

## The metabolic role of the gut microbiota in health and rheumatic disease: mechanisms and interventions

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**Abstract** | The role of the gut microbiome in animal models of inflammatory and autoimmune disease is now well established. The human gut microbiome is currently being studied as a potential modulator of the immune response in rheumatic disorders. However, the vastness and complexity of this host–microorganism interaction is likely to go well beyond taxonomic, correlative observations. In fact, most advances in the field relate to the functional and metabolic capabilities of these microorganisms and their influence on mucosal immunity and systemic inflammation. An intricate relationship between the microbiome and the diet of the host is now fully recognized, with the microbiota having an important role in the degradation of polysaccharides into active metabolites. This Review summarizes the current knowledge on the metabolic role of the microbiota in health and rheumatic disease, including the advances in pharmacomicrobiomics and its potential use in diagnostics, therapeutics and personalized medicine.

The human body harbours approximately  $10^{14}$  microbial cells, which account for most of the DNA content present within our bodies; the proportion of DNA from *Homo sapiens* origin is estimated to be <1% of this genomic material<sup>1</sup>. The gut microbiome, which is defined as the total genomic content of the complex microbial communities (the microbiota) inhabiting the gastrointestinal tract, has a critical role in the steady-state immune homeostasis of the host<sup>2</sup>. Importantly, alterations in the microbiome have been associated with a variety of intestinal and extra-intestinal disorders, including cardiovascular disease, obesity, multiple sclerosis and inflammatory arthritis<sup>3–8</sup>. The evidence linking the microbiome and human disease is rapidly expanding.

In addition to expressing microorganism-associated molecular patterns (MAMPs), which are directly sensed by pattern-recognition receptors to modulate the immune response, the gut microbiota secrete large amounts of hormone-like metabolites (molecules <1 kDa)<sup>9</sup>. Metabolomics approaches (BOX 1), which enable the systematic study of microbial and host metabolites (that is, the metabolome), promise to unravel key pathways modulating inflammatory diseases in the near future.

In this Review, we provide an up-to-date overview on how changes in the composition of intestinal microbial communities (caused by diet, intake of probiotics or antibiotics, for example) might have an important role in health and in the pathogenesis of rheumatic

diseases via changes in the metabolome. Although other mucosal sites and their microbiomes are of relevance to the understanding of rheumatic disease pathogenesis, here we focus on the intestine as it is by far the largest human bioreactor and also because the majority of the studies in autoimmunity in the context of the microbiome have been focused on this organ. Similarly, the roles of the virome and mycobiome in health and disease are intriguing, but beyond the scope of this Review. We also discuss insights gained in the past few years into how specific metabolites produced by gut bacteria (rather than taxonomic differences in the microbiota) can modulate host immunity. Finally, we examine the novel field of pharmacomicrobiomics (drug–microbiome interactions) and its potential therapeutic applications.

### The gut microbiota and its metabolites

The human intestinal microbiome has three fundamental roles. First, it helps the mammalian intestinal immune system to mature and has an important protective role in the defence against invading pathogens (which explains why two-thirds of human immune cells reside in the lamina propria). In fact, animals raised in germ-free environments fail to achieve features of normal immune maturation<sup>10</sup>. Second, gut microbial communities provide nutritional support to their host, as they have unique capabilities to synthesize essential vitamins and amino acids that maintain mammalian health.

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**Key points**

- Emerging data implicate the human microbiome in the pathogenesis of inflammatory arthritides
- Mucosal sites exposed to high load of bacterial antigens (i.e. gut) may represent the initial site of tolerance break in rheumatoid arthritis, psoriatic arthritis and related diseases
- Microbial and dietary metabolites (e.g. SCFAs and MCFAs) have immunomodulatory properties that could be exploited for the treatment of rheumatic disorders
- Pharmacomicrobiomics is a novel field of research that investigates the effect of variations within the human microbiome on drugs and could facilitate precision medicine in cancer and autoimmunity

Last, the intestinal microbiota has a vital metabolic function, as exemplified by its unique enzymatic capability of degrading complex polysaccharides derived from the diet. While our gut commensals extract nutrients from food we ingest to guarantee their survival, they concomitantly generate by-products that ultimately maintain local gut homeostasis and prevent systemic inflammatory responses.

These metabolites of the gut microbiota can be grouped into several major categories according to their biological functions. These include short-chain and medium-chain fatty acids (SCFAs and MCFAs, respectively) produced by fermentation of dietary fibres and carbohydrates; secondary bile acids converted from primary bile acids; metabolites generated from meat-derived choline and L-carnitine; and other lipids including conjugated fatty acids and cholesterol (reviewed previously<sup>11</sup>). In addition, the gut microbiota contributes to the production of vitamins K and B12, and folate<sup>12</sup>, as well as to the production of indole derivatives (for example,  $\gamma$ -aminobutyric acid), which affect the levels of brain-derived neurotropic factor in the central nervous system<sup>13</sup>.

