occludens 1 and 2 and cingulin are nontransmembrane proteins found in tight junctions at sites of cell-to-cell contact[[222](#_ENREF_222)]. They are bound to occludin, and they probably couple cells to the cytoskeleton[[222](#_ENREF_222),[223](#_ENREF_223)]. Actin and myosin filaments anchor cells together by calcium-dependent adhesion molecules (E-cadherins) in an adherens junction, and intermediate filaments are anchored to desmosomes and help bind cells[[222](#_ENREF_222)]. Multiple cellular signaling pathways affect the assembly and sealing of the junctions[[223](#_ENREF_223)], and they are cell-type specific with protein kinase C modulating occludin and zona occludens 1[[222](#_ENREF_222)].

*Escherichia coli* and *Clostridia difficile* can dissociate the binding proteins and increase intestinal permeability by opening a paracellular route[[37](#_ENREF_37),[224](#_ENREF_224)] (Table 2). The TLRs on the intestinal epithelial cells can modulate the integrity of the intestinal barrier, possibly by influencing the expression of molecular mediators that can affect the structure or function of the binding proteins[[225](#_ENREF_225),[226](#_ENREF_226)]. Activation of TLR2 increases the phosphorylation of isoforms of protein kinase C, and this action has been associated with enhanced expression of zona occludens and the sealing of tight junctions[[225](#_ENREF_225)]. Conversely, activation of TLR4 reduces the expression of phosphorylated occludin and increases intercellular permeability[[226](#_ENREF_226)]. Bacterial ligands derived from different microbial species may influence intestinal permeability through TLR signaling and the translocation of microbial products through a porous intestinal barrier may contribute to a systemic autoreactive response[[227-229](#_ENREF_227)].

Another mechanism by which the microbiome may influence the systemic immune response is by the active transport of bacterial antigens across the mucosal barrier by M cells within Peyer’s patches[[230](#_ENREF_230)]. Whereas immune cells can be activated in the intestine and migrate to the liver or peripheral lymph tissue by translocation, they may also be activated in the systemic circulation by translocated or actively transported bacterial components that are presented by antigen presenting cells and recognized as foreign antigens by circulating naïve CD4+ T helper lymphocytes[[39](#_ENREF_39),[117](#_ENREF_117),[118](#_ENREF_118),[193](#_ENREF_193)].

**IMPLICATIONS OF THE INTESTINAL MICROBIOME IN AUTOIMMUNE HEPATITIS**

The intestinal microbiome has been implicated in the pathogenesis of diverse inflammatory liver diseases, including NASH, PSC and PBC[[48](#_ENREF_48),[51](#_ENREF_51),[54](#_ENREF_54),[59](#_ENREF_59),[62](#_ENREF_62)], and the pathogenic pathways of autoimmune hepatitis have been incompletely defined[[2](#_ENREF_2)]. The principal target antigen remains unclear in most patients with autoimmune hepatitis, and conventional corticosteroid therapies have been unable to consistently induce sustained treatment-free remissions[[231](#_ENREF_231),[232](#_ENREF_232)]. Progressive hepatic fibrosis occurs in 25% of patients[[233](#_ENREF_233)], and emerging drug therapies and molecular interventions can suppress the immune response but not eliminate the disease[[234](#_ENREF_234)].

The intestinal microbiome is a reservoir of antigens and activated immune cells that could initiate, exacerbate, or perpetuate autoimmune hepatitis[[30-34](#_ENREF_30)]; dysbiosis has been demonstrated in experimental and human autoimmune hepatitis[[69](#_ENREF_69),[70](#_ENREF_70)]; atypical pANCA, manifested in most patients with autoimmune hepatitis, cross-react with an antigen found in intestinal bacteria[[53](#_ENREF_53)]; and the permeability of the intestinal mucosal barrier has been increased in patients with the disease[[70](#_ENREF_70)]. Investigations of the microbiome in autoimmune hepatitis may discover new antigens and suggest new therapies that might eliminate the primary antigen or the supplemental antigens that sustain or advance the disease.

