Integration of microbiome and epigenome to decipher the pathogenesis of autoimmune diseases

Beidi Chen, Luxi Sun, Xuan Zhang

Department of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Clinical Immunology Center, Chinese Academy of Medical Sciences and Peking Union Medical College, The Ministry of Education Key Laboratory, Beijing, 100730, China

School of Medicine, Tsinghua University, No.1 Tsinghua Yuan, Beijing, 100084, China

Abstract

The interaction between genetic predisposition and environmental factors are of great significance in the pathogenesis and development of autoimmune diseases (AIDs). The human mucosa is the most frequent site that interacts with the exterior environment, and commensal microbiota at the gut and other human mucosal cavities play a crucial role in the regulation of immune system. Growing evidence has shown that the compositional and functional changes of mucosal microbiota are closely related to AIDs. Gut dysbiosis not only influence the expression level of Toll-like receptors (TLRs) of antigen presenting cells, but also contribute to Th17/Treg imbalance. Epigenetic modifications triggered by environmental factors is an important mechanism that leads to altered gene expression. Researches addressing the role of DNA methylation, histone modification and non-coding RNA in AIDs have been increasing in recent years. Furthermore, studies showed that human microbiota and their metabolites can regulate immune cells and cytokines via epigenomic modifications. For example, short-chain fatty acids (SCFAs) produced by gut microbiota promote the differentiation of naïve T cell into Treg by suppressing histone deacetylases (HDACs). Therefore, we propose that dysbiosis and resulting metabolites may cause aberrant immune responses via epigenetic modifications, and lead to AIDs.

With the development of high-throughput sequencing, metagenome analysis has been applied to investigate the dysbiosis in AIDs patients. We have tested the fecal, dental and salivary samples from treatment-naïve rheumatoid arthritis (RA) individuals by metagenomic shotgun sequencing and a metagenome-wide association study. Dysbiosis was detected in the gut and oral microbiomes of RA patients, but it was partially restored after treatment. We also found functional changes of microbiota and molecular mimicry of human antigens in RA individuals.

By integrating the analysis of multi-omics of microbiome and epigenome, we could explore the interaction between human immune system and microbiota, and thereby unmasking specific and more sensitive biomarkers as well as potential therapeutic targets. Future studies aiming at the crosstalk between human dysbiosis and epigenetic modifications and their influences on AIDs will facilitate our understanding and better managing of these debilitating AIDs.

© 2017 Elsevier Ltd. All rights reserved.
1. Introduction

Autoimmune diseases (AIDs) such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), are characterized by breaking of immune tolerance which leads to the accumulation of autoreactive lymphocytes and excessive production of autoantibodies. The pathogenesis of AIDs is still not clear, though a few studies have revealed some related genetic susceptibility loci. According to epidemiological studies, the concordance rate of monozygotic twins with SLE or RA is 10%-40% [1], which suggests the involvement of non-genetic factors such as environmental triggers are also important in the pathogenesis and development of AIDs.

There is a latent phase in RA patients with only serum elevation of RA-related autoantibodies but without clinically evident synovitis, which indicates that RA may originate from an extra-articular location, for example, a mucosal site [2,3]. Mucosal surfaces of human closely interact with the exterior environment, and commensal microbiota at the gut and other human mucosal cavities play a crucial role in the development and regulation of immune system [3]. Microbiome is the collective of microbial genomes that reside in an environmental niche. The gene number of human microbiome, especially gut microbiome, is much bigger than the human genome [4,5]. With the development of high throughput sequencing (HTS) and the establishment of metagenomics, metagenome-wide association study (MGWAS) has been widely used in studies of human diseases. Recent works indicates that human microbiome are closely related to many diseases, including AIDs such as SLE [6-8], RA [9-13], inflammatory bowel disease (IBD) [14], type 1 diabetes mellitus (T1D) [15]. Epigenetics are stable heritable traits regulating gene expression which cannot be explained by changes in DNA sequences. Epigenetic modifications are implicated in the pathogenesis of many complicated diseases, including AIDs [16-18]. Furthermore, as an environment cue inside human body, human microbiome is also possible to influence gene expression by epigenetic modifications, which turns out to be a way of crosstalk between microbiome and host cells.

Techniques of microbiome and epigenome have already been applied in AIDs research, but studies combining them together in exploring AIDs pathogenesis have not really started yet. Epigenetic modifications could lead to transcriptional respond of human genes to environmental cues. Therefore, human microbiome, as an important environmental factor closely related to human body, may play a crucial role in AIDs induction and aggravation via epigenetic modifications (Fig. 1). In this review, we will focus on the crosstalk between human microbiome and immune system, the microbiome changes in AIDs and their possible pathogenic immunological mechanisms. Further, the possible mechanism of how human microbiome mediate the pathogenesis of AIDs through epigenetic machinery will be discussed.

2. Human microbiome and immune system

The gut of fetus is almost sterile. Colonization of microbes in human gut starts at birth from maternal microbiota of genital tract, colon and the overall environment [19]. Important determinants of the gut microbiotic composition for infants include delivery mode, feeding type, gestational age, infant hospitalization stay, and antibiotic use [20]. The population and species of gut microbiota are small in neonates, but they will be well established in the next 2 years and remains relatively constant throughout life [21]. Although the gut microbiome can alter along with environmental triggers such as diet changes, the gut microbiome changes resulted from transient antibiotic use showed a considerable indigenous recovery potential, and were genetically regulated [22]. Local mucosal cells, related microbiota and their metabolites should be treated as a whole. The metabolic enzymes produced by gut microbiota are crucial in human metabolites digestion and utilization [23]. Gut microbiota help further digest exogenous undigested food by anaerobic glycolysis in colon, and degrade endogenous chemical compounds secreted by host cells and microbial cells. Intestinal epithelial cells (IECs) are the main channels for the crosstalk between host cells and microbiome, as well as the impact of microbiome on host immune function.