Multiple lines of investigation indicate that the gut microbial metabolome profoundly affects normal physiology and disease (FIG. 1). In rheumatic diseases, this influence can either be direct, through interference with

the biology of dendritic cells, macrophages and T cells, or indirect, through modulation of energy metabolism and induction of obesity, a risk factor for rheumatoid arthritis (RA), psoriatic arthritis (PsA) and osteoarthritis<sup>14–16</sup>. Alterations in dietary habits and/or manipulation of the intestinal microbial communities could indirectly alter the downstream production and concentration of a variety of metabolites, which, in turn, could exert local and systemic immune-modulating effects.

**SCFAs and MCFAs**

The role of SCFAs (for example, acetate, butyrate and propionate) has been studied in inflammation and immune-mediated diseases. SCFAs act via two principal mechanisms: by signalling through G protein-coupled receptors (GPCRs) (for example, GPR43, GPR41 and GPR109A), and by inhibiting histone deacetylases (HDACs) and thus permitting gene transcription<sup>17</sup>. Studies in mice show that altering the composition of the intestinal microbiota with antibiotics at birth (that is, within a critical developmental window) has long-lasting metabolic consequences<sup>18</sup>. These consequences include reduced bone mineral density and increased body fat mass<sup>18</sup>, which are linked to the metabolic syndrome. The effects of the gut microbiota on the harvest of energy from the diet (that is, absorption of SCFAs acetate and propionate and thus caloric extraction from the diet) and on host adiposity are mediated by GPR41 (REF. 19). In this context, GPR41-deficient mice extract fewer calories from the diet and excrete more acetate and propionate<sup>19</sup>.

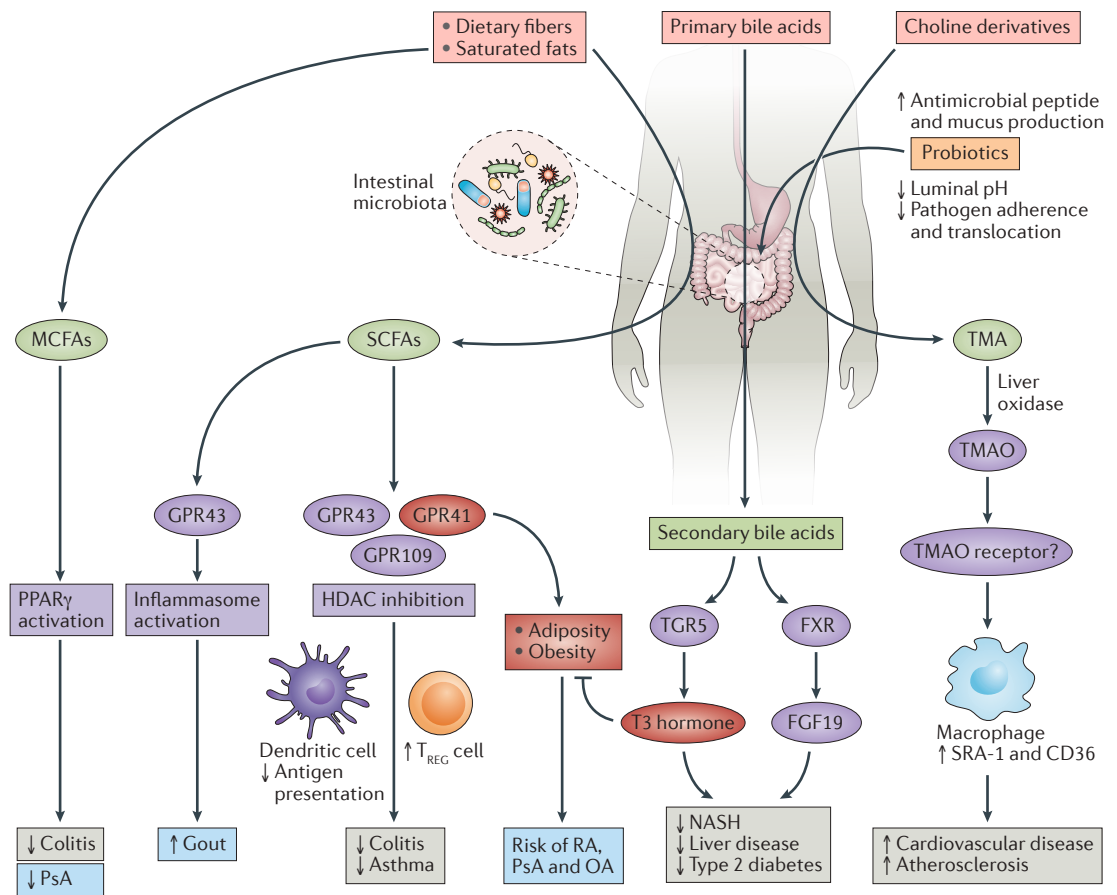
Acetate, produced by fermentation of fructose by Bifidobacteria, protects mice from lethal intestinal infections and stimulates the colonic epithelium to improve epithelial integrity<sup>20</sup>. Butyrate is also a direct substrate used by the colonic epithelium to provide energy<sup>21</sup>. The expression of GPR43 and GPR109A receptors in non-haematopoietic, probably colonic epithelial cells is required for the protective effects of SCFAs against colitis in mice. These protective effects are mediated by activation of the NLRP3 inflammasome and caspase-1, which promotes production of mature IL-18 (REF. 22). These observations are of potential clinical relevance, as SCFA-producing probiotics or even metabolites that activate GPR43 could possibly be delivered to the colon to exert their immunoprotective effects.

