

REVIEW ARTICLE

Gut microbiome in type 1 diabetes: A comprehensive review

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Funding information

National Natural Science Foundation of China, Grant/Award Numbers: 81500600, 81670716 and 81461168031; Central South University, Grant/Award Number: 502211703; National Science and Technology Infrastructure Program, Grant/Award Number: 2015BAI12B13

Summary

Type 1 diabetes (T1D) is an autoimmune disease, which is characterized by the destruction of islet β cells in the pancreas triggered by genetic and environmental factors. In past decades, extensive familial and genome-wide association studies have revealed more than 50 risk loci in the genome. However, genetic susceptibility cannot explain the increased incidence of T1D worldwide, which is very likely attributed by the growing impact of environmental factors, especially gut microbiome. Recently, the role of gut microbiome in the pathogenesis of T1D has been uncovered by the increasing evidence from both human subjects and animal models, strongly indicating that gut microbiome might be a pivotal hub of T1D-triggering factors, especially environmental factors. In this review, we summarize the current aetiological and mechanism studies of gut microbiome in T1D. A better understanding of the role of gut microbiome in T1D may provide us with powerful prognostic and therapeutic tools in the near future.

KEYWORDS

environmental factors, genetics, gut microbiome, immune system, type 1 diabetes

1 | INTRODUCTION OF TYPE 1 DIABETES

Type 1 diabetes (T1D) is an organ-specific autoimmune disease due to T lymphocytes and other immune cell infiltrating and attacking pancreatic β cells, resulting in the destruction of β cells and progression to insulin deficiency.¹ Type 1 diabetes is consisted of 2 subtypes. The majority of T1D is the autoimmune type (T1A), with a smaller proportion being the non-autoimmune type, also known as the idiopathic type 1 diabetes (T1B). In clinical practice, islet cell autoantibodies, including anti-insulin autoantibody (IAA), glutamic acid decarboxylase antibody (GADA), anti-protein tyrosine phosphatase like protein (IA-2A) and zinc transporter 8 antibody (ZnT8A) are used to diagnose T1A and identify high-risk subjects.²⁻⁴ Epidemiologically, incidence of T1D increases at a rate of 3% to 5% per year.⁵ Moreover, multiple factors influence the susceptibility to T1D, including genetic and environmental factors. Although gender is a very important factor in a variety of autoimmune diseases (women are generally more

susceptible than men), there is no gender difference in the prevalence of T1D in humans.⁶⁻⁸

With regard to genetic factors, more than 50 T1D susceptibility genes have been uncovered by familial linkage analysis and genome-wide association studies. Notably, not every gene contributes to T1D susceptibility equally. Particularly, the HLA-DR and -DQ genes account for approximately 40% to 50% of the disease risk.⁹⁻¹¹ Other susceptibility genes, including PTPN22, IL2Ra, and CTLA4 are also interrelated closely with T1D.¹²⁻¹⁴

Environmental factors are considered to be another essential modulator of T1D.^{15,16} In the islets of newly diagnosed diabetic children, an abundance of IFN- γ and HLA could be found, indicating that viral infection may be involved in T1D. Subsequent studies demonstrated that viruses, especially, enterovirus and Coxsackie B virus, may speed up the progression of T1D, potentially by directly inducing insulinitis or activating immune system through molecular mimicry of islet autoantigens.^{17,18} Diet is another environmental factor

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influencing disease susceptibility. In non-obese diabetic (NOD) mice, gluten-free diet delayed diabetes, and early dietary exposure to cow's milk proteins accelerated T1D, which is possibly because of the fact that albumin in cow's milk is a molecular mimic of ICA-1, a surface protein on β cells.¹⁹ Vitamin D is thought to be the protective factor against T1D, and the deficiency of vitamin D may increase the risk of T1D.^{20,21} In addition, antibiotics have been used to treat infections for more than 50 years. In the last few years, researchers believed that the abuse of antibiotics is correlated with the increased incidence of T1D, but its exact role is still controversial. While some literature suggested antibiotics usage in early life increased T1D susceptibility, another study reported antibiotics protected from T1D in Bio-Breeding diabetes-prone (BB-DP) rat or in NOD mice by modulating gut microbiome.²²⁻²⁴ Mikkelsen and colleagues showed that antibiotics usage within 1 year had nothing to do with the risk of developing T1D.²⁵