**EVALUATING THE INTESTINAL MICROBIOME IN AUTOIMMUNE HEPATITIS**

Traditional stool culture techniques are limited in assessing the intestinal microbiome mainly because anaerobic organisms are difficult to culture[[235](#_ENREF_235)] and some microbial species may elude detection by conventional protocols[[235](#_ENREF_235),[236](#_ENREF_236)]. A common method for studying the diversity of the intestinal microflora has been to sequence the 16S ribosomal ribonucleic acid (rRNA) gene[[235](#_ENREF_235),[237](#_ENREF_237)]. The 16S rRNA gene is present in all prokaryotic cells; it has highly variable regions interspersed with highly conserved regions; and its sequences are unique to the major groups of prokaryotic organisms[[237](#_ENREF_237)]. These signature sequences can be used to reconstruct the phylogeny of the intestinal microbiome[[236](#_ENREF_236)].

Primers are designed that are complementary to the universally conserved regions that flank the variable regions, and the bacterial species and their proportions in the microbiome are determined[[237](#_ENREF_237),[238](#_ENREF_238)]. The variable regions are amplified by polymerase chain reaction (PCR), and the PCR products are purified for sequencing[[81](#_ENREF_81)]. Sequencing results are compared to established annotated datasets[[235](#_ENREF_235)]. The sequencing protocol misidentifies or omits a critical residue in only 1% of procedures[[237](#_ENREF_237)]. Ambiguities resulting from the sequencing determinations are the most common errors, and they may reflect inadequacies in the available datasets[[235](#_ENREF_235),[237](#_ENREF_237)]. Only known bacterial sequences or sequences closely homologous to known bacterial sequences can be analyzed[[235](#_ENREF_235)].

Publicly available databases are available and continue to evolve for analyses of 16S rRNA gene sequences[[236](#_ENREF_236),[239](#_ENREF_239)]. Quantitative Insights Into Microbial Ecology (QIIME)[[240](#_ENREF_240)] and Mothur, a comprehensive software package that integrates several algorithms from pre-existing software[[241](#_ENREF_241)], are open-source tools that can be used to describe and compare microbial communities. Short 16S rRNA sequences can be organized into OTUs, and a motif-based hierarchical method (MtHc) can analyze massive metagenomic datasets with high accuracy[[242](#_ENREF_242)]. The Human Microbiome Project provides a catalogue of bacterial species, and it will help define the nature of the intestinal microbiome, the factors that affect its composition, distribution and evolution, and the relationship of the intestinal microflora to human health and disease[[236](#_ENREF_236),[243](#_ENREF_243),[244](#_ENREF_244)].

Further advances in the techniques used to reconstruct the composition of the human intestinal microbiome include microarray technology, fingerprinting techniques such as determination of the terminal restriction fragment length polymorphisms (T-RFLP), and next-generation sequencing (NGS)[[79](#_ENREF_79),[245-247](#_ENREF_245)]. Microarray hybridization of deoxyribonucleic acid (DNA) provides a high throughput platform that consists of several thousand probes that can detect nucleic acid sequences simultaneously[[246-248](#_ENREF_246)]. Unknown microbial sequences and uncharacterized microbial populations are undetected by the microarray techniques, and uncertainties about the existence and importance of undiscovered microbial populations are the major limitations of this method[[247](#_ENREF_247)[248](#_ENREF_248)].

The Human Gut Chip (HuGChip) has 4441 probes that includes 2442 probes specific for known microbes and 1919 probes that are explorative for unknown microbes[[247](#_ENREF_247)]. Probes with overlapping similarities are becoming more sensitive to microbial species that are less abundant[[249](#_ENREF_249)], and probes with explorative designs are being coupled to probes with microbial specificity in an effort to identify microorganisms with uncharacterized sequences[[247](#_ENREF_247),[250](#_ENREF_250)]. Essential for the design of useful explorative probes is the correct anticipation of genetic variations within the microbial community and the construction of probes with high sensitivity and specificity[[249](#_ENREF_249)].

Currently, intestinal ecosystems are being studied mainly by 16S rRNA sequencing[[236](#_ENREF_236)]. This technique is useful in identifying the microbial species that constitute the intestinal microbiome, determining the evolution and transition of the microbial community (phylogeny) and quantitating the microbial diversity[[236](#_ENREF_236)]. The challenge is to define the functions of the microbiome, and whole genome sequencing (WGS) may provide these insights[[236](#_ENREF_236),[251](#_ENREF_251)]. Array-based next generation sequencing (NGS) can analyze the whole genome, exons, and regions of interest, and it may emerge as the lens by which to understand the function of the microflora[[79](#_ENREF_79)].