Importantly, butyrate induces the differentiation of regulatory T ( $T_{reg}$ ) cells *in vitro* and in the colonic lamina propria *in vivo*, and ameliorates the development of colitis induced by adoptive transfer of CD4<sup>+</sup> CD45RB<sup>hi</sup> T cells in immunocompromised mice<sup>23–25</sup>. Induction of  $T_{REG}$  cell differentiation occurs via signalling through GPR43 expressed by  $T_{REG}$  cells and through increased acetylation of histone H3 at the *Foxp3* locus<sup>23–25</sup>. Propionate also induces  $T_{REG}$  cells to some extent. However, whether acetate, which does not inhibit HDACs, has a similar effect on  $T_{REG}$  cell differentiation remains controversial<sup>23–25</sup>.

GPR43-deficient mice have exacerbated colitis, which indicates a critical role for this receptor in regulating inflammation<sup>26</sup>. GPR43-deficient animals, similar to germ-free wild-type mice, have exacerbated arthritis induced by transfer of arthritogenic serum derived from

**Box 1 | Approaches to metabolomics**

Metabolomics studies either use an untargeted approach to comprehensively profile all metabolites present in a sample, and potentially discover previously uncharacterized metabolites, or use a targeted approach to accurately quantify a pre-defined subset of metabolites. Metabolomic phenotyping relies on either nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry, usually in combination with liquid or gas chromatography<sup>121,122</sup>. Mass spectrometry is generally more sensitive than NMR, enabling detection of metabolites with very low concentrations. NMR is more expensive than mass spectrometry and requires larger sample volumes, but enables fast detection of polar metabolites and is nondestructive to the sample<sup>121,122</sup>. NMR or mass spectrometry spectra enter a workflow involving peak detection and deconvolution, and compound quantification and identification, followed by complex statistical analyses and comparisons with exhaustive databases of metabolites and metabolic pathways. Furthermore, web-based libraries of predefined metabolite sets covering various pathways, disease states and biocompartments can be used to identify patterns of metabolite changes in a biologically meaningful context<sup>123</sup>. Advances in analytical modalities and data analysis in metabolomics promise to have a great effect on the development of personalized approaches to disease diagnosis and treatment, and on drug discovery and development by the pharmaceutical industry<sup>122,124</sup>.



**Figure 1 | Immune and disease-modulating capabilities of intestinal microbial metabolites and probiotics.** The intestinal microbiota converts dietary fibres into short-chain and medium-chain fatty acids (SCFAs and MCFAs), primary bile acids into secondary bile acids, and choline derivatives into trimethylamine (TMA). SCFAs act through various G protein-coupled receptors (GPCRs) to inhibit histone deacetylases (HDACs) and alter the biology of regulatory T (T<sub>REG</sub>) cells and dendritic cells or to activate the inflammasome. Secondary bile acids activate the transmembrane GPCR TGR5 and the farnesoid X receptor (FXR), inducing the T3 thyroid hormone and fibroblast growth factor 19 (FGF19), respectively. These pathways and end products modulate a variety of inflammatory, metabolic and autoimmune diseases. Probiotics support the host's immune system, enhance intestinal barrier function and limit enteric pathogens. The dark blue colour boxes highlight rheumatic diseases that might be affected by the intestinal microbiota. These diseases include gout, psoriatic arthritis (PsA), rheumatoid arthritis (RA) and osteoarthritis (OA). NASH, nonalcoholic steatohepatitis; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; SRA-1, scavenger receptor A-1; TMAO, TMA N-oxide.

K/BxN mice. The K/BxN serum-transfer arthritis can be partly inhibited by oral administration of acetate<sup>26</sup>. The observation of exacerbated serum-transferred arthritis in germ-free mice is clearly distinct from findings in T-cell-mediated mouse models of arthritis, in which germ-free mice are protected<sup>3,5</sup>. In contrast to mice with K/BxN serum-transferred arthritis, however, GPR43-deficient mice have reduced severity of gouty arthritis induced by intra-articular injection of monosodium urate (MSU) crystals, owing to microbiota-dependent acetate-induced inflammasome activation and IL-1 $\beta$  production<sup>27</sup>. Intriguingly, butyrate specifically inhibited class I HDACs and decreased production of IL-1 $\beta$ , IL-6 and IL-8 induced by MSU crystals and palmitic acid in peripheral blood mononuclear cells from healthy donors and patients with gout<sup>28</sup>. Taken together, these data

provide compelling evidence for an arthroprotective role of SCFAs. Our and other research groups are currently investigating the effects of various SCFA formulations on the amelioration of inflammatory arthritis and skin disease in animal models.