2 | GUT MICROBIOME AND TYPE 1 DIABETES

2.1 | Introduction of gut microbiome

Gut microbiome contains approximately 500 to 1000 different bacterial species and 100 trillion (10^{14}) bacteria residing in gastrointestinal tract. Because of the symbiotic relationship between gut microbiome and our body, gut microbiome also is called commensal bacteria.²⁶ Microbes in the gut and intestine are generally divided into Gram-positive (G+) and Gram-negative (G-) populations.^{27,28} Usually, gastrointestinal tracts are dominated by 4 bacterial phyla based on 16S rRNA sequencing, including *Firmicutes*, *Bacteroides*, *Proteobacteria*, and *Actinobacteria*.²⁹ *Firmicutes* and *Bacteroidetes* constitute the most abundant phyla in the adult's gut and intestine, and *Actinobacteria* is predominant in the gut of breast-fed infants.³⁰ *Bifidobacterium* is the most abundant bacteria in *Actinobacteria* and considered to be probiotic microorganisms. Here we quote the definition of microbiota and microbiome by Samantha and Whiteside.³¹ Microbiota refers to microorganisms in a particular environment and can be detected by 16S rRNA sequencing. However, microbiome refers to a larger range, including not only 16S rRNA region but also the whole bacterial genome and products.³¹

2.2 | The role of gut microbiome in T1D

Gut microbiome maintains a constantly dynamic and homeostatic condition. However, at the same time, it can be affected by multiple factors. During childbirth, gut microbiome in infants is largely determined by delivery mode. A study showed that gut microbes in vaginally delivered infants were closer to the mother's vaginal microbiome. In contrast, bacteria in the gut of infants who were delivered through C-section were similar to those on mother's skin surface.³² Additionally, Biasucci and colleagues demonstrated that caesarean delivery affected the early biodiversity of intestinal bacteria of the newborn, indicating that the mode of delivery could influence the composition of intestinal bacteria substantially.³³ After childbirth, gut microbiome is largely affected by diet, antibiotics usage, medicine, and even pH value of drinking water. For example, high-fat and high-sugar diet affected the incidence of diabetes by causing dysbiosis of

gut microbiota, particularly the decreased *Bifidobacteria* spp.³⁴ In NOD mice, treatment with a broad-spectrum antibiotic altered the composition of gut microbes and decreased the proportion of regulatory T cells (Tregs) in the intestinal lamina propria, resulting in an increased incidence of T1D.²² On the contrary, Hu and colleagues reported that NOD mice received with antibiotics were protected from T1D development. In particular, when mothers were treated with antibiotics, the offspring could also be protected from T1D with an unknown mechanism.³⁵ Another study showed that although antibiotic treatment in NOD mice changed insulin sensitivity as well as the composition of gut microbes, gut permeability was not altered in the following 8 weeks.³⁶ Therefore, the impact of antibiotic treatment on gut microbiome and T1D is far from conclusive and may depend on the specific antibiotic used and time window of usage. Proton pump inhibitors are mainly used to depress gastric acid production and treat peptic ulcers. Treatment of proton pump inhibitors lowered gut microbial diversity.³⁷ The pH value of drinking water also influenced the composition and diversity of intestine bacteria.^{38,39} In conclusion, multiple factors can affect gut microbiome. The dysregulation of gut microbiome is closely involved in the pathogenesis of T1D.⁴⁰

Gut microbiome is regarded as another essential modulator of T1D susceptibility in recent years, illustrated by a very quickly growing number of studies reported. In 2008, Wen and colleagues found that specific pathogen free (SPF) condition protected myeloid differentiation primary response gene88 (MyD88)-deficient NOD mice from T1D. However, germ-free (GF) environment reversed the disease protection in those mice, indicating that gut microbiome might be a critical factor in T1D pathogenesis.⁴¹ The following studies showed that *Bacillus cereus* could delay T1D onset and decrease disease incidence in GF NOD mice and BB-DP rats.⁴² Bacterium *A. muciniphila* protected NOD mice from T1D, especially during infancy, suggesting that early postpartum was a critical time for the microbial protective role to take place.²⁴ However, some peptides secreted by certain bacteria in the gut mimicked pancreatic autoantigen and activated diabetogenic CD8 T cells to accelerate insulinitis in NOD mice.⁴³ Similarly, *Citrobacter rodentium* was shown to promote the development of T1D.²⁹ All these studies demonstrated that gut microbiome may be a pivotal modulator in T1D pathogenesis.