**DEVELOPING TREATMENT STRATEGIES TO INVESTIGATE**

The intestinal microbiome can be manipulated by dietary adjustments [[37](#_ENREF_37), [90](#_ENREF_90), [252](#_ENREF_252)], probiotic preparations[[197](#_ENREF_197),[253](#_ENREF_253),[254](#_ENREF_254)], supplements of vitamin A and retinoic acid [[255](#_ENREF_255), [256](#_ENREF_256)], antibiotics[[169](#_ENREF_169),[257](#_ENREF_257)], intestinal re-colonization[[33](#_ENREF_33),[107](#_ENREF_107),[109](#_ENREF_109),[110](#_ENREF_110)], pharmacological agents that decrease intestinal permeability[[258-260](#_ENREF_258)], molecular interventions that block TLR signaling and the production of pro-inflammatory cytokines[[261](#_ENREF_261)], molecular interventions (polysaccharide A) that stimulate anti-inflammatory responses[[105](#_ENREF_105), [262](#_ENREF_262)], and short chain fatty acids that may modulate signaling pathways that affect gene expression, intestinal barrier integrity, and inflammatory responses[[200](#_ENREF_200)] (Table 3).

Antibiotics (tetracycline and minocycline) have reduced disease activity in rheumatoid arthritis, especially in seropositive patients with disease of short duration[[257](#_ENREF_257)]. Probiotic supplements containing *Bifidobacterium bifidum* have promoted the expansion of Tregs in cell cultures[[253](#_ENREF_253)], and probiotics enriched with strains of *Lactobacillus* alone or in combination with retinoic acid have prevented the development of type 1 diabetes in NOD mice[[254](#_ENREF_254),[263](#_ENREF_263)]. Gelatin tannate has been used in a murine model of acute colitis to protect the mucosal barrier, alter the composition of the microbiota, and decrease inflammatory activity[[259](#_ENREF_259)]. Gelatin tannate has also been evaluated in LPS-stimulated cell cultures, and it has inhibited the expression of the intercellular adhesion molecule-1 (ICAM-1) and reduced the production of IL-8 and TNF-α in a dose-dependent fashion[[260](#_ENREF_260)]. Oligodeoxynucleotides designed to block TLR7 signaling have improved tests and reduced activity in a murine model of lupus nephritis and lung injury[[261](#_ENREF_261)]; polysaccharide A has induced IL-10 producing Tregs in experimental autoimmune encephalitis[[105](#_ENREF_105),[262](#_ENREF_262)]; and short chain fatty acids have modulated intestinal signaling pathways, inhibited histone deacetylases, regulated gene expression, and increased the integrity of the intestinal barrier[[200](#_ENREF_200),[264](#_ENREF_264)].

Manipulations of the intestinal microbiota have also shown promise in animal models and patients with liver disease. In rats with carbon tetrachloride-induced cirrhosis, antibiotic therapy and probiotic supplements have decreased systemic endotoxin levels and improved liver tests[[265](#_ENREF_265)], and in rats with ischemic/re-perfusion liver injury, probiotic supplements with *Lactobacillus* have reduced the production of pro-inflammatory, pro-fibrotic cytokines and improved liver tests[[266](#_ENREF_266)] (Table 3). The intestinal microbiota have been implicated in the pathogenesis of PSC[[267](#_ENREF_267),[268](#_ENREF_268)], and a small randomized clinical trial has indicated that treatment with vancomycin or metronidazole can improve serum alkaline phosphatase and bilirubin levels and decrease pruritus[[269](#_ENREF_269)]. Clarification of the role of the intestinal microbiome in autoimmune hepatitis is necessary to direct investigational strategies that would help develop ancillary interventions to improve the outcome of this disease.

Manipulations of the intestinal microbiota, especially with antibiotics, may have adverse consequences which must be defined in animal and human studies and counter-balanced against potential benefits (Table 3). The intestinal microbiome performs important digestive and detoxification functions, produces nutrients and short chain fatty acids that can affect intestinal integrity, defends against invading pathogens, and influences the innate and adaptive immune responses within and outside the intestine[[30](#_ENREF_30),[270](#_ENREF_270),[271](#_ENREF_271)]. Dysbiosis associated with antibiotics, especially in early age, may perturb immune tolerance for the microflora and predispose to other immune-mediated diseases (asthma, celiac disease, and type 1 diabetes)[[165](#_ENREF_165),[166](#_ENREF_166),[175](#_ENREF_175),[272](#_ENREF_272)]. Antibiotic manipulations may also favor the emergence of drug-resistant pathogenic or immunogenic microflora [[169](#_ENREF_169)]. The optimal nature and duration of the manipulations that might impact on the intestinal microbiome are uncertain, and the durability of the responses are unclear. Much work needs to be done to establish microbiome manipulation as a way forward in autoimmune hepatitis, but observations already made in diverse systemic immune-mediated diseases and the unmet needs in the management of autoimmune hepatitis justify rigorous evaluation of this possibility.