The dietary sources, microbial degradation pathways and mechanistic effects of MCFAs remain insufficiently studied. MCFAs have known antibacterial properties and seem to activate peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ )<sup>29</sup>, which ameliorates colitis in animal models<sup>30</sup> and human Crohn disease<sup>31</sup>. The relevance of MCFAs in rheumatic disease remains to be elucidated, although our and other research groups have observed decreased levels of MCFAs in the intestinal lumen of patients with psoriasis<sup>32</sup> and inflammatory bowel disease (IBD)<sup>33</sup>, and *HLA-B27* transgenic rats<sup>34</sup>.

### Bile acid metabolites

The key role of bile acids in liver injury and regeneration, diabetes and other metabolic diseases is increasingly being appreciated<sup>35</sup>. Bile acids exert their effects through two main receptors: farnesoid X receptor (FXR) and the transmembrane GPCR TGR5. In the liver and intestine, FXR controls several important metabolic pathways and activation of this receptor results in reduction of bile acid synthesis, lipogenesis and gluconeogenesis, while hepatocyte regeneration is enhanced<sup>36</sup>. Fxr-deficient mice spontaneously develop hepatocellular carcinoma and other degenerative features in the liver<sup>37</sup>. Many important actions of FXR are mediated through the enterokine fibroblast growth factor 19 (FGF19), which signals through FGF receptor 4 on hepatocytes<sup>38</sup>. TGR5 signalling in skeletal muscle and brown adipose tissue regulates metabolism and energy homeostasis via activation of cAMP-dependent iodothyronine deiodinase 2, which converts the thyroid hormone T4 to the active thyroid hormone T3 intracellularly, thereby decreasing blood glucose levels and increasing energy expenditure<sup>39</sup>. In Kupffer cells and macrophages, TGR5 activation inhibits lipopolysaccharide-induced cytokine production<sup>40</sup>. Not surprisingly, agonists of bile acid receptors — particularly FXR — are currently being tested as drug candidates for the treatment of nonalcoholic steatohepatitis (NASH) and hypercholesterolaemia<sup>41</sup>.

Primary bile acids entering the intestinal lumen undergo deconjugation, dehydroxylation, epimerization, and oxidation by bacterial enzymes such as 7 $\alpha$ -dehydroxylase<sup>35</sup>. These modifications affect the solubility of bile acids as well as their binding affinity to FXR and thus affect the metabolic pathways discussed above<sup>35</sup>. Therefore, the modulation of the gut microbiota could be a strategy for personalized management of NASH<sup>42,43</sup>. The microbiota of humans with obesity contains a decreased proportion of Bacteroidetes compared with that of lean individuals<sup>44</sup>. In addition, the severity of NASH is associated with a specific pattern of gut microbial dysbiosis<sup>45</sup>. These perturbations in the gut microbiota affect bile acid metabolism, for instance by altering intestinal 7 $\alpha$ -dehydroxylating bacteria<sup>35</sup>. This pattern of dysbiosis could lead to Toll-like receptor 4 activation and low-grade hepatic inflammation and the metabolic syndrome<sup>46–49</sup>. Given that adiposity, obesity and the metabolic syndrome are risk factors for some rheumatic diseases, bile acid metabolism by the gut microbiota is likely to represent a relevant pathway. However, research in this area is still awaited.

### Choline and L-carnitine metabolites

The gut microbiota converts dietary choline and L-carnitine to trimethylamine (TMA), which is further metabolized by the flavine monooxygenase system in the liver to produce the toxic metabolite TMA-N-oxide (TMAO)<sup>50,51</sup>. Choline metabolites generated by the gut microbiota are associated with NASH and cardiovascular disease<sup>50–53</sup>. Plasma levels of TMAO are correlated with an increased risk of major adverse cardiovascular events in humans<sup>54</sup>. Mechanistically, dietary choline and TMAO upregulate the expression of scavenger receptors CD36 and SRA-1

on macrophages to promote foam cell formation, and also reduce macrophage reverse cholesterol transport<sup>51,53</sup>. These observations suggest a potential causative relationship between choline metabolites generated by the microbiota and the pathogenesis of atherosclerosis and cardiovascular disease. This may be relevant in light of the increased risk of cardiovascular disease in patients with rheumatoid arthritis. However, no data on TMAO levels in patients with rheumatic diseases is available yet.

### Metabolic modulation by microbiota

#### Diet

The notion that certain dietary habits could prevent or ameliorate rheumatic diseases is an ancient one. Fermented fish oil, for example, is considered to be the civilized world's first health elixir<sup>55</sup>. Roman soldiers are also said to refuse to march without their daily ration of *garum* (which was made of cod liver oil). Multiple epidemiological studies have interrogated the therapeutic properties of a variety of diets and their potential effects on inflammatory arthritis<sup>56–59</sup>. The majority of these studies have been focused on RA (TABLE 1). Diet could affect RA and other inflammatory diseases by altering the composition of the microbiome and the downstream production of immune-modulating metabolites.