Studies using germ-free (GF) mice and specific pathogen-free (SPF) mice unmasked the influence of commensal bacteria on the structure and function of immune system. Compared with SPF mice, GF mice have smaller independent lymphoid follicles [24] and Peyer’s patches [25], and decreased intestinal secretory IgA and plasma cells [26], while the invariant natural killer T cells (iNKT) were significantly increased at the gut and lung of GF mice [27]. Besides, in GF mice, the helper T cells 17 (Th17) of intestinal lamina propria and the regulatory T cells (Treg) of colon lamina propria were both downregulated to a lower level [28,29]. Normal gut microbiota can promote the differentiation of regulatory B cells (Breg) in spleen as well as mesenteric lymph nodes by IL-15 and IL-6 production, while Breg restrained excessive inflammation via IL-10 secretion [30]. Not only can gut microbiota regulates local mucosal immunity, it also influences systemic immunity and the formation of peripheral lymphoid organs. For example, the serum IgA level decreased in GF mice [31]. In another study, commensal fungi drove migration of CD103+ RALDH4+ dendritic cells (DC) to the peripheral lymph nodes after birth in mice. By producing large amount of retinoic acid, these cells directed the homing of lymphocytes to both gut-associated lymphoid tissues (GALT) and peripheral lymph nodes. Moreover, some of these DCs...
also functioned in maintaining the volume of secondary lymphoid organs in adult mice [32]. This study provided a new interpretation of the connection between gut microbiome and systemic AIDs.

The microbiota influences host immune system mainly in two ways, that is, by microbial metabolites or by microbial components. Among metabolites, short-chain fatty acids (SCFAs) are most frequently studied, which have been suggested to strongly associate with immune system. SCFAs are mostly produced from undigested complex carbohydrate by gut microbiome at host colon, most of which are butyrate, propionate and acetate [33]. Both human IECs and microbiota themselves use SCFAs as crucial energy sources. SCFAs also have other important physiological functions, which includes promoting cell differentiation [34], apoptosis [35], and anti-inflammation [36–40]. However, there was also study indicating that by recruiting neutrophils, SCFAs were able to aggravate inflammation [41]. Another intriguing function of SCFAs was that it could promote the expression of mucin in intestinal epithelial goblet cells, thereby enhancing mucosal immunity by improving the protective function of IECs [42,43]. Other bacterial metabolites that were capable of affecting host immune system included ligands of aryl hydrocarbon receptor and polyamines [44]. Apart from metabolites, there are a lot of bacterial components with immunomodulatory function. Take polysaccharide A (PSA) as an example. Introduction of Bacteroides fragilis, a bacterial PSA, to GF mice could correct the pre-existing Th1/Th2 imbalance and direct lymphoid organogenesis [45]. PSA produced by B. fragilis also directly promoted Treg differentiation and further immunologic tolerance via interaction with Toll-like receptor (TLR) 2 [46].

Fig. 1. Dysbiosis, epigenetic changes and aberrant immune responses in autoimmune diseases. Human dysbiosis and epigenetic changes have mutual effect on each other, and they can result in aberrant immune responses, including increased production of pro-inflammatory and reduction of anti-inflammatory cytokines as well as dysregulation of immunocytes. Of note, immunological changes can in turn affect microbiome and human epigenome. Genetically predisposed individuals with aberrant immune responses may further lead to autoimmune diseases.

3. AIDs and human microbiome

3.1. Microbiome changes in AIDs patients and animal models

Studies using animal models of AIDs indicate that the pathogenesis of AIDs is related to gut dysbiosis. It is suggested that the production of antinuclear antibodies (ANAs) detected in SLE patients, was influenced by the commensal gut microbiota, especially increased colonization with segmented filamentous bacteria (SFB), and IL-17 receptor signaling [47]. Autoimmune arthritis was markedly attenuated in the K/BxN arthritis mouse model under GF conditions, accompanied by reductions of serum autoantibody titers and Th17 cells from intestinal lamina propria [48]. While introduction of SFB into those GF animals could restore the number of lamina propria Th17 cells and the production of serum autoantibodies, and could aggravate arthritis as well. However, for mice model of lupus, whether they were raised under GF condition or not didn’t influence disease severity [49]. Dietary choice which is an impact factor of gut microbiome, has also been studied in AIDs animal models. A study has examined the impact of drinking water pH on immune response and gut microbiome in a spontaneous mouse model of SLE [50]. The result revealed that the composition of gut microbiome was significantly different between mice given acidic versus neutral pH water, and the neutral group developed nephritis more quickly than the acidic group. In addition, immune responses in the gut mucosa of mice given neutral pH water were dominated by Th17 and Th9-associated factors. Although it was mentioned that SFB could affect ANAs abundance [47], the observation of this study suggested that the presence of SFB had no effect on Th17 and lupus incidence [50]. In young, female lupus-prone
mice, significant decrease of *Lactobacilli* and increase of *Lachnospiraceae* as well as overall diversity were found compared with healthy controls. Furthermore, the enhancement of *Lachnospiraceae* in those mice was associated with an earlier onset of and/or more severe lupus [51]. After oral administration of retinoic acid to lupus-prone mice, the recovery of downregulated *Lactobacillus* and improvement of symptoms were observed. In conclusion, gut dysbiosis is strongly associated with new-onset untreated RA individuals [12]. Increase in *Prevotellacopri* abundance was associated with *Bacteroides* reduction and a loss of reportedly beneficial bacteria in those patients. Likewise, Maeda et al. showed that patients with early RA