**OVERVIEW**

Dysbiosis has already been described in experimental and human autoimmune hepatitis[[69](#_ENREF_69),[70](#_ENREF_70)]; antibodies reactive to antigens homologous to bacterial antigens (atypical pANCA) have been commonly present in patients with the disease[[53](#_ENREF_53),[65](#_ENREF_65)]; and increased permeability of the intestinal mucosal barrier has been demonstrated[[70](#_ENREF_70)]. The intestinal microbiome is an available source of immune stimulatory antigens, products, and immune cells that already have been implicated in multiple systemic immune-mediated (rheumatoid arthritis, diabetes, multiple sclerosis, inflammatory bowel disease)[[35](#_ENREF_35),[38](#_ENREF_38),[40-43](#_ENREF_40)] and chronic liver diseases (NASH, PBC)[[51](#_ENREF_51),[54](#_ENREF_54)]. The role of the intestinal microbiome in the occurrence and behavior of autoimmune hepatitis warrants rigorous evaluation.

The sequencing of the 16S rRNA gene can be used to characterize the intestinal microbial population and determine disease-specific dysbioses[[236](#_ENREF_236)]. Microarrays comprised of thousands of microbial probes can be applied to enhance comprehension of the “pathological” components of the intestinal microbiome[[247](#_ENREF_247),[249](#_ENREF_249)]. Whole genome sequencing and next generation sequencing of genomic regions specific for the disease can define the disease-related metagenome[[79](#_ENREF_79)], and associations between gut-derived micro-organisms and the immune-mediated mechanisms of autoimmune hepatitis can then be evaluated in experimental models.

Adjunctive forms of therapy may emerge to complement current immunosuppressive regimens. Individual bacterial species within the microbiome may be manipulated by diet, designer probiotics, re-colonization methods, antibiotics, selected vitamin supplements, and pharmacological agents that decrease intestinal permeability[[236](#_ENREF_236)]. Molecular interventions that block TLR signaling or modulate signaling pathways may also evolve to reduce pro-inflammatory cytokine production, limit unfavorable gene expression, and strengthen the integrity of the intestinal barrier[[200](#_ENREF_200),[261](#_ENREF_261)].

Major obstacles to the performance of these studies are the limited number of publicly available tools to analyze the microbial metagenome, especially in translational settings[[239](#_ENREF_239)], the multiplicity of environmental factors (diet, antibiotics, sanitation) that can affect variations of the microbiome among communities and between individuals[[273](#_ENREF_273),[274](#_ENREF_274)], and uncertainties about the roles of luminal and mucosal microbiota in directing the immune response[[81](#_ENREF_81),[275](#_ENREF_275)].

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**Figure 1 Interactions between the intestinal microbiome and the liver.** Dysbiosis can generate microbe-associated molecular patterns (MAMPs) that activate toll-like receptors (TRLs) in the intestine. Activated TLRs can stimulate the transcription factor, nuclear factor-kappa B (NF-κB), in macrophages and generate pro-inflammatory cytokines. They can also increase the expression of the major histocompatibility complex (MHC) on antigen presenting cells (APCs) and sensitize CD4 lymphocytes to bacterial ligands. The activated lymphocytes can proliferate as T helper (Th) 1, Th2, and Th17 cells. The dysbiosis can also generate short chain fatty acids, endotoxin, lipopolysaccharide (LPS), and bacterial components that can serve as antigenic ligands. Tight junctions within the intestinal mucosa may weaken with the dysbiosis and allow paracellular translocation of lymphocytes, bacterial ligands and endotoxin. These gut-derived elements can then enter the portal vein and be delivered to the liver. The bacterial ligands within the liver can activate TLRs within hepatocytes, hepatic stellate cells, Kupffer cells, and sinusoidal epithelial cells and generate pro-inflammatory cytokines and reactive oxygen species (ROS) that can produce damage-associated molecular patterns (DAMPs) that activate TLRs in a self-amplification loop (upper right corner blow-up). The hepatic TLRs can also contribute to the sensitization of CD4 lymphocytes to bacterial ligands and self-antigens that resemble bacterial ligands (molecular mimicry). Concurrently, the bacterial ligands and gut-derived endotoxin can activate the non-obese diabetes-like receptor (NLR) of inflammasomes within hepatocytes and hepatic stellate cells (upper left corner blow-up). The release of caspase 1 can generate interleukin (IL) 1β and IL-18 and promote tissue injury and the immune response. The net effect is to increase hepatic inflammation and liver damage and predispose to autoimmunity and hepatic fibrosis.