2.3 | Taxonomic changes of gut microbiome in T1D pathogenesis

Taxonomic study of gut microflora is usually performed by 16S rRNA sequencing to identify the classification of phylum, family, genus, and species of gut microbes. Taxonomic changes of gut microbiome in T1D have been uncovered by a number of longitudinal as well as cross-sectional case-control studies in humans and experiments in animals (Table 1). In 2011, a longitudinal case-control study was carried out by Knip and colleagues to explore the relationship between gut microbiome and T1D. The 16S pyrosequencing data showed that children with positive islet-autoantibodies had higher *Bacteroidetes*/*Firmicutes* ratio and lower Shannon diversity existed in the gut microbiome.^{44,45} Further studies indicated that *Bacteroides dorei* and *vulgatus* were significantly accumulated in children with high risk

TABLE 1 The summary of changes in gut microbiome and possible effects in T1D

Changes in Gut Microbes	Possible Effects or Function	Refs
<i>Clostridium perfringens</i> ↑ <i>Dialister invisus</i> ↑ <i>Gemella sanguinis</i> ↑ <i>Bifidobacterium longum</i> ↑ <i>Prevotella</i> ↓ Akkermansia ↓ <i>Bifidobacterium adolescentis</i> ↓ <i>Roseburia faecis</i> ↓ <i>Faecalibacterium prausnitzii</i> ↓	Intestinal integrity ↓ Gut permeability ↑ Inflammation ↑ T1D predisposition ↑	29, 48, 50, 57
<i>Leptotrichia goodfellowii</i> ↑ <i>Bacillus cereus</i> ↑ <i>Enterobacter mori</i> LMG 25706 ↑	Molecular mimicry Activate diabetogenic T cells T1D risk ↑	43
<i>Bacteroidetes/Firmicutes</i> ↑	Occurs before and at the Diagnosis of T1D	45
<i>Bacteroides dorei</i> ↑ <i>Bacteroides vulgatus</i> ↑ <i>Faecalibacterium prausnitzii</i> ↓	Involved in multiple autoimmune diseases Disruption of the epithelial layer? Manipulation of immune system?	46, 47
<i>Bifidobacteria</i> ↓ <i>Bacteroides</i> genus ↑	Butyrate ↓ Bacterial translocation ↑ T1D predisposition ↑	48
<i>Bacteroides adolescentis</i> ↓ <i>Roseburia faecis</i> ↓	Regulatory T cells ↓ T1D risk ↑	48, 49
Diversity ↓	SCFAs ↓ Inflammation ↑ T1D predisposition ↑	54, 55

of T1D^{46,47} and associated with autoantibody positivity.^{48,49} The abundance of short-chain fatty acids (SCFAs)-producing bacteria as well as lactate-producing bacteria was reduced in T1D patients.⁵⁰ The reduced number of *Lactobacillus* and *Bifidobacterium* could also be observed at the onset of T1D.⁵¹ Similar results were replicated in animals, illustrated by the fact that more *Lactobacillus* and *Bifidobacterium* could be found in bio-breeding diabetes-resistant rat but less in T1D-prone BB-DP rats.⁵²

Several cross-sectional case-control studies have been performed to reveal the differences of gut microbiome between T1D patients and healthy control subjects. When compared with healthy subjects, more phylum *Bacteroidetes* and lower abundance of 2 dominant *Bifidobacterium* species were found in T1D children.⁴⁷ Mejía-León and Barca compared gut microbiome in patients with newly diagnosed T1D, patients with T1D duration, and healthy controls. The results showed that that newly diagnosed T1D patients had an increased abundance of *Bacteroides*, whereas healthy control possessed more *Prevotella*.⁵³

The changes in the composition of gut microflora in T1D result in the altered diversity of gut microbes. Several studies recruited children with islet autoantibody positivity and later progressed to clinical diagnosis of T1D and found the reduced diversity of intestinal bacteria before the disease onset.⁵⁴ Similarly, reduced diversity of gut microbes was found in diabetic Sprague-Dawley rats with Streptozotocin injection.⁵⁵ All these data demonstrated that alteration in gut bacteria is closely related to T1D in both humans and animal models.