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>Sequencing technology employed</th>
<th>Dysbiotic characteristics</th>
<th>Other findings</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>16S rRNA gene amplification</td>
<td>Firmicutes/Bacteroidetes↓, Synergistetes↓</td>
<td>Enhanced oxidative phosphorylation and glycan utilization pathways in microbiota.</td>
<td>Hevi 2014 [8]</td>
</tr>
<tr>
<td>SLE</td>
<td>16S rRNA gene amplification</td>
<td>Firmicutes↓, Bacteroidetes↑, microbial diversity and abundance↑</td>
<td>Genera Rhodococcus, Eggerthella, Klebsiella, Prevotella, Eubacterium, Flavonifractor and Incertaeagens increased, Dialister and Pseudobutyribrio decreased.</td>
<td>He 2016 [7]</td>
</tr>
<tr>
<td>RA</td>
<td>16S rRNA gene amplification</td>
<td><em>Prevotella copri</em>: <em>Prevotella</em> negatively correlated with Bacteroides</td>
<td><em>Prevotella copri</em> negatively correlated with presence of predisposing genes.</td>
<td>Scher 2013 [12]</td>
</tr>
<tr>
<td>RA</td>
<td>Lactobacillus-specific PCR amplification</td>
<td>Lactobacillus↑, microbial diversity and abundance↑</td>
<td>None</td>
<td>Liu 2013 [13]</td>
</tr>
<tr>
<td>RA</td>
<td>16S rRNA gene amplification</td>
<td><em>Actinobacteria</em>↑, microbial diversity↑</td>
<td><em>Collinsella</em> correlated closely with IL-17A production and could alter gut permeability and disease severity.</td>
<td>Chen 2016 [10]</td>
</tr>
<tr>
<td>RA</td>
<td>16S rRNA gene amplification</td>
<td><em>Prevotella copri</em> dominance</td>
<td>Autoreactive SKG mouse T cells were activated by human dysbiotic microbiota, causing joint inflammation.</td>
<td>Maeda 2016 [9]</td>
</tr>
</tbody>
</table>

SLE: systemic lupus erythematosus; RA: rheumatoid arthritis.
carried gut microbiota dominated by *Prevotella copri* [9]. Our group tested the fecal, dental and salivary samples from treatment-naive RA individuals by metagenomic shotgun sequencing and a metagenome-wide association study [11]. Dysbiosis was detected in the gut and oral microbiomes of RA patients, and there was a concordance between the microbiome of gut and oral cavity. Specifically, *Haemophilus* spp. were depleted in individuals with RA at all three sites and negatively correlated with serum autoantibodies titers, whereas *Lactobacillus salivarius* was over-represented in individuals with RA at all three sites and was especially larger in amount in very active RA patients. Changes were also observed in the functional pathway of RA microbiota, including the redox environment, transport and metabolism of iron, sulfur, zinc and arginine, and molecular mimicry of related autoantigens was also detectable. Alterations in the gut, oral microbiome could be helpful in differentiating RA patients from healthy controls and stratify individuals according to their response to therapy, and is helpful in the diagnosis and prognostic prediction of RA patients. A study of microbiome intervention showed that after orally administered with probiotic *Lactobacillus casei* 01 for weeks, RA patients had a significantly lower disease activity score [56]. In addition, serum pro-inflammatory cytokines, including TNF-alpha, IL-6 and L-12, significantly decreased in the probiotic group, whereas the serum level of regulatory cytokine IL-10 significantly increased. In another study, probiotic capsule containing *Lactobacillus acidophilus*, *Lactobacillus casei* and *Ridibacterium bifidum* was introduced to RA patients [57]. Compared with the placebo group, probiotic group resulted in a lower disease activity score with a significant decrease in serum insulin levels, B cell function as well as serum high-sensitivity C-reactive protein (hs-CRP) level. Treatment of arthritis rat model with probiotic *Bacillus coagulans* and probiotic inulin could lead to clinical improvement as well as inhibition of fibrinogen, serum amyloid A and TNF-alpha production [58]. Mice with collagen-induced arthritis (CIA) had partial depletion of commensal bacteria and more serious arthritis symptoms after oral administration with antibiotic enrofloxacin [59]. In a microbial transplantation study, researchers inoculated stool samples from RA patients and healthy controls into GF arthritis-prone SKG mice respectively. SKG mice harboring microbiota from RA patients developed more severe arthritis when treated with zymosan [9]. Those studies involved microbial interventions with probiotic, prebiotic, antibiotic and/or fecal transplantation provided supporting evidence for the role of dysbiosis in AIDs pathogenesis.