**Table 1 Key Requirements for gut-derived systemic immune response**

|  |  |  |
| --- | --- | --- |
| **Key requirement** | **Features** | **Mechanisms** |
| Activation of TLRs | Intestinal receptors responsive to MAMPs and DAMPS[[34](#_ENREF_34),[47](#_ENREF_47),[48](#_ENREF_48),[120](#_ENREF_120)]  Signaling dependent on MyD88[[124](#_ENREF_124),[125](#_ENREF_125)]  Activates NF-κB[[123](#_ENREF_123),[125](#_ENREF_125),[126](#_ENREF_126)]  Favors T lymphocyte activation[[41](#_ENREF_41)]  Modulates actions of Tregs[[141](#_ENREF_141),[142](#_ENREF_142)]  Present in hepatocytes, HSCs, Kupffer cells, sinusoidal epithelial cells, BEC[[48](#_ENREF_48)] | Increases pro-inflammatory cytokines[[126](#_ENREF_126)]  Upregulates class II MHC[[127](#_ENREF_127)]  Increases co-stimulatory molecules[[41](#_ENREF_41), [127](#_ENREF_127)]  Promotes pathogen-specific responses[[142](#_ENREF_142)]  LPS activates TLR4[[123](#_ENREF_123),[134](#_ENREF_134)]  Sequences in bacteria activate TLR9 [[135](#_ENREF_135)]  TLR4 in HSCs promote fibrosis[[46](#_ENREF_46),[144](#_ENREF_144)]  Implicated in other liver diseases[[58](#_ENREF_58),[148](#_ENREF_148)] |
| Stimulation of inflammasomes | Protein complexes that release pro-inflammatory IL-1β and IL-18[[111-113](#_ENREF_111)]  NLRs sense microbial products[[156](#_ENREF_156)]  Upregulated in Kupffer cells, hepatocytes, and sinusoidal epithelial cells[[113](#_ENREF_113)]  Activation by highly diverse ligands[[112](#_ENREF_112)] | Upregulated in hepatocytes by LPS[[113](#_ENREF_113)]  Activates pro-caspase 1[[156](#_ENREF_156)]  Promotes hepatic fibrosis[[50](#_ENREF_50)]  Shapes innate and adaptive immunity [[112](#_ENREF_112), [160](#_ENREF_160)]  Implicated in NAFLD[[51](#_ENREF_51)]  Activation separate from TLRs[[112](#_ENREF_112),[155](#_ENREF_155)] |
| Emergence of dysbiosis | Microflora differ from commensals[[116](#_ENREF_116)]  Dysbiosis varies in specific diseases[[116](#_ENREF_116)]  Less bacterial diversity common[[170](#_ENREF_170)]  Antibiotics most frequent basis[[165](#_ENREF_165),[175](#_ENREF_175)]  Uncertain cause or effect of disease[[116](#_ENREF_116)] | Can activate TLRs and NLRs[[116](#_ENREF_116),[173](#_ENREF_173)]  Genetic factors may affect composition[[177](#_ENREF_177)]  Gender-related compositional differences[[179](#_ENREF_179)]  May affect gender-related autoimmunity[[180](#_ENREF_180)]  Present in AIH and experimental NASH[[47](#_ENREF_47),[69](#_ENREF_69)] |
| Molecular mimicry | Microbial and self-homologies[[33](#_ENREF_33),[185](#_ENREF_185)]  Cross-reacting antibodies [[53](#_ENREF_53), [57](#_ENREF_57), [184](#_ENREF_184)]  Promiscuous activity of effectors [[186](#_ENREF_186)]  Epitope spread [[187](#_ENREF_187)] | pANCA react with bacterial antigen [[53](#_ENREF_53)]  AMA cross-reacts with *Escherichia coli* [[56](#_ENREF_56), [57](#_ENREF_57)]  Increasingly distant homologues targeted [[187](#_ENREF_187)] |
| Breech of intestinal mucosal barrier | Gut-derived products enter system [[195](#_ENREF_195)]  Translocation prime basis [[46](#_ENREF_46), [195](#_ENREF_195)]  Active transport also possible [[230](#_ENREF_230)] | Gut-derived lymphocytes in lymph nodes [[118](#_ENREF_118)]  Microbial components in peripheral blood [[195](#_ENREF_195)]  Activates TLRs and NLRs [[123](#_ENREF_123), [130](#_ENREF_130)]  Implicated in NASH and diabetes [[197](#_ENREF_197), [198](#_ENREF_198)] |