2.4 | Mechanism of gut microbiome in T1D pathogenesis

Gut microbiome is numerous, diverse, and dynamic. It is critical to determine its role in T1D predisposition and pathogenesis. However,

the mechanism is very complicated and remains elusive so far. To our knowledge, gut microbiome may exert function by affecting intestinal permeability, molecular mimicry, and modulating innate and adaptive immune system.

2.4.1 | Intestinal permeability

The intestinal barrier maintains mucosa permeability and separates the luminal antigens from the interior of the body. When intestinal barrier is disrupted, intestinal permeability may increase. Studies showed that when intestinal barrier is compromised, pancreatic-draining lymph node T cells, particularly diabetogenic CD8+ T cells, would be activated and proliferate, promoting insulinitis. It has been reported that intestinal permeability in children with T1D was significantly higher than that in controls.⁵⁶ Maffei and colleagues chose 10 healthy subjects and 10 children at risk for T1D to participate in a case-control study to examine the relationship between intestinal permeability and T1D. The results showed that 3 bacteria, including *Dialister invisus*, *Gemella sanguinis*, and *Bifidobacterium longum*, were associated with altered intestinal permeability in T1D.⁵⁷ In addition, many other studies revealed that the changes of certain microbes, such as *Clostridium perfringens*, *Dialister invisus*, *Gemella sanguinis*, and *Bifidobacterium longum*, were related to compromised gut integrity and increased T1D risk.^{29,48,50,57} When the intestinal permeability is increased, intestinal toxins, food antigens, and infection factors may translocate from gastrointestinal lumen to intestinal mucosal components, and finally to the pancreatic lymph nodes to induce or exacerbate T1D.²⁹

2.4.2 | Molecular mimicry

Considering the various and numerous bacteria in the gut, it is not easy to estimate the amount of bacterial proteins or metabolites produced. Of note, a number of bacterial proteins have been

demonstrated to share the similar molecular structure with self-antigen in pancreas. An illustration is Mgt protein of *Leptotrichia goodfellowii* and islet-specific glucose-6-phosphatase-related protein (IGRP). IGRP protein is a member of G6Pase family and specifically expressed in pancreas. IGRP₂₀₆₋₂₁₄ peptide (VYLKTNVFL) is an important epitope that can activate diabetogenic NY8.3 T cells. *Leptotrichia goodfellowii* is a member of *Fusobacteria*. Interestingly, although *Fusobacteria* usually composes a small portion in the gut microbiome (<0.1%), it is closely related to autoimmune disease, inflammatory bowel diseases (IBDs), and skin ulcers.⁵⁸⁻⁶⁰

A fragment of Mgt protein in *L. goodfellowii* (267-275, TYLKTNVFT) shares sequence similarity with IGRP₂₀₆₋₂₁₄. Experimentally, Mgt₂₆₇₋₂₇₅ activated NY8.3 T cells and accelerated T1D in NOD mice. In addition to *L. goodfellowii*, *Flavobacteriia* bacterium, *Bacillus cereus*, and *Enterobacter mori* LMG 25706 also possessed homologue peptides of IGRP₂₀₆₋₂₁₄, which were functional in vitro and in vivo.⁴³ These findings illustrate that certain diabetogenic microbes exist in the gut and can induce or speed up the occurrence of T1D through molecular mimicry.

Notably, molecular mimicry is an important mechanism of gut microbiome not only for T1D but also for many other autoimmune diseases. Systemic lupus erythematosus is an autoimmune disease characterized by multi-system damage. Unlike T1D, B cells and autoantibodies play an essential role in disease pathogenesis. Hevia and colleagues recruited 20 remission patients and matched healthy control. They found a lower *Firmicutes/Bacteroidetes* ratio in the gut microbes from Systemic lupus erythematosus patients, but the exact mechanism was not further studied.⁶¹ Following studies demonstrated that *B. fragilis*, a member of *Bacteroides* bacteria phylum, can recognize and stimulate VH4-34-encoded IgG+ autoreactive B cells.⁶² Interestingly, molecular mimicry does not always exacerbate autoimmune diseases. *Bacteroides* express integrase that contains a low-avidity mimotope for IGRP₂₀₆₋₂₁₄. This mimotope helps to recruit diabetogenic CD8+ T cells to gut and further suppress IBD by targeting gut dendritic cells.⁶³ Therefore, molecular mimicry generally is a very important mechanism for gut microbiome to induce autoimmune diseases, but its exact role may depend on different diseases and scenarios.