Beyond the known association of dysbiosis of gastrointestinal tract and AIDs, oral microbiota changes have also been observed in AIDs patients, in particular RA patients. The coexistence of RA and periodontal disease (PD) has long been reported. The compositional change of the oral microbiota was found in CIA mice [60]. Subgingival *Tannerella forsythia* and supragingival *Streptococcus anginosus* were two characteristic pathogenic bacteria in early RA [61]. The oral microbial profile of new-onset RA patients was different from healthy controls, and it was probably due to the severity of PD rather than RA. The presence and abundance of *Porphyromonas gingivalis* were also correlated with the severity of PD [62]. As mentioned above, we also carried out a metagenomic analysis and MGWAS of dental and salivary samples from RA patients, and we found concordance between the gut and oral microbiomes [11]. Specifically, *Haemophilus* spp. were depleted and *Lactobacillus salivarius* was over-represented in oral cavity of RA individuals. Moreover, renal involvement and autoantibodies appearance were more frequent in SLE patients with nasal carriage of *Staphylococcus aureus* [63]. Although there lacks compositional analysis of lower respiratory tract microbiome in AIDs patients, some other experiments suggested that lung may be the early site of autoimmune-related injury, probably resulting from microaspiration of oral pro-inflammatory microbiota [64]. It is conceivable that microbiome in oral cavity and respiratory tract may have some connection with AIDs as in gastrointestinal tract, but more work needs to be done to substantiate this possibility.

### 3.2. Immunopathological mechanisms of dysbiosis in promoting AIDs

Although studies about AIDs and human microbiome have been increasing in recent years, whether dysbiosis is the cause or the consequence of AIDs is still controversial. Some argued that, for genetic susceptible individuals, dysbiosis probably exert a crucial role in both the onset and the outcome of SLE, so a vicious circle exists between dysbiosis and autoimmunity [65]. However, the exact immunopathological mechanisms by which human microbiome induce and aggravate AIDs remain inconclusive. Herein we summarize some possible mechanisms (Table 2).

#### 3.2.1. Treg/Th17 and TLR dysregulation

There are growing numbers of studies about the Treg/Th17 imbalance present in SLE and RA recently. For instance, selective depletion of Treg cells in C57BL/6 mice ended up with an exacerbation of delayed-type hypersensitivity arthritis, which could be counteracted by IL-17 monoclonal antibody [91]. IL-17, a pro-inflammatory cytokine which participates in autoimmunity, is mainly produced by Th17 [92,93]. Furthermore, numerous studies have confirmed the important role of human microbiome in Treg/Th17 imbalance. It was mentioned above that the severity of arthritis and the number of Th17 cells from intestinal lamina propria were both reduced in the *K/BxN* arthritis mouse model under GF conditions, but these could be restored after intake of SFB [48]. Inoculating fecal samples from RA patients into GF arthritis-prone SKG mice led to increase of intestinal Th17 cells, while co-culturing naive T cells of SKG mice and *Prevotellacopri*-stimulated DC resulted in IL-17 over-expression when treated with arthritis-related autoantigens [9]. Gut dysbiosis resulted from antibiotic use caused IL-17A enhancement in mesenteric lymph node cells and aggravated arthritis in CIA mice [59]. Ex vivo analysis of blood samples showed enlarged Th17 and Foxp3(+) IL-17(+) populations in anti-dsDNA positive SLE patients without Treg difference compared to controls, suggesting a possible Treg-Th17 trans-differentiation [6]. Although studies above suggested that gut dysbiosis may mediate the initiation of AIDs mainly via Th17 cells and related cytokines, there was also a research suggesting that autoimmune arthritis was regulated by gut microbiota through follicular helper T (Tfh) cells rather than Th17 cells in *K/BxN* mice [94]. Likewise, a more recent study also showed that SFB increased systemic Tfh cell number and autoantibody responses which exacerbated autoimmune arthritis via driving Thfh cells from Peyer’s Patch to systemic sites and promoting their differentiation [66]. Therefore, despite the fact that most studies supported the role of microbiota in Treg/Th17 imbalance of AIDs, further explorations in this area are still awaited.

TLRs, locating at the surface of antigen presenting cells, are crucial pattern recognition receptors that recognize pathogen associated molecular pattern of microorganisms. TLR-2 and TLR-4 are of most importance in microbiome mediated RA. In mice injected with streptococcal cell wall to develop joint inflammation, TLR-2-deficiency led to neither joint swelling nor inhibition of cartilage matrix synthesis. Myeloid differentiation factor 88 (MyD88), a Toll/IL-1R domain, played an indispensable part in this process [67]. IL-1 receptor-deficient mice developed acute paw swelling when treated with lipopolysaccharide (LPS), a TLR-4 ligand, but it did not sustain in TLR-4 mutant mice, suggesting that TLR-4 signaling could circumvent IL-1 receptor in mediating
3.2.2. Autoantigen overproduction by microbial enzymes

Extracellular proteolysis can be catalyzed by cytokines and protease produced by innate immunocytes, giving rise to “remnant epitopes” which can be recognized by major histocompatibility complex class I [70]. It should be noticed that human microbiota is a huge source of various kinds of proteolytic enzymes. For example, supernatants from Porphyromonas gingivalis culture were effective at breaking down the extracellular matrix found in both arthritic joints and oral mucosa, such as fibrinogen, fibronecctin and type I collagen [71]. Thus, proteolytic enzymes produced by microbiota can act as a supplement for host enzymes, and create more “remnant epitopes” serving as autoantigens which play important role in the pathogenesis of AIDs [72].

Except for the newly made “remnant epitopes”, enzymatic antigen modification may be another way of generating autoantibodies. Anti-citrullinated protein antibody (APCA) is a common and relatively characteristic autoantibody in RA. Citrullination is a post-translational modification in which arginine is processed into citrulline. Human protein can be catalyzed into citrullinated protein by peptidyl-arginine-deiminases (PAD), and the new epitopes generated may result in APCA production and autoimmune responses by breaking immune tolerance [73]. Porphyromonas gingivalis is the only bacteria known to date that has the ability to produce PAD [74]. Degradation of the proteins and citrullination of resulting residues were seen after incubating wild-type Porphyromonas gingivalis with fibrinogen or alpha-enolase, and knock-out of PAD gene led to loss of protein citrullination [75].