AMA: Antimitochondrial antibodies; BEC: Biliary epithelial cells; DAMPS: Damage-associated molecular patterns; HSCs: Hepatic stellate cells; IL: Interleukin; LPS: Lipopolysaccharide; MAMPs: Microbe-associated molecular patterns; MHC: Major histocompatibility complex; MyD88: Myeloid differentiation factor 88; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; NF-κB: Nuclear factor kappa B; NLRs: Non-obese diabetes-like receptors; pANCA: Atypical perinuclear anti-neutrophil cytoplasm antibodies; TLRs: Toll-like receptors; Tregs: Regulatory T cells. Superscripted numbers in brackets are references.

**Table 2 Microbial mechanisms for breeching intestinal barrier**

|  |  |  |
| --- | --- | --- |
| **Microbial Effect** | **Features** | **Mechanisms** |
| Translocation | Migration of gut-derived products[[195](#_ENREF_195),[224](#_ENREF_224)]   * Tight junctions weakened[[218](#_ENREF_218)] * Increased intestinal permeability[[195](#_ENREF_195), [218](#_ENREF_218)] * Paracellular migration[[37](#_ENREF_37),[224](#_ENREF_224)]   Consequences[[192](#_ENREF_192)]   * LPS and CpG delivered to liver[[123](#_ENREF_123), [130](#_ENREF_130), [195](#_ENREF_195)] * Activated immune cells translocate[[118](#_ENREF_118),[193](#_ENREF_193)] * Translocated microbial antigens activate peripheral immune cells[[185](#_ENREF_185)] * TLRs and NLRs activated[[123](#_ENREF_123),[130](#_ENREF_130)] | Gut-derived SCFA affect tight junctions[[200](#_ENREF_200)]  Butyrate strengthens intestinal barrier[[203](#_ENREF_203)]   * Induces mucin synthesis[[201](#_ENREF_201), [203](#_ENREF_203)] * Reduces bacterial translocation[[204](#_ENREF_204)] * Increases peripheral Tregs[[205](#_ENREF_205)] * Inhibits NF-κB and inflammation[[207](#_ENREF_207)]   Lactate strengthens intestinal barrier[[37](#_ENREF_37)]   * Fermented to butyrate[[215](#_ENREF_215),[216](#_ENREF_216)]   Low butyrate- and lactate- producing bacteria associated with weak barrier[[217](#_ENREF_217),[218](#_ENREF_218)] |
| Increased mucosal permeability | Intestinal epithelial cells bound together by junctional complex of proteins[[222](#_ENREF_222),[223](#_ENREF_223)]   * Occludin main component[[222](#_ENREF_222)] * Zona occludens couples cytoskeleton[[222](#_ENREF_222)] * Cingulin contacts cell[[222](#_ENREF_222)] * Actin and myosin anchor cells[[222](#_ENREF_222)] * Intermediate filaments bind cells[[222](#_ENREF_222)]   Signaling pathways seal junction[[223](#_ENREF_223)]  Protein kinase C modulates occluding[[222](#_ENREF_222)] | TLRs affect molecular mediators[[225](#_ENREF_225),[226](#_ENREF_226)]  Signaling pathways disrupted[[223](#_ENREF_223)]  Junctional binding proteins dissociated[[224](#_ENREF_224)]  Paracellular migration routes formed[[37](#_ENREF_37),[224](#_ENREF_224)]  *E. coli* and *C. difficile* key effectors[[37](#_ENREF_37)] |
| Active transport | Bacterial antigens actively transported across intestinal barrier[[230](#_ENREF_230)] | M cells in Peyer’s patches capable of active transport[[230](#_ENREF_230)] |

Superscripted numbers in brackets are references. Cpg: Un-methylated cytosine-phosphorothioate-guanine oligonucleotide; LPS: Lipopolysaccharide; NF-κB: Nuclear factor kappa B; NLRs: Non-obese diabetes-like receptors; SCFA: Short chain fatty acids; TLRs: Toll-like receptors; Tregs: Regulatory T cells.