2.4.3 | Innate immune system

Multiple lymph cells infiltrate into the pancreas during early insulinitis, including macrophages. Pancreatic macrophages reside in intra-islet vessels and are in close contact with β cells.⁶⁴ Pancreatic macrophages capture dense core and present islet granules to islet reactive CD4+ T cells, contributing to the destruction of β cells.⁶⁵ Ferris and colleagues found that macrophages in NOD islets possessed an activated state, presenting with the elevated sensitivity to stimuli and increased expression of inflammatory transcripts, like Cxcl9, Ccl5, and Cd40. More importantly, injection of lipopolysaccharide (LPS), a layer of lipid polysaccharide in the outermost layer of gram negative bacteria, resulted in rapid inflammation in the pancreas, indicating that islet macrophages can sense and respond to gut microbiome and accelerate T1D.⁶⁴

Gut microbiome possesses multiple pathogen-associated molecular patterns, such as LPS, lipoproteins, peptidoglycan, and their nucleic

acids. The reorganization of pathogen-associated molecular patterns depends on pattern-recognition receptors in the host, particularly toll-like receptors.⁶⁶ Gut microbes can trigger different toll-like receptors to induce both pro-diabetogenic and anti-diabetogenic signals. Toll-like receptor-induced signals are mediated by signalling adaptors, such as MyD88, to promote cellular responses to LPS.^{67,68} Myeloid differentiation primary response gene88-deficient NOD mice in SPF environment were protected from diabetes. Surprisingly, T1D was developed in the GF Myd88-negative NOD mice, indicating that the T1D-protective effect required signals from gut microbiome.⁶⁹

2.4.4 | Adaptive immune system

In addition to innate immunity, the interaction between gut microbes and adaptive immunity is essential for the development and pathogenesis of T1D. Some particular gut bacteria have the capacity to regulate T cell subsets and function. *Listeria* can induce Th1 response, and segmented filamentous bacteria can augment Th17 responses. The altered schaedler flora and consortia of Clostridia have the capability to induce regulatory T cells.^{70,71} Furthermore, altered gut microflora can increase the number of type 1 regulatory T (Tr1) cells in the intestine. These Tr1 cells can migrate into the periphery, inhibiting the activation of effector T cells and decreasing diabetes incidence.⁷²

So far the exact mechanism for the bacteria mentioned above to promote the differentiation of naïve T cells into different helper T cells is largely unknown. Short-chain fatty acids, which are secreted by gut microbes, have been proved to exert an important role. Short-chain fatty acids, including acetate, propionate, and butyrate, are produced by bacterial fermentation of cellulose that cannot be digested by host enzymes.⁷³ Short-chain fatty acids may support epithelial cell integrity as well as the function of adaptive immune cells (e.g., promoting peripheral regulatory T-cell generation).^{74,75} Mariño and colleagues reported that mice dieted with acetate and butyrate were protected from T1D. They further demonstrated that acetate markedly decreased the frequency of autoreactive T cells in lymphoid tissues and butyrate increased the number and function of regulatory T cells. Acetate and butyrate also maintained intestinal integrity and decreased the concentration of diabetogenic cytokines in the serum, like IL-21.⁷⁶ As mentioned earlier, SPF rather than GF Myd88 negative NOD mice showed delayed onset of T1D. Correspondingly, the concentration of acetate and butyrate in the serum of SPF Myd88 deficient NOD mice was much higher when compared with those in GF transgenic NOD mice. To further explore the potential mechanism, GF Myd88-deficient NOD mice were fed with acetate or butyrate for 5 weeks. The reduced frequency of autoreactive T cells and the increased number of Treg cells were found in the spleen and colon, suggesting that SCFAs produced by gut microbes are essential in disease protection.⁷⁶ *Bacteroides fragilis* could induce Treg cells and enhance its suppressive function, which also required butyrate.^{77,78}