3.2.3. Molecular mimicry

Immunizing DR4-IE-transgenic mice with Porphyromonas gingivalis enolase induced the production of antibodies to human alpha-enolase and the occurrence of arthritis [76]. Infection with Epstein–Barr virus (EBV) has long been implicated with SLE, and study revealed that EBV antigens shared structural molecular mimicry with common SLE antigens and functional molecular mimicry with critical immune-regulatory components [77]. Anti-dsDNA antibodies from SLE serum were capable of binding to *Burkholderia* bacterial cytosome B 561 partial sequence ARVL-WRATH and transcription regulator protein sequence RAGTDEFG [78]. Sjögren’s syndrome Antigen A (SSA/Ro60) can lead to generation of anti-Ro60 antibodies, which are commonly seen in AIDs such as SLE and Sjögren’s syndrome. Ro60-reactive T cells from HLA-DR3 transgenic mice could be activated by human oral, intestinal, skin and vaginal bacteria [79]. The autoantigen U2 snRNP B’ possessed a superfamilial protein domain that shares 83% similarity with the mannan sequence of *Saccharomyces cerevisiae*, and anti-S. cerevisiae autoantibodies were highly expressed in both SLE and RA [80]. All studies above indicate that cross reactions caused by molecular mimicry may be one of the inducing factors of AIDs occurrence.

### Table 2

**Immunopathological mechanisms of dysbiosis in promoting Autoimmune Diseases.**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>① Treg/Th17 &amp; TLR dysregulation</td>
<td>[9, 48, 59, 66]</td>
</tr>
<tr>
<td>Dysbiosis dysregulates TLRs on antigen presenting cells and leads to Treg/Th17 imbalance.</td>
<td>[69]</td>
</tr>
<tr>
<td>② Autoantigen overproduction</td>
<td>[70–75]</td>
</tr>
<tr>
<td>The proteolytic enzymes produced by microbiome cleave the host protein and produce “remnant epitopes” serving as autoantigen. Host protein can also be modified by microbial enzymes, giving rise to new autoantigens.</td>
<td></td>
</tr>
<tr>
<td>③ Molecular mimicry</td>
<td>[76–80]</td>
</tr>
<tr>
<td>Some microbial components resemble self-peptides, which results in cross reaction and activation of autoreactive T and B cells.</td>
<td></td>
</tr>
<tr>
<td>④ Microbial translocation</td>
<td>[81–87]</td>
</tr>
<tr>
<td>Microbial components/metabolites translocate into circular system and are transported to specific organs and tissues, causing immunopathological injuries.</td>
<td></td>
</tr>
<tr>
<td>⑤ Others</td>
<td>[6, 30, 88–90]</td>
</tr>
<tr>
<td>B cells over-activation and protective IgM down-regulation; curli/DNA composites production; superantigen production, etc.</td>
<td></td>
</tr>
</tbody>
</table>

Treg: regulatory T; Th17: helper T 17; TLR: Toll-like receptor.
synthesis or secretion of anti-phosphorylcholine IgM in B1 cells [6]. Amyloid fibers, also called curli, is a major protein of the extracellular matrix produced by bacteria, and a component of bacterial biofilm. Curli is able to bind extracellular DNA and form curli/DNA composites. When administered with curli-DNA composites, lupus-prone and wild-type mice both went through immune activation and developed autoantibodies. However, when infected with curli-producing bacteria, lupus-prone mice presented higher autoantibody titers compared to controls [89]. Moreover, many bacteria and viruses can produce superantigen, which nonspecifically activates immune responses. Study demonstrated that the relapse of Wegener’s granulomatosis, an AIDs with renal involvement, was closely correlated with a previous upper respiratory infection of Staphylococcus aureus that produces superantigen [90].

4. Dysbiosis, epigenetic modifications and AIDs

Epigenetic modifications, including DNA methylation, histone modification and noncoding RNAs (ncRNA), play important parts in various life processes, such as cell growth, differentiation, aging, and even immune responses. They participate in the occurrence and development of many diseases including AIDs [16-18]. Abnormal epigenetic changes have been noted in a variety of immune cells as well as pathological cells in AIDs compared with healthy controls, such as different types of T cells, B cells, monocytes, macrophages and fibroblasts [18]. Given that environmental signals can affect gene expression via epigenetic mechanisms, this may also be a crucial way of how human microbiome interact with host gene expression. Advanced study deciphering the interaction mechanisms between human microbiota and epigenetics, and its dysregulation in the pathogenesis and progression of AIDs worth considerable attention. Numerous studies have demonstrated that the compositional and/or functional changes of human microbiome have various effects on the status of human epigenetics. On one hand, microbial metabolites can affect the activity of epigenetic enzymes or act as the substrates necessary for epigenetic modifications. On the other hand, the ncRNAs or epigenetic enzymes of microbiome can translocate into host cells, influencing the expression of host genes. It should also be noticed that epigenetic changes in human body may also lead to dysbiosis.