**Table 3 Treatment considerations for investigation of gut-derived immune responses**

|  |  |  |
| --- | --- | --- |
| **Treatment Consideration** | **Nature** | **Findings** |
| Dietary adjustments | Animal protein, saturated fats[[90](#_ENREF_90)]  High carbohydrate diets[[90](#_ENREF_90)]  Low fat high fiber diet[[90](#_ENREF_90)] | *Bacteroides,* Firmicutes (including *Clostridia*), and *Prevotella* favored by different dietary regimens [[37](#_ENREF_37), [90](#_ENREF_90)] |
| Probiotic preparations | *Bifidobacterium bifidum*[[253](#_ENREF_253)]  *Lactobacillus* strains[[254](#_ENREF_254),[263](#_ENREF_263),[266](#_ENREF_266)]  Lactobacillus rhamnosus[[276](#_ENREF_276)]  *Anaerostipes caccae*[[277](#_ENREF_277)] | Expands Tregs in cell culture[[278](#_ENREF_278)]  Prevents diabetes in NOD mice [[263](#_ENREF_263)]  Improves liver tests in rat model [[266](#_ENREF_266)]  Increases tight junction proteins [[276](#_ENREF_276)]  Consumes lactate and produces butyrate[[277](#_ENREF_277)] |
| Vitamin A and retinoic acid | Retinoic acid supplement [[255](#_ENREF_255)]  Dietary vitamin A [[256](#_ENREF_256)] | Restores *Lactobacilli* in lupus model [[255](#_ENREF_255)]  Regulates cytokine in lupus model [[256](#_ENREF_256)]  Induces IL-10-producing Tregs [[279](#_ENREF_279)] |
| Antibiotics | Tetracycline, minocycline [[257](#_ENREF_257)]  Vancomycin, metronidazole [[269](#_ENREF_269)] | Reduces activity in RA [[257](#_ENREF_257)]  Improves tests and pruritus in PSC [[269](#_ENREF_269)] |
| Re-colonization  Fecal transplantation | *Bacteroides fragilis* [[107](#_ENREF_107)]  *Clostridia species* [[109](#_ENREF_109), [110](#_ENREF_110)] | Induces Tregs in colitis model [[107](#_ENREF_107), [109](#_ENREF_109), [110](#_ENREF_110)] |
| Intestinal barrier protectors | Gelatin tannate [[258-260](#_ENREF_258)] | Enhances mucus barrier [[258](#_ENREF_258), [259](#_ENREF_259)]  Reduces activity in murine colitis [[259](#_ENREF_259)]  Alters composition of microbiota [[259](#_ENREF_259)]  Limits inflammatory effects of LPS [[260](#_ENREF_260)]  Inhibits IL-8 and TNF-α in LPS cells [[260](#_ENREF_260)] |
| TLR inhibitors | Oligodeoxynucleotides blocking TLR7 signaling [[261](#_ENREF_261)] | Improves tests and reduces activity in murine model of lupus nephritis [[261](#_ENREF_261)]  Improves autoimmune lung injury [[261](#_ENREF_261)] |
| Molecular interventions | Polysaccharide A [[105](#_ENREF_105), [262](#_ENREF_262)] | Induces IL-10 producing Tregs [[105](#_ENREF_105), [262](#_ENREF_262)]  Protects against EAE in mice [[105](#_ENREF_105)] |
| Short chain fatty acids | Acetate, proprionate, butyrate [[200](#_ENREF_200)] | Modulates gut signaling pathways [[200](#_ENREF_200)]  Inhibits histone deacetylases [[200](#_ENREF_200), [264](#_ENREF_264)]  Regulates gene expression [[200](#_ENREF_200)]  Enhances gut integrity [[200](#_ENREF_200)] |

Superscripted numbers in brackets are references. EAE: Experimental autoimmune encephalitis; IL: Interleukin; LPS: Lipopolysaccharide; NOD: Non-obese diabetes; PSC: Primary sclerosing cholangitis; RA: Rheumatoid arthritis; TLR: Toll-like receptor; TNF-α: Tumor necrosis factor-alpha; Tregs: Regulatory T cells.