In addition to T cells, SCFAs are able to influence B cell activity.⁷⁹ Recently, Kim and colleagues demonstrated that SCFAs regulated the energy metabolism of B cells in the gut, and promoted B cells differentiating into plasma cells and memory B cells through BCR-activating antigens, resulting in more IgG and IgA production.^{80,81} Meanwhile, IgA can coat a substantial amount of gut microbes to

protect from exotic pathogens and maintain the homeostasis of the intestine. This is essential for bacteria colonization in the gut of neonates. Therefore, IgA can be used as a marker for colonization of gut microbiome.⁷⁰

3 | GUT MICROBIOME AS A BIOMARKER FOR T1D

We have shown that gut microbiome is closely related to the occurrence and development of T1D. As mentioned earlier, the composition of intestinal microflora is altered before the onset of T1D, including a reduced microbial diversity and increased *Bacteroidetes* in subjects with pre-T1D.⁹ Specifically, the abundance of *B.dorei* in *Bacteroidetes* is increased and can be a useful predictor for T1D in Finland. Furthermore, those *Bacteroides* strains are resistant to common antibiotics and correlated with high-protein and high-fat diets.⁴⁶ However, an important issue is that the composition of gut microbes largely depends on environmental factors (e.g., geographic location, eating habits, and sanitary conditions). Therefore, the results found in Finland may not be able to apply to other geographical locations. In addition, we need to evaluate the advantage of gut microbiome in T1D prediction, when compared with islet autoantibodies that have already been used in the clinical practice to predict T1D. The value of gut microbiome in T1D prediction requires further studies.

4 | T1D TREATMENT AND GUT MICROBIOME

Besides disease prediction, the application of gut microbiome to treat T1D seems promising. In animals, the incidence of T1D in disease-prone mice could be dramatically reduced when cohorting with normal mice or orally gavage with faecal samples from healthy mice.⁴³ Oral probiotic administration could also prevent diabetes development in NOD mice.⁸² Interestingly, a recent study reported that accurate regulation of gut microbiome by using tungstate, which could specifically inhibit molybdenum-cofactor-dependent microbial respiratory pathways, relieved IBD with minimal side effects.⁸³ The transformation or precision editing of gut microbiome may be applied to treat T1D in the future. In humans, several clinical trials using probiotics to treat T1D are undergoing, which are summarized in Table 2.

The modulation of gut microbiome may be a valuable approach for T1D treatment. Nevertheless, T1D treatment with drugs might affect gut microbiome the other way round. Currently, insulin is commonly used for glycemic control in T1D patients, while certain immunomodulators and traditional Chinese medicine are in the pre-clinical or clinical trials. Their effects on gut microbiome are discussed below.

4.1 | Insulin

The previous literature showed that several drugs commonly used in the treatment of type 2 diabetes, such as metformin, liraglutide, and saxagliptin, had an impact on the composition of gut microbiome.^{84,85}

Insulin administration is commonly used in patients with T1D, but whether insulin treatment can affect gut microbiome is still unknown.

TABLE 2 The summary of gut microbiome in clinical trials of type 1 diabetes

NCT No.	Trial Name	Interventions	Intervention Model	Outcome Measures	Estimated Enrollment	Sponsors	Status
NCT03423589	Modulation of type 1 diabetes susceptibility through the use of probiotics	VSL#3	Single group assignment	Transcriptional analysis, gut microbiota	30 participants	Medical College of Wisconsin	Recruiting
NCT02903615	Optimizing health in type 1 diabetes	Novel diet: Prebiotic fibre focus, lower carbohydrate, Mediterranean-style. Standard diet	Parallel assignment	Glucose control	20 participants	Garvan Institute of Medical Research	Recruiting
NCT02605148	TEFA family prevention: Gluten-free diet to preserve Beta-cell function (TEFA)	Gluten free diet with probiotics, omega 3 fatty acid, vitamin D supplement	Parallel assignment	β -cell function, glucose control	60 participants	Lund University	Recruiting
NCT02442544	Prebiotic fibre supplement in T1DM children	Prebiotic, Placebo	Parallel assignment	Glucose control, gut microbiota	38 participants	University of Calgary	Active, not recruiting
NCT03032354	Probiotics in newly recognized type 1 diabetes	<i>Lactobacillus rhamnosus</i> GG and <i>Bifidobacterium lactis</i> BB12, placebo	Parallel assignment	β -cell function	96 participants	Medical University of Warsaw	Active, not recruiting