4.1. The crosstalk of microbiome and epigenetics and its effect on immune system

The relationship between microbiome and human epigenetic changes has been studied for years. For instance, the blood DNA methylation profile of pregnant women were associated with their gut microbial composition [95]. Several studies showed that alterations of commensal microbiota and their metabolites were able to affect human epigenetic status [Table 3]. Colonization of neonatal GF mice with a conventional microbiota reduced the methylation level of Cxcl16 gene and its expression, which protected them from mucosal invariant natural killer T (iNKT) cells accumulation in local mucosa, and ameliorated the pathology of IBD and allergic asthma [27]. The results indicated that gut commensals protected neonates from immune mediated diseases by regulating DNA methylation. The DNA methylation level of TLR4 gene was significantly higher in IECs than in splenic cells, whereas it was lower in IECs of GF mice than in those of control mice. Therefore, the presence of gut microbiota led to down-regulation of TLR4 expression and reduction of immune responses, suggesting that alterations of TLR4 gene methylation status by existence of gut microbiota can induce immune tolerance [96]. Another potential mechanism of immune tolerance was that gut microbiota increased macrophage secretion of IL-10, which in turn suppressed IL-12 p40 production through the histone deacetylation function of histone deacetylase (HDAC) 3 [97]. Owing to the reduction of trimethylation of lysine 4 of histone protein 3 (H3K4me3), many inflammatory factors including type 1 interferons (IFN-I) of mononuclear phagocytes was down-regulated in GF mice. In other words, the depletion of gut microbiota could compromise NK cell priming and antiviral immunity [98]. Colonization of GF mice with the microbiota from pathogen-free mice revealed that some microRNA (miRNA) expression were altered in the ileum and the colon of colonized mice. Among the dysregulated miRNA, mmu-mir-665 downregulated the expression of ABCC3 in murine macrophages [99]. Given that proteins encoded byABCC3 gene are capable of transporting various molecules across extra- and intra-cellular membranes, the results suggested that gut microbiota may compromise the phagocytic function of macrophage by affecting the level of miRNA. Another study identified that 16 out of 334 detectable miRNA were differently expressed in the caecum of GF and conventional male mice, and many of their target genes were intestinal barrier-related genes encoding for functional and mucus layer proteins, indicating that intestinal immuno-barrier function can be regulated by gut microbiota through miRNA alterations [100]. In addition, a group of scientists demonstrated the efficacy of intestinal long non-coding RNAs (lncRNA) expressed profile in discriminating different gut microflora [101]. Although the results pointed out the possible relationship between gut microbiota and local lncRNA, the exact causality should be further explored.

Microbial metabolites are crucial in mediating the regulation of epigenetic modifications in human body. (1) The activity of epigenetic enzymes can be affected by microbial metabolites, for example, SCFAs are important HDAC inhibitors. In particular, butyric acid has been shown to have a broad HDAC inhibitory effect [102]. SCFAs were able to suppress inflammation through inhibiting the HDAC within peripheral blood mononuclear cells (PBMCs) [39,40], macrophages [37] and DCs [38,102]. Treatment of LPS-stimulated PBMC and leucocytes with SCFAs resulted in inactivation of nuclear factor-kB (NF-kB) and down-regulated expression of TNF-alpha through HDAC inhibition [39,40]. Apart from innate immune cells, the number and immunosuppressive function of Treg cells can also be promoted by the HDAC inhibitory ability of SCFAs, maintaining the immune homeostasis of colon [102-104]. The regulation of Treg size and function in colon by SCFAs was dependent on Ffar2, a G-protein-coupled receptors (GPCR). The combination of the ligand with its receptor repressed the expression of HDAC6 and HDAC9 in Treg [104]. As for possible mechanism, treatment of naive T cells with butyrate enhanced histone H3 acetylation in the promoter and conserved non-coding sequence regions of the Foxp3 locus, leading to differentiation into Treg. How butyrate promoted acetylation, whether by HDAC inhibition or by other mechanisms remained elusive [103]. In addition, butyrate could modulate high-fat diet-induced skeletal muscle mitochondrial adaptation, obesity and insulin resistance through nucleosome positioning [105]. Aside from affecting enzymatic activity, gut microbiota contributed to the metabolism of epigenetic enzymes co-factors, thus indirectly influenced various enzymes that participated in epigenetic processes [106]; (2) Gut metabolites serve as indispensable substrates of human epigenetic modifications. For example, folate and choline were reported both to be methyl donors [107,108], which played a significant role in DNA and histone methylations. Young women aged 20–30 with low folate or choline diet had reduced global DNA methylation in white blood cells [109]. Beside folate intake, gut microbiota produce folate which are absorbed by host colon, thus having a strong impact on the level of host folate [110]. Ingestion of probiotic Bifidobacterium led to a significant increase of folate concentration in human feces [111]. Studies showed that choline metabolism was also in control of gut
microbiota [112,113]. Taken together, compositional or functional changes of the methyl group metabolism related microbiota may disturb immune responses through altering methylation level of immune cells. Gut microbiota are also important source of acetyl donors [106].

The short ncRNA and epigenetic enzymes produced by microbiome can even be secreted out of the bacterial cells to modulate host gene expression. HTS analysis revealed that most extracellular RNA components of Escherichia coli was in the size range between 15 and 40 nucleotides and stemmed from specific intracellular RNAs which have important functions [114]. Surprisingly, not only could these extracellular RNA pair with bacterial genome, they were also able to align with human genome in regions of 14 different chromosomes. Interestingly, most of the matched regions were histone marks, DNAseI hypersensitivity clusters or intronic regions with elevated transcription levels in different cell lines [115]. These findings indicated that the extracellular short chain RNAs could probably act as miRNAs to regulate human gene expressions. An analogous study found that ncRNAs produced by Escherichia coli were capable of regulating host gene expressions, with mechanism similar to RNA interference [116]. Mycoplasma CG- and GATC-specific DNA methyltransferases could translocate into host nucleus as well and selectively methylated the host genome [117].