The Mediterranean diet consists of high amounts of fruit, vegetables, whole grains, fish and olive oil, low amounts of red meat and moderate amounts of alcohol. Multiple randomized controlled trials (RCTs) have shown a Mediterranean diet intervention has beneficial effects on inflammation and physical function among patients with existing RA when compared with a Western diet<sup>60,61</sup>. However, a prospective analysis that used data from the Nurses' Health Study showed no significant association of a Mediterranean diet with the risk of incident RA in women<sup>62</sup>. Curiously, modest associations were observed between increased legume intake and higher risk of developing RA, and between long-term moderate alcohol drinking and reduced risk of developing RA<sup>63</sup>. Beneficial effects were also found to be associated with an increased fatty acid intake but were not associated with the levels of plasma antioxidants<sup>64</sup>. The plasma levels of vitamin C, retinol and uric acid (all reportedly antioxidants enriched in Mediterranean diets), however, were inversely correlated with RA disease activity<sup>65</sup>. Noticeably, vegetarian and Mediterranean diets have been linked to increased production of health-promoting SCFAs<sup>66,67</sup> which, as discussed, prevent the activation of effector T cells and the occurrence of undesirable local and systemic inflammatory responses<sup>23–25</sup>. Whether or not the benefits of Mediterranean-type diets in RA can be explained by alterations in gut microbiota, leading to a SCFA-driven immune-modulation, remains to be elucidated<sup>67</sup>.

In a population-based study in Sweden, intake of oily fish was associated with a modestly decreased risk of developing RA<sup>68</sup>. Similar population-based studies also supported the hypothesis that oil-derived omega fatty acids could help prevent RA<sup>69,70</sup>. A dose–response meta-analysis, for instance, showed a non-statistically

Table 1 | Dietary studies in rheumatoid arthritis and psoriatic arthritis

Disease	Study design	Dietary intervention	Outcome	Refs
RA	Population-based	Oily fish consumption	Modest decrease in RA incidence	68
RA	Dose-response meta-analysis	Fish consumption	Non-statistically significant inverse association between fish consumption and RA	57
RA	Population-based	Alcohol consumption	Moderate consumption was inversely correlated with RA incidence	58
RA	Dose-response meta-analysis	Alcohol consumption	Low to moderate consumption was inversely correlated with RA incidence	59
RA	Prospective	Alcohol consumption	Moderate consumption was inversely correlated with RA incidence in women	64
RA	Prospective	Mediterranean diet	No effect on RA incidence	63
RA	RCT	Mediterranean diet	Functional improvement and decreased disease activity	62
RA	RCT	Mediterranean diet	Decreased disease activity	61
PsA	Prospective	Weight loss (Mediterranean diet)	≥5% of weight loss predicted achievement of minimal disease activity in patients treated with TNF inhibitor	60

PsA, psoriatic arthritis; RA, rheumatoid arthritis; RCT, randomized controlled trial.

significant inverse association between fish consumption and RA<sup>56</sup>. Other studies have also shown fish oil has beneficial effects in patients with RA, both in established disease<sup>71</sup> and as an adjuvant intervention to triple-DMARD therapy in early RA<sup>72</sup>. The inverse association between fish consumption and RA has been mainly attributed to the abundance of long-chain n-3 polyunsaturated fatty acids (PUFAs) in fish<sup>73</sup>. Indeed, two members of the n-3 PUFA family, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are metabolized to competitive inhibitors of n-6 PUFAs (precursors of prostaglandins and leukotrienes) and suppress the production of inflammatory cytokines<sup>74</sup>. These effects may explain, at least partially, the role of fish oil consumption and n-3 PUFAs in ameliorating systemic inflammation<sup>75</sup>. Studies in animal models have suggested that dietary extra-virgin olive oil prevents collagen-induced arthritis via downregulation of TNF, IL-17 and matrix metalloproteinases<sup>76</sup>, which is perhaps due to the effects of phenolic compounds such as hydroxytyrosol that are present in olive oil<sup>77</sup>. Another intriguing explanation for the inverse association between fish and olive oil and RA derives from studies showing that the microbiota from animals fed a diet rich in saturated fats led to TLR-driven inflammation via production of CCL2, a mediator of macrophage chemotaxis. Strikingly, the gut microbiota of mice fed with fish oil promoted anti-inflammatory responses when transplanted into obese animals, which suggests a possible biologic mechanism for saturated lipid-induced, microbiota-driven modulation of metabolic inflammation<sup>78</sup>.

Findings from several studies suggest that increased consumption of fruits and vegetables is associated with reduced risk of RA<sup>69</sup>. This effect is perhaps mainly due to the antioxidant content of fruits and vegetables. The large prospective Iowa Women's Health Study did not show a significant association between fruit intake and the risk of RA, but did show a modest inverse association between consumption of cruciferous vegetables and the risk of this disease<sup>79</sup>.

Studies correlating red meat intake and RA have been conflicting. A prospective study found that a high intake of meat was associated with an increased risk of RA<sup>80</sup>, but the Nurses' Health Study found no evidence of such an association<sup>81</sup>. The potential link between red meat consumption and RA has been proposed to be mediated by iron, which is present at high levels in red meat and has been shown to accumulate in rheumatoid synovial membranes and aggravate inflammation<sup>82</sup>.