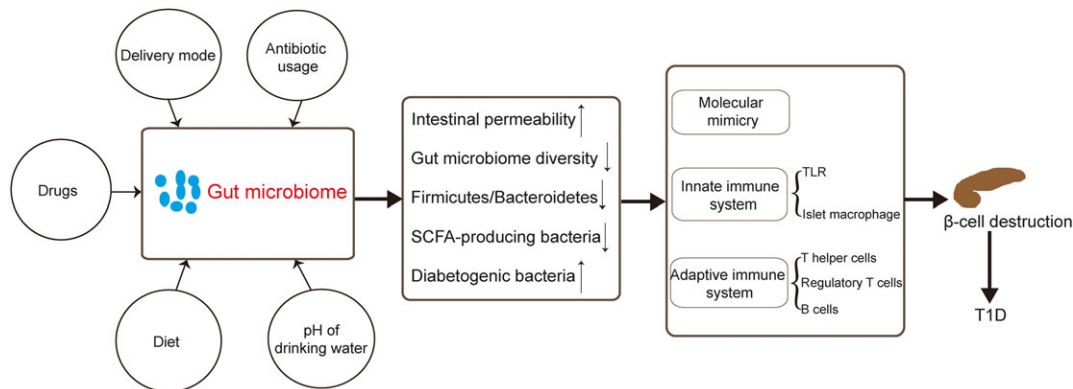


FIGURE 1 The role of gut microbiome in type 1 diabetes. Multiple factors can influence gut microbiome, resulting in an increase in intestinal permeability, a decrease in gut microbiome diversity, and *Firmicutes/Bacteroidetes* ratio. Consequently, these changes may destroy islet β cells through molecular mimicry, excessive activation of innate immunity, and adaptive immunity

A recent article investigated it by using the NOD mouse model. From 4 to 9 weeks of age, mice were given by gavage porcine insulin twice a week, followed by once a week of insulin for 21 weeks. Faeces were collected and gut microflora composition was determined by 16 s RNA sequencing. As a result, they found that oral insulin administration had no effect on gut microbiota.⁸⁶ The possible reason could be that most of insulin was degraded when it passed through the intestine, causing its low bioavailability. However, in clinical practice, insulin is commonly subcutaneously injected in T1D patients. So far, whether insulin administration can affect gut microbiome in T1D patients has not been reported yet.

4.2 | Immunomodulators

Since T1D is an autoimmune disease, several immunomodulators were accessed for their potential in T1D immunotherapy. Among those, the anti-CD20 chimeric mAb (rituximab that depletes most of B cells), the humanized anti-CD3 mAbs (teplizumab and oteplizumab), glutamic acid decarboxylase 65 (to induce antigen specific tolerance), DiaPep277 (the peptide corresponding to the human heat shock protein 60₄₃₇₋₄₆₀), and abatacept (CTLA-4-Ig fusion protein that inhibits T cell activation) have been evaluated in clinical trials.⁸⁷⁻⁹² Although most of immunomodulators showed only modest success in clinical studies, their effects on gut microbiome have not been known yet.

In mouse models, several studies suggest that certain immunomodulators can alter gut microbiome. In allogeneic skin graft mouse, teplizumab administration protected against disease by increasing IL-10-producing T cells. Interestingly, those T cells were required to migrate into the intestine to exert the protective function. If the migration was blocked, the treatment effects of teplizumab were abolished, indicating that gut microbiome might be involved in teplizumab-mediated disease protection.⁹³ The following study found that alteration of gut microbiome during anti-CD3 mAb treatment or cohousing experiments significantly boosted intestinal IL-10-producing Tr1 cells and decrease T1D incidence.⁷² Further studies may be required to clarify how gut microbiome is altered after anti-CD3 mAb treatment.

4.3 | Traditional Chinese medicine

Traditional Chinese medicine, also called herbal medicine (HM), has been used in worldwide for a thousand years. Most HMs are taken orally, which may preferentially alter gut microflora. An interplay between HMs and gut microbes might exist. On one hand, gut bacteria play an essential role in HM therapy by biotransforming HM chemicals into effective smaller units that can be better absorbed.⁴⁶ On the other hand, HM chemicals improve the composition of intestinal flora and pathological condition. So far, most HMs have not been studied in T1D yet, except very few reports. *Ganoderma lucidum* could drop the increased *Firmicutes/Bacteroidetes* ratio to normal level.⁹⁴ Berberine, a plant benzylisoquinoline alkaloid, showed the capability to protect NOD mice from T1D by modulating immune system.⁵³ Artemisinin and Danzhi Jiangtang Capsule could improve the function islet β cells by increasing β -cell mass and reducing pancreatic β -cell apoptosis, respectively.^{95,96} However, whether and how gut microbiome is altered in HMs-mediated disease protection is unknown yet.