The interplay between microbiome and human epigenetics is bidirectional, that is, human epigenetic changes can in turn result in dysbiosis. miRNAs exist in host feces in the form of extracellular vesicles, and they can enter bacterial cells and specifically regulate bacterial gene expression. Neonatal mice failed to develop conventional gut microbiota when their Dicer, the cleaving enzyme needed for miRNA formation, were knocked out [118]. After IEC-specific deletion of HDAC3, decrease of basal expression of antimicrobial defense related genes, large loss of Paneth cells of gut wall, and the alteration of gut microbiota composition were observed. However, re-derivation of those mice into GF conditions showed that the changes mentioned above were largely restored in the absence of commensal microbiota. The findings indicated that the gut immune disorders resulting from a loss of HDAC3 in IECs were mediated by commensal microbiota [119]. Even microbiome themselves have similar mechanisms of epigenetic regulation, which have important influence on microbial gene expression. Methylation at a specific recognition site of self-genome prevent the cleavage by the cognate restriction enzyme in bacteria, whereas nonmethylated foreign DNA are cleaved. In this sense, DNA methylation act as a protective immune system in bacteria [120]. The role of epigenetic modiﬁcation regulated by microbiome in the occurrence and development of human diseases is getting increasing interest (Table 4). For instance, a food contaminating mycotoxin named ochratoxin A can trigger autism-like disorder via miRNA modulation in predisposed male individuals [122]. Moreover, DNA hypomethylation of arginine vasopressin promoter in the medial amygdala caused by toxoplasma gondii infection led to behavior changes in male rats [123]. Incubations of human fecal slurry with pectin revealed a large amount of butyrate in the fermentation supernatants, and co-culture of the supernatants

### Table 3

Epigenetic changes and immune function manifestations in mice with gut microbial alterations.

<table>
<thead>
<tr>
<th>Epigenetic change</th>
<th>Detected gene/cell/site</th>
<th>Related microbial condition</th>
<th>Immune function manifestation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA hypomethylation</td>
<td>CxxC16 gene in colon and lung tissue</td>
<td>Colonization of neonatal GF mice with conventional microbiota</td>
<td>Diminished mucosal iNKT cells, ameliorated pathology of IBD and allergic asthma</td>
<td>Olszak 2012 [27]</td>
</tr>
<tr>
<td>DNA hypermethylation</td>
<td>TLR4 gene in IECs PBMCs, DCs, macrophages and Treg cells</td>
<td>Gut microbiota presence SCFAs presence</td>
<td>Fulfilled immune tolerance Promoted anti-inflammation</td>
<td>Takahashi 2011 [96]</td>
</tr>
<tr>
<td>Histone acetylation</td>
<td>histone H4 of IL12b promoter in macrophages</td>
<td>Gut microbiota presence</td>
<td>Suppressed IL-12 p40 production of macrophage and fulminated immune tolerance</td>
<td>Kobayashi 2012 [97]</td>
</tr>
<tr>
<td>Histone deacetylation</td>
<td>H3K4me3 in DCs</td>
<td>GF condition</td>
<td>Downregulation of many inflammatory factors, and compromise of NK cell priming and antiviral immunity</td>
<td>Ganal 2012 [98]</td>
</tr>
<tr>
<td>miRNA</td>
<td>ileum and colon tissue</td>
<td>Colonization of GF mice with the microbiota from pathogen-free mice</td>
<td>Downregulation of ABCG3 in murine macrophages and dysregulation of the metabolism of xenobiotics and endogenous toxins</td>
<td>Dalmasso 2011 [99]</td>
</tr>
<tr>
<td>miRNA</td>
<td>Caecum tissue</td>
<td>GF condition</td>
<td>Alteration of Intestinal immuno-barrier function and homeostasis</td>
<td>Singh 2012 [100]</td>
</tr>
<tr>
<td>IncRNA</td>
<td>Intestinal tissue</td>
<td>IncRNA discriminated different gut microbes</td>
<td>Not studied yet</td>
<td>Liang 2015 [101]</td>
</tr>
</tbody>
</table>

GF: germ-free; iNKT: invariant natural killer T cell; IBD: inflammatory bowel disease; IEC: intestinal epithelial cell; PBMC: peripheral blood mononuclear cell; DC: dendritic cell; Treg: regulatory T cell; SCFA: short-chain fatty acid; NK: natural killer cell; miRNA: microRNA; lncRNA: long non-coding RNA.
Table 4
Other diseases in which crosstalk between dysbiosis and epigenetic modifications might be involved.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Epigenetic modification and microbial changes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon tumor</td>
<td>Administration of prebiotic Bifidobacterium and Lactobacillus decreased histone acetylation, increased DNA methylation, and reduced IBD-triggering factors activation; AIEC infection up-regulated miR-30C and miR-130A in human intestinal epithelial T84 cells and mouse enterocytes, leading to reduction of autophagy-related proteins and enhancement of inflammatory responses.</td>
<td>Ghadimi 2012 [125]</td>
</tr>
<tr>
<td>IBD</td>
<td>The pathological changes and gut dysbiosis in virus-induced LEW1.WR1 rat model could be reversed by HDAC inhibitors; The lipolytic activity of 3T3-L1 adipocytes was related to the HDAC inhibitory function of SCFAs.</td>
<td>Nguyen 2014 [127]</td>
</tr>
<tr>
<td>T1D</td>
<td>The pathological changes and gut dysbiosis in virus-induced LEW1.WR1 rat model could be reversed by HDAC inhibitors; The lipolytic activity of 3T3-L1 adipocytes was related to the HDAC inhibitory function of SCFAs.</td>
<td>Hara 2014 [128]</td>
</tr>
<tr>
<td></td>
<td>The lipolytic activity of 3T3-L1 adipocytes was related to the HDAC inhibitory function of SCFAs.</td>
<td>Rumberger 2014 [129]</td>
</tr>
</tbody>
</table>