The mechanisms by which dietary habits could modulate RA disease activity remain a matter of debate. A monoclonal anticitrullinated peptide antibody (ACPA; CCP-Ab1) derived from a patient with RA has been reported to crossreact with various autoantigens but also with numerous plant and microbial proteins<sup>83</sup>. This finding suggests that multiple environmental factors, including microorganisms and diet, might trigger the generation of ACPAs that then crossreact with various citrullinated autoantigens through molecular mimicry to induce RA.

Beyond the known association between weight loss and improvement of psoriatic disease outcomes, studies looking at the effects of specific diets in other autoimmune rheumatic diseases are either lacking or very small to provide significant conclusions. It is conceivable that dietary interventions (that is, Mediterranean diet) in related disorders (for example, spondyloarthritis) will have similar beneficial effects as in RA, although more work is needed to address this possibility.

### Probiotics and prebiotics

The composition of the gut microbiota could be modulated through the use of probiotics containing living bacteria or prebiotics containing non-digestible supplements that promote the growth of selected bacteria. Several studies have investigated the potential benefits of probiotics in RA and allied conditions. A RCT in patients with RA found that *Lactobacillus casei* significantly decreased disease activity when compared with placebo, and also lowered the levels of TNF, IL-6 and IL-12 while raising the levels of IL-10 (REF. 84). A prior pilot study of administration of *L. casei* to patients with RA reported a similar effect<sup>85</sup>. However, administration of other *Lactobacillus* strains was not efficacious in another small study involving patients with this disease<sup>86</sup>. By contrast, a study in which mice deficient for the IL-1 receptor antagonist were used as a model of spontaneous arthritis, our research group showed that the *Lactobacillus* genus was sufficient to trigger disease when the bacteria were given to germ-free animals<sup>3</sup>. The evidence for a role of probiotics in the prevention of *Clostridium difficile* colitis<sup>87</sup> and treatment of Crohn disease or ulcerative colitis<sup>88</sup> remains

similarly controversial. The reasons for this uncertainty are likely to be multifactorial, and include the possibilities that ingested microorganisms are destroyed by the low pH of the gastric environment, that probiotics and prebiotics have little or no biologic benefits, and the difficulties in defining mixtures of highly-effective bacterial strains. These issues are also likely to challenge the design and evaluation of similar studies conducted in rheumatic diseases.

### Faecal microbiota transplantation

Faecal microbiota transplantation (FMT), or bacteriotherapy, has been used to reverse dysbiosis by re-establishing the full community of gut microorganisms in a recipient individual by colonoscopy transference of stools from a close healthy donor. FMT has been proven to be highly effective (and even curative) in antibiotic-resistant *C. difficile* infectious (CDI) colitis<sup>89</sup>. In spite of these notable outcomes, the use of FMT has intrinsic challenges, including appropriate donor selection and screening (for example, viral serology), how to define a healthy microbiota, stool preparation and methods of administration<sup>90</sup>. One advance in this area includes the use of oral, capsulized, frozen FMT, an approach that showed encouraging results in an open-label trial involving patients with CDI<sup>91</sup>. FMT has also been applied to the treatment of autoimmune diseases, most notably IBD. Preliminary case reports in patients with Crohn disease or ulcerative colitis were promising, reporting high long-term remission rates<sup>92,93</sup>. However, several subsequent studies in children and adults with IBD showed mixed results. When all studies were combined in a meta-analysis<sup>94</sup>, the clinical remission rate achieved with FMT in patients with IBD was calculated to be ~45%. Young patients and patients with Crohn disease had the best outcomes, with 64.1% and 60.5% of patients achieving clinical remission, respectively. Two RCTs of FMT in patients with IBD patients were reported in the past year. The first RCT revealed a statistically significant difference in remission rates among patients with ulcerative colitis, with 24% and 5% of treated and control patients achieving remission, respectively<sup>95</sup>. The second trial, conversely, showed no significant differences between treated and untreated patients with IBD<sup>96</sup>. Interestingly, however, the microbiota of the responders to FMT therapy was similar to that of their healthy donors in the second study, which suggests that the efficacy of this approach might depend on the colonization capabilities of donor microbial communities.

Despite these encouraging studies, FMT has not yet been used in patients with rheumatic disease. However, the results of the studies in IBD are relevant when discussing potential future applications of this approach in inflammatory arthritides, particularly given the stronger association between SpA and Crohn disease when compared with ulcerative colitis. If better outcomes with FMT are achieved in Crohn disease, it is expected that other extra-intestinal manifestations of SpA would also respond to this approach. Although mechanistic evidence supports the therapeutic potential of FMT, it is not clear whether this strategy could be adapted for

rheumatic diseases. Careful trial design coupled with taxonomic, metagenomic and metabolomic analyses will be essential in this area. To truly understand its potential immune-modulating effects, FMT should first be attempted in previously untreated patients with new-onset disease or in those with advanced disease who are not responding to any other therapy.

### Other potential strategies

Other microbiome-altering therapeutic strategies have been studied. These range from ecosystem-level interventions to single-target approaches that include individual species or their metabolites. Methods that are based on the use of live organisms attempt to modulate intestinal ecology through oral administration of either single strains of bacteria or a defined bacterial consortium, bypassing the need for FMT-dependent techniques. Animal studies have shown successful treatment of IBD-like disease by administration of either *Bacteroides fragilis*<sup>97</sup> or a cocktail of Clostridia<sup>98</sup>, which both induced colonic T<sub>REG</sub> cells. Intriguingly, multiple studies have shown that the efficacy of some anticancer immunotherapies is highly dependent on the presence of specific microbiota<sup>99,100</sup>, which suggests that probiotics containing these strains might be beneficial in the treatment of cancer and perhaps also autoimmune disease.

Other sophisticated approaches exploit bacterial-derived bioactive molecules with immune-modulating properties, including polysaccharides, structural proteins and SCFAs. *B. fragilis*-derived polysaccharide A is sufficient for the induction of T<sub>REG</sub> cells (and concomitant suppression of IL-17 production) in the lamina propria, protecting mice from developing colitis<sup>101</sup>. Similarly, Clostridia-induced butyrate promotes the differentiation of T<sub>REG</sub> cells *in vitro* and *in vivo*, and ameliorates colitis in mice<sup>24</sup>. Nevertheless, the effectiveness, tolerability and long-term effects of these compounds are yet to be elucidated<sup>102</sup>. Multiple pharmaceutical companies, both new and established ventures, have been investing in research and discovery in both pre-clinical studies and clinical trials in the field of microbiomics<sup>103</sup> (TABLE 2). Commercialization of drugs that influence the human microbiota and/or its metabolites, however, will not be free of scientific and regulatory challenges<sup>104</sup>. This is largely due to many unknowns in systems for delivery of bacterial organisms to desirable target intestinal regions, viability concerns and current discussion about whether microorganisms can constitute patentable intellectual property.

### Pharmacomicrobiomics

Within the umbrella concept of precision medicine, the study of drug-microbiome interactions — that is, pharmacomicrobiomics — has gained traction following the launch of the Human Microbiome Project<sup>105</sup>. The overall premise of pharmacomicrobiomics is that intestinal microbial communities could be manipulated to improve drug efficacy and reduce adverse drug reactions.

Gut microorganisms can have an influence on xenobiotics (foreign compounds not normally found within mammals, including therapeutic drugs and diet-derived

Table 2 | Microbiota-based approaches in the pipeline for inflammatory and autoimmune disease

Company	Therapeutic approach and indication (product name)	Development stage	Large Pharmaceutical Partner/s
AvidBiotics	Targeted anti-bacterial non-antibody proteins (Avidocin)	Preclinical	
CIPAC	Standardized approach to FMT for CDI colitis and IBD	Undisclosed	
Enterome	Biomarkers for IBD and NASH based on microbiome signatures (EP-8018)	Preclinical	<ul style="list-style-type: none"> <li>• Danone</li> <li>• Abbvie</li> </ul>
4D Pharma (formerly GT Biologics)	Therapies derived from microbiome molecules for IBD	Preclinical	
Rebiotix Inc.	Prescreened stool offered to health providers for FMT (microbiota restoration therapy) for CDI colitis (RBX2660)	Phase II (FDA Breakthrough Therapy designation)	
Osel Inc.	Single strains of native and genetically engineered bacteria for specific urogenital and gastrointestinal disease (Lactin V)	Phase II	
Second Genome	Application of microbiome science for discovery of new therapies for diseases such as IBD (SGN-1019)	Phase I	<ul style="list-style-type: none"> <li>• Janssen</li> <li>• Pfizer</li> </ul>
Seres Health	Therapeutics to catalyze restoration of a healthy microbiome in CDI colitis (SER-109)	Phase III (FDA Orphan Drug designation)	<ul style="list-style-type: none"> <li>• Nestle</li> <li>• Publicly traded</li> </ul>
Symbiotix	<i>Bacteroides fragilis</i> -derived polysaccharide-A (PSA) for IBD and multiple sclerosis	Preclinical	
Vedanta Biosciences	Immunomodulating therapies based on the human microbiome, such as a Clostridia cocktail for IBD (VE-202)	Phase I/II	Johnson & Johnson

CDI, *Clostridium difficile* infectious; FMT, faecal microbiota transplantation; IBD, inflammatory bowel disease; NASH, non-alcoholic steatohepatitis. Adapted from Olle, B. Medicines from microbiota. *Nat. Biotechnol.* **31**, 309–15 (2013)<sup>104</sup>.

bioactive metabolites) via a few general mechanisms. The first set of such processes involves direct microbial interference with xenobiotics leading to the generation of end products that are different from the original pro-substance or pro-drug. Examples of this type of process include: microbial production of bioactive compounds (for example, bacterial biotransformation of hydroxycinnamates into anti-inflammatory cinnamoyl esterases); microbial detoxification of xenobiotics (for example, digoxin inactivation by the gut commensal bacterium *Eggerthella lenta*<sup>106,107</sup>; and direct binding of microorganisms to xenobiotics (for example, physical attachment of *Helicobacter pylori* to levodopa (L-DOPA), decreasing the bioavailability of the drug<sup>108</sup>). The second set of mechanisms involves indirect effects of host-microbial interactions on xenobiotics. Several examples have been described: enterohepatic cycling of xenobiotics (for example, cleavage of NSAIDs by  $\beta$ -glucuronidases, which has enteropathic effects<sup>109</sup>); alteration of host gene expression in response to microbial colonization (for example, upregulation of hepatic expression of CYP450 genes<sup>110</sup>); production of intermediate metabolites by gut microorganisms (for example, TMA-containing metabolites, as described above)<sup>51,53,54,111</sup> and competition between microbial metabolites and xenobiotics for binding sites in host enzymes (for example, microbial-dependent inhibition of 5-fluorouracyl catabolic enzyme by the antiviral sorivudine<sup>112</sup>).

Pharmacomicrobiomic mechanisms were also revealed for a few analgesic and immunosuppressive drugs. For example, the bioavailability of acetaminophen was markedly associated with the concentration of p-cresol sulfate, a co-metabolite of the human gut microbiota<sup>113</sup>. The gut microbiota was also found to modulate the immune effects of cyclophosphamide<sup>114</sup>. This drug alters the composition of the microbial community of the small intestine of cancer-bearing mice and induces the translocation of Gram-positive bacteria into secondary lymphoid organs, where these bacteria stimulate immune responses driven by T helper 17 (T<sub>H</sub>17) cells and T helper 1 (T<sub>H</sub>1) cells. Under germ-free conditions, however, these animals have reduced T<sub>H</sub>17 responses and their tumours become resistant to cyclophosphamide, which suggests that the gut microbiota can help shape the anticancer (and potentially antirheumatic) immune response to this drug.

The emerging interest in pharmacomicrobiomics<sup>115</sup> has also been extended to the study of other drugs utilized for the treatment of rheumatic diseases. The pro-drug sulfasalazine, the first rationally designed medication for RA, consists of an anti-inflammatory 5-aminosalicylic acid (5-ASA) molecule connected to a sulfapyridine through an N–N double bond. The drug remains inactive until it reaches the distal gut, where azoreductases encoded by the gut microbiome cleave the N–N double bond to release active 5-ASA. Faecal assays

showed full conversion of sulfasalazine to its constituent molecules in conventionally raised animals, whereas germ-free animals excreted the pro-drug<sup>116</sup>.

Short-term exposure to certain xenobiotics substantially affected the physiology and composition of the gut microbiota, and the expression of genes from the gut microbiome<sup>117</sup>. Interestingly, both antibiotics and host-targeted drugs rapidly alter the activity of the gut microbiome to increase the expression of genes involved in drug metabolism, drug resistance and response to stress<sup>117</sup>. In the case of sulfasalazine, the drug induced the expression of microbiome gene clusters annotated as encoding thioredoxins and nitrate reductases<sup>117</sup>. In the past few years, the concept of the microbiome cloud was introduced, which reflects the temporal and spatial uncertainty of defining an individual's microbiome composition, emphasizing the challenges in defining a core human microbiome<sup>118</sup>. Hence, classifying microbiomes into different metabolotypes might be more relevant to functional and pharmacomicrobiomic studies than simpler taxonomic approaches<sup>118</sup>.

This knowledge is of high relevance to the efficacy, resistance and adherence to medications in the rheumatologic clinical setting, because the magnitude of response to drugs such as methotrexate and other oral DMARDs is known to have a high and unpredictable interindividual variability<sup>119</sup>. Differences in the bioavailability and efficacy of therapeutic agents could at least partially be driven by disparities in the prevalence and/or expression of xenobiotic-induced genes that affect

drug metabolism, small-molecule transport and/or the production of protective molecules<sup>120</sup>. Future studies will require advanced bioinformatic tools to accelerate the discovery of drug–microbiome interactions and correlate them with clinical data.

## Conclusions

The local intestinal and systemic immune responses to diet and derived metabolites have been studied for centuries. Over the past decade, technological advances in microbiomics and metabolomics coupled with a renaissance of mucosal immunology studies have led to novel and exciting discoveries. Our growing fundamental knowledge about how diet affects the composition of microbial communities and enzymatic conversion of by-products is changing our understanding of the symbiotic relationship between the microorganisms that populate our body cavities and the biologic responses required for host adaptation. This understanding is being further exploited for diagnostic, biomarker discovery and therapeutic purposes. Much remains to be elucidated, but we have reason to expect an accelerated expansion in the use of big data in metabolomics, metagenomics and pharmacomicrobiomics for personalized medicine in rheumatic and autoimmune diseases. For these efforts to succeed, a comprehensive approach that incorporates researchers from a variety of disciplines, including clinical rheumatologists, immunologists, microbiologists and computational biologists, will be required.

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#### Author contributions

All authors researched data for the article and substantially contributed to discussion of content, writing and review/editing of the manuscript before submission.

#### Competing interests statement

The authors declare no competing interests.