5 | CONCLUSION AND PERSPECTIVE

Currently, the role and mechanism of gut microbiome in T1D attract more and more attention. A rapid growing number of publications have been reported in recent years. Although great progress has been made, many details are still missing and a large number of questions need to be answered.

Genome-wide association studies have identified more than 50 T1D-related susceptibility genes. Among them, HLA genes contribute to disease susceptibility substantially in both humans and NOD mice. Replacement of NOD IA-g7 with C57BL/6 E α 16 prevented T1D in transgenic NOD mice, because of the altered antigen presentation for islet autoantigens. However, a recent article reported that the disease protection in E α 16/NOD mice also depended on gut microbiome, illustrated by the fact that GF or antibiotics-treated E α 16/NOD lost the disease protection. These data indicate that an interplay between T1D susceptibility genes and gut microbiome may exist and play a crucial role in the pathogenesis of T1D.⁹⁷

Notably, many susceptibility genes (e.g., IL-10, IFI1, TNFAIP3, PTPN2, and FUT2) are shared between T1D and IBD.^{98,99} Whether

those genes have an impact on gut microflora in T1D is unknown. If the impact exists, how to establish the link between susceptibility genes, altered gut bacteria, and T1D pathology requires further studies.

To sum up, T1D is an autoimmune disease triggered by both genetic and environmental factors. In past decades, a substantial progression has been made to clarify the genetics risk factors by using genome-wide association studies. But genetics alone is not sufficient to explain why the prevalence of T1D increases at a rate of 3% to 5% per year, when considering genetics in population to be relatively stable. In recent years, the importance of environmental factors in T1D, especially gut microbiome, has been realized. Growing attention has been paid to clarify the taxonomic and functional changes of gut microbiome in the disease pathogenesis, and the interplay between gut microbiome with immune system, which is becoming the hotspot of both research and clinical trials in the field.

Gut microbiome, especially SCFAs, are important to maintain intestinal barrier and immune homeostasis. Evidence so far has demonstrated that dysbiosis of gut microbiome increases T1D predisposition. In humans or animals, the decreased diversity of gut microbiome occurs before disease onset and remains after the diagnosis of T1D. *Bacteroidetes* and *Firmicutes* are dominant in the faecal samples. The ratio of *Firmicutes* to *Bacteroidetes* is decreased in T1D patients. *Dialister invisus*, *Gemella sanguinis*, and *Bifidobacterium longum* are associated with intestinal permeability. Once the balance of gut microflora is disrupted, the compromised intestinal mucosa permeability may lead to outer or bacterial antigen leakage, boosting excessive immune response or activating autoreactive T cells by molecular mimicry (Figure 1).

In light of these results, gut microbiome may be a critical modulator in T1D predisposition and pathogenesis. Continuous studies are required to obtain a better understanding on the roles and mechanisms of gut microbiome in T1D, which may provide a valuable tool to predict and treat T1D in the future.

ACKNOWLEDGEMENTS

We thank Ms Yani Peng for language embellishment of the manuscript. This work is supported by the National Natural Science Foundation of China (grant no. 81500600, 81670716, and 81461168031), the National Science and Technology Infrastructure Program (2015BAI12B13), and the Graduate innovation project of Central South University (grant no. 502211703).

CONFLICT OF INTEREST

Authors Zheng P, Li Z, and Zhou Z declare that they have no conflict of interest.

ETHICAL APPROVAL

This review article cites the peer-reviewed articles of our group and other groups. Therefore, the study designs are detailed in the primary articles, and this review does not include a study design with the direct ethical statements of the human study or animal study designs. For our own published studies cited in this article, all animal studies go through the office of human studies or the animal studies offices to confirm the ethical treatment of humans and animals in research.

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How to cite this article: Zheng P, Li Z, Zhou Z. Gut microbiome in type 1 diabetes: A comprehensive review. *Diabetes Metab Res Rev.* 2018;34:e3043. <https://doi.org/10.1002/dmrr.3043>