HDAC: histone deacetylase; IBD: inflammatory bowel disease; AIEC: adherent-invasiveEscherichia coli; T1D: type 1 diabetes mellitus; SCFA: short-chain fatty acid.

Fig. 2. Mechanisms of gut microbiota regulation of human epigenome and immune responses. Homeostasis (left-hand side), the gut microbiota and their metabolites have critical roles in modulating human epigenetic modifications and the development of intestinal immunity. Gut microbiota convert undigested dietary components into various metabolites, for example, short-chain fatty acids (SCFAs), which have broad effects on host immune system through various G protein-coupled receptors (GPCRs) to inhibit histone deacetylases (HDACs) within peripheral blood mononuclear cells (PBMC), phagocytes and dendritic cells. SCFAs promote gut homeostasis by enhancing epithelial barrier function and immune tolerance. The mechanisms include suppression of the production of nuclear factor-kB (NF-kB) and TNFα; promote differentiation of naïve immune cells into regulatory T (Treg) cells and B (Breg) cells; increase the expression of fork head box P3 (FoxP3) and the production of anti-inflammatory cytokines IL-10. Beneficial gut microbiota also affect immune related genes by producing ncRNAs and epigenetic enzymes to interfere gene expression or metabolites serve as indispensable substrates of human epigenetic modifications; Autoimmune disease (right-hand side), alterations in the composition of gut microbiota (termed as dysbiosis), e.g. suppression of probiotics and overgrowth of harmful pathogenic bacteria, are observed. Dysbiosis leads to imbalanced immune status through insufficient production of immune protective metabolites and/or invasion of pathogenic bacteria and accumulation of their metabolites. Altered gut microbiota results in possible impaired epithelial barrier function; decreased methylation level and diminished inhibition of HDACs; increased production of NF-kB and TNFα; hyperactivation of T helper 1 (Th1), Th17 cells Th1 cells and secretion of inflammatory cytokines. The detailed mechanisms of how host immune system affect microbiota via epigenetic modification remains to be explored.

with several colon tumor cell lines showed very strong HDAC inhibitory activity in the nuclear extracts of the cell lines [124], suggesting that HDAC inhibitory function of microbial metabolites had a great effect on colon tumor cells. Although some argued that human microbiome may also be responsible for producing epigenetic changes in SLE [125], no direct evidence has yet been provided. There were already several studies addressing the relationship between dysbiosis and epigenetic changes in some
AIDs, including IBD [126,127] and T1D [128,129]. LPS-induced and NF-kB-mediated activation of IBD-causing factors (IL-17, IL-23 and CD40) in IECs were diminished by the administration of prebiotic Bifidobacterium and Lactobacillus, and the decrease of histone acetylation as well as slightly increase of DNA methylation were also found, without knowing whether these epigenetic changes participated in the above mechanism [126]. Adherent-invasive Escherichia coli (AIEC) infection up-regulated miR-30C and miR-130A in human intestinal epithelial T84 cells and mouse enterocytes through activation of NF-kB, leading to reduction of autophagy-related proteins and enhancement of intracellular AIEC as well as inflammatory responses. More importantly, an inverse correlation between the level of miR-30C and miR-130A and these autophagy-related proteins was also found in the ileal biopsy samples of patients with Crohn’s disease, suggesting that the role of human microbiome regulation of miRNA expression and autophagy might be crucial in IBD pathogenesis [127]. As for T1D, the pathological changes and gut dysbiosis in virus-induced LEW1. WR1 rat model could be reversed by HDAC inhibitors [128]. In addition, the lipolytic activity of 3T3-L1 adipocytes was shown to be in relation with the HDAC inhibitory function of SCFAs [129]. Although the association between microbiome and epigenetics in AIDs such as T1D has been strongly suggested, potent evidences are still needed for the causality.

5. Conclusion and prospect

In conclusion, human microbiome is closely associated with host immune system. Growing evidence has suggested that the compositional and functional changes of human microbiome are capable of regulating the development and function of immune system via epigenetic mechanisms, which may break the immune homeostasis and finally result in the development of AIDs (Fig. 2). By integrating the analysis of multi-omics of microbiome and epigenome, we could explore the interaction between human immune system and microbiota, and thereby unmasking specific and more sensitive biomarkers as well as potential therapeutic targets. Future studies aiming at the crosstalk between human dysbiosis and epigenetic modifications and their influences on AIDs will facilitate our understanding and better managing of these debilitating AIDs.

Competing financial interests

The authors declare no competing financial interests.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (81325019, 81630044, 81273312, 81601432, 81550023), the National Science Fund for Distinguished Young Scholars of China (813250046), National Key Research and Development Program: “Precise Medical Research” (2016YFC0903900), and CAMS Innovation Fund for Medical Sciences (2016-12M-1-003).